AIDS

and Other Manifestations of HIV Infection

FOURTH EDITION

EDITED BY

Gary P. Wormser

AIDS and Other Manifestations of HIV Infection

Fourth Edition

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AIDS and Other Manifestations of HIV Infections

Fourth Edition

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Preface

In 1981, when AIDS was Þrst recognized, cases were rare and the disease was regarded mostly as a curiosity. By the mid-1980s, the etiologic agent, HIV, had been discovered and partially characterized, allowing the enormity of the pandemic to begin to be appreciated. It became accepted that HIV infection was a chronic illness whose natural history may span more than a decade, with symptoms arising transiently within the Þrst two months for some patients, and during the last few years of life for almost all patients. The most common method of transmission is sexual, with populations who have traditionally been at greater risk for sexually transmitted diseases disproportionately affected. Because of this, it affects a much younger population than do most other chronic diseases.

In the early 1980s, management was relegated to the diagnosis and treatment of the late opportunistic infections. With the discovery and development of antiretroviral therapies, the clinical approach has changed dramatically. HIV-infected persons are now placed under medical supervision as soon as they are diagnosed, with or without symptoms. Equipped with an expanding array of diagnostic tests (e.g. T-cell subsets, HIV viral load testing, HIV resistance testing, *Pneumocystis carinii* direct ßuorescent assays) and an even wider variety of new antiretroviral drugs (reverse transcriptase inhibitors, protease inhibitors, and others), the health care practitioner has more to offer these patients than ever before. Objective reductions in mortality and improvements in quality of life have resulted, which are truly remarkable achievements. However, chronic antiretroviral therapy has posed new challenges in managing drug toxicity, adherence and prevention of the emergence of drug resistance.

The purpose of this book is to chronicle the emerging story of HIV infection and to describe its diverse manifestations and consequences. It is hoped that the Fourth Edition of *AIDS and Other Manifestations of HIV Infection* will provide a comprehensive overview of the biologic properties of the etiologic viral agent, its clinicopathological manifestations, the epidemiology of its infection, and present and future therapeutic and preventive options. Particular importance is given to providing a rigorous scientiÞc foundation as a basis for understanding the pathobiology of HIV.

Chapters have been extensively revised in accordance with the rapidly expanding knowledge base, and are well referenced. The section on clinical manifestations has been expanded in the Fourth Edition and now includes new chapters on the: cardiovascular, renal, and dermatologic manifestations of HIV infection. In-depth discussions of particular clinical issues are principally done on a system-by-system basis for the major systems involved. New chapters have also been added to the Fourth Edition on molecular diagnostic techniques (to the Etiologic Agent Section), the role of host genetic variation in HIV infection and its manifestations (to the Immunology of HIV Infection Section), discovery and development of new HIV medicines, analysis of HIV dynamics using mathematical models, toxicities of antiretroviral therapy, HIV drug susceptibility testing, practical therapeutics, the global impact of HIV and AIDS (to the Treatment and Prevention Section), reßecting the increasing importance of these topics as a basis for improving the care of HIV-infected patients worldwide.

It is my hope and intention that the Fourth Edition of *AIDS and Other Manifestations of HIV Infection* will provide a reference source for essential information needed by members of the healthcare and scientiÞc community, and that it will enable healthcare workers to render the highest level of care possible to their patients.

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DEDICATION

To the memory of John Wormser, Ruth Wormser, and Julius and Martha Lowenstein.

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I

BACKGROUND AND EPIDEMIOLOGY

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AIDS and Other Manifestations of HIV Infection
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Chapter 1

The Epidemiology of HIV and AIDS

Patricia L. Fleming

The emergence and dissemination of a new infectious disease agent worldwide within the span of two decades at the end of the twentieth century has presented unprecedented medical, social, and political challenges. The human immunodeÞciency virus (HIV) and the syndrome of opportunistic illnesses that characterize late-stage HIV disease, known as the acquired immunodeÞciency syndrome (AIDS), have claimed over 20 million lives worldwide and the Joint United Nations Program on AIDS (UNAIDS) estimates that there were more than 40 million HIV-infected persons living worldwide in 2001 (1). In the United States (U.S.), the Centers for Disease Control and Prevention (CDC) estimates that there were 850,000Ð950,000 prevalent cases of HIV/AIDS in 2000 (2). More than 467,000 deaths of persons with AIDS have occurred since the epidemic was Þrst recognized in 1981 (3Ð5). Despite recent successes in treating HIV that have increased survival and substantially decreased death rates (6,7), HIV remains a devastating illness, without cure, that mainly affects young adults. Its social and economic tolls threaten to destabilize some countries in the developing world and it continues to be a costly and controversial disease in the U.S.

Understanding the epidemiology of HIV provides an important foundation for clinicians in recognizing risk behaviors associated with clinical manifestations suggestive of HIV infection in their patients, and encouraging acceptance of HIV testing, adoption of risk-reduction strategies to prevent further transmission, and treatment to prevent opportunistic illnesses and delay disease progression. At the start of the third decade of the HIV pandemic, and following the terrorism of September 11, 2001 and the subsequent bio-terrorism, the government and the public have a heightened awareness of the crucial role of public health preparedness in assuring well-being.

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Disease surveillance, prevention and control are in the headlines. Health care providers have a renewed appreciation of their responsibility to report cases of notiÞable diseases to public health authorities. This chapter reviews the historical context of the epidemic, summarizes the global epidemic, presents HIV/AIDS surveillance data to characterize affected populations, describes trends in the incidence and prevalence of HIV and AIDS, describes clinical manifestations and the impact of treatment, and identiÞes emerging issues that challenge the ability to prevent and control HIV.

BACKGROUND AND CONTEXT

The HIV pandemic is arguably the most compelling public health crisis of the post-World War II generation. In the post-World War II era, infectious diseases were on the wane. Common bacterial infections were readily treated with available antibiotics; ubiquitous childhood diseases (e.g. polio, measles, mumps) were preventable with vaccination; even the scourge of smallpox was eradicated worldwide. The epidemiology and clinical management of chronic diseases such as heart disease and cancers and an emerging interest in environmental health were ascendant public health priorities.

In 1981, with the Þrst reports of what came to be called AIDS, a new awareness of the threat of emerging infectious diseases arose. Within two years, the epidemiologic evidence suggested that a new infectious agent was responsible for cases of unusual opportunistic illnesses, indicative of severe immunosuppression. The pattern of AIDS case reports suggested that this disease agent was likely transmitted through sexual contact (homosexual and heterosexual), sharing of drug injecting paraphernalia, contamination of the blood supply, and perinatally from mother to child (8Ð13). By 1983, a new retrovirus was isolated from AIDS patients and identiÞed as the causative agent (14,15) An antibody test was developed that enabled diagnosing HIV infection early in the disease course and

permitted screening of the blood supply (16). By March of 1985, universal screening of the blood supply, coupled with voluntary donor deferral and heat-treatment of blood components, had virtually eliminated new HIV infections through transfusions or the receipt of blood products by hemophiliacs. During the Þrst decade, the U.S. government and all state and territorial health departments began to conduct AIDS case surveillance to monitor the outcome of HIV infection and its impact on the population. Anonymous serologic surveys of the prevalence of HIV antibodies in some populations were implemented using blood routinely collected for other purposes in a large number of hospitals and clinics serving high-risk clients in diverse geographic settings, and in selected accessible populations such as military applicants and women giving birth. These surveys revealed that HIV was more widespread and more prevalent than AIDS case data suggested (17).

Many warning signs of the potential for the emergence or re-emergence of great pandemics caused by infectious agents were accumulating during the latter half of the twentieth century: increasing population pressures, migration and urbanization, political or social upheaval, and especially in western countries, the sexual revolution and drug-culture. Emerging from obscure African origins, HIV was introduced into susceptible populations in North America and western Europe and spread rapidly during the latter part of the 1970s and early 1980s. In the western countries, its spread was most rapid among homosexual men and drug-injectors. The epidemic had spread worldwide by the late 1980s. In Africa, the epidemic was termed ÒPattern IIÓ, i.e. heterosexually-acquired HIV predominated, as opposed to the homosexual and drug injecting associated epidemics in North America and Western Europe (i.e. ÒPattern IÓ). In Asia, Latin America and the Caribbean, heterosexual and drug-use associated transmission led to rapid spread and recently, a drug-use associated epidemic has emerged in eastern European nations (18,19).

Despite the warning signs of fertile ground for an infectious disease epidemic, HIV continues to confound medical and public health practitioners worldwide. As yet, there is no cure, and no vaccine. The ability to mount effective prevention and control efforts is complicated by social taboos, fear and prejudice. In the U.S., because HIV is spread principally through sex and sharing of drug-injection paraphernalia, and disproportionately affects racial/ethnic minority populations and homosexual men, discussions of HIV prevention inevitably touch on sensitive cultural topics. There is not one standard or paradigm for providers or communities to adopt in communicating behavioral risk reduction messages or implementing programs to promote HIV prevention. Yet, HIV remains eminently preventable through behavior change.

The complexity of the virus itself, its mechanism of action, and its effects on the immune system have stimulated giant leaps forward in research in basic science, immunology, and virology. This knowledge has rapidly advanced HIV clinical management and inßuenced medical practice far beyond HIV. The epidemic has also changed how affected communities inßuence government. New coalitions of communities, government, academics, and private industry have mobilized funding, deÞned research priorities, stimulated a growing pipeline of drugs for treatment, revised rules for eligibility for services and beneÞts, and communicated effective strategies for HIV prevention and control.

GLOBAL EPIDEMIOLOGY

UNAIDS estimates that approximately Þve million persons were newly infected with HIV during 2001 and about three million deaths were attributed to AIDS worldwide (1). Many countries, particularly some in Africa, Asia and eastern Europe are now experiencing rapid spread of HIV (20). Because of the long incubation period from infection to end-stage disease, about 10 years in the absence of treatment, these countries are still in relatively early stages of the epidemic with the peak of HIV-related morbidity and mortality yet to come. As the world goes through a period of rapid HIV spread with a large gap between the number of new cases and the number of deaths of prevalent cases, HIV prevalence worldwide is expected to escalate for the foreseeable future.

Sub-Saharan Africa accounts for over 70% of prevalent cases of HIV worldwide (Fig. 1.1). Political, cultural and economic barriers to HIV prevention and control are likely to combine to assure a decade or more of human misery in this region of the world. Already, many countries in Africa have experienced large numbers of AIDS deaths among adults in their prime years of productivity with resultant decreases in life expectancies and negative economic impact. The epidemic is believed to be transmitted principally through heterosexual contact with a large proportion of the infections in women, high rates of HIV among infants, and a growing number of orphaned children.

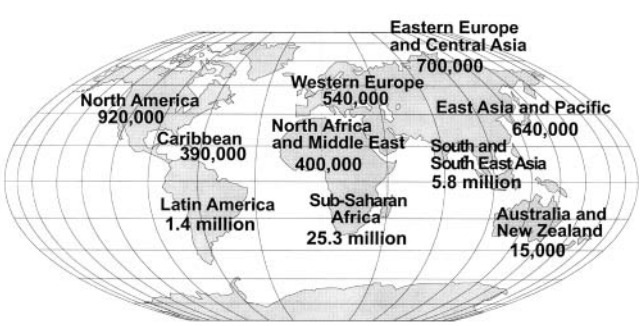


FIG. 1.1. Number of living adults and children estimated to be infected with HIV and AIDS – worldwide, 2000. Source: Joint United Nations Program on AIDS.

Other geographic areas have not yet been as severely affected (Fig. 1.2). In Latin America and the Caribbean, the epidemiologic pattern is more similar to that in the U.S., i.e. a mixed population infected by sex between men and between men and women as well as through needle-sharing among drug-users. Resources invested in HIV prevention and treatment have shown some successes in some Latin American countries. In contrast, in resource poor India, China, and other countries in southern and eastern Asia, there are rapidly emerging epidemics (1,19,20). In many of these countries, there is the potential for explosive sexual spread through populations that have high background rates of sexually transmitted diseases. Some Asian countries with entrenched drug cultures experienced the emergence of the epidemic among drug-injectors. If the epidemic continues to grow rapidly in this heavily populated region of the world, the human toll will be devastating. In addition to sexual, perinatal and drug-use associated transmission, inadequate screening for HIV antibodies and contamination of the blood supply contribute to the epidemic in many developing countries. Despite hopeful advances in short-course therapy during labor and delivery to prevent mother-to-child transmission, logistical, economic and policy barriers challenge efforts to reduce mother-to-child transmission in many developing countries. Finally, breastfeeding continues to pose a transmission risk in developing countries (21).

The U.S. rate of 14.9 reported AIDS cases per 100,000 population in 2001 is by far the highest in the industrialized world; the rates in adult (13 years) men and women, respectively, were 28.1 and 9.1 (5). In 1993, when the U.S. expanded the AIDS case deÞnition to include laboratory-initiated reporting of a measure of severe immunosuppression, European countries elected not to adopt the immunologic criterion and only adopted the added clinical conditions. Thus, trends in AIDS incidence in Europe and the U.S. are not directly comparable after 1992. Nevertheless, the marked disparity between U.S. rates and those among the western European countries highlights the severity of the epidemic in the U.S. Among the European countries with the highest numbers of cumulative AIDS cases through 2000 are Spain (59,466), France (53,095) and Italy (47,503) compared to 774,467 cases reported in the U.S. through 2000 (18,22). For cases diagnosed in 2000, the western European countries with the highest AIDS incidence rates per 100,000 were: Portugal 10.4; Spain 6.3; Italy 3.2; Switzerland 2.8; France 2.6; Luxemburg 2.4; Greece 1.2; United Kingdom 1.1 (18). These countries have complex and variable epidemics in that drug-use associated HIV predominates in some, while sexual transmission among men predominates in others. The epidemic curves have been similar to the U.S., with a rapid increase in infections in the early to mid-1980s, a peak in AIDS incidence in the early l990s, and dramatic declines in AIDS incidence and deaths during 1996 and 1997 following the introduction of highly active anti-retroviral treatment (HAART) including protease inhibitors. Many of the developed countries are implementing HIV case reporting and studies of HIV incidence to supplement their AIDS surveillance data. Evidence suggests that there is a resurgence of sexually transmitted diseases including gonorrhea and syphilis among men who have sex with men which may presage new increases in HIV infection rates in western Europe and the U.S. (23Ð25).

In contrast, the emerging epidemics in the eastern European countries have largely been concentrated in injecting-drug users. The highest AIDS incidence rates per 100,000 population were reported from Romania (2.5) and Ukraine (1.2) (18). However, these countries do not yet have well established HIV/AIDS surveillance systems, and the recency of the HIV epidemic has not yet resulted in a large proportion of infected persons having AIDS.

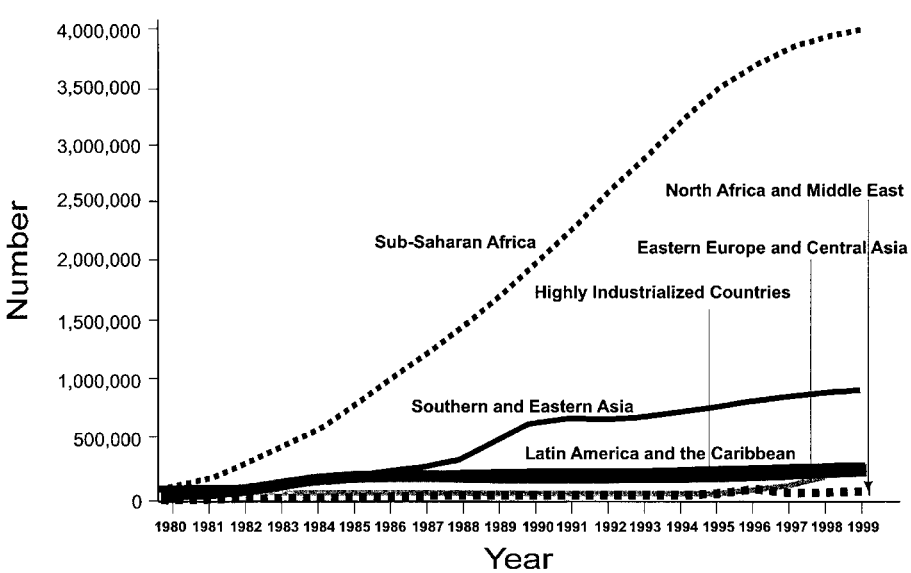


FIG. 1.2. Estimated number of new HIV infections, by region and year—worldwide, 1980–1999. Source: Joint United Nations Program on AIDS.

Efforts to prevent spread from drug injectors to their heterosexual partners are essential in this region.

In the U.S., recent attention to the economic threat and destabilizing political potential from the HIV epidemic has resulted in mobilizing resources to provide assistance. U.S. government agencies are working in collaboration with the ministries of health in African and Asian countries to build capacity for epidemic surveillance, deliver short-course treatments in labor and delivery for perinatal prevention, provide training to improve clinical management of HIV infection, and conduct behavioral surveillance and research to improve efforts in primary HIV prevention. A consortium of U.S. government and academic institutions is engaged in clinical trials of treatment interventions that can be delivered in the context of developing world infrastructure. However, perhaps the most important effort of all, i.e. vaccine research and development, has yet to yield a highly effÞcacious vaccine. This elusive prevention tool is most critical to stemming the further spread of HIV. Regardless of any future success in developing vaccines or in bringing new drugs to market, the current burden of 40 million HIV-infected persons worldwide marks a disease toll of historic proportions.

HIV/AIDS EPIDEMIOLOGY IN THE UNITED STATES

Case DeÞnition and ClassiÞcation System

Case DeÞnition

Following the initial reports of *Pneumocystis carinii* pneumonia (PCP) and KaposiÕs sarcoma (KS) among young homosexual men in Los Angeles and New York (3,4), a working case deÞnition was established to identify other cases of unusual opportunistic illnesses or malignancies that were previously known to occur principally in persons with immunosuppressive disorders. Health care providers were alerted to report cases of unusual opportunistic illness, termed AIDS, in previously healthy persons to local health authorities. The earliest AIDS data collection forms included conditions such as cytomegalovirus infection, toxoplasmosis of the central nervous system, and gastrointestinal candidiasis (excluding oral thrush) in addition to PCP and KS. Data on non-speciÞc signs and symptoms thought to be attributable to the opportunistic illnesses were collected such as skin lesions, lymphadenopathy, fever, diarrhea, oral thrush, weight loss. In 1982, history of ÒIV drug usageÓ and history of blood transfusion were collected but sexual behaviors were not. By 1983, the AIDS case report form was revised to include: a more extensive list of opportunistic illnesses (e.g. tuberculosis, nocardiosis); conditions or symptoms associated with but not speciÞc for AIDS such as amebiasis, disseminated herpes zoster, persistent bone marrow dysfunction, arthralgialmyalgia; conditions preceding or coexisting with AIDS such as HodgkinÕs disease, chronic hepatitis, chronic renal failure, insulin-dependent diabetes mellitus; and an extensive social and risk history including drug use and sexual orientation. The epidemiologic characteristics of persons with the new syndrome were consistent with sexual transmission or spread by a blood-borne agent. They were inconsistent with air-, food-, water-borne or insect vector transmission. Thus, even prior to the identiÞcation of HIV as the etiologic agent, disease surveillance and case investigations had made clear that this new condition was transmitted via sex between men or between men and women, through receipt of contaminated blood via needle-sharing among drug-users, or transfusion of blood or blood products, and perinatally from mother to child (3,4,8Ð13).

With the isolation of HIV and the availability of a serologic antibody test, it was feasible to revise the case deÞnition to include laboratory test results indicative of HIV infection and to restrict the clinical conditions indicative of AIDS to those that were highly speciÞc to immunosuppression in the absence of any other cause. Thus, the 1985 AIDS case deÞnition thus included opportunistic clinical conditions deÞnitively diagnosed based on culture, biopsy or radiologic evidence (e.g. PCP deÞnitively diagnosed) and conditions requiring additional laboratory evidence indicative of HIV infection (26). Because the epidemic initially was detected among homosexual men, as the epidemic spread into racial/ethnic minority communities, including drug-users and their (mostly female) heterosexual partners, there were concerns that the AIDS case deÞnition disproportionately captured clinical manifestations common in white gay men. In 1987, the AIDS case deÞnition was expanded to include additional conditions (e.g. wasting, dementia) that were more characteristic of injecting drug-users, and presumptive diagnoses of opportunistic illnesses in the presence of serologic evidence of HIV infection (e.g, PCP presumptively diagnosed). The 1987 AIDS case deÞnition for adults and adolescents (13 years old) included 23 opportunistic conditions and that for children, 24 conditions (27). In 1993, the AIDS case deÞnition for adults was expanded to include three additional clinical conditions and laboratory evidence of severe immunosuppression in persons with documented evidence of HIV infection, i.e. invasive cervical cancer, pulmonary tuberculosis, recurrent pneumonia, and <200 CD4 T-lymphocytes per microliter of blood (28,29). The addition of the immunologic criterion reßected the widespread use of this laboratory measure in clinical practice to monitor disease progression. These changes effectively deÞned the onset of AIDS approximately 18 to 24 months earlier in the clinical course than the previous deÞnition and necessitated the use of statistical adjustments to monitor temporal trends in AIDS incidence (30). The pediatric case deÞnition was not changed in 1993.

Beginning in 1985, several states initiated reporting of HIV cases in addition to AIDS cases. Although proponents of HIV case reporting argued for expansion of the AIDS

TABLE 1.1. *2000 revised surveillance case deÞnition for HIV infection (35)*

This revised de nition of HIV infection, which applies to any HIV (e.g. HIV-1 or HIV-2),is intended for public health surveillance only. It incorporates the reporting criteria for HIV infection and AIDS into a single case de nition. The revised criteria for HIV infection update the de nition of HIV infection implemented in 1993 (29); the revised HIV criteria apply to AIDSde ning conditions for adults (29) and children (27,37) which require laboratory evidence of HIV. This de nition is **not** presented as a guide to clinical diagnosis or for other uses (27,29).

I. In adults, adolescents, or children aged greater than or equal to 18 months,[a] a reportable case of HIV infection must meet at least one of the following criteria:

Laboratory Criteria

- Positive result on a screening test for HIV antibody (e.g. repeatedly reactive enzyme immunoassay), followed by a positive result on a con rmatory (sensitive and more speci c) test for HIV antibody (e.g. Western blot or immuno uorescence antibody test)

or

- Positive result or report of a detectable quantity on any of the following HIV virologic (non-antibody) tests: HIV nucleic acid (DNA or RNA) detection (e.g. DNA polymerase chain reaction (PCR) or plasma HIV-1 RNA[b]; HIV p24 antigen test, including neutralization assay; HIV isolation (viral culture)

OR

Clinical or Other Criteria (if the above laboratory criteria are not met)

- Diagnosis of HIV infection, based on the laboratory criteria above, that is documented in a medical record by a physician

or

- Conditions that meet criteria included in the case de nition for AIDS[cd] (27,29,37)

II. In a child aged less than 18 months, a reportable case of HIV infection must meet at least one of the following criteria:

Laboratory Criteria

De nitive

- Positive results on two separate specimens (excluding cord blood) using one or more of the following HIV virologic (nonantibody) tests: HIV nucleic acid (DNA or RNA) detection; HIV p24 antigen test, including neutralization assay, in a child greater than or equal to 1 month of age; HIV isolation (viral culture)

or

Presumptive

A child who does not meet the criteria for de nitive HIV infection but who has:

- Positive results on only one specimen (excluding cord blood) using the above HIV virologic tests and no subsequent negative HIV virologic or negative HIV antibody tests

OR

Clinical or Other Criteria (if the above deÞnitive or presumptive laboratory criteria are not met)

- Diagnosis of HIV infection, based on the laboratory criteria above, that is documented in a medical record by a physician

or

- Conditions that meet criteria included in the 1987 pediatric surveillance case de nition for AIDS[d] (27,37)

III. A child aged less than 18 months born to an HIV-infected mother will be categorized for surveillance purposes as "not infected with HIV" if the child does not meet the criteria for HIV infection but meets the following criteria:

Laboratory Criteria

De nitive

- At least two negative HIV antibody tests from separate specimens obtained at greater than or equal to 6 months of age

or

- At least two negative HIV virologic tests[e] from separate specimens, both of which were performed at greater than or equal to 1 month of age and one of which was performed at greater than or equal to 4 months of age

AND

NO other laboratory or clinical evidence of HIV infection (i.e. has not had any positive virologic tests, if performed, and has not had an AIDS-de ning condition)

or

Presumptive

A child who does not meet the above criteria for de nitive "not infected" status but who has:

- One negative EIA HIV antibody test performed at greater than or equal to 6 months of age and NO positive HIV virologic tests, if performed

or

- One negative HIV virologic test[e] performed at greater than or equal to 4 months of age and NO positive HIV virologic tests, if performed

or

- One positive HIV virologic test with at least two subsequent negative virologic tests,[e] at least one of which is at greater than or equal to 4 months of age; or negative HIV antibody test results, at least one of which is at greater than or equal to 6 months of age

TABLE 1.1. *Continued*

AND

NO other laboratory or clinical evidence of HIV infection (i.e. has not had any positive virologic tests, if performed, and has not had an AIDS-de ning condition)

OR

Clinical or Other Criteria (if the above de nitive or presumptive laboratory criteria are not met)

- Determined by a physician to be "not infected", and a physician has noted the results of the preceding HIV diagnostic tests in the medical record

AND

NO other laboratory or clinical evidence of HIV infection (i.e. has not had any positive virologic tests, if performed, and has not had an AIDS-de ning condition)

IV. A child aged less than 18 months born to an HIV-infected mother will be categorized as having perinatal exposure to HIV infection if the child does not meet the criteria for HIV infection (II) or the criteria for "not infected with HIV" (III).

[a] Children aged greater than or equal to 18 months but less than 13 years are categorized as "not infected with HIV" if they meet the criteria in **III**.

[b] In adults, adolescents, and children infected by other than perinatal exposure, plasma viral RNA nucleic acid tests should NOT be used in lieu of licensed HIV screening tests (e.g. repeatedly reactive enzyme immunoassay). In addition, a negative (i.e. undetectable) plasma HIV-1 RNA test result does not rule out the diagnosis of HIV infection.

[c] AIDS-de ning conditions for adults and adolescents 13 years and older include the following conditions (27,29) Candidiasis of bronchi, trachea, or lungs; Candidiasis, esophageal; Carcinoma, invasive cervical;* CD4 T-lymphocyte count < 200 cells/microliter or percent < 14;* Coccidioidomycosis, disseminated or extrapulmonary;* Cryptococcosis, extrapulmonary; Cryptosporidiosis, chronic intestinal; Cytomegalovirus disease (other than in liver, spleen or nodes); Cytomegaloviris retinitis;* HIV encephalopathy;* Herpes simplex: chronic or bronchitis, esophagitis or pneumonitis; Histoplasmosis, disseminated or extrapulmonary,* Isosporiasis, chronic intestinal;* Kaposi's sarcoma; Lymphoma, Burkitt's;* Lymphoma, immunoblastic;* Lymphoma primary in brain;* *Mycobacterium avium complex* or *M. kansasii*, disseminated or extrapulmonary, *M. tuberculosis*, pulmonary,* *M. tuberculosis*, disseminated or extrapulmonary;* Mycobacterium of other species, disseminated or extrapulmonary;* *Pneumocystis carinii* pneumonia, Pneumonia, recurrent;* Progressive multifocal leukoencephalopathy; Salmonella septicemia, recurrent;* Toxoplasmosis of brain: Wasting syndrome due to HIV.*

* Conditions that are AIDS-de ning in the presence of laboratory evidence for HIV infection (35); other listed conditions require diagnosis by de nitive methods if laboratory tests for HIV were not performed or gave inconclusive results and the patient had no other cause of immunode ciency.

[d] AIDS-de ning conditions for children less than 13 years old include the conditions listed above and: Bacterial infections, multiple or recurrent (including Salmonella septicemia)* and Lymphoid interstitial pneumonia or pulmonary lymphoid hyperplasia (27,37); but they **exclude** the conditions above that were added to the adult/adolescent case de nition in 1993: invasive cervical carcinoma, extrapulmonary), *M. tuberculosis*, recurrent pneumonia, and CD4 T-lymphocyte count < 200 cells/microliter or percent < 14.

* See asterisk under footnote c above.

[e] HIV nucleic acid (DNA or RNA) detection tests are the virologic methods of choice to exclude infection in children aged less than 18 months. Although HIV culture can be used for this purpose, it is more complex and expensive to perform and is less well standardized than nucleic acid detection tests. The use of p24 antigen testing to exclude infection in children aged less than 18 months is not recommended because of its lack of sensitivity.

An original paper copy of the document containing this de nition (35) can be obtained from the Superintendent of Documents, U.S. Government Printing Of ce (GPO), Washington, D.C. 20402–9371; telephone (202)512–1800 Contact GPO for current prices.

case de nition to include all persons infected with HIV in 1993 [or even earlier], it was not until the dramatic declines in AIDS cases and deaths which occurred following the introduction of HAART that a national debate earnestly addressed the need to monitor infected persons at all stages of disease (31–34). As of January 1, 2000, CDC implemented a revised integrated HIV and AIDS case de nition for adults and children that recognizes one disease with multiple stages and incorporates current laboratory measures of HIV for adults and children (Table 1.1). This de nition was published together with CDC recommendations for nationwide HIV case surveillance (35).

Classi cation System

As the AIDS case de nition was revised to incorporate advances in clinical, diagnostic and laboratory practices, it was also necessary to revise the HIV disease classi cation systems. The current classi cation system for adults and adolescents was introduced in 1993 (29). It incorporates stage of disease as measured by symptoms, and stage of immunosuppression as measured by CD4 count. A 9-cell grid is organized into three clinical stages (asymptomatic or acute HIV infection, symptomatic, AIDS-de ning conditions) and three immunologic categories (CD4 □ 500 cells per microliter of blood, 200–499, and < 200). For infants and children < 13 years old, a 12-cell classi cation system was introduced in 1994 (36,37). The immunologic criteria for children are divided into three age-speci c categories characterized as none, moderate, or severe immunosuppression. The clinical categories are none, mild, moderate, or severe symptoms.

HIV/AIDS Surveillance Methods

Case Reporting and Seroprevalence Surveys

The HIV/AIDS surveillance system represents the collective efforts of health department staff at county, city

and state/territorial levels, physicians, nurses and other health care providers, affected communities and the CDC to develop and maintain an accurate, timely, representative, and conÞdential system of reporting information about those who are affected by the epidemic. When the Þrst cases of AIDS were detected, the surveillance system only identiÞed severe morbidity, which we now understand represents only the end-stage of HIV disease. Thus, as the epidemiology of HIV was revealed and as laboratory advances enabled improved testing and diagnostics, it became possible to expand reporting criteria to include asymptomatic and mildly symptomatic stages of disease.

AIDS is a notiÞable disease in all states and territories, meaning that each jurisdiction has legal requirements that health care providers notify health authorities when a person is diagnosed as having AIDS. In implementing laws, rules, or regulations requiring disease reporting, states enact legal requirements for conÞdentiality of the surveillance data and penalties for inappropriate use or disclosure (38). AIDS cases are reported to local health departments by health care providers, facilities and laboratories using a standard case report form and case deÞnition. These reports are forwarded to state or territorial health departments for aggregation and dissemination of summary data. In addition to passive reporting by providers, health departments conduct active surveillance by visiting facilities and soliciting case reports, completing case report forms to reduce the burden on providers, providing training and feedback to reporting sources, and monitoring completeness of case reporting. At the state level, the data are used to track changes in the local epidemic and identify emerging populations at risk. States then forward data to CDC which is responsible for analysis and dissemination of the national data (Fig. 1.3). The national database is protected under a federal assurance of conÞdentiality which prohibits the release of HIV/AIDS surveillance data for non-public health purposes. The CDCÕs database does not contain individual patient or physician identifying data and is only linked to identifying data maintained at the state or territorial level by state-assigned numbers and codes. In cases requiring follow up investigation to identify potential new or unusual modes of HIV transmission, CDC scientists must work in collaboration with local health authorities.

Following the availability of the HIV antibody test, efforts to monitor HIV in the population were focused on large-scale anonymous unlinked serologic surveys. CDC and states invested surveillance resources in establishing a network of seroprevalence surveys (39). Populations targeted for seroprevalence surveys were those thought to be at high-risk, such as drug-users entering treatment and clients of sexually-transmitted disease clinics. In addition, accessible populations such as military service applicants, or those in which HIV testing was required such as Job Corps entrants, were sources of epidemiologic data on the extent of HIV in the population. While these data were useful to estimate the prevalence of HIV in selected populations in the U.S., they were able to collect only limited demographic and other epidemiologic data. Only one serologic survey, the survey of childbearing women which anonymously tested blood specimens routinely collected from newborns for other health purposes, provided population-based estimates of HIV prevalence.

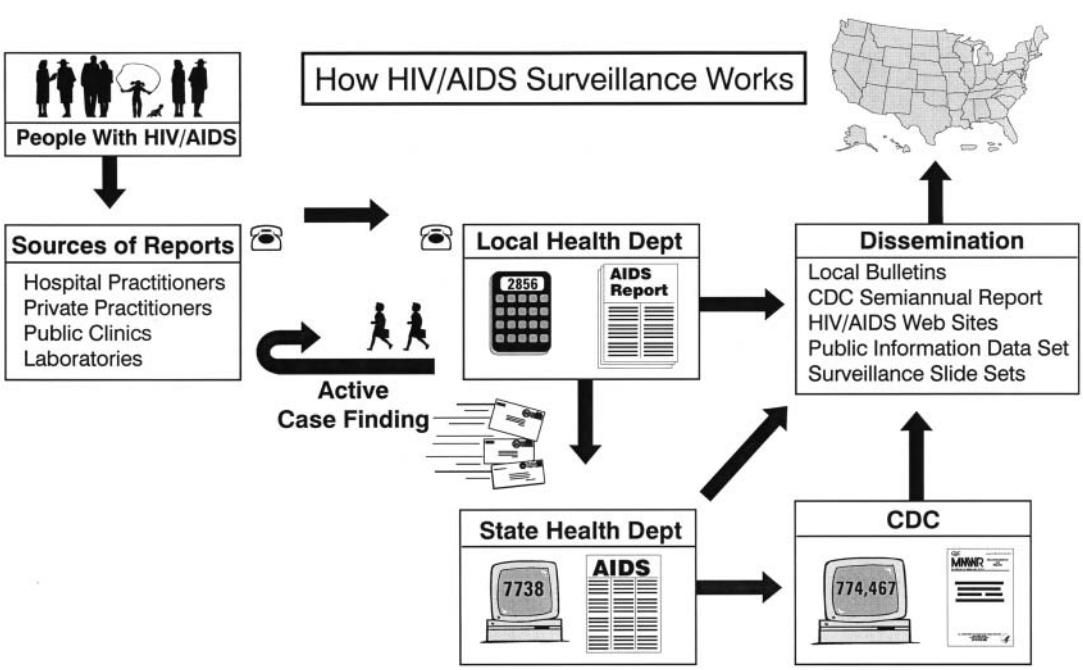


FIG. 1.3. Core HIV/AIDS surveillance information ow. Source: CDC, Division of HIV/AIDS Prevention.

From this survey, the prevalence of HIV in women delivering liveborn infants in the U.S. was derived (40,41). Implemented in the late 1980s, after HIV incidence had already peaked in the U.S., the serologic surveys tracked relatively stable seroprevalence levels in most of the target populations through the mid-1990s (42). Most national seroprevalence surveys were discontinued in the mid-1990s. Although some states continue these surveys, it is now challenging to interpret whether changes in seroprevalence represent increasing or decreasing HIV incidence or increasing survival of persons with HIV who are on HAART.

During the late-1980s, in response to the growing epidemic, Congress allocated resources to improve and standardize AIDS surveillance. CDC and state and territorial health departments responded by improving the quality, completeness and timeliness of AIDS case reporting (43,45). As a result of the high quality and comparability of the data across jurisdictions, during the 1990s, the AIDS surveillance data became the basis for allocating nearly $2 billion in federal resources for patient care and treatment to states and hard-hit cities. The epidemiologic characteristics of AIDS cases also became an important basis for allocating resources for HIV prevention.

However, with the advent of HAART, by the mid-1990s, AIDS surveillance data were clearly inadequate to represent the extent of the epidemic in the U.S. Together with the discontinuation of the seroprevalence surveys, the national HIV/AIDS surveillance program was in jeopardy. Because resources for prevention and care are tied to surveillance data, broad political, public health and community efforts combined to support an expansion of surveillance crtieria to include all persons with HIV, regardless of stage of disease. These efforts led to the 2000 integrated HIV and AIDS case definition for adults and children and the recommendation that all states implement HIV case reporting in addition to AIDS (35).

While case reports are extremely valuable for monitoring the number and characteristics (e.g. sex, racial-ethnic group, age group, mode of HIV transmission, geographic distribution, clinical status and CD4 count or viral load at diagnosis of HIV or AIDS) of persons who are known to be infected with HIV or to have progressed to AIDS, they do not represent undiagnosed HIV-infected persons or those tested anonymously. Thus, epidemiologists use case surveillance data in conjunction with data from other sources including seroprevalence surveys in selected populations, studies of HIV incidence, death certificates, service databases such as test results from publicly funded counseling and testing sites, and behavioral surveys (7,46–52).

Prior to 1993, AIDS surveillance data reliably estimated trends in HIV incidence and the cumulative number of HIV infections by the application of statistical back-calculation models which estimated that HIV incidence peaked at over 100,000 infections annually in the mid-1980s and declined to between approximately 40,000 to 60,000 infections annually in the early 1990s (53–55). However, during the l990s, this method became unuseable because of changes to the AIDS case definition and the impact of HAART on AIDS incidence. It has since been necessary to estimate HIV incidence indirectly by synthesizing data from multiple sources; CDC has estimated that HIV incidence remained relatively stable, at approximately 40,000 annual infections, during the mid- and late-nineties (35,47). Technical developments have enabled estimating recent seroconversions, within a window period of about 130 days, from blood samples of persons newly testing HIV positive (56). Because direct measures of HIV incidence in the population are crucial to efficient targeting of prevention resources, detecting new trends in populations at risk, and monitoring the effectiveness of prevention programs, HIV incidence monitoring in conjunction with HIV/AIDS case reporting are current priority surveillance strategies.

Adjustments to Surveillance Data

The process of case reporting involves a time lag from when a laboratory or clinical diagnosis is confirmed until a report is forwarded in turn to local, state, and federal levels. Case reports in a given time period are useful to monitor the performance of the reporting system. However, in order to monitor trends in AIDS incidence in the population, the data are statistically adjusted to account for reporting delays. The adjusted data do not represent counts of persons with AIDS, rather they represent estimates of AIDS incidence taking into account variations in reporting delays by demographic and geographic variables. Nearly all cases are reported to CDC within a year of diagnosis, thus, estimates for the most recent quarter-years are subject to the greatest adjustment (5,57).

Similarly, there are lags in obtaining complete data on mode of HIV transmission. These data are important to understanding epidemiologic patterns, detecting emerging communities at risk, and guiding prevention program priorities. However, unless a patient divulges risk behaviors, or providers elicit and record a thorough behavioral risk history, cases often are reported without any behavioral data. The number and proportion of such cases has steadily increased reflecting the increased volume of cases reported after the 1993 definition change, increased reliance on laboratory reporting, and the growth of the heterosexual epidemic wherein patients may not have primary HIV risk behaviors (58). With the adoption of laboratory-initiated HIV case reporting, behavioral data are usually lacking until a patient is obtaining on-going medical care and health departments conduct a labor intensive follow up review of medical records, or interview the provider or the patient. To address the lack of behavioral risk data on a large proportion of cases, CDC and states are implementing a variety of more efficient

strategies including follow up studies on a sample of cases and estimating the distribution of behavioral risks using statistical models based on behavioral surveys (59). In addition, for persons who were infected heterosexually, who may not have a partner with a primary risk or recognize their partnersÕ risks, efforts are underway to develop a standardized deÞnition of high-risk heterosexual sex so that states will classify persons with multiple sex partners or a history of sexually transmitted diseases in a consistent way. Finally, standardized statistical methods are applied at the national level to estimate the true risk distribution by adjusting for unreported risk information using historical patterns of reclassiÞcation after follow up investigations of persons initially reported without risk (60).

HIV/AIDS Surveillance Data

Through December 31, 2001, state and territorial health departments have reported 816,149 persons with AIDS to the CDC; 81.6% were adult and adolescent men, 17.3% women, and 1.1% infants and children (5). Although the annual number of reported AIDS cases declined dramatically in the latter half of the 1990s coincident with the widespread use of HAART, the number of reported AIDS cases has remained stubbornly over 40,000 cases per year. During 2001, 43,158 AIDS cases were reported to CDC (5). Through December 31, 2001, deaths of persons with AIDS numbered 467,910, or 57.3% of cumulative reported AIDS cases. In addition, 174,026 reports of persons diagnosed with HIV (without an AIDS diagnosis), of whom 3,923 (2.3%) were infants and children, have been received from 39 areas that have implemented conÞdential (i.e. reporting by patient name) HIV infection case surveillance. These latter reports are not necessarily reßective of the characteristics of all persons diagnosed with HIV in the U.S. However, additional data will become available in future from the remaining geographic areas which have either implemented HIV case reporting using a patient code, or a hybrid name-to-code system, or are expected imminently to implement HIV reporting (61). Ultimately, the national HIV/AIDS case reporting system should include data on all persons known as having HIV or AIDS in the U.S.

Demographic Characteristics

From statistical models based on the distribution of ages at AIDS incidence in the absence of treatment, it has been estimated that the vast majority of new HIV infections occur among adults < 30 years old, with as many as half of all infections occurring in those under 25 (55,62). These young ages represent the highest risk of acquiring or transmitting HIV because rates of partner change and of sexually transmitted infections that potentiate HIV spread are highest in young adult age groups (63). Following infection, a small proportion of individuals progress rapidly to AIDS (64). However, the majority of AIDS cases are diagnosed in their mid-thirties, consistent with models of the natural history of HIV which estimate the median incubation period from infection to AIDS-deÞning opportunistic illnesses to be about 10 years (Fig. 1.4).

Although the epidemic in the U.S. has disproportionately affected men since the beginning, the proportion of adult AIDS cases reported annually that are female has increased steadily from less than 10% during the 1980s to 26% in 2001 (Fig. 1.5) (5). As the heterosexual epidemic has grown in the U.S., CDC has estimated that at least one-quarter of new HIV infections are now occurring in women (CDC, unpublished data). The proportion of reported HIV cases that are female was 32% in 2001 reßecting the characteristics of the epidemic in the HIV reporting states, which under-represent states in which the epidemic has predominantly affected men who have sex with men (MSM) and injecting drug-users (IDU).

The proportion of AIDS cases that are racial/ethnic minorities (including blacks, Hispanics, American Indians/

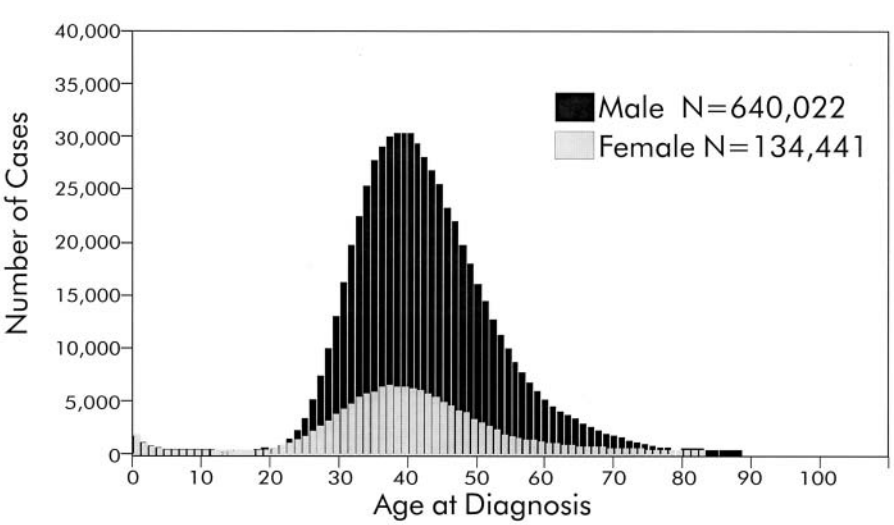


FIG. 1.4. AIDS cases by age and sex, reported 1981–2000, United States. Source: CDC, Division of HIV/AIDS Prevention.

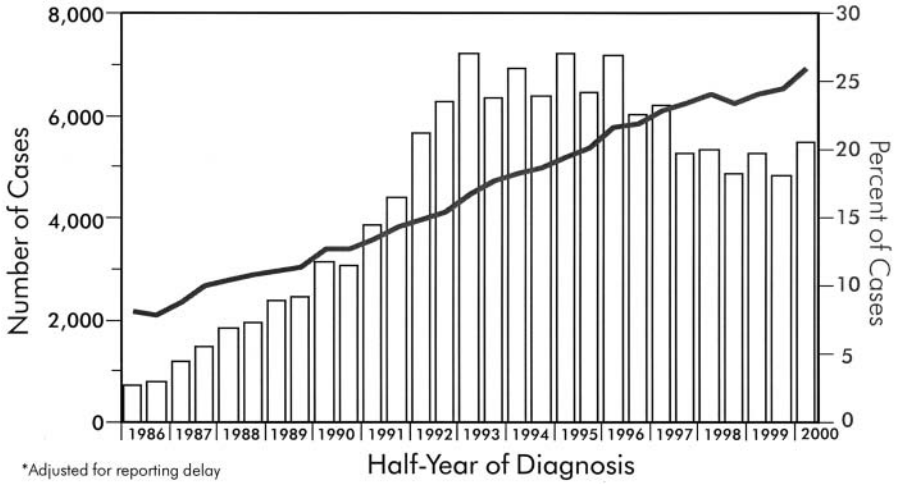


FIG. 1.5. IDS incidence in women (bars—left scale) and percentage of total AIDS cases (line—right scale), January 1986–June 2000, United States. Source: CDC, Division of HIV/AIDS Prevention.

Alaska Natives, and Asians/PaciÞc Islanders) has also increased steadily over time (Fig. 1.6). Of the 42,983 adults reported with AIDS in 2001, 69% were racial/ethnic minorities. Among women with AIDS, the proportion is even higher, 82% in 2001. The disproportionate impact of the drug-use associated epidemic on racial/ethnic minorities, including drug-injectors and their heterosexual partners, is reßected in rates that are many times higher than those in whites. Among adult men, the rates of reported AIDS cases per 100,000 males in 2001 were: whites 13.7; blacks 109.2; Hispanics 43.3; Asian/PaciÞc Islander 8.6; American Indian/Alaska Native 18.8. For adult women, the rates were 2.4, 47.8, 12.9, 1.5, and 4.9, respectively (5).

AIDS cases have been reported from every state, however, among men, the epidemic is concentrated mainly on the east and west coast, in large urban areas with the highest concentrations of MSM. Among IDU, both male and female, the epidemic has largely affected urban centers along the east coast. HIV-infected heterosexual partners of drug-users tend also to reside in east coast states. Thus, for men, states with the highest reported AIDS rates per 100,000 in 2001 are bicoastal, whereas for women, the highest rates are mainly on the east coast (Figs. 1.7 and 1.8, respectively). While the number of AIDS cases reported from smaller urban and rural areas has increased as HIV was disseminated throughout the nation, the proportionate distribution of cases by urbanicity has remained remarkably stable (Fig. 1.9). The rate of reported AIDS cases in 2001 was 2 times higher in metropolitan areas with populations greater than 500,000 population (19.0) than in those with $<$500,000 (9.5) and 3.3 times higher than in non-metropolitan areas (5.8). Even within the most densely populated metropolitan areas, the central counties are more heavily affected than the outlying counties with a rate of 20.4 versus 4.9 (5).

Trends by Mode of HIV Transmission

The dynamics of HIV transmission are quite complex at both the individual and population levels. The risk of

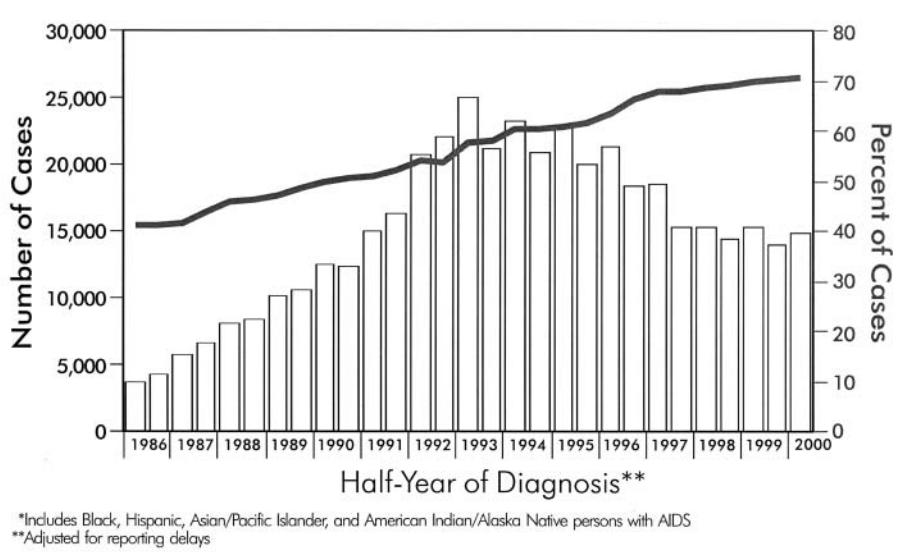


FIG. 1.6. AIDS incidence in racial/ethnic minorities (bars—left scale) and percentage of total AIDS cases (line—right scale), January 1986–June 2000, United States. Source: CDC, Division of HIV/AIDS Prevention.

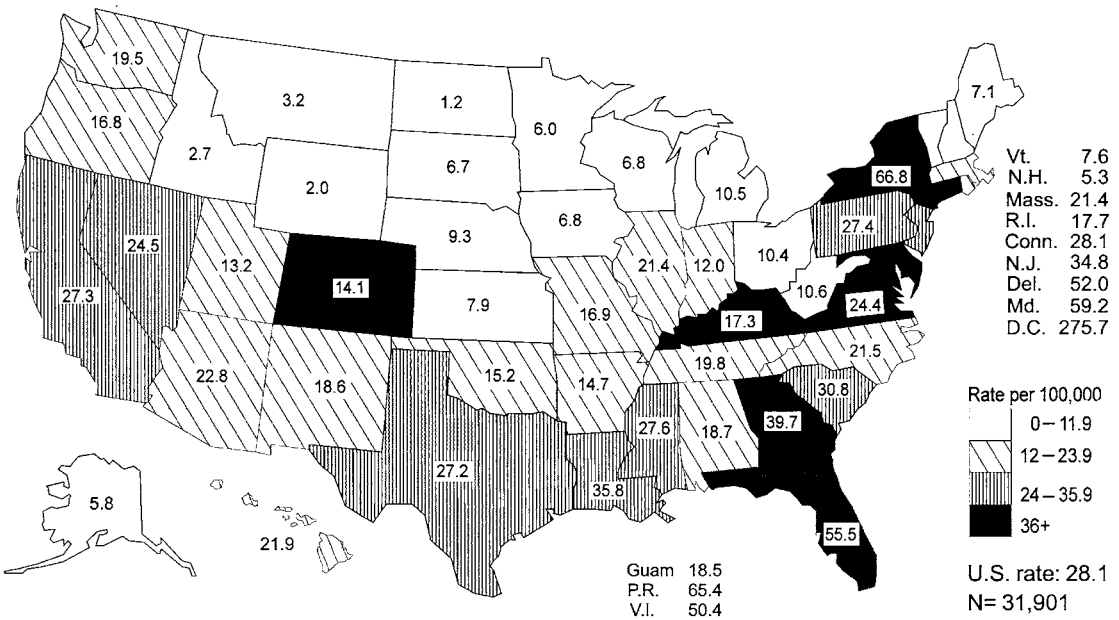


FIG. 1.7. Male adult/adolescent annual AIDS rates per 100,000 population for cases reported in 2001, United States. Source: CDC, Division of HIV/AIDS Prevention.

contracting HIV varies by mode of exposure to the virus (65Ð75). Numerous published studies have quantiÞed HIV transmission risks; some of these studies are summarized in Table 1.2. At the individual level, factors that determine the risk of infection include the per contact risk of becoming infected if exposed, the probability that a partner of unknown status may be infected which is determined by the prevalence of HIV in that population, and the frequency of practicing the risky behavior. Numerous individual factors may mitigate the risk including genetic factors, the use of microbicides or barrier protection. Others may potentiate infection, for example, the presence of ulcerative sexually-transmitted infections or traumatic sex. The stage of disease of the infected contact and whether treatment has reduced levels of circulating virus may also affect the risk of transmission (76). For clinicians counseling patients about HIV prevention, it is important to understand that the epidemiologic

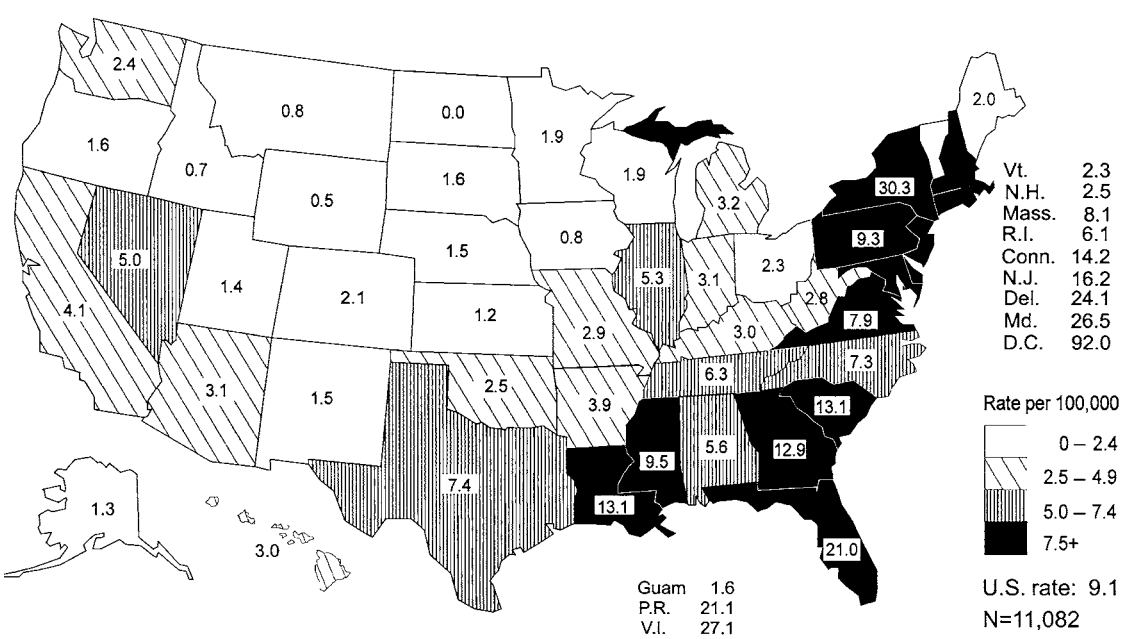


FIG. 1.8. Female adult/adolescent annual AIDS rates per 100,000 population for cases reported in 2001, United States. Source: CDC, Division of HIV/AIDS Prevention.

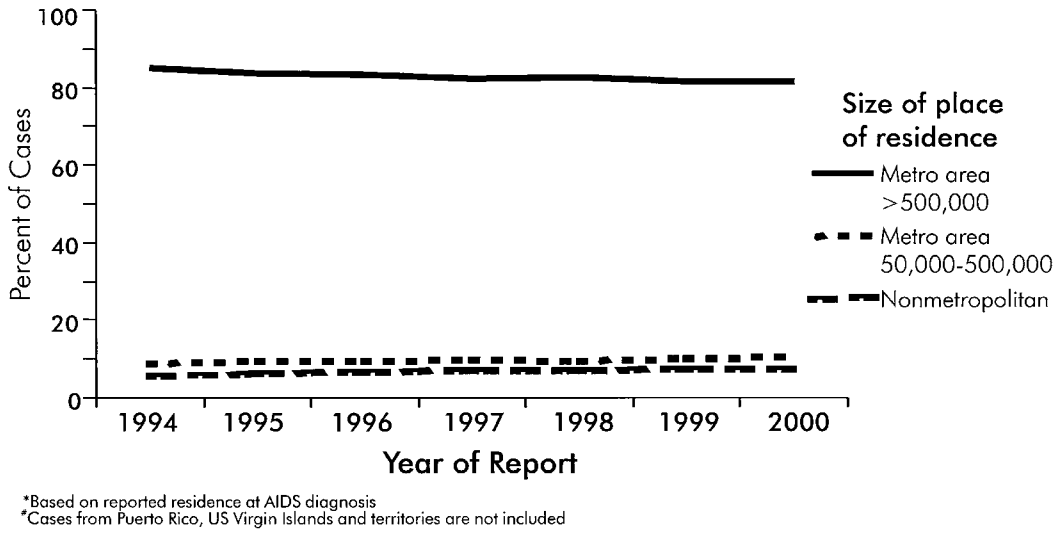


FIG. 1.9. Percent distribution of adult/adolescent AIDS cases by size and place of residence, 1994–2000, United States. Source: CDC, Division of HIV/AIDS Prevention.

risks summarized in Table 1.2 represent population probabilities and are not necessarily applicable at the individual level. That is, because any single exposure may result in infection, individuals need to avoid high risk behavior to prevent transmitting or acquiring HIV.

The early burst of HIV in the U.S. population occurred when the virus was introduced into susceptible populations with high rates of behaviors conducive to viral spread. The Þrst reports of AIDS in homosexual men in 1981 suggested that this population was at the vanguard of the Þrst wave of HIV transmission in the U.S. In retrospect, these early cases represented only a small proportion of men who were infected during the late 1970s and progressed relatively rapidly to AIDS. As HIV spread, AIDS appeared in IDU, recipients of blood and blood products, and infants born to drug-injecting women. A second wave of susceptibles was infected through heterosexual spread of HIV from drug-users to their male or female partners or from bisexual men to their female partners. Thus, a number of factors facilitated rapid spread of HIV during the 1980s: the existence of bridge populations (e.g. men who engage in sex with other men and inject drugs through sharing of paraphernalia represented a bridge for spread of the epidemic from the MSM community as did bisexual men), geographic mobility of at-risk and infected populations, high rates of risky sex and drug-using behaviors, and a large size demographic cohort in their twenties and thirties when the virus was introduced, i.e. the generation born between 1946 and 1964 known as the Òbaby boomÓthat was in the ages of peak sex and drug-using behavioral risks.

Exposure to contaminated blood poses a high risk of infection but that varies by the route of blood exposure. Early in the HIV epidemic, highly efÞcient transmission occurred to transfusion recipients of blood donated by HIV-infected persons. Hemophiliacs who had multiple

TABLE 1.2. *Reported estimates of per-contact HIV transmission risk from selected studies*

Risk category	Probability of transmission	Source[a]
Recipients of HIV + transfusions	89.5%	Donegan, 1990
Mother-to-child transmission		Connor, 1994
Without zidovudine	25%	
With zidovudine	8%	
MSM, overall[b]	0.01–5.1%	Vittinghoff, 1999
IDU (Connecticut)	0.67%	Kaplan, 1992
Heterosexual, overall[b]	0.03–0.23%	Downs, 1996; Peterman, 1988
Needlestick	0.13–0.70%	Henderson, 1990

[a] For complete citation, see “References”
[b] Included within the range are estimates for unprotected insertive and receptive anal, oral, or vaginal intercourse.
MSM = Men who have Sex with Men.

exposures to contaminated blood products through receipt of clotting factors also experienced high rates of HIV infection. In recent years, more sensitive HIV viral detection tests have enabled the identiÞcation of blood donated during the Òwindow periodÓ following infection but prior to developing antibodies. Thus, the risk of infection through receipt of blood has been virtually eliminated (77). Sharing of syringes and other drug-injecting equipment can expose multiple users to contaminated blood and the repetitive nature of exposures in drug-addicted populations assured high probabilities of infection in this population. High rates of transmission occurred in large cities in the northeast and the drug-associated epidemic remains concentrated in urban areas along the east coast with high rates also in Puerto Rico. Occupational transmission through exposure to HIV-infected blood has occurred much less frequently, and has been principally through needlesticks (78). The risk of infection per exposure is inßuenced by the size of the needle bore and the depth of the wound. Recently, this risk has been reduced through post-exposure prophylactic antiretroviral therapy (79).

Epidemiologic studies suggest that unprotected receptive anal intercourse is among the most efÞcient modes of sexual transmission. Sexual transmission by vaginal, anal, or oral routes is facilitated by high risk behaviors including multiple partners and anonymous sex, ulcerative sexually transmitted diseases, and the use of non-injecting drugs or alcohol which are associated with lower rates of condom use. With rising background HIV prevalence rates in the population, high prevalence of risky sexual behaviors, and high STD rates, rapid spread of HIV occurred in MSM populations in the early epi-centers in the northeast and on the west coast as the virus spread geographically through the susceptible population. With each successive cohort of young men who come of age, prevalence in the population will be determined in part by the prevalence of risky behaviors and the mixing of populations across age groups. Heterosexual transmission from male or female (mostly male) injecting drug-users to their male or female (mostly female) sex partners triggered the epidemic of heterosexually-acquired HIV. While the act of receptive anal intercourse does not necessarily pose a differential risk by sex per contact with an infected partner, the role of unprotected anal intercourse has apparently played a greater role in the MSM epidemic than in the heterosexual epidemic because of differences in the prevalence of this behavior in MSM versus heterosexual populations in the U.S. and because the prevalence of HIV is higher among MSM than among the general heterosexual population. Vaginal intercourse is thought to be relatively less efÞcient for female-to-male transmission than for male-to-female. However, as with transmission among MSM and IDU, rates of heterosexual transmission are affected by background prevalence in the population, prevalence of risky sex (multiple partners, anonymous sex, unprotected sex), sexual mixing patterns and sexually transmitted diseases. Sorting out the role of prostitution in the heterosexual epidemic in the U.S. has been challenging in that many commercial sex workers are also drug-injecting women who may have multiple routes of acquiring and transmitting HIV.

Transmission vertically from mother to child occurs at a rate of about 25% (80). In 1994, results of clinical trials were published indicating that maternal zidovudine (ZDV) treatment prenatally and intra-partum, together with post-partum treatment of the neonate resulted in a reduced rate of transmission to about 8% (80) Subsequent recommendations for ZDV to reduce perinatal transmission coupled with universal counseling and voluntary HIV testing of pregnant women have dramatically reduced the incidence of perinatally-acquired HIV in the U.S. (81Ð83). Currently, the transmission rates have been reduced to as low as 2% or less with the addition of maternal antiretroviral treatment during pregnancy for maternal health, improved obstetrical practices including Caesarian-section and rapid testing in labor and delivery, and the emergence of effective short-course interventions in labor and delivery for women who have little or no prenatal care (84Ð87).

Other categories of transmission risks have been reported but they occur much less frequently in the population and they involve unusual sexual behaviors or exposures to blood or body ßuids. For example, there have been reports of sexual transmission to very young children, deliberate self-inoculations, exposure to concentrated virus in laboratory settings, and other unusual exposures to blood (60,88,89). Conversely there have been no credible reports of transmission by bites between children, mosquito bites, or other common potential routes of exposure. The results of standard investigations of potential cases of unusual transmission have reassured the public that there are no reported risks from sources other than blood and sexual exposure (60,90). Providers should alert health departments to any cases of potential unusual routes of transmission so that they may investigate in collaboration with the CDC and issue revised prevention guidelines if appropriate.

Hierarchical Risk ClassiÞcation

To monitor trends in the population by mode of transmission, the HIV/AIDS case report form collects data on multiple modes of exposure. However, in order to summarize data for persons reported as having multiple risks, CDC adopted a hierarchical risk classiÞcation system. For any individual who has multiple risks, it is difÞcult to determine with certainty which exposure mode accounted for the infection (5). Fourteen percent of 807,075 AIDS cases reported through December, 2001 were reported with two or more transmission modes, 76% with one, and 10% with no risks reported (5). Thus, the hierarchy classiÞes persons having multiple modes as

most likely infected by the one listed highest in the hierarchy. One exception is the category of MSM who are also IDU. Because both modes represent high risk of exposure to HIV, men with both are classiÞed as such rather than presuming that one is more likely than the other to be the source of the HIV infection. The hierarchy of modes of HIV exposure for adults/adolescents is: MSM, IDU, MSM and IDU, hemophilia/coagulation disorder, heterosexual contact with a person with HIV/AIDS or with a person who has a primary risk (e.g. bisexual male, IDU), receipt of blood or blood products or tissue (5). The distribution of transmission modes for men and women with AIDS who were diagnosed during 2000 is shown in Fig. 1.10.

Men who have Sex with Men (MSM)

MSM have always accounted for the largest proportion of AIDS cases. MSM accounted for 40% of the estimated 41,311 adults diagnosed with AIDS during 2001 (Table 1.3) (5). Cohort studies, begun in 1983 in cities hit early by the MSM epidemic, stored baseline sera from volunteers. Later, after the antibody test was available, these specimens revealed seroprevalence rates as high as over 40% (91). These studies, plus statistical models of AIDS incidence, suggest that the epidemic in gay men spread very rapidly early in the l 980s and that HIV incidence had peaked by the mid-1980s (53Ð55). AIDS incidence peaked approximately 10 years later in MSM, in the early 1990s, in association with the expansion of the AIDS case deÞnition. Dramatic declines in AIDS and deaths occurred during 1996Ðl998, attributed in part to the introduction of HAART and in part to the expected declines reßecting the epidemic curve (Fig. 1.11). Trends in AIDS incidence have stabilized during l999 through 2001. However, characteristics of MSM with AIDS have shifted, with racial/ethnic minority MSM representing an increasing proportion of AIDS cases (Fig. 1.12) (92). While the shift to minority MSM may in part reßect differentials in access to care and treatment, reports of resurgent STD incidence and of high HIV seroconversion rates among MSM in several cities, particularly among racial minorities, have resulted in a new focus on prevention needs of all MSM (46,93).

At the end of 2000, nearly 338,000 persons were living with HIV/AIDS of whom 45% were MSM. AIDS prevalence by risk group for men is shown in Fig. 1.13. Although most seroprevalence surveys have been discontinued, the data available from 1993 through 1997 highlight the high HIV prevalence rates among black MSM in STD clinics, compared to Hispanics and whites (Fig. 1.14).

Injecting Drug Users (IDU)

The epidemic in drug-using populations has been disproportionately concentrated among racial/ethnic minorities, especially black and Hispanic men and women on the east coast. Seroprevalence surveys have documented large differentials in HIV prevalence among IDU entering drug treatment in cities on the east coast versus the west coast (Fig. 1.15). HIV incidence peaked among IDUs in the mid-1980s (53,54). As the high HIV prevalence in minority drug injectors contributed to the heterosexual spread of HIV, heterosexually acquired infections began to account for an increasing proportion of AIDS cases in minority women and men. The age at initiation of drug-injecting generally lags several years behind initiation of non-injecting drug use as well as

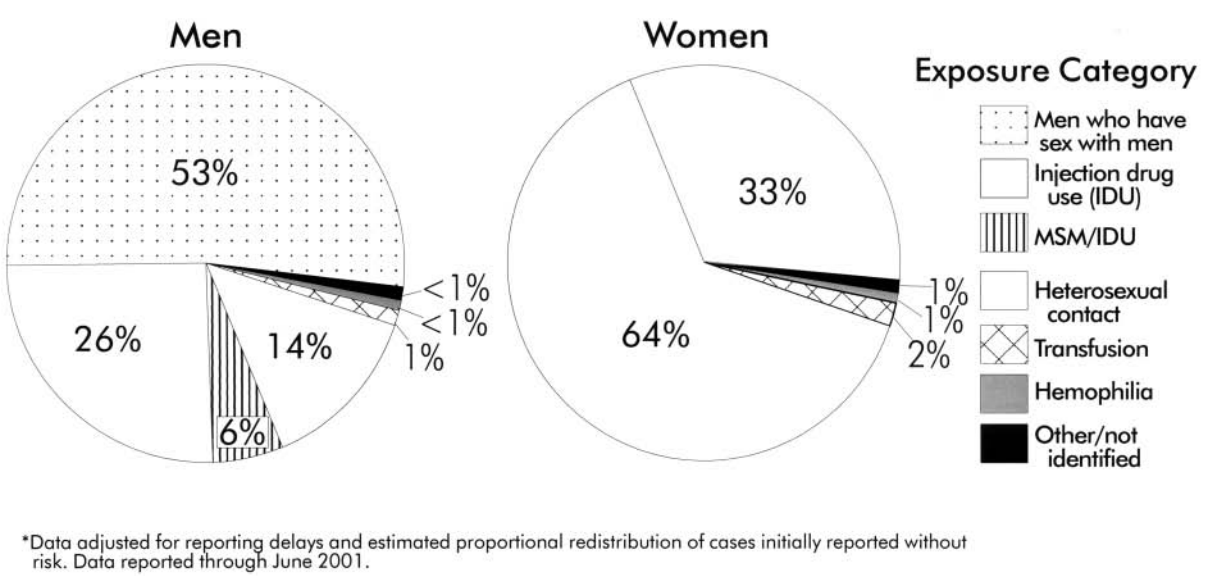


FIG. 1.10. Estimated AIDS incidence among adults/adolescents diagnosed in 2000, by sex and exposure category, United States. Source: CDC, Division of HIV/AIDS Prevention.

TABLE 1.3. *Estimated adult/adolescent AIDS incidence,[a] by sex, exposure category, and year of diagnosis, 1996, 1998, 2001, United States*

Exposure Category[b]	1996		1998		2001	
(Men > 13 years)	N	(%)[c]	N	(%)	N	(%)
MSM	26,081	(42.9)	17,315	(40.4)	16,453	(39.8)
IDU	12,804	(21.1)	8,556	(20.0)	7,280	(17.6)
MSM&IDU	3,479	(5.7)	2,312	(5.4)	1,839	(4.4)
HET	4,596	(7.6)	3,981	(9.3)	4,555	(11.0)
Other/NIR	628	(1.0)	407	(1.0)	374	(0 9)
Male subtotal	47,588	(78.3)	32,571	(76.0)	30,501	(73.8)
(Women > 13 years)						
IDU	5,282	(8.7)	3,708	(8.7)	3,410	(8.3)
HET	7,570	(12.4)	6,289	(14.7)	7,066	(17.1)
Other/NIR	365	(0.6)	263	(0.6)	333	(0.8)
Female subtotal	13,217	(21.7)	10,260	(24.0)	10,809	(26.2)
Total	**60,805**		**42,832**		**41,311**	

[a] Data are adjusted for delays in reporting of cases and of exposure category; data reported to CDC through June 30, 2002.
[b] Exposure categories include: MSM (men who have sex with men); IDU (injecting-drug users); HET (heterosexual contact with a partner in a primary risk category (i.e. IDU, bisexual man, HIV positive recipient of blood/blood products), or a partner known to have HIV infection or AIDS); other/NIR (receipt of blood/blood products, rare or unusual transmission modes or persons having no identi ed risk).
[c] Column percentages may not total 100 due to rounding.

behind age at initiation of high-risk sexual activity. Trends in HIV seroprevalence among IDU entering drug treatment centers by age group (Fig. 1.16) and sex (Fig. 1.17) illustrate the highest rates in older cohorts and the similarity in rates for men and women. IDU accounted for about 27% of 338,000 persons living with HIV/AIDS as of 2000 and 26% of persons diagnosed with AIDS in 2001 (Table 1.3). Declines in AIDS cases and deaths were observed during 1996 and 1997, although the rate of decline was not as great as for MSM (Fig. 1.11).

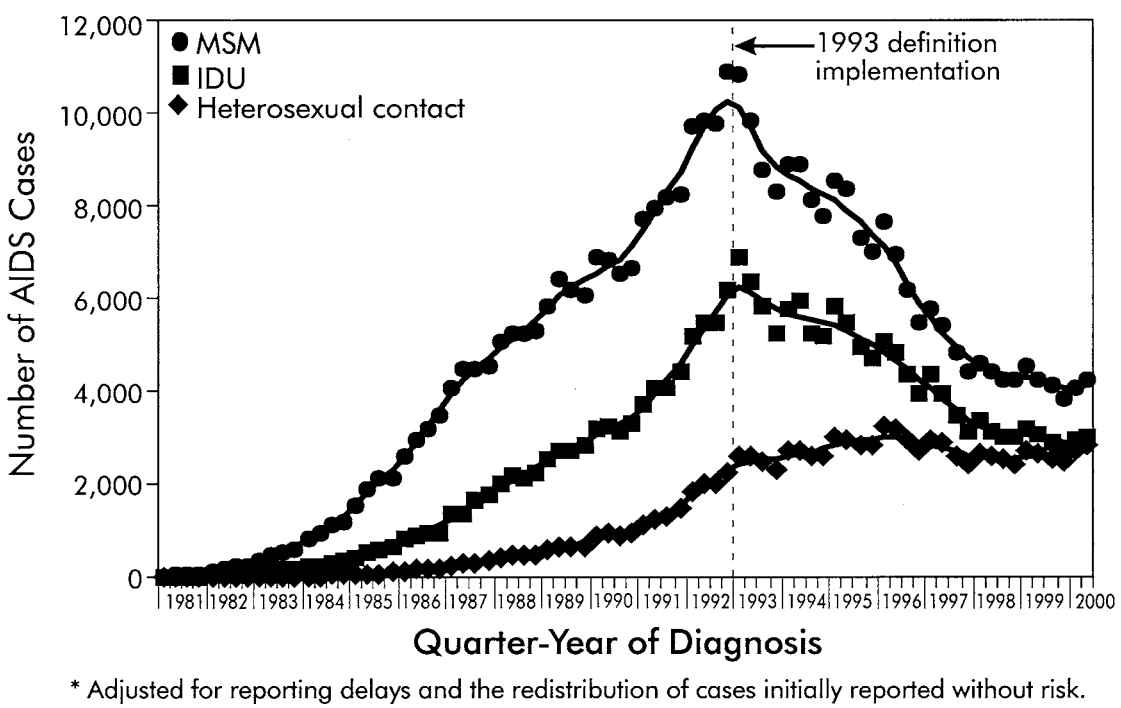
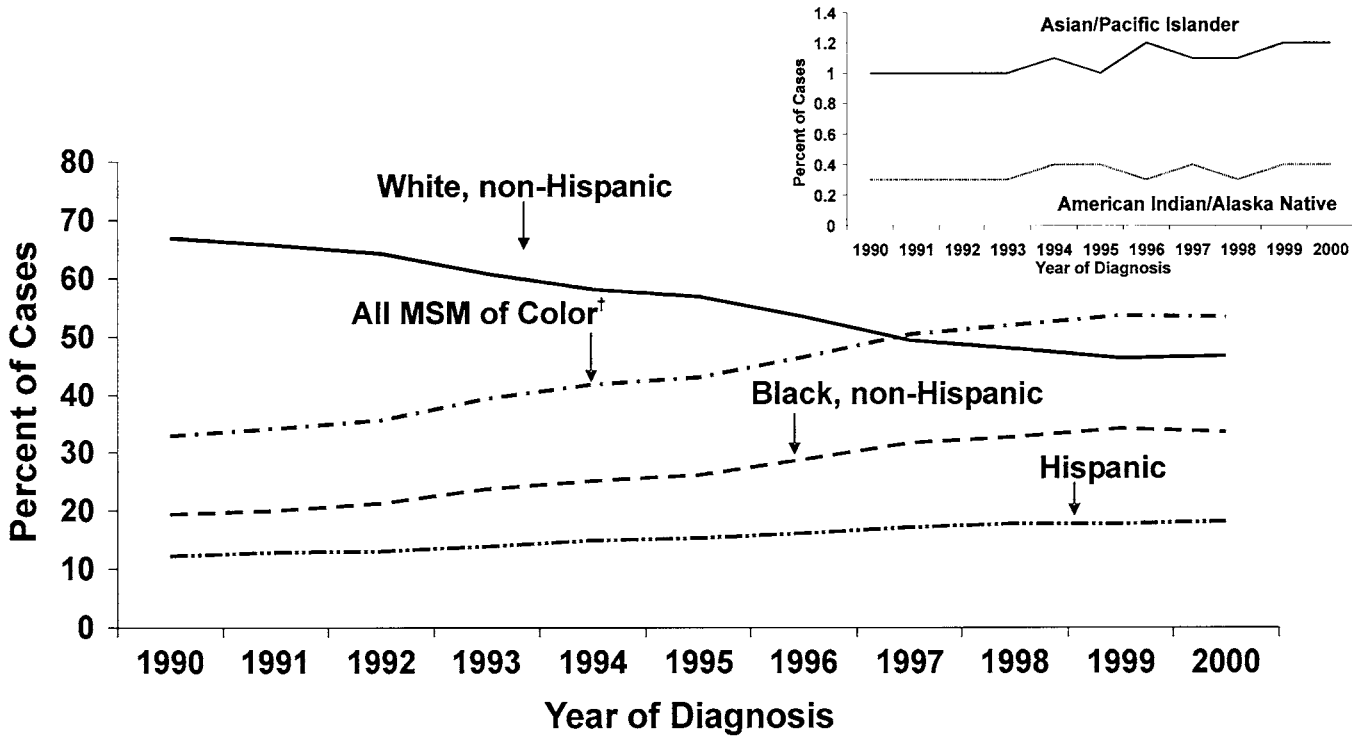


FIG. 1.11. Estimated number of AIDS cases among men who have sex with men (MSM), injecting drug users (IDU) and persons exposed through heterosexual contact, by quarter year of diagnosis, 1981–2000, United States. Source: CDC, Division of HIV/AIDS Prevention.

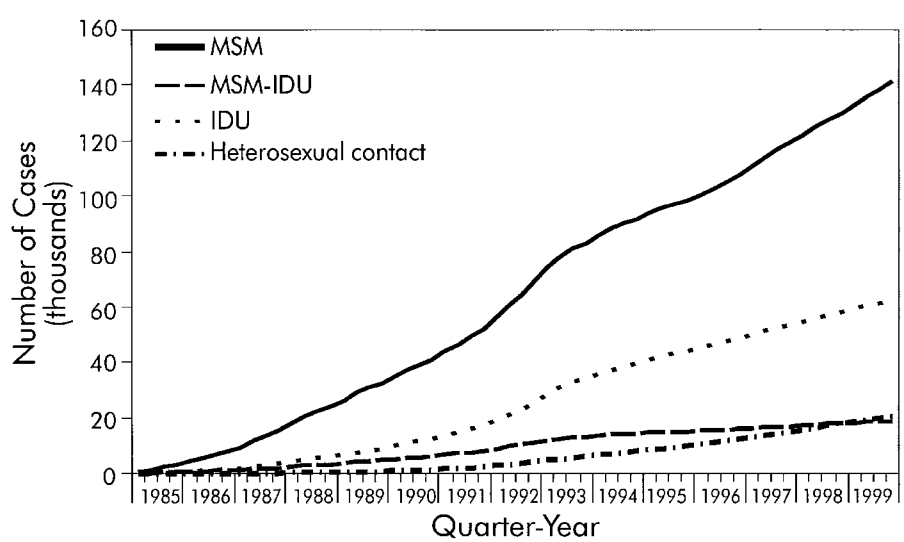


*Estimated number of AIDS diagnoses adjusted for reporting delays and unreported risk/exposure; data reported to CDC through June 2001.
†Men of Color who have sex with men (defined as non-Hispanic black, Hispanic, American Indian/Alaska Native and Asian/Pacific Islander men ≥13 years of age who have sex with men).
Total N=297,622

FIG. 1.12. Proportion of AIDS cases among men who have sex with men by race/ethnicity and year of diagnosis, January 1990–December 2000, United States. Source: CDC, Division of HIV/AIDS Prevention.

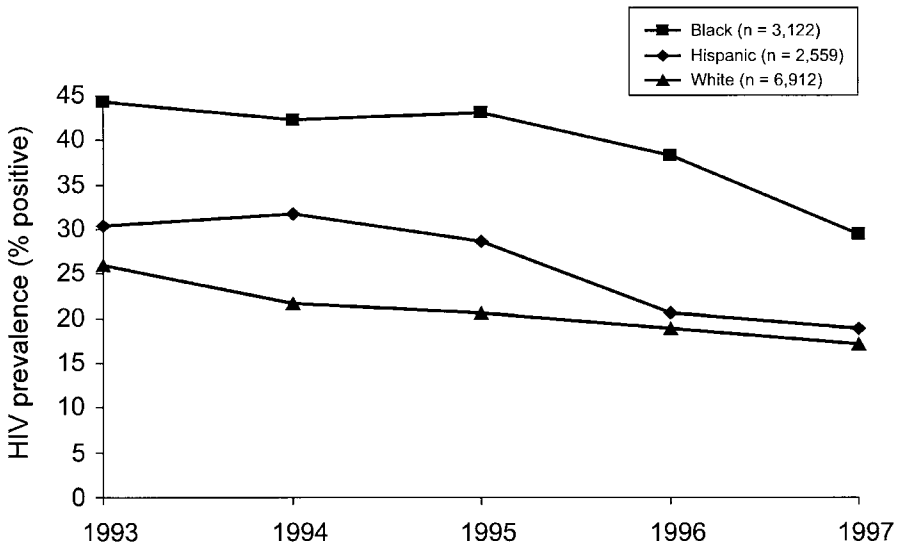
Heterosexually-Acquired Infections

The extent of the heterosexual epidemic in the U.S. has met with controversy. Some claim that the risk of heterosexual transmission is overstated in order to alarm the public and garner attention and resources to a disease that has not affected mainstream America (94). Others claim that the extent of the heterosexual epidemic has not been recognized and that prevention resources are not being appropriately targeted (95). Classifying infected persons by a behavioral risk when they are not members of a primary risk group (i.e. MSM, IDU, infected transfusion



*Adjusted for reporting delays and unreported risk

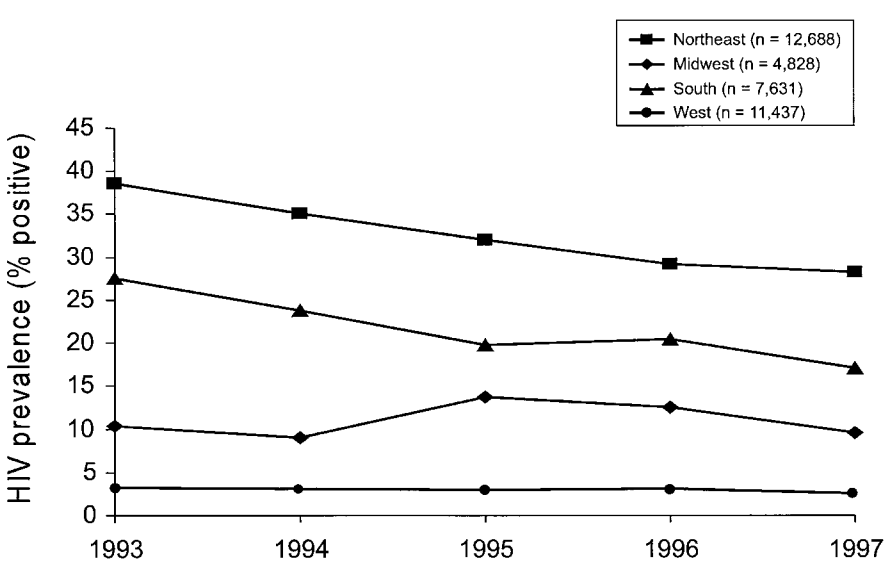
FIG. 1.13. Estimated AIDS prevalence among men, by risk exposure, 1985–1999, United States. Source: CDC, Division of HIV/AIDS Prevention.



Note. Standardized to 1993 STD clinic population by region and age group.

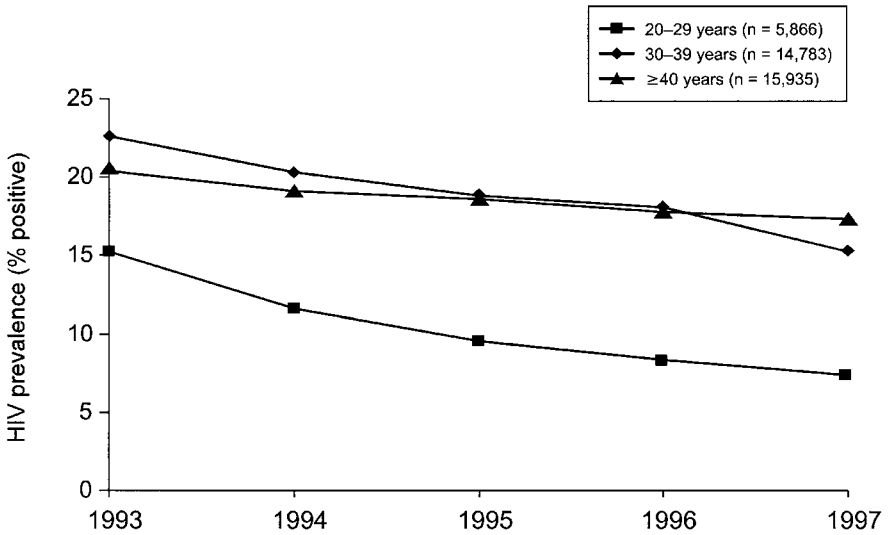
FIG. 1.14. HIV prevalence among men who have sex with men at sexually transmitted disease clinics, by race/ethnicity, 1993–1997. Source: CDC, Division of HIV/AIDS Prevention.

recipient, hemophiliac) is complicated. Obviously, for sexually active infected persons for whom one can deÞnitively rule out any exposure since 1978 through needle-sharing, male-male sex, or history of transfusion prior to 1985, they most likely were infected heterosexually (barring rare modes and infrequent occupational exposures). However, CDC has deÞned heterosexual-contact cases based on evidence that one or more sex partners is in a primary risk group or is *known* to have tested positive or to have AIDS. Most case reports are completed by infection control practitioners or health department staff from abstraction of medical records and heterosexual risk factors may not be documented unless the provider inquired about the risk behaviors or infection status of current and prior partners. Many bisexual men and drug-injectors may conceal their primary risk behavior such that heterosexual partners may not know or recognize their partnerÕs risk (96). Prevalence in the general heterosexual population is low, and heterosexual sex is a highly prevalent behavior, thus, heterosexual sex per se is not classiÞed as a risky behavior. Early in the epidemic, many partners had not been tested or had not progressed to clinical illness such that a partnerÕs antibody status was likely unknown. Among heterosexuals, identifying high risk sexual behaviors such that an infected person can be classiÞed as having heterosexually-acquired HIV, has been the subject of several studies (59,96). These studies have found that with detailed case investigation, one or more sex partners having primary risks or positive HIV test results can generally be identiÞed. However, there is an



Note. Standardized to 1993 clinic population by sex, race/ethnicity, and age group.

FIG. 1.15. HIV prevalence among injection drug users entering drug treatment centers by region, 1993–1997. Source: CDC, Division of HIV/AIDS Prevention.

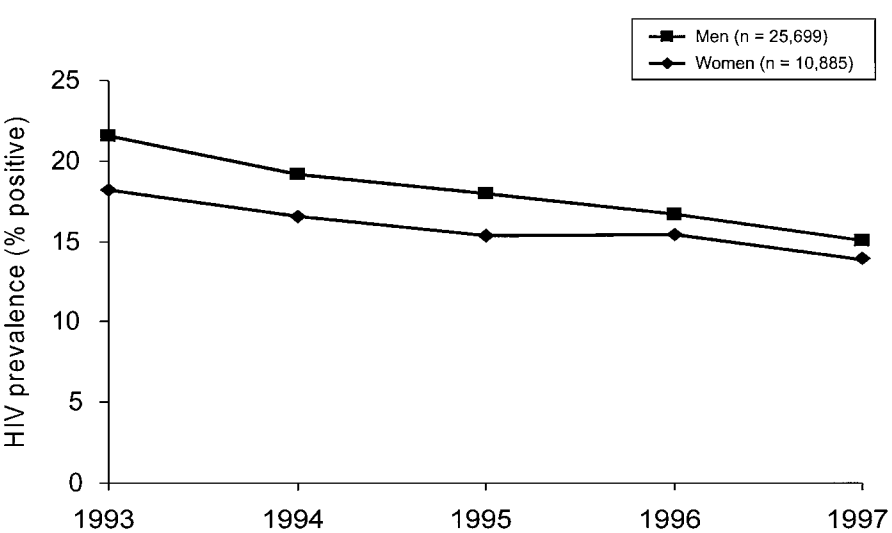


Note. Standardized to 1993 clinic population by region, sex, and race/ethnicity.

FIG. 1.16. HIV prevalence among injection drug users entering drug treatment centers by age group, 1993–1997. Source: CDC, Division of HIV/AIDS Prevention.

important group that denies behavioral risk factors and cannot identify partners having a primary behavioral risk or partners known to be HIV-infected. Such persons probably acquired HIV through secondary heterosexual transmission (i.e. transmitted sexually from persons who themselves were infected through high-risk heterosexual contact). A number of factors may be associated with the growth of a secondary heterosexual epidemic. Among the racial/ethnic minorities disproportionately affected by the IDU epidemic, high rates of non-injection drug use, notably crack cocaine, are also associated with large numbers of partners, anonymous sex, exchange of sex for drugs or money and high rates of sexually transmitted diseases (49,96Ð98). Whether the prevalence of HIV in a sub-population, speciÞcally racial/ethnic minority groups in the U.S., can achieve high enough levels to be self-sustaining and become endemic against a backdrop of high prevalence of risky heterosexual behaviors is a major epidemiologic question in the U.S. Prevention interventions targeted to heterosexual men and women who engage in high risk behaviors are needed to forestall this scenario.

Among prevalent AIDS cases at the end of 2000, 19% were heterosexual-contact cases. Women account for the majority of heterosexual-contact AIDS cases (Table 1.3); the distribution of prevalent AIDS cases by risk exposure is shown for women in Fig. 1.18. Despite overall declines in AIDS incidence in other major risk groups, slight increases have been reported in the number of heterosexual AIDS cases in the last few years and they accounted for



Note. Standardized to 1993 clinic population by region, race/ethnicity, and age group.

FIG. 1.17. HIV prevalence among injection drug users entering drug treatment centers by sex, 1993–1997. Source: CDC, Division of HIV/AIDS Prevention.

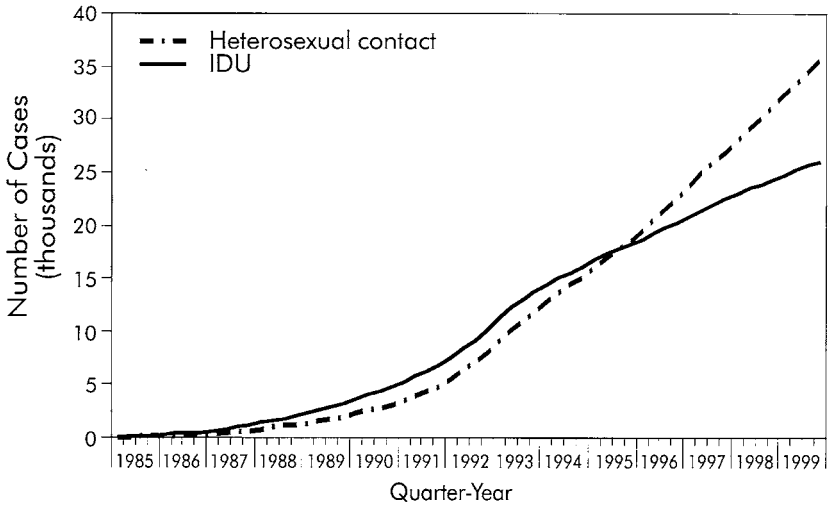
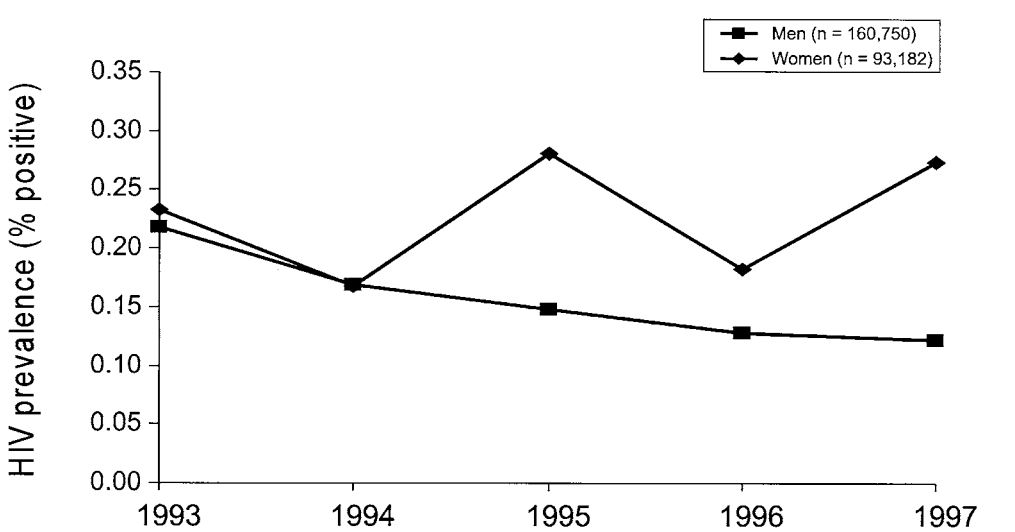


FIG. 1.18. Estimated AIDS prevalance among women, by risk exposure, 1985–1999, United States. Source: CDC, Division of HIV/AIDS Prevention.

about 28% of incident AIDS cases during 2001 (Figure 1.11) (Table 1.3). Whether this represents increases in new HIV infections in recent years in this population, or lack of access to testing and treatment, cannot be determined from the available data. However, there is some evidence of increases in new HIV cases in some states among cohorts of young women who are most likely to be recently infected (99). On the other hand, HIV prevalence among young men and women entering the Job Corps, and among military applicants suggest that prevalence in young, mostly heterosexual populations may be stable or decreasing (Figs. 1.19 and 1.20, respectively). Data from HIV reporting states showed stable numbers of new HIV diagnoses among young adults during 1994 to 2001 suggesting that HIV incidence in the youngest age groups is likely to have been fairly stable in these states in recent years (Fig. 1.21).

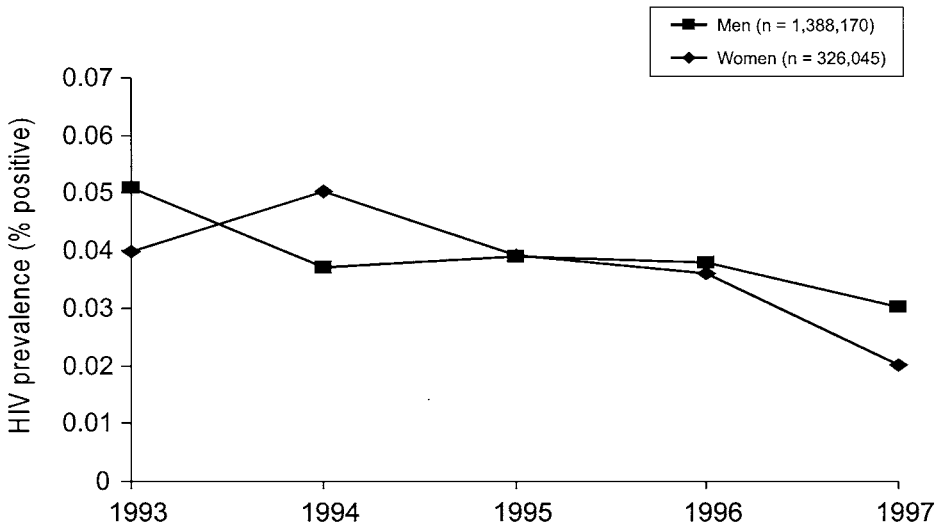
Perinatally-Acquired Infections

The perinatal AIDS epidemic peaked at above 900 cases in 1992, and declined more than 80% through 1999 (Fig. 1.22). The declines in AIDS have been inßuenced by the introduction of prophylaxis against AIDS-deÞning opportunistic illnesses including PCP, HAART for children which is maintaining infected children longer AIDS-free, but mostly, the use of ZDV and other antiretroviral therapy for pregnant women which reduced the number of infected children born each year (100Ð104). Reductions in HIV transmission rates from 25% to $<2\%$ have been reported in a number of sources (84,85,104). Thus, the pool of children at risk of AIDS has dropped dramatically. Recently, CDC estimated that 280Ð370 HIV-infected children were born in 2000, representing a signiÞcant decline from the estimated peak of 1,760 HIV infected



Note. Standardized to 1993 population of Job Corps entrants by region, race/ethnicity, age group, and metropolitan statistical area.
Source of data: US Department of Labor.

FIG. 1.19. HIV prevalence among Job Corps entrants by sex, 1993–1997. Source: CDC, Division of HIV/AIDS Prevention.



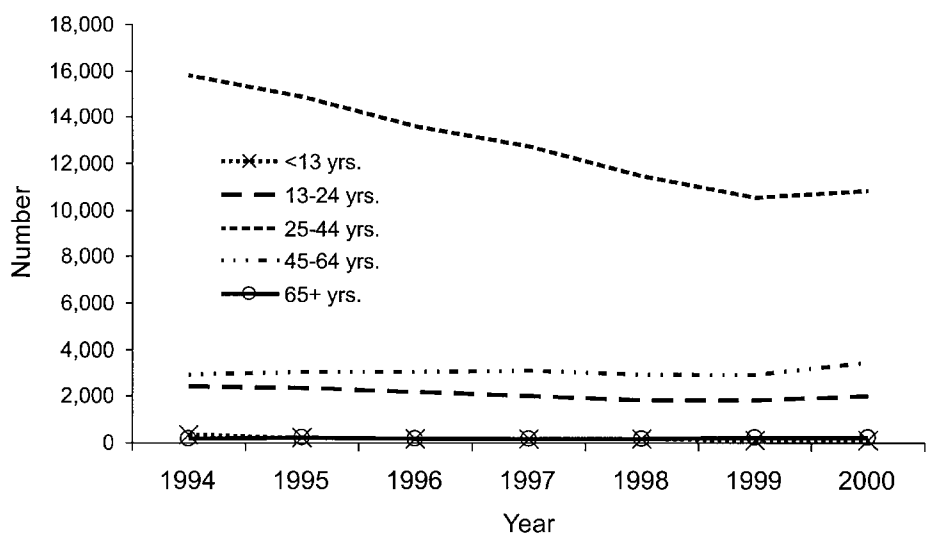
Note. Standardized to 1993 population of military applicants by region, race/ethnicity, age group, and metropolitan statistical area.
Source of data: US Department of Defense.

FIG. 1.20. HIV prevalence among military applicants by sex, 1993–1997. Source: CDC, Division of HIV/AIDS Prevention.

children born during 1991 (105). These declines reßect the successful implementation of public health guidelines for universal offering of voluntary testing to pregnant women, ZDV and other antiretroviral treatments which have reduced transmission rates, and improved obstetrical practices for HIV-infected women. To further reduce the number of infected children born each year, programmatic efforts are centered on outreach to high-risk women to promote access to prenatal care, testing and treatment.

HIV/AIDS Prevalence in the U.S.

Following the peak of infections in the mid-1980s, the prevalence of AIDS began to rise gradually as infected persons progressed inexorably to AIDS. However, prior to HAART, survival following an AIDS diagnosis was poor with most persons dying within two years (6). With the HAART-associated increases in survival of AIDS patients on treatment (Fig. 1.23), death rates with HIV as the



*Includes all new HIV diagnoses with and without AIDS. Adjusted for reporting delays and redistribution of exposure for cases reported without information on mode of exposure.

+Alabama, Arkansas, Arizona, Colorado, Idaho, Indiana, Louisiana, Michigan, Minnesota, Mississippi, Missouri, New Jersey, Nevada, North Carolina, North Dakota, Ohio, Oklahoma, South Carolina, South Dakota, Tennessee, Utah, Virginia, West Virginia, Wisconsin, and Wyoming.

FIG. 1.21. Estimated number of HIV diagnoses, by age group and year of diagnosis—25 states, 1994–2000. Source: CDC, Division of HIV/AIDS Prevention.

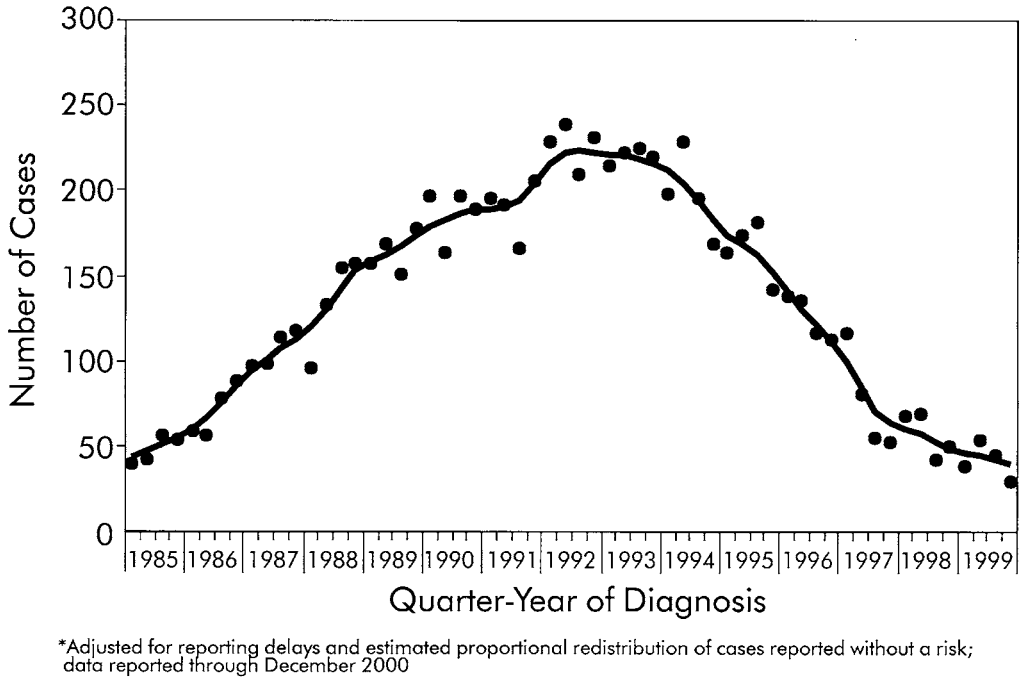


FIG. 1.22. Perinatally-acquired AIDS cases by quarter year of diagnosis, 1985–1999, United States. Source: CDC, Division of HIV/AIDS Prevention.

underlying cause have declined. Among adults 25Ð44, it was the leading cause of death from 1993 through 1995 and dropped to the Þfth cause in 1998 (Fig. 1.24). As survival of persons with HIV/AIDS has improved, deaths due to HIV infection decreased and the proportions of deaths of persons with HIV/AIDS that were caused by other conditions increased (7). The prevalence of AIDS has steadily increased as deaths declined more rapidly than AIDS incidence (Fig. 1.25). If survival gains continue, and infections are not reduced, overall HIV prevalence (including AIDS) can be expected to rise. During 1998 through 2000, the number of deaths annually was less than half the estimated number of persons newly infected, as well as less than half of the number of new AIDS cases. If these patterns continue, HIV prevalence will rise, and the proportion of infected persons who are AIDS-free will rise. Factors that can change the current dynamics include: a rise or decline in annual infections from the estimated

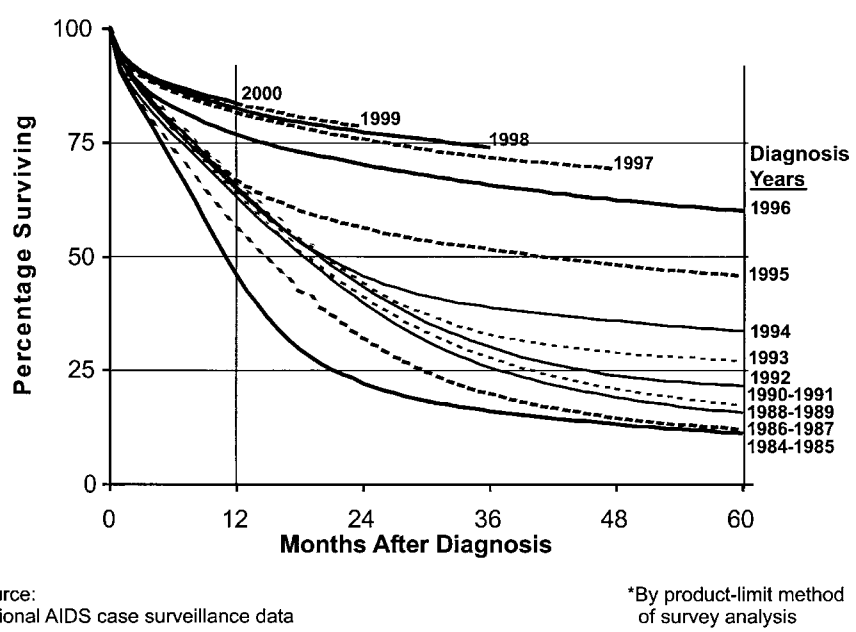


FIG. 1.23. Cumulative proportion of AIDS patients surviving, by months after diagnosis of the first AIDS-defining opportunistic illness, for different years of diagnosis of the opportunistic illness. Source: CDC, Division of HIV/AIDS Prevention.

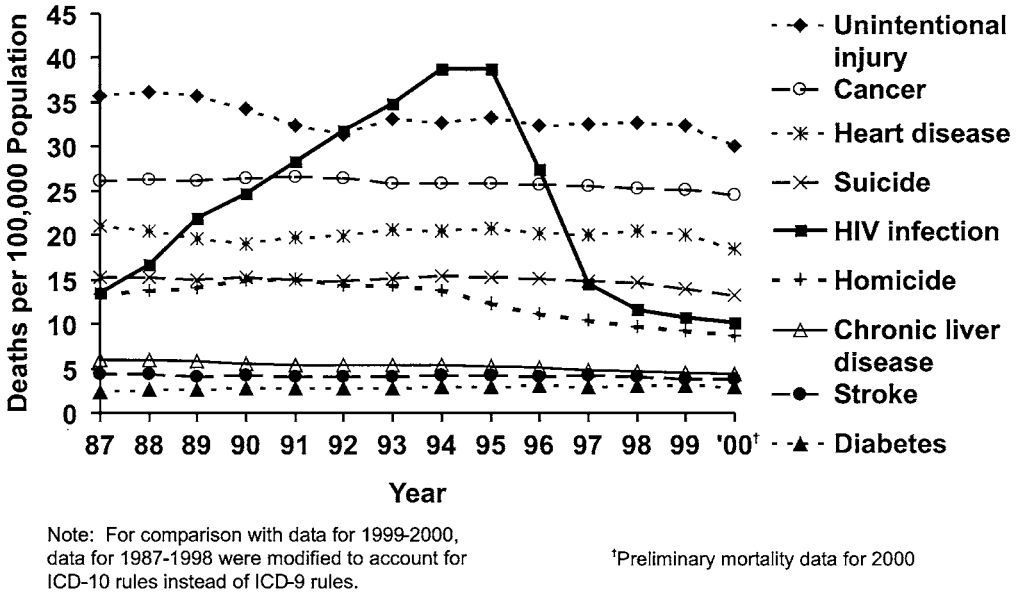


FIG. 1.24. Trends in annual rates of death from leading causes of death among persons 25–44 years old, 1987–2000, United States. Source: CDC, National Center for Health Statistics.

stable rate of approximately 40,000 per year during the l990s; a rise in deaths due to the emergence of treatment resistant viral strains, aging of the infected cohort with competing causes of mortality, serious or life-threatening side effects of treatment, declines in offering or accepting timely HIV testing, or decreases in access to treatment; a further decline in deaths due to improved therapies, and better access to early testing and treatment. As of 2000, CDC estimates that there have been from 1.3 to 1.4 million cumulative HIV infections: approximately 450,000 cumulative deaths and an estimated three-fourths of the 850,000Ð950,000 persons living with HIV/AIDS had been diagnosed with HIV/AIDS (2).

Clinical Manifestations and Impact of Treatment

The clinical course of HIV is characterized by an acute viral illness following infection, an incubation period of

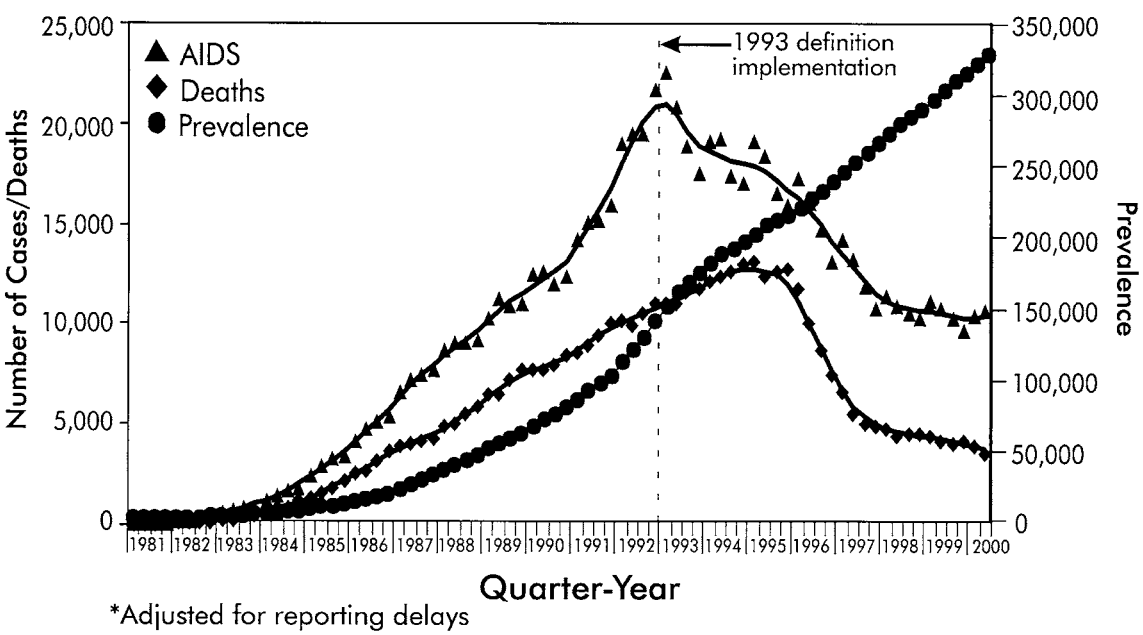


FIG. 1.25. Estimated AIDS incidence, deaths, and prevalence, by quarter year of diagnosis/death, 1981–June 2000, United States. Source: CDC, Division of HIV/AIDS Prevention.

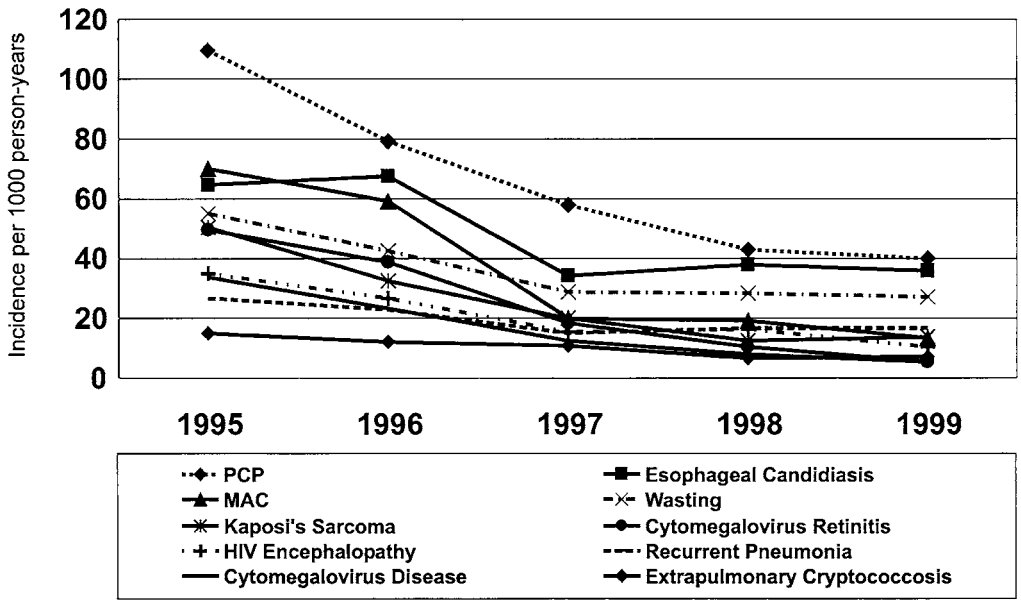


FIG. 1.26. Annual trends in the 10 most common incident opportunistic illnesses, Adult/adolescent Spectrum of Disease (ASD) project, 1995–1999. Source: CDC, Division of HIV/AIDS Prevention.

variable duration which may be marked by no or mild nonspeciÞc clinical symptoms during which viral load increases and the CD4 count decreases, severe immunosuppression which is associated with the onset of AIDS-deÞning conditions, and death. Because the AIDS epidemic was Þrst recognized based on the appearance of unusual clinical symptoms in previously healthy young men, early surveillance efforts concentrated on describing the spectrum of illnesses that were associated with HIV-induced immunosuppression. AIDS cases were reported based on the occurrence of an initial AIDS-deÞning condition such that AIDS incidence reliably tracked trends in the initial AIDS OIs. However, with the inclusion of the immunologic criterion in the 1993 case deÞnition, AIDS surveillance data no longer reliably tracked speciÞc AIDS-related clinical conditions. Instead, large cohorts or multi-site surveillance projects are relied on to track trends in the incidence and prevalence of speciÞc HIV-related conditions in the HIV-infected population, and to examine the impact of treatment on the occurrence of opportunistic illnesses. One such project, the Adult/Adolescent Spectrum of Disease Project, has conducted reviews of medical records of persons with HIV or AIDS every six months until death or loss to follow up since 1990 in over 100 clinical facilities in 11 geographic areas. In 1995, the last year before the widespread use of HAART, among persons having one or more AIDS-deÞning conditions, the most frequently occurring initial opportunistic illnesses were *Pnemocystis carinii* pneumonia (35%), esophageal candidiasis (14%), Kaposiõ sarcoma (13%), wasting syndrome (7%), and *Mycobacterium avium* complex (7%) (106). Pulmonary tuberculosis, which was added to the AIDS case deÞnition in 1993 to reßect the intersection of the TB and AIDS epidemics, occurred in 4% of persons with AIDS. The incidence per 1,000 person years of observation of any AIDS-deÞning condition was 269 in 1995; by 1996 and 1997, incidence had decreased to 215 and 148, respectively. This trend was directly associated with increased proportions of subjects who were prescribed triple combination antiretroviral therapy containing a protease inhibitor. The declines in the incidence of the most commonly occurring opportunistic illnesses were greatest from 1995 through 1998 followed by a stabilizing trend, similar to that observed in national AIDS surveillance data (107) (Fig. 1.26). The beneÞcial impact of antiretroviral therapy in preventing or delaying the onset of severe AIDS-deÞning illnesses is reßected in improved AIDS-free survival times and in decreased risk of mortality among patients with AIDS (6,107Ð109).

Acute primary HIV infection is associated with a rapid rise is plasma viremia. Although there are no large-scale population-based studies from which estimates can be derived, in small studies, more than half of newly infected persons experience an acute retroviral syndrome (ARS). This illness is characterized as ßu-like and is marked by fever, lymphadenopathythy, rash, and myalgia. Onset occurs within a few weeks of infection and the syndrome generally resolves with two weeks (110,111). Many persons with ARS who seek care may not be offered HIV testing, therefore, it is important that clinicians be alert to a history of HIV risk behaviors in persons presenting with such symptoms. Timely HIV diagnosis may improve opportunities to prevent further HIV spread as infectiousness may be high during this stage; as well, early identiÞcation of HIV offers the best options for clinical management and treatment consistent with current guidelines (102,112,113). Following ARS, seroconversion occurs as antibody levels begin to rise and nearly all HIV-

infected persons have detectable antibody 6 months after infection (114). Recent testing technology can distinguish between positive antibody tests of long duration and those in the window period of seroconversion, thus identifying incident HIV infections among all persons with positive antibody tests (56).

Progressive immune dysfunction follows infection but only a small proportion of infected persons develop AIDS OIs within the Þrst few years (64). The proportion progressing to AIDS increases with time and is estimated to follow a normal distribution with a median incubation period of approximately 10 years. Regular monitoring of CD4 counts and viral load are crucial tools for detecting progressive disease in advance of the appearance of severe clinical symptoms. However, as most persons living with HIV now have been diagnosed, and as opportunities to receive treatment increase, AIDS cases increasingly represent persons who were not diagnosed previously or those for whom treatment regimens may be failing due to resistance or adherence problems. Recent reports of side effects of long-term treatment with protease inhibitors suggest that the spectrum of clinical conditions occurring in persons with HIV may be much changed in the treatment era compared to the natural history era (7,115,116).

The rapid evolution of the treatment armamentarium has led to on-going updates of treatment recommendations via the internet to provide clinicians with up-to-date recommendations. Published guidelines for HIV treatment for adults/adolescents (113), children (103), and pregnant women (104) are routinely reviewed and updated based on the strength of evidence ranging from clinical trials to observational studies. They are disseminated widely and rapidly Òon-lineÓ http://www:hivatis.org/trtg;dlns.html #Adult or http://www: hivatis.ors/trtsdlns.html#Pediatric

EMERGING ISSUES

The third decade of the HIV pandemic looms ahead with innumerable challenges. Advances in prevention science and a growing number of treatment regimens have raised new research, surveillance and programmatic issues (117,118). Behavioral risk reduction has been achieved in many at-risk populations, notably safer sex and injecting practices among MSM and IDUõ, respectively, but as people with HIV are diagnosed earlier and survive longer on treatment, sustaining safer sex and drug-using behaviors among infected persons will require ongoing programs to reduce further transmission. Reports of on-going risky sexual behaviors among infected persons emphasize the need for research studies to identify effective interventions, programs to support risk reduction, and monitoring of behavioral risk factors (119). Behavioral surveillance efforts in at-risk and infected populations have lagged disease surveillance efforts. Rapid clinical advances have been accompanied by reports of side effects of treatment as well as the threat of transmitting drug-resistant viral strains (116,120). Efforts to develop effective treatments with less complex dosing and fewer side effects are needed to promote adherence and decrease the risk of developing drug-resistance. The intersection of disease prevention and control comes in efforts to develop new rapid testing technologies to promote testing acceptance and knowledge of infection status, referrals to care and treatment for positive persons, and surveillance efforts to implement a nationwide HIV reporting system with the capacity to characterize incident infections and use these data to target prevention resources to the highest risk communities (121Ð124). Increasingly, surveillance data are relied on for prevention and treatment services planning, evaluation, and resource allocation. Ultimately, Þelding an effective vaccine may offer the best hope of HIV prevention and control worldwide.

The public health surveillance system for HIV/AIDS is widely recognized as among the best disease surveillance systems in the world. As the need for accurate, timely, and high quality data on persons with HIV/AIDS continues to grow in response to the growing array of prevention and treatment programs, expanded support for and commitment to public health surveillance will be required by all levels of government, health care providers and affected communities. As one of the costliest epidemics in human history, the HIV epidemic will no doubt ultimately be contained. The cost of that containment in human lives will be determined by political, social and economic forces that will either enable or thwart the delivery of effective and compassionate prevention and control measures worldwide.

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Chapter 2

Epidemiology of Pediatric HIV Infection

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The Þrst cases of the acquired immunodeÞciency syndrome (AIDS) in children were reported to the Centers for Disease Control and Prevention (CDC) in 1982. Since that time nearly 9,000 cases of AIDS in children aged less than 13 years and over 5,000 deaths in children under 15 years have been reported (1). Perinatal transmission of the human immunodeÞciency virus (HIV) accounts for 91% of the pediatric AIDS cases and almost all new HIV infections in children (1). Based on data from the National Survey of Childbearing Women, conducted during 1989Ð1995, the CDC estimates that approximately 6,000 to 7,000 infants are born each year to human immunodeÞciency virus (HIV)-infected women in the United States (2). Considerable advances in the understanding of the pathogenesis, diagnosis, treatment, monitoring, and prevention of HIV infection in children have changed the epidemiology of pediatric HIV infection in United States.

EPIDEMIOLOGY IN CHILDREN

In the United States, 8908 (1.2%) of the 774,467 cases of AIDS reported to the CDC as of December 2000, occurred in children aged under 13 years and another 4061 cases occurred in adolescents aged 15 to 19 years. Children in the United States have acquired HIV infection primarily through two routes: perinatally (mother-to-child) and by transfusion of blood or blood products (Table 2.1). In most developed countries, transmission of HIV through blood products virtually ended in 1985, when universal screening of blood donations for HIV antibody began (3,4). Trends in pediatric AIDS cases therefore reßect predominantly trends in perinatally acquired cases. The number of perinatal AIDS cases reported each year continued to increase through 1992, leveling off in 1993 and declining dramatically throughout the late 90s (5) (Fig. 2.1). From 1993 to 2000, an 81% decline in perinatal AIDS was observed, largely attributable to the widespread implementation of recommendations for zidovudine (AZT) therapy to HIV-infected pregnant women; combination antiretroviral therapy for the womanÕ9 own care and obstetrical interventions such as scheduled cesarean delivery. Some of the decline is also attributable to improved treatments that delay the onset of AIDS-deÞning illness for HIV-infected children, such as prophylaxis for *Pneumocystis carinii* pneumonia (PCP), a common AIDS-deÞning condition.

The demographic characteristics of children acquiring HIV through these various routes differ markedly. Characteristics of children with perinatally acquired AIDS parallel those of women with HIV infection and AIDS in the United States (Fig. 2.2). The HIV epidemic among women is concentrated in the Northeast and in the South, especially in New York, Florida, Texas, California and New Jersey. Black and Hispanic women are disproportionately affected by the HIV epidemic, with the rates of AIDS for females reported in 2000 being highest in black, non-Hispanic at 45.9 per 100,000, followed by Hispanic at 13.8 per 100,000 compared with white, non-Hispanic at 7.9 per 100,000 (1). In the United States, most of these children are from large eastern urban areas (Table 2.2), and they are largely of minority race and ethnicity (Table 2.3). The male-to-female ratio in these children is 1:1, indicating that mothers transmit equally to male and female infants. Mothers of these children have most commonly acquired HIV infection through injecting drug use (39%) or heterosexual transmission (36%) (Table 2.1).

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TABLE 2.1. *Reported estimates of per-contact HIV transmission risk from selected studies*

Exposure category	2000 Number	%	Cumulative 1982–2000 Number	%
Perinatally acquired (by mother's risk factor	177	90	8133	91
Intravenous drug user	40		3172	
Heterosexual contact	79		2969	
Transfusion recipient	2		153	
Risk unknown	56		1839	
Transfusion acquired	2	1	382	4
Hemophilia	1	1	237	3
Other/unknown	16	8	156 *	2
Total	196	100	8908	100

* Includes 26 children with sexual abuse as risk factor.

In the United States, since the implementation of heat treatment for clotting factors in 1984 and nationwide screening of blood donors in 1985, in conjunction with self-deferral of blood-donations from high-risk individuals, the incidence of HIV infection attributable to HIV transmission through blood and blood products has been virtually eliminated (6,7). As of December 2000, only two children in the United States developed AIDS following the receipt of blood screening negative for HIV antibody at the time of donation, after which the donor seroconverted (1). Children infected by transfusion account for 4% and children with hemophilia or other clotting disorders for 3% of cumulative pediatric AIDS cases (1). Most children with transfusion-acquired AIDS were transfused in infancy because of perinatal problems; the demographics of these cases reßect characteristics of children with increased perinatal morbidity (7,8). The racial distribution of children infected from blood products is more reßective of the United States population. The male-to-female ratio differs between these groups (Table 2.3). This is consistent with the Þnding in a study of blood-transfusion practices, that male infants are more likely to receive transfusions than females (9) and that blood products are used for clotting disorders, such as hemophilia, which are most

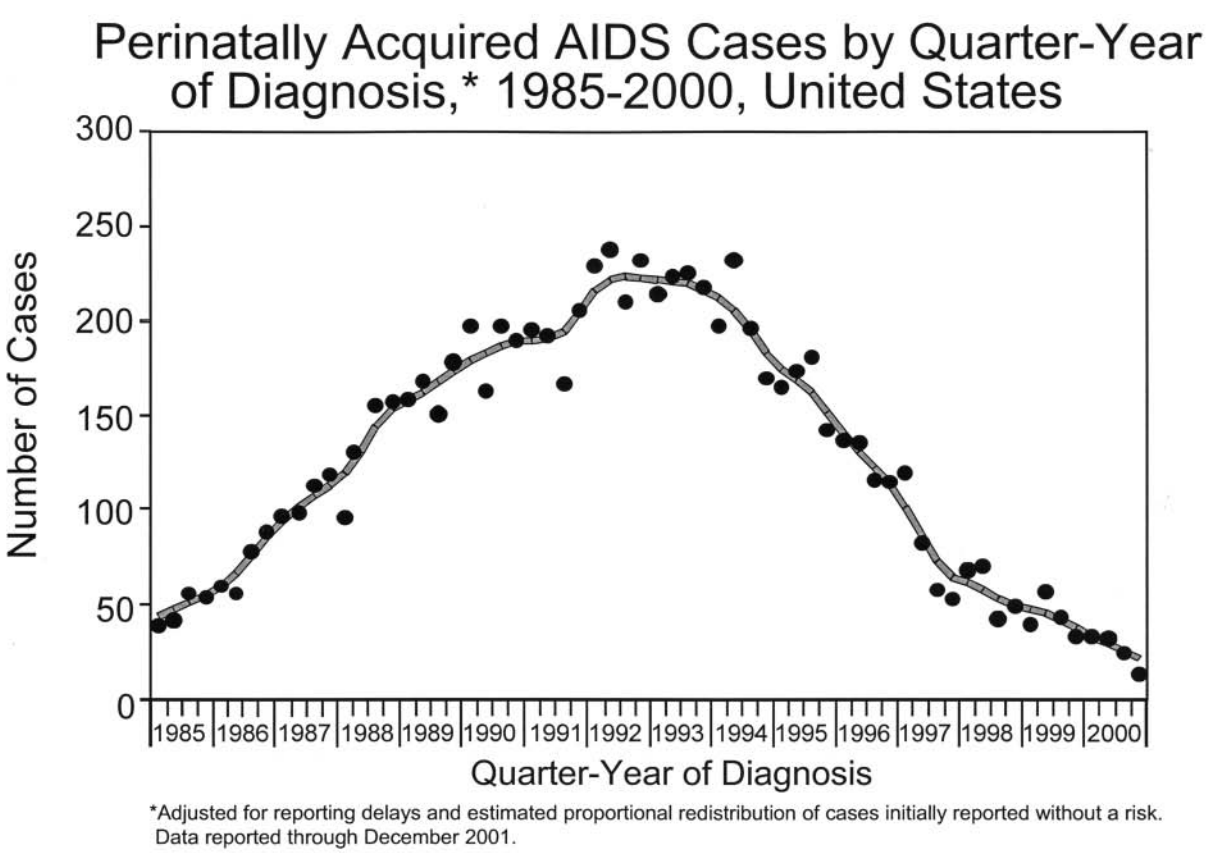

FIG. 2.1. Perinatally acquired AIDS cases by quarter-year of diagnosis, 1985–2000, United States.

commonly sex-linked genetic disorders. Nearly all patients with transfusion-associated AIDS received their contaminated transfusion in or before 1985 and thus are now adolescents and young adults. Similarly patients with hemophilia, diagnosed with AIDS as children are now largely young adults.

With the implementation of routine testing for HIV in pregnancy, recommended since 1995, most HIV-exposed babies should be identiÞed before birth. However, infants born to women who have not been tested for HIV, may be Þrst recognized when they present with symptoms. Untreated, 50% of HIV-infected children will develop serious HIV-associated illness by 5 years of age and will have an AIDS deÞning illness by 6.6 years (10). As of December 2000, 180 adolescents who acquired HIV perinatally were not diagnosed with AIDS until 13 years of age or older and are counted as adolescent or adult cases (1).

It was estimated, using data from the Pediatric Spectrum of Disease Project (PSD) and the CDC HIV/AIDS surveillance databases, that in 1999 in the United States there were 10,000 children and youth living with HIV of whom 2400 were adolescents (11). Improvement in survival of children with HIV/AIDS is largely due to two factors: the use of combination antiretroviral therapy and prevention of PCP. Without antiretroviral therapy, a bimodal progression pattern has been reported in children infected by perinatal transmission. About 10% to 20% of infected infants developed rapidly progressive disease and died within the Þrst few years of life (10,12). In the remaining children however, the disease advanced more slowly and they survived well beyond school age with a course similar to that seen in adults (10,12). The annual number of deaths from pediatric AIDS has declined each year from 586 in 1994 to 107 in 1999 (1). Initially the effect of antiretroviral therapy on survival in children with AIDS was limited (4,13). But with current antiretroviral therapy, survival has improved by about 70% and the annual mortality rate decreased from 5.3% to 0.7% (14,15). In the early 1990s the most common cause of death was pneumocystosis, accounting for about 25% of deaths, but by 1998, it accounted for only 6.2 to 8.8% of deaths (16).

The incidence of AIDS in children has also been reduced by these two factors: the use of combination antiretroviral therapy and prevention of PCP. PCP was also the most common AIDS deÞning condition (ADC) in children and infants pre-1995, accounting for about one-third of ADCs. Since 1996, the proportion of ADCs due to PCP has fallen to about 25% (17). This change in

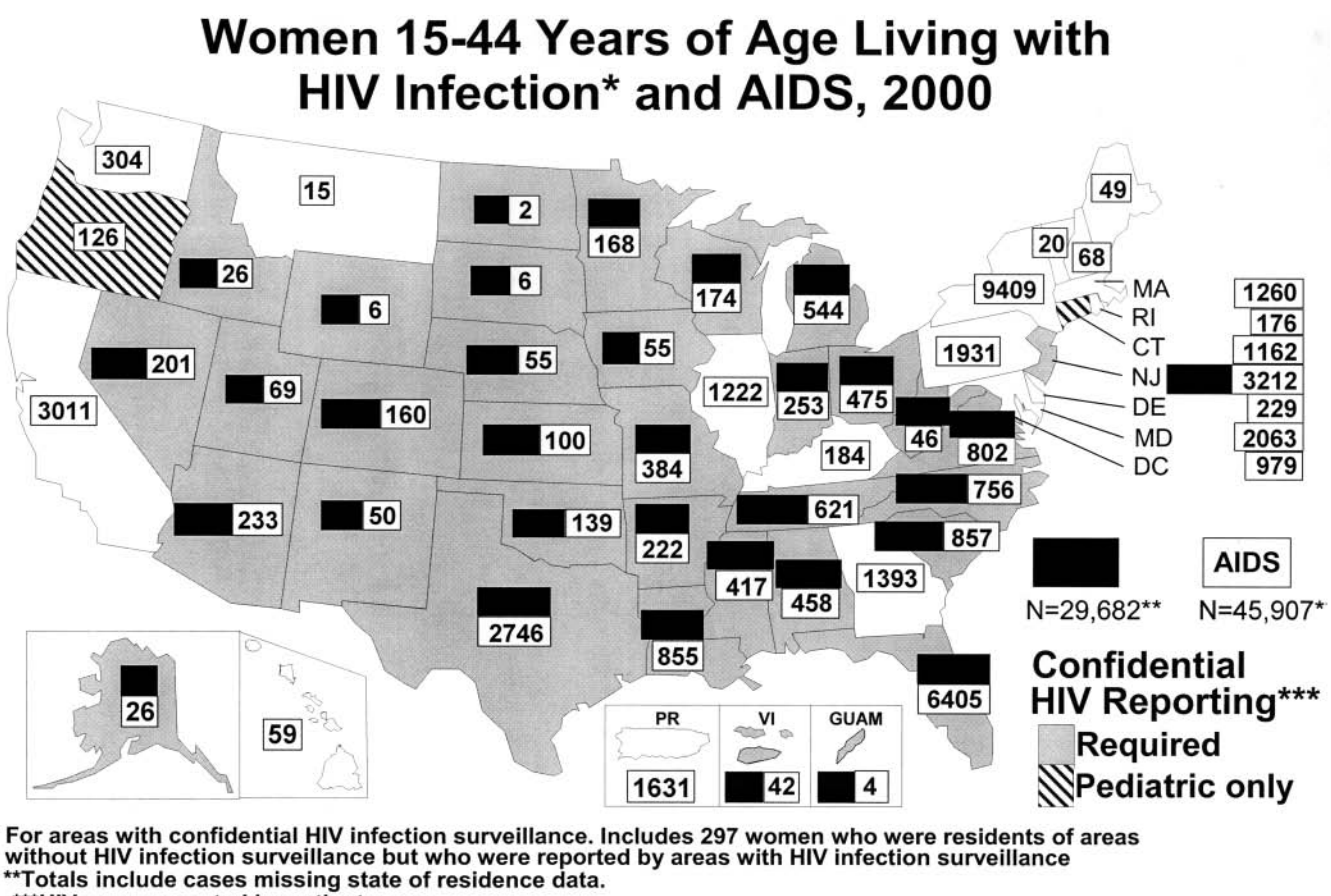

FIG. 2.2. Women 15–44 years of age living with HIV infection and AIDS, 2000.

TABLE 2.2. *Geographic distribution of children (aged < 13 years old) reported to the CDC through December 31, 2000, United States, cumulative AIDS cases and currently living with HIV/AIDS*[1]

Area of residence	Cumulative AIDS cases	Living with HIV/AIDS
New York	2242	503
Florida	1402	640
New Jersey	751	499
California	608	155
Puerto Rico	388	89
Texas	380	361
Pennsylvania	325	152
Maryland	301	129
Illinois	269	107
Georgia	211	84
Massachusetts	206	96
Connecticut	176	132
District of Columbia	171	84
Virginia	169	130
Louisiana	125	137
Ohio	121	90
North Carolina	116	130
Michigan	107	96
Other U.S. States	823	596
Total[2]	8908	4314

[1] Includes HIV infection not AIDS for persons reported from areas with con dential HIV reporting.
[2] Includes data from United States (50 states and the District of Columbia) and from U.S. dependencies, possessions and independent nations.

incidence of PCP occurred after implementation of guidelines to start PCP prophylaxis for all infants born to mothers with HIV-infection and to discontinue when the infant is conÞrmed to be not-infected (18).

The successes of antiretroviral therapy and prevention of opportunistic infections, such as PCP, has led to HIV infection becoming more of a chronic illness than a rapidly fatal one. This means that many more infected infants and children will be living to be adolescents and adults. This brings with it additional issues for care of adolescents with HIV, such as acceptance of complex treatment regimens and the potential toxicity of long term drug therapy, as well as the many psychosocial issues for adolescents with a chronic illness, especially one that is transmissible.

TABLE 2.3. *Demographic characteristics of children (aged < 13 years) with AIDS, cumulative data reported to the CDC as of December 31, 2000 by transmission categoryÑUnited States*

Demographic characteristic	Transmission category			
	Perinatal *n* = 8133	Transfusion acquired *n* = 382	Hemophilia *n* = 237	Other/unknown *n* = 156
	No. (%)	No. (%)	No. (%)	No. (%)
Sex				
Female	4103 (50)	141 (38)	7 (3)	86 (55)
Male	4030 (50)	241 (62)	230 (97)	70 (45)
Race				
White	1173 (14)	189 (49)	159 (67)	27 (17)
Black	5010 (63)	89 (23)	34 (14)	98 (63)
Hispanic	1875 (23)	93 (24)	38 (16)	26 (17)
Other[a]	61 (< 1)	11 (3)	5 (2)	5 (3)
Unknown	14 (< 1)	0	1 (< 1)	0

[a] Asian/Paci c Islander/American Indian/Alaska Native.
CDC, Centers for Disease Control and Prevention.

MODES OF TRANSMISSION IN CHILDREN

Perinatal Transmission

Prospective studies of mother-to-infant transmission in both developed and developing countries found transmission rates of 13% to 40% (19–22). Transmission rates in developed countries, such as the United States, are generally lower, between 15% and 25%, whereas rates in developing countries, such as those in Africa, are generally higher between 25% and 40% (23). Differences in transmission rates from study to study reßect differences in factors that affect the likelihood of mother-to-infant transmission in the populations under study.

Mother-infant transmission of HIV can occur during pregnancy (*in utero*), during labor and delivery (intrapartum); and through breast-feeding (postpartum). *In utero* transmission has been demonstrated by identiÞcation of the virus in fetal tissue and amniotic ßuid (24–26). Increasing evidence indicates that most perinatally infected infants acquire their infection close to or during labor and delivery (intrapartum period). Among infants who are not breast-fed, the proportion of infections occurring *in utero* is estimated to be approximately 25–40% and 60–75% intrapartum (27,28). The absolute risk for *in utero* transmission is estimated to be approximately 5–6%, and for intrapartum 13 –18% (29,30).

A large body of literature has described risk factors associated with perinatal HIV transmission. The pathogenic basis for transmission is affected by a number of factors, some of which are not fully understood. A number of studies have shown that maternal viral load is a critical determinant of perinatal HIV transmission (31–33). Other factors associated with an increased risk of transmission include low maternal CD4 counts, advanced maternal illness, vaginal delivery, premature delivery, and longer duration of rupture of membranes (22,27,29,34–38). There is also evidence to suggest that mother-child discordance of HLA and HLA supertypes is associated with decreased risk of HIV transmission (39,40). There is less conclusive evidence that the following factors affect perinatal HIV transmission: viral genotype or phenotype, viral strain diversity, chorioamnionitis, sexually transmitted infections, fetal scalp electrodes, and maternal factors such as, illicit drug use, cigarette smoking, anemia, nutritional status (27,31).

Evidence for HIV transmission through breast milk was Þrst documented in case reports of mothers who were infected subsequent to giving birth and transmitted HIV to their breast-fed infants (41–43). HIV has been isolated from breast milk (44,45). In addition, studies of mothers who were infected during or before pregnancy showed an increase in the maternal-infant transmission rate among those who breast-fed compared with those who did not. A meta-analysis of six studies indicated an additional risk of about 14% (46). In a randomized trial of the effect of formula feeding versus breast feeding on perinatal HIV transmission, the frequency of breast milk transmission of HIV was 16.2% (47). Factors affecting HIV transmission from breast milk are currently a topic of research in order to determine strategies to reduce perinatal HIV transmission in the developing world. It appears that most HIV transmission occurs during the early weeks to months of breast-feeding, estimated as 66% in the Þrst 6-weeks of life, or 0.7% per month in the Þrst six months, 0.3% per month from 12 to 18 months and 0.2% per month from 18 to 24 months (48). In one study, from South Africa, it was found that HIV transmission was lower in those infants exclusively breast-fed compared to those with mixed breastfeeding (hazard ratio (HR) = 0.56) (49). Further studies are needed to conÞrm this observation. Other factors associated with postnatal HIV transmission are maternal nipple lesions, mastitis, maternal CD4 count, maternal seroconversion during breast-feeding and infant oral thrush (50).

Transfusion-Associated Transmission

Transfusion of blood or blood components from an HIV-infected donor is a highly efÞcient mode of transmission. More than 90% of persons receiving an HIV-seropositive unit of blood acquire the infection (51,52). Both cellular and plasma components of whole blood have transmitted HIV infection. Immunoglobulin preparations including Rh factor have not transmitted HIV because the fractionation process used to prepare these products effectively removes HIV by partitioning and inactivation (53).

The CDC has estimated that between 1978 and 1985, about 12,000 transfusion recipients in the United States who survived their underlying disease acquired transfusion-associated HIV infection (54). Persons receiving multiple transfusions between 1978 and 1985 were at greater risk. For this period, HIV seroprevalence rates of 4% to 8% were detected in leukemic (54), hemodialysis (55), and other patients receiving multiple transfusions (56). In the cumulative data reported to CDC as of December 31, 2000, of the children less than 13 years of age diagnosed with AIDS, 382 cases (4%) had transfusion acquired infection (1).

Since donor screening was implemented in March 1985, two pediatric cases of AIDS following infection acquired from blood that was screened as HIV negative have been reported to the CDC as of December 31, 2000 (1). With donor screening using questionnaires and laboratory tests for HIV antibodies and p24 antigen, the chances of HIV transmission from a unit of blood have been reduced to between 1 in 450,000 and 1 in 660,000 (4). In 1999, U.S. Blood Banks implemented Nucleic Acid AmpliÞcation Testing (NAT) of all donated units of blood; this has further reduced the risk of an HIV-infected unit of blood being transfused to about one in two to three million (57).

HIV Infection from Blood Products

Historically, both Factor VIII and IX concentrates have transmitted HIV (53). In the United States up to 80% of persons with hemophilia receiving large-pool, non-heat-treated factor products from plasma donated before HIV screening, were infected with HIV. More severe hemophilia and greater factor usage have been associated with a greater risk of HIV infection. Persons with hemophilia treated exclusively with cryoprecipitate appear to have had a lower risk of exposure to HIV and subsequent infection (58). In the cumulative data reported to CDC as of December 31, 2000, of the children less than 13 years diagnosed with AIDS, 237 cases (3%) were reported among children with hemophilia (1).

Sexual transmission from HIV-infected men with hemophilia to their female sex partners, with subsequent perinatal transmission to their infants, has also occurred in some of these families. About 10% to 20% of the long-term female sex partners of HIV-infected men with hemophilia have acquired HIV infection through heterosexual contact (59). As of December 31, 2000, the CDC has received reports of 101 children with AIDS whose mothers acquired HIV infection from men who had received contaminated blood or blood-products (26 of whom had hemophilia) (60).

Other Routes of Transmission

Sexual transmission of HIV infection is the most common route of transmission among adolescents and adults but is not a commonly reported risk for infection among children in the United States. However, several cases of sexual transmission of HIV by sexual abuse have been reported among children in the United States (61–63). In 1998, using population-based HIV and AIDS surveillance, 26 children were identified who were sexually abused by an abuser with confirmed ($N=17$) or suspected ($N=9$) HIV infection (63). Although this number is small, since it is likely a minimum estimate, it indicates that HIV transmission from sexual abuse is a public health problem.

Transmission of HIV also has occurred through transplantation of bone, kidney, liver, heart, pancreas, and possibly skin (64). HIV infection acquired through artificial insemination also has been reported (65,66). The U.S. Public Health Service (USPHS) recommends that all tissue and organ allograft donors as well as blood and semen donors be evaluated for risks associated with HIV infection and tested for HIV infection (60). To date no pediatric cases, in which the transplanted organ was identified as the source of HIV infections, have been reported to CDC.

Early in the AIDS epidemic, concern was raised about transmission of HIV through casual contact in settings such as schools, day-care facilities, and families; however, evidence indicates that transmission in these settings is extremely rare or non-existent. No cases of transmission within the school or day-care setting have been reported. In at least 18 studies of more than 1300 household contacts of HIV-infected persons, transmission within households has not been found despite close personal contact and sharing of items likely to be soiled with blood and body secretions of the infected family member (67,68). Rare cases of transmission within household settings have been reported (69,70). One instance of transmission between two children living in the same household, documented by molecular epidemiology, was reportedly due to unrecognized blood contact between the children during bleeding episodes (70). Another instance of apparent transmission between two siblings was not well documented, as molecular techniques were not available at the time of occurrence (71). Two instances of transmission between brothers who were receiving intravenous infusion of factor products at home also have been reported (72,73). Transmission in both instances was most likely due to blood contact with infected blood. Four other cases involving unprotected exposure to blood or blood-containing fluids while providing home nursing care also have been reported (69). These cases, although quite rare, highlight the need for precautions to minimize skin, mucous membrane and percutaneous contact with blood in households and other settings, especially when health care is being provided.

Biting has received much attention as a possible mode of HIV transmission, but there is little evidence that biting transmits HIV (67). In a study of the family members of children with transfusion-associated HIV infection, nine HIV-infected children bit their contacts, and seven contacts bit an HIV-infected child. There was no evidence of infection in the contacts (74). Similarly, no evidence of infection was found in 30 health care workers bitten and scratched by a brain-damaged adult with HIV infection (75) or in a woman who was bitten severely while attending an HIV-infected patient who was having a seizure (76). Further, four children bitten several times by an HIV-infected child over a 2-week period were seronegative 6 months after the incident (77). Transmission involving biting has been suspected in six cases (67,71,78–82). In only one case was the biter a child. This case, however, is lacking in evidence to confirm this route of transmission because of lack of availability of molecular technology at that time (71). In the other cases, the bites were by adults and were severe with blood exchange.

The CDC and the American Academy of Pediatrics (AAP), as well as a number of state health and education departments, have recommended that most children with HIV infection be allowed to attend school and day care (83,84) and that these children should not be excluded from these settings solely on the basis of their HIV infection. Persons caring for children should be trained in, and adhere to proper infection-control technique (85). It

should be kept in mind, however, that the vast majority of HIV-infected persons do not transmit the virus except by the well-recognized routes, most commonly, sexual contact, sharing of contaminated needles, and mother-to-child transmission.

PREVENTION OF HIV INFECTION IN CHILDREN

The 1994 Pediatric AIDS Clinical Trials Group (PACTG) protocol 076 demonstrated that AZT therapy administered to selected HIV-infected pregnant women and their newborn infants reduced the rate of perinatal HIV transmission from 25% to 8% and was the Þrst major prevention breakthrough in the HIV epidemic (86). In 1994, the USPHS published guidelines for the use of AZT to reduce perinatal HIV transmission (87), and in 1995 the USPHS published guidelines for universal, routine HIV counseling and voluntary testing of pregnant women (88). These guidelines are updated regularly (89,90). Updated guidelines reßect the data extending the efÞcacy of AZT to all HIV-infected women, including those with advanced disease and prior AZT therapy and data on obstetrical interventions such as scheduled cesarean delivery. The guidelines also address issues about new standard treatment regimens for adults in the context of pregnancy (89). Data from additional clinical trials and observational studies have found transmission rates as low as 5% with AZT alone (91,92). In the late 1990s, highly active combination antiretroviral therapy became available that could decrease viral load below the level of quantitation and is now standard treatment of HIV-infected individuals including pregnant women (89). In several observational studies, the perinatal HIV transmission rate has been found to be lower with maternal combination therapy than with AZT monotherapy, that is, in the range of 1Ð3% (93,94). In a meta-analysis of observational studies, maternal viral load below 1,000 copies per ml at the time of delivery was found to be associated with a risk of perinatal transmission of 1% (95).

Consistent with the evidence that most fetal and newborn HIV infections are acquired near or during delivery, recent clinical trials have shown that abbreviated antiretroviral regimens focused on the peripartum period are also effective in decreasing the risk of perinatal transmission (93,96). A short course of AZT, administered late in pregnancy and intrapartum decreased the perinatal HIV transmission rate by 50% in the non-breast-feeding cohort of women in Thailand (97) and by 37% in a breast-feeding cohort of women in West Africa at 3 months and 26% at 24 months (98). A study in breast-feeding women in Uganda demonstrated that a single oral dose of nevirapine administered to the women at the onset of labor followed by a single oral dose administered to the infant decreased the risk of HIV infection by 41% compared with a very short AZT regimen. This difference remained through 18 months of life (99,100). In another African trial, a short course of AZT and lamivudine (3TC) was compared to placebo in breast-feeding women. This intervention of antenatal AZT + 3TC followed by 1 week postpartum AZT + 3TC to both mother and baby reduced the risk of infant infection by 63% at 6 weeks (to 5.9% transmission). However at 18 months, the transmission rate was 14.9% in the AZT + 3TC study arm compared to 22.2% placebo arm and conÞdence intervals overlapped. In other words, the efÞcacy of the AZT + 3TC regimen was no longer statistically signiÞcant at 18 months of age in this breast-feeding population (101,102).

Use of postpartum antiretroviral prophylaxis alone has not been evaluated in a randomised clinical trial. In an observational study in New York, administration of AZT for six weeks to newborns was associated with a signiÞcant decrease in transmission if the drug was initiated within 24 hours of birth (103). However, in another observational study from North Carolina, administration of AZT only to the newborn was not associated with a decrease in the risk of HIV transmission (92).

Obstetrical interventions have the potential to decrease perinatal transmission. Both a metanalysis of observational studies and a randomised clinical trial indicated that the perinatal transmission rate is lower for women delivered by elective Cesarean delivery compared to those delivered vaginally (35,36). The American College of Obstetricians and Gynecologists (ACOG) recommends that HIV-infected women with an HIV viral load of 1,000 copies per ml or greater be counseled regarding the potential beneÞt of scheduled cesarean delivery (104). They also state that data are insufÞcient to demonstrate a beneÞt for women with HIV viral load $< 1{,}000$ copies per ml. Other obstetrical interventions such as those to decrease the duration of rupture of membranes would potentially also contribute to decreasing HIV transmission (34). Vaginal lavage intrapartum has been proposed as an intervention to reduce perinatal HIV transmission. So far, a safe and effective cleansing agent has not been found (105,106).

The use of HIV vaccines in the perinatal period and later in infancy to protect against breast feeding transmission of HIV is theoretically appealing if an effective and immunogenic product could be developed. Studies using passive antibody have so far been inconclusive (107). Phase 1 studies of perinatal HIV vaccine candidates are currently underway (108,109). A model of peripartum antiretrovirals paired with an infant HIV vaccine series is a promising approach which requires further study for use in developing countries where many HIV-infected women breastfeed into the second year.

The American College of Obstetricians and Gynecologists, AAP and USPHS have all made recommendations, for universal, routine prenatal HIV education and HIV testing (90,110). Women who are identiÞed as HIV-infected need to be counseled on ways to prevent

transmission to their infant, including treatment of maternal disease (using combinations of antiretroviral agents as needed), use of AZT prophylaxis, elective cesarean delivery and avoidance of breast-feeding. Despite widespread implementation of these guidelines, perinatal HIV infection has not been eliminated, about 300 new cases of perinatally acquired HIV are estimated to occur each year in the United States. The major barrier to prevention is that some women do not receive adequate prenatal care. Compared with the general population, HIV-infected women are much less likely to receive prenatal care. In one report, overall 2% of pregnant women but 15% of HIV-infected women did not receive prenatal care (111). If perinatal HIV infection is to be eliminated then approaches must be developed to address the needs of women who receive little or no prenatal care. Approaches to reaching these women and to increase voluntary counseling and testing, include provider social marketing, community outreach, case management and provider training.

INTERNATIONAL PERSPECTIVE

HIV infection has become a worldwide pandemic, The World Health Organization (WHO) estimates that 40 million people, of whom 2.7 million are children (< 15 years) are living with HIV/AIDS (112). In 2001, 800,000 children worldwide became infected with HIV and 580,000 children died of AIDS. At least 1,700 new perinatal HIV infections occur each day with a rate of more than one infection every minute. The largest numbers of infected children live in sub-Saharan African countries. Because the virus is spread primarily through sexual contact, devastating potential exists for the spread of infection to sexually active women and for transmission of the virus from these women to their children.

Two patterns of pediatric HIV infection now exist, one in the industrialized countries, such as the United States, Canada and European countries, and another in resource-poor countries. In the industrialized countries, there are effective programs for prevention of perinatal HIV infections (113,114). In contrast, in resource poor countries, there is a perinatal HIV epidemic and HIV/AIDS has become one of the leading causes of death in children. Affordable and acceptable approaches to HIV counselling and testing in pregnancy, peripartum antiretroviral prophylaxis and reduction of transmission from breast-feeding in resource-poor settings are urgently needed (115).

CONCLUSIONS

In the United States, the epidemiology of pediatric HIV infection has changed dramatically over the past decade. The incidence of pediatric HIV infection has fallen because transfusion-acquired infection has been virtually eliminated and perinatal infections dramatically reduced. However, the epidemic among young women and adolescents, who become infected through sexual transmission and drug use, continues. Clearly, prevention of HIV infection in young women would prevent perinatal HIV transmission. However, much progress has been made in reducing perinatal HIV transmission. For further decreases in perinatal HIV transmission, health care providers must increase the uptake of prenatal care and HIV testing among all pregnant women including late presenting women whose HIV status is unknown at delivery.

For children infected with HIV, the current management of HIV infection has markedly improved their survival. Many children, both infected and uninfected, will outlive their HIV-infected parents and the death of their parents may leave them orphaned. It is estimated that by the end of 2000, about 80,000 children and adolescents in the United States will be orphaned by parental death caused by HIV infection (116,117). Health care providers now must find ways to provide complex care to children in families frequently from disadvantaged backgrounds and in which one or both parents may also be chronically ill or deceased.

With the improvement in survival, the focus of care for the HIV-infected child has changed from acute to chronic care and from infants to children of all ages including adolescents. The drugs currently available are however not a panacea. For optimal viral suppression at least 90% adherence to therapy is required and for many, sustaining that level of adherence is difficult. In addition, the drug regimens may be complex and expensive and drug toxicity and resistance become an increasing problem with prolonged therapy. Those caring for children with HIV infection continue to be challenged to find the best approach to provide comprehensive medical and psychosocial management for children with this complex disease.

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Chapter 3

The Genetic Diversity and Global Molecular Epidemiology of HIV

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The human immunodeÞciency virus (HIV) and its most severe clinical manifestation, the acquired immunodeÞciency syndrome (AIDS), have produced a pandemic of unprecedented proportions. By the end of the year 2001, the Joint United Nations Programme on HIV/AIDS (UNAIDS) estimated that there were over 40 million persons infected and living with HIV worldwide (1). Despite the rapid ascertainment of the diseaseÕs epidemiology, the discovery of the etiologic agent itself and ways to prevent its spread, and the development of diagnostic tests and antiretroviral therapies, it is clear that HIV will continue to be a global problem well into the 21st century. Nevertheless, continuing advances in molecular biology, immunology, and epidemiology have contributed greatly to the understanding of HIV. This chapter will summarize the global molecular epidemiology of HIV including an overview of its genetic diversity and related implications.

GENETIC DIVERSITY OF HIV

Human immunodeÞciency virus is a primate retrovirus of the Lentivirinae family, a group that shares the ability to infect their host chronically, and progressively damage the hostÕs immune system. Genetic variation of HIV is extremely high as a consequence of the rapid turnover of HIV virions (2). Individuals infected with HIV maintain a substantial viral burden during the entire course of infection (3,4). Related strains share genetic similarity, as expressed by the sequence of component nucleotides, and methods have been developed to infer the phylogenetic relationships among different strains (5Ð7). Two viral types have been characterized in humans: HIV type 1 (HIV-1) and HIV type 2 (HIV-2). Both of these viruses most likely originated as cross-species (zoonotic) transmissions from two non-human primate reservoirs, namely chimpanzees (*Pan troglodytes troglodytes*) and sooty mangabeys (*Cercocebus atys*), respectively (8Ð10). The initial epicenters of HIV-1 and HIV-2 infection appear to have been in Central Africa and West Africa, respectively. In contrast to HIV-1, which has spread extensively around the world, HIV-2 is still primarily found in persons from West Africa (11,12).

Based on viral genetic sequences, HIV-1 isolates have been classiÞed into a number of subtypes (alternatively termed clades or genotypes) (Fig. 3.1). These subtypes, designated by letters A, B, C, D, F, G, H, J, and K, constitute the major group of HIV-1 or group M (13,14). Phylogenetic analyses of viral sequences from numerous HIV-1 isolates worldwide have revealed two additional groups of viruses, namely groups O (outlier) and N (non-M, non-O) (Fig. 3.1). The vast majority of HIV-1 strains identiÞed worldwide, which is responsible for the global pandemic, belong to group M. Besides the various subtypes within group M, more detailed analyses have revealed additional complexities in the classiÞcation of strains (15). For example, some subtypes are further subdivided into sub-subtypes (16). Other strains can only be classiÞed within a group but fail to cluster with known subtypes (17).

Distinguishing among HIV variants is further complicated by the potential for coinfection (18Ð20) and genetic recombination between distinct viral strains (21Ð23). Recombination was initially detected when phylogenetic analyses of full genome sequences collected from different regions of the world, documented that some HIV genomes consisted of mosaic structures with two or more different

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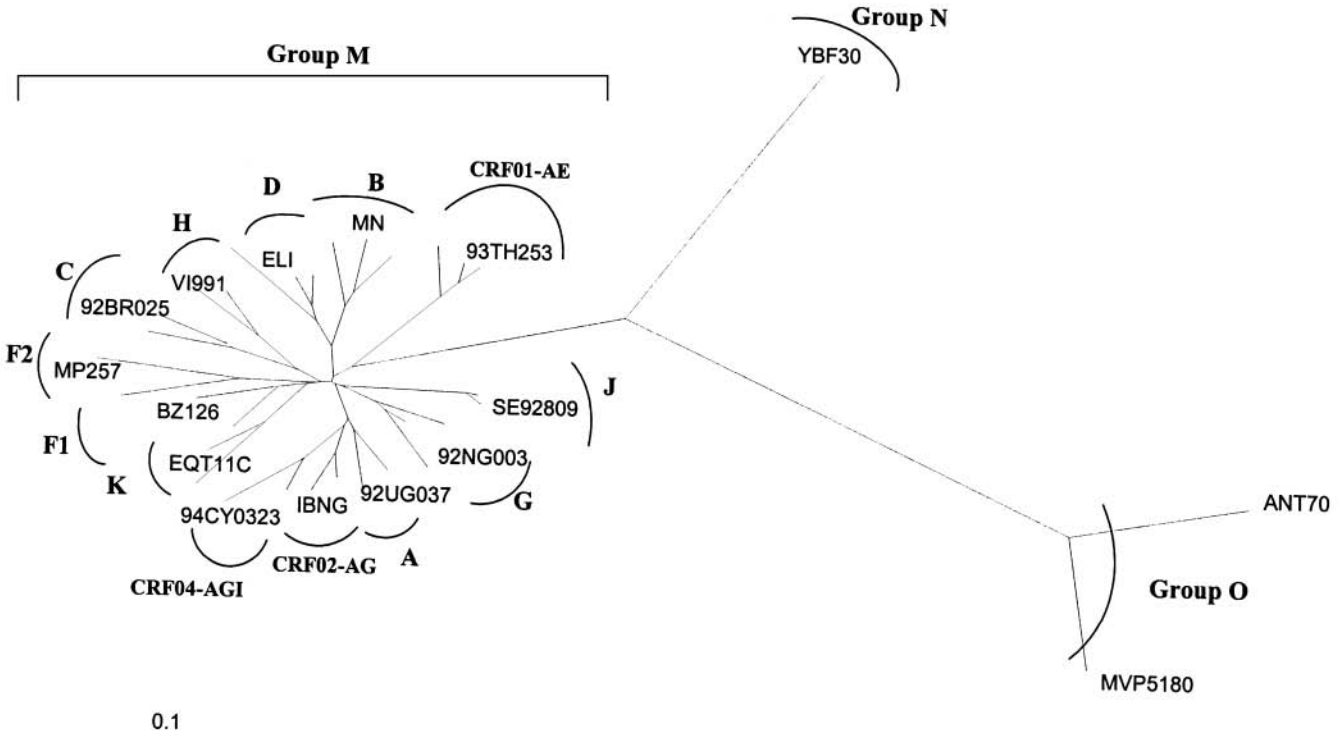

FIG. 3.1. Simpli ed phylogenetic tree of HIV-1 presents the relationship among group M. group N, and group O. Group M comprise nine basic subtypes A, B, C, D, F (F1 and F2), G, H, J, K, and three CRFs (CRF01-AE, CRF02-AG, CRF04-AGI). The *env* gp41 sequences were used for construction of the tree by the neighbor joining method with the Kimura two-parameter algorithm. The distance scale represents 10% divergence.

viral subtypes (22,24–26). Recent data indicate that co-infection between different subtypes of HIV-1 is more common than previously thought, especially in regions where a number of different HIV strains circulate (18,19,27). Recently, intersubtype HIV-1 superinfection has been documented in two individuals where the immune responses elicited by the primary infecting viral strain did not protect against subsequent reinfection with viral strains at a different subtype (27a). Dual infection and recombination between divergent HIV-1 strains of group M and group O have been documented (28,29).

As with HIV-1, a mosaic HIV-2 genome was reported, indicating that coinfection with genetically divergent strains and recombination can occur in HIV-2-infected individuals (8,30). While dual infections with HIV-1 and HIV-2 have been reported, no recombination between these viruses has been found (20,31).

Many recombinant viruses appear to be unique in that they have been found only in isolated individuals without evidence of epidemic spread. However, a few recombinant viruses have been designated as "circulating recombinant forms" (CRF) and account for a signi cant amount of HIV-1 transmission in some areas (15,17). For example, CRF01-AE represented strains that were initially described as subtype E based on *env* sequences but were later determined to be recombinants of subtypes A and E (26). Several CRFs have been reported from diverse regions around the world (32–37). Ongoing research aims to elucidate the biologic and epidemiologic signi cance of coinfection and recombination on HIV genetic diversity (14).

In the early 1990s, divergent strains of HIV-1 were reported and provisionally categorized as group O (38,39). The public health signi cance of these divergent strains was originally highlighted by reports that some strains were not reliably detected by many of the antibody screening tests then in use (40,41). However, unlike HIV-1 group M, which has spread globally, group O has had limited geographic dissemination. Outside West Central Africa (Cameroon, Gabon, and Equatorial Guinea) group O viruses have been found sporadically in other parts of the world including Western Europe and the United States in individuals of West Central African origin or among those with African contacts (42–44). HIV-1 group N was rst reported in 1998 and is so far represented by only a few viral isolates from Cameroonian patients (45).

HIV-2 was rst identi ed in West Africa and there is evidence that such viruses were present in this geographic region as early as the 1960s (11,46). As with HIV-1, HIV-2 has also been classi ed into several subtypes (A–G), although based on a much smaller number of sequenced isolates (8,30,47).

Although nucleotide sequences have been determined from thousands of HIV isolates, this is much less than the

tens of millions of HIV-infected persons in the world and not necessarily representative of the actual distribution of HIV strains. International collaborative efforts have been expanded to collect, characterize, and classify systematically HIV isolates from around the world (14). Figure 3.2 illustrates geographically where the HIV-1 subtypes and HIV-2 have principally been recognized (14). Based on available data, the greatest diversity of HIV strains has been found in sub-Saharan Africa. In contrast, most HIV isolates reported from Europe and the Americas are subtype B. However, multiple independent introductions of various HIV strains into different regions of the world have increased the complexity of the epidemiologic picture.

GLOBAL MOLECULAR EPIDEMIOLOGY

The pandemic of HIV/AIDS reßects many coexisting subepidemics in different regions and populations (14,48,49). As described in the chapter entitled Global Impact of HIV, there are many important epidemiologic, sociodemographic, behavioral, and biologic factors that contribute to the differential spread of HIV.

The vast majority of HIV infections in the world is transmitted sexually, most often through heterosexual intercourse (48). Notable exceptions occur, particularly among certain populations in industrialized countries in North America, Europe, and the PaciÞc Basin, where transmission has predominantly occurred through male-to-male sexual contact and injecting drug use. The areas characterized by these latter transmission modes have typically had a predominance of HIV-1 subtype B, though not all areas with subtype B predominance have featured these transmission modes, whereas the other subtypes appear to predominate in most of the areas where heterosexual transmission prevails (14).

In regions such as sub-Saharan Africa and some Caribbean and Latin American countries where transmission occurred primarily via heterosexual intercourse, the highest rates of HIV-1 infection have been among female commercial sex workers or prostitutes, their male sexual contacts, and young adults in urban areas. This pattern has occurred in some areas where subtype B has predominated, but to a greater extent has been associated with subtypes other than B, notably A, C, D, and CRF01-AE (previously referred to as subtype E) (14). Based on known distributions of the major subtypes, it has been estimated that subtype C represents the largest number of HIV-1 infections worldwide (who.int/emc-hiv/global_reprt/index.html).

In contrast to the wide dispersion of HIV-1 group M subtypes, the distribution of the more divergent HIV-1 group N and O strains, as well as HIV-2, remains more geographically limited (11,12,39,41,50). Given the wide diversity of HIV-1 and HIV-2 subtypes recognized from non-systematic sampling, both a wider distribution of recognized strains and the existence of yet more divergent variants seem likely. The potential for difÞcult-to-detect divergent HIV strains to enter human populations remains a public health concern.

MOLECULAR EPIDEMIOLOGY BY REGION

Africa

Africa has been the region most severely affected by the HIV/AIDS epidemic. The high prevalence of HIV in many parts in Africa, the wide distribution of HIV-1 and HIV-2 subtypes (Fig. 3.2), and the genetic variation within subtypes, suggest that HIV has been on that continent for a relatively long time compared with other regions (14,51,52). However, extensive demographic changes with shifts from rural to urban areas and numerous population displacements from migrant labor and civil disturbances over recent decades, may have facilitated the spread of HIV in many areas.

Heterosexual HIV transmission is the major mode of spread among adults, with very little transmission recognized from male-to-male sexual exposure or injection drug use. A number of explanations have been proposed for the relative efÞciency of heterosexual transmission and the rapid spread of HIV-1 in sub-Saharan Africa. HIV-1 infection in Africa has been associated with increased numbers of heterosexual partners, a history of prostitution among women, and sexual contact with prostitutes among men (53Ð55). The role of STDs in augmenting transmission of HIV-1 has received considerable attention (56). A number of sexually transmitted infections, especially genital ulcer disease, have been associated with HIV-1 infection in both men and women in various studies (57,58). In addition, lack of circumcision appears to be a risk factor for HIV infection, either independently or perhaps due to an association between genital ulcer disease and an intact foreskin (59Ð61). HIV-1 subtypes other than B (relatively little subtype B has been recognized in Africa) have been suggested as contributing to the heterosexually-transmitted epidemics in Africa (and Asia) based on ecologic patterns of infection, but documentation that the non-B subtypes are actually transmitted more readily by this route than subtype B is lacking (62,63).

As noted previously, the distribution of HIV-2 infection as well as the more divergent HIV-1 groups N and O strains appears to be limited geographically primarily to West Africa (11,12) and Central Africa, particularly Cameroon (39,41,50), respectively. Retrospective serologic studies indicate that persons from a number of West African countries were infected with HIV-2 as early as the 1960s (46). Nevertheless, in most areas, the prevalence of HIV-2 remains low and does not appear to be increasing over time, in contrast to the continued rise in rates for HIV-1 in the same areas.

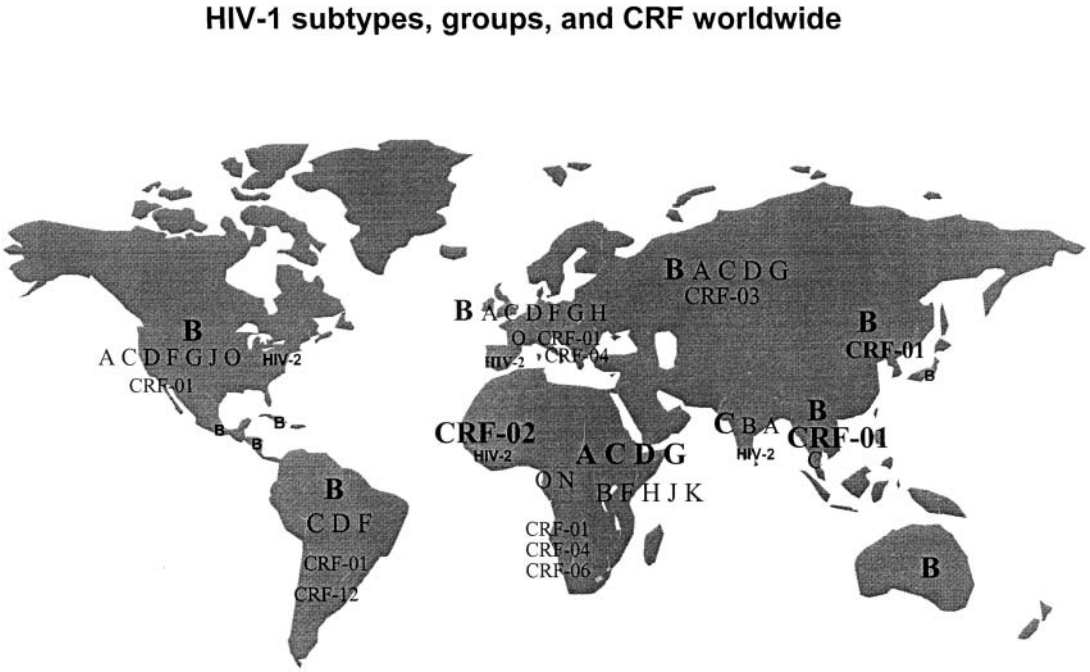

FIG. 3.2. Distribution of HIV-1 group M (subtypes A, B, C, D, F, G, H, J, and K), groups N and O, and circulating recombinant forms (CRF) throughout the world. Letters in bold represent major prevalent strains in speci c geographic regions, This map is not an exhaustive list of all reported strains and does not include reports of single cases nor recently imported cases.

Americas

Since the Þrst recognition of AIDS in 1981 in the United States (64), HIV-1 infection spread rapidly in this region in the early 1980s, primarily associated with male-to-male sexual exposure and injecting drug use, and subsequently by heterosexual transmission (65,66). As in North America, most Latin American and Caribbean countries experienced rapid spread of HIV-1 among homosexual and bisexual men early in the epidemic (66). However, the proportion of reported AIDS cases resulting from heterosexual transmission has increased dramatically since the early 1980s in most Latin American countries and heterosexual transmission appears now to be the predominant route of transmission in a number of countries (66).

The resulting geographic distribution of AIDS cases is far from homogenous either among or within countries. The great diversity of the countries and populations in the region has led to substantial variations in the patterns and rates of HIV-1 infection and AIDS. In most countries, HIV-1 infection has been concentrated in urban areas (67). Smaller numbers of persons with HIV-2 infection have been reported from the Americas, notably in the United States and Brazil, without evidence to date of local epidemic spread (12). While the vast majority of previously characterized isolates in the Americas have been HIV-1 subtype B, the occasional presence of HIV-2 (12,68,69) and other HIV-1 subtypes (70Ð73), indicates that multiple introductions of HIV have occurred and probably continue to occur.

Europe and the Former Soviet Republics

As in North America, groups predominantly affected early in the epidemic, especially in northern Europe, were homosexual and bisexual men and IDUs. However, transmission patterns vary widely within the region (48). In contrast, heterosexual IDUs account for the majority of AIDS cases in southern European countries. In addition to the HIV patterns resulting from local transmision, several western European countries have had appreciable numbers of persons with of HIV infection who came from or were infected in developing countries, especially in Africa. The HIV strains involved extend well beyond the well-characterized subtype B of Europe and North America and include non-B HIV-1 subtypes and HIV-2 (14,74,75). Thus far, wider spread of these strains, including those HIV-1 subtypes associated with the primarily heterosexually spread epidemics in Africa, has not been recognized beyond the imported cases themselves and their direct contacts.

In Eastern Europe and the former Soviet republics, HIV-1 has emerged as a major public health problem in recent years. Earlier in the epidemic, outbreak investigations of nosocomial HIV-1 transmission in Romania and the former Soviet Union demonstrated the potential for localized epidemics resulting from unscreened transfusions or the improper use of needles and syringes to administer multiple therapeutic injections (76Ð78). Following major political and economic changes in Eastern Europe and the former Soviet Union, massive social upheaval from economic difÞculties and ethnic and

religious conßicts have created circumstances which have facilitated the spread of HIV-1 and other sexually transmitted infections as well as infectious diseases like tuberculosis (34,79Ð81).

Asia and the PaciÞc

It appears that extensive spread of HIV-1 did not begin until the late 1980s in the worldÕ most populous region and that reported numbers of HIV and AIDS do not adequately reßect the dramatic increases of HIV-1 in many Asian countries such as India and China, which each have populations larger than all of Africa (1,48).

The HIV epidemic in Thailand, clearly documented by excellent surveillance, illustrated the potential for rapid HIV-1 transmission in Asia. Extensive HIV transmission began around 1988 with rapid increases in HIV-1 seroprevalence among IDUs, as evidenced by the prevalence among persons attending drug treatment centers in Bangkok increasing from 1% to approximately 40% by the end of 1988 (82). The second phase of the epidemic, which was later shown by molecular epidemiological studies to be distinct from the Þrst (83Ð85), occurred primarily among persons at heterosexual risk (82,86). High incidence resulted in marked increases in HIV-1 prevalence and the female commercial sex industry was quickly recognized as being a major source of transmission (87). The increased infectiousness that occurs in the primary phase of HIV infection and the large number of clients served by prostitutes, compounded by prevalent sexually transmitted diseases, are likely to have contributed greatly to extensive transmission and the rapidly expanding HIV epidemic (88). The many men who were infected by sexual contact with prostitutes transmitted HIV in turn to their other female sex partners (89Ð91) resulting in a subsequent increase in maternal-infant HIV transmission (92).

The dramatic epidemic documented in Thailand illustrates the potential for HIV transmission in other Asian countries through both sexual and drug-related spread. Molecular epidemiological studies have been useful in showing the association between population movements and the introduction of different HIV strains (82,93). Cross-border opiate traffÞc between countries in the region and drug use involving the sharing of injection equipment pose a substantial risk for HIV-1 transmission (94,95). Studies in China have documented the rapid spread of HIV-1 among injecting heroin users in the southwestern provinces bordering Southeast Asia (96,97). Since the mid-1990s, HIV has spread extensively beyond the initial risk groups and regions (98,99)[1].

In India, sharp increases in HIV seroprevalence have been noted, especially among female prostitutes and patients attending STD clinics in some urban areas (100,101). Even by 1995, it was estimated that over 1.7 million Indians were infected with HIV-1 (48). The problem was further compounded by the potential for HIV transmission via transfused blood since professional blood donors provide over half the blood for Indian hospitals (102,103). Due to the high HIV seroprevalence among some groups of blood donors and the resultant HIV risk, India has expanded blood screening programs in a number of cities. While most HIV detected in India has been type 1, smaller numbers of persons with HIV-2 infection have also been reported (12).

Implications of HIV Genetic Diversity

HIV genetic diversity impacts a number of important areas. For example, there are important questions on epidemiologic transmission patterns as well as the intriguing possibility that genetic and phenotypic differences in HIV affect transmissibility, pathogenicity, and responses to therapy and vaccines. In addition, genetic recombination of different strains or subtypes during co-infection may allow for the formation of viral hybrids with altered pathogenic or transmissibility properties.

Diagnostics, Virus Detection, and Screening Strategies

The high genetic variation of HIV has important implications for the sensitivity and speciÞcity of diagnostic tests. The isolation of HIV-1 in 1983 and the development of an antibody test soon thereafter were major breakthroughs. However, the subsequent identiÞcation of HIV-2 required signiÞcant modiÞcation of diagnostic tests. Somewhat analogous to the discovery of HIV-2 has been the characterization of the highly divergent HIV-1 group O and group N strains. Reports that infections with these viral strains were not well detected by commonly used HIV screening tests in Europe and North America have raised concerns about test sensitivity for blood transfusion safety as well as for individual diagnosis (40,41). Although earlier development and evaluation of different diagnostic tests were based primarily on subtype B strains from North America and Europe, more recent tests, including rapid tests, are much more sensitive in detecting divergent strains like group O as well as group M subtypes other than subtype B (104). Similarly, recent versions of assays to quantitate viral load have improved sensitivities to the major HIV-1 subtypes compared with earlier assays which were primarily based on subtype B sequences (105,106). In addition to antibody tests and viral load assays for screening and diagnosis, new serologic testing strategies to detect recent infection and to estimate incidence have also demonstrated different performance characteristics depending on subtype (107).

Active surveillance for and characterization of the prevailing HIV strains are essential to validate the sensitivity of HIV tests in clinical practice and research use. This surveillance may well be particularly challenging

because infections with as yet unrecognized highly divergent strains may be difficult to detect using available diagnostic tests.

Transmission and Transmissibility

As outlined earlier, molecular epidemiology has helped to elucidate the epidemiologic and historical aspects of transmission at the community (108), national (78,109),and international levels (110,111). As mentioned earlier, molecular characterization documented the recent, independent introductions of different HIV subtypes in a number of countries (72,83,112,113).

Once identified, genotypic differences among isolates can be used to evaluate community transmission patterns and epidemiologic linkage among cases (108,114). A well-known molecular investigation of HIV transmission involved the case of an HIV-infected dentist who apparently transmitted the virus to six of his patients (115–117). Nucleotide sequence analysis showing strong similarities between the viral strains from the dentist and those of his patients, was a powerful tool in identifying the epidemiologic link among these individuals. Subsequent investigations have used this approach to either support or refute other putative cases of transmission (118–121).

In addition to the well-recognized sociodemographic and behavioral factors, it has been hypothesized that virologic strain differences in transmissibility may play a role in the spread and therefore in the epidemiologic patterns of HIV infection (63,122). Studies concerning HIV-1 and HIV-2 have provided the clearest evidence of differences in transmissibility of viral strains. Although HIV-2 shares the same modes of transmission as HIV-1 (123), prospective cohort studies have shown less efficient rates of sexual transmission (124,125) and substantially lower mother-to-infant transmission rates for HIV-2 than HIV-1 (123,126).

There is also preliminary evidence from mother-infant pairs suggesting selective transmission of certain maternal HIV-1 variants (127,128) and suggestive evidence for differential transmissibility of two different subtypes through sexual contact (129). More detailed prospective studies with controls for potentially confounding factors are needed to more fully examine strain-specific differences in transmissibility.

Clinical Manifestations

Although the clinical and immunologic manifestations of HIV infection are addressed in other chapters of this book, it is important to realize that the wide spectrum of clinical conditions described in North America does not necessarily reflect the typical clinical picture of HIV disease in other parts of the world (130). Relatively little information is available on the natural history of HIV infection in many parts of the world, including the chronological order and the stages of immune deficiency at which different opportunistic diseases occur. It has been hypothesized that HIV infection may progress more rapidly among persons in developing than in industrialized countries (131). However, an apparently poor outcome may reflect initial diagnosis of HIV/AIDS late in the course of infection, lack of access to medical care, or death from prevalent opportunistic infections such as tuberculosis that occur relatively early in the course of HIV infection, rather than any appreciable differences in the rate of decline of immunologic function (131–133).

Although various biological, including host immunological, factors may hasten HIV pathogenesis and disease progression, studies do not suggest significant differences in natural history of HIV-1 infection by sex or racial/ethnic group (134–136). However, there is *in vitro* evidence that different strains may vary in pathogenic potential. For instance, HIV isolates vary in their cellular host range and tropism (137,138), and some studies have shown that these properties are associated with specific viral genetic and phenotypic changes (139–143). Additional evidence suggesting that pathogenicity is viral strain specific comes from small studies of persons with transfusion-acquired HIV infection, in which the rate of disease progression in recipients correlated with the rate of progression in the corresponding donors (144,145). While much of the research on clinical progression has involved North American and European subtype B viruses, work has expanded in recent years to characterize the biological properties of other HIV subtypes (63,146–149).

Studies from West Africa show that persons with advanced HIV-2 infection have clinical manifestations similar to those among persons with HIV-1 infection (150,151). However, the rate of immunologic deterioration and disease progression resulting from HIV-2 infection appears to be appreciably slower than that from HIV-1 or HIV-1/HIV-2 dual infection (151–157) Although little is known about intratype differences in pathogenesis, there are a few reports suggesting the possibility that disease progression may be faster for certain HIV-1 or HIV-2 subtypes (8,63,148,149,158). However, since disease progression and natural history are also influenced by non-viral factors, prospective studies are needed to assess accurately the pathogenic effects of different HIV subtypes (63).

Viral Resistance to Therapy

Given the high genetic variation of HIV, drug-resistant strains are present even in the absence of therapy. When therapy is initiated, selection begins for these drug-resistant strains (159–161) and replacement of previously sensitive variants can occur rapidly (162). As with many other aspects of HIV characterization, efforts are increasing to describe resistance mutations among strains other

than HIV-1 subtype B from the Americas and Europe (163,164). Since drug-resistant strains of HIV can be effectively transmitted, resulting in "primary" resistance in the newly infected person (118,165–167), it has become necessary to conduct surveillance for drug-resistant HIV strains, as is done for gonorrhea, tuberculosis, and malaria.

Vaccines

As with early diagnostic tests for HIV-1, initial vaccine candidates were primarily based on subtype B (168). However, the expanding knowledge of HIV genetic diversity coupled with the urgent need to develop vaccines for subtypes other than B in developing countries where the majority of infections occur has led to a number of new vaccine candidates which are described elsewhere in this book (Progress in the development and testing of HIV vaccines).

To the extent that vaccine efficacy is strain- or subtype-specific, the ability to evaluate them in areas of highest HIV incidence, where multiple HIV strains may be present, and the later applicability of the vaccine may pose considerable challenges. Currently, it is unclear to what extent the various HIV-1 subtypes or clades represent different immunotypes for host humoral and cellular responses (168). Ongoing assessment of vaccine candidates will clearly depend on continued surveillance and characterization of viral variants as well as detailed evaluation of host immune responses to these strains.

CONCLUSION

As the HIV/AIDS pandemic enters the twenty-first century, the pace of HIV transmission is increasing in sizeable parts of the world. Epidemiologic data suggest that for many parts of the world, especially in developing countries, there will be continued high rates of transmission and increases in HIV/AIDS. Large parts of Africa have already been devastated by the epidemic, and the coming years will bring an increased burden of AIDS-related death. As the Americas, Europe, and Africa began to come to terms with AIDS in the 1980s and early 1990s, Asia, with over three billion people, will be the new arena for HIV infection in the next millenium. In many countries, AIDS is or will likely become the leading cause of death in adults and one of the leading causes of infant and child mortality. The resulting socioeconomic consequences for communities will be considerable.

As understanding of the significance of HIV genetic diversity increases, knowledge of the frequency and distribution of different strains will play an important role in a timely and effective response to the HIV pandemic. At present, data regarding HIV subtypes are mostly limited to studies conducted for purposes other than surveillance. The expansion of epidemiologic surveillance for different strains of HIV and attempts to collect representative specimens from different populations will provide useful information to the public health community on the danger of emerging infectious agents such as HIV (14). Strong public and political support is necessary to rally the resources to monitor global HIV strain diversity for surveillance, prevention and vaccine development activities. In an age of rapid population movements and extensive interactions between communities, the challenge of HIV underscores the importance of well-coordinated clinical, laboratory, and epidemiologic efforts to stop its spread.

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Chapter 4

Introduction to Retroviruses

Stephen P. Goff

Human immunodeficiency virus type 1 (HIV-1), the causative agent of acquired immune deficiency (AIDS), is a lentivirus, a member of a subfamily of retroviruses with complex regulation of viral gene expression and replication. HIV-1 is an unusual retrovirus. Whereas most retroviruses are relatively benign and rarely kill the infected cell, HIV-1 is cytopathic and cytotoxic, and high levels of viral gene expression often result in the death of the infected cell and even neighboring cells. The mechanisms of cell killing and the basis for the remarkable pathogenicity of the virus remain to be elucidated. But unusual as it is, HIV-1 is still a retrovirus, and as such its life cycle is similar to that of the simple retroviruses. This chapter will review the fundamentals of retrovirus structure and replication and so provide the basic context in which to understand the biology of this agent. The most broadly conserved aspects of the life cycle will be the focus.

The retrovirus family is a large and diverse group of viruses found in all vertebrates. These viruses replicate through an extraordinary and unique life cycle, differentiating them sharply from other viruses. The virion particles generally contain a genomic RNA, but upon entry into the host cell, this RNA is reverse transcribed into a DNA form of the genome that is integrated into the host chromosomal DNA. The integrated form of the viral DNA, the provirus, then serves as the template for the formation of viral RNAs and proteins that assemble progeny virions. These features of life cycleÑespecially the re verse ßow of genetic information from RNA to DNA, and the establishment of the DNA in an integrated form in the host genomeÑare the deÞning hallmarks of the retro viruses. This life cycle also accounts for many of their diverse biological activities. Creation of proviral DNA confers on the viruses a powerful ability to maintain persistent infection in the face of the host immune response; and to enter the germ line, permitting vertical transmission of virus.

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TAXONOMY

The genera of the retroviruses have recently been formalized and given new names by the International Committee on Taxonomy of Viruses. While older classiÞcation schemes utilized morphological criteria, more modern systems use genome organization as deÞning criteria. The alpharetroviruses, betaretroviruses, and gammaretroviruses are considered Òsimple Ó retroviruses, while the deltaretroviruses, epsilonretroviruses, lentiviruses and spumaviruses are considered Òcomplex Ó. The simple viruses encode only the Gag, Pro, Pol, and Env gene products; the complex viruses encode these same gene products but also an array of small regulatory proteins with a range of functions. The properties of the viruses belonging to each of these genera are summarized brießy below. Representative members of each genus are listed here (Table 4.1).

VIRION STRUCTURE

Retrovirus virions are initially assembled and released from infected cells as immature particles containing unprocessed Gag and Gag-Pol precursors of the proteins that eventually make up the mature virus. The immature virion morphology is spherical, with a characteristic electron-lucent center. The virions have been described as a Òprotein vesicle Ó, to suggest some ßuidity in the interactions between the individual Gag proteins that make up the particle. Upon maturation, the precursor proteins are cleaved, and the structure and morphology of the virion changes drastically (1). The mature retrovirus particle is a spherical structure, roughly 100 nm in

TABLE 4.1. *Retrovirus genera*

New name	Examples	Morphology
Alpharetrovirus	Avian leukosis virus (ALV) Rous sarcoma virus (RSV)	C-type
Betaretrovirus	Mouse mammary tumor virus (MMTV) Mason-P zer monkey virus (MPMV) Jaagsiekte sheep retrovirus (JSRV)	B, D-type
Gammaretrovirus	Murine leukemia viruses (MuLV) Feline leukemia virus (FeLV) Gibbon ape leukemia virus (GaLV) Reticuloendotheliosis virus (RevT)	C-type
Deltaretrovirus	Human T-cell lymphotropic virus (HTLV)–1,–2 Bovine leukemia virus (BLV) Simian T-cell lymphotropic virus (STLV)–1,–2,–3	—
Epsilonretrovirus	Walleye dermal sarcoma virus Walleye epidermal hyperplasia virus 1	—
Lentivirus	Human immunode ciency virus type 1 (HIV -1) Human immunode ciency virus type 2 Simian Immunode ciency viruses (SIV) Equine infectious encephalitis virus (EIAV) Feline immunode ciency virus (FIV) Caprine arthritis encephalitis virus (CAEV) Visna/maedi virus	rod/cone core
Spumavirus	Human foamy virus (HFV)	immature

diameter. The size of the virions in a given preparation is not highly homogeneous but rather varies over a fairly wide range, suggesting that a discrete, highly ordered structure may not exist. After processing of the Gag precursor during virion maturation, the CA protein collapses to form a more ordered paracrystalline core, and in the case of HIV-1 the conical core is probably a well-deÞned structure (2). But even after maturation the overall diameter of the virion is heterogeneous and suggestive of considerable disorder. The virions exhibit a buoyant density in sucrose in the range of 1.16Ð1.18 g/ml. The sedimentation rate of the particles is typically about 600 **S**. The virions are sensitive to heat, detergent, and formaldehyde.

Virion Proteins

The stoichiometry of the various viral gene products in the virion are not very Þrmly established, but estimates suggest that about 1,500 to perhaps 2,000 Gag precursors are present per particle. After processing, all cleavage products are thought to be retained, suggesting equimolar presence of these proteins in the mature virions. Levels of the Pol proteins are typically about one-tenth to one-twentieth of the Gag proteins, corresponding to about 100 to 200 molecules per virion. The levels of the Env proteins are quite variable among the viruses. For the simple retroviruses such as the murine leukemia viruses, the levels of Env are close to that of Gag; perhaps 1200 monomers, or 400 trimers, are present per virion. For the lentiviruses the levels of Env per virion are much lower, possibly as low as ten trimers per virion.

Nomenclature

The cleavage of Gag, Pol and Env precursors forms the products in the mature infectious virions. These proteins are named by convention by a two-letter code: MA for matrix or membrane-associated protein; CA for capsid; NC for nucleocapsid; PR for protease; DU for dUTPase; RT for reverse transcriptase; IN for integrase; SU for surface protein; and TM for transmembrane protein (3). The localization of these proteins in the mature virion is not known with great precision, but a highly schematic version of the generic retrovirion can be drawn (Fig. 4.1).

Arrangment of Virion Components

The genomic RNA is highly condensed in the virion by its association with the nucleocapsid protein, NC. The complex is contained within a protein core largely composed of the capsid protein CA, another Gag gene product. The shape of the core is different among the various retroviral genera, and indeed is a distinguishing feature of the genera. In most of the viruses the core is roughly spherical, but in some cases can be either conical or cylindrical. In all the viruses the core is surrounded by a roughly spherical shell consisting of MA, which in turn is surrounded by the lipid bilayer of the virion envelope. The virion membrane contains the envelope glycoprotein, with the TM subunit present as a single-pass transmembrane protein anchor, and the SU subunit as an entirely extravirion protein bound to TM. The envelope proteins for those viruses examined closely have been found to reside in the membrane as trimers.

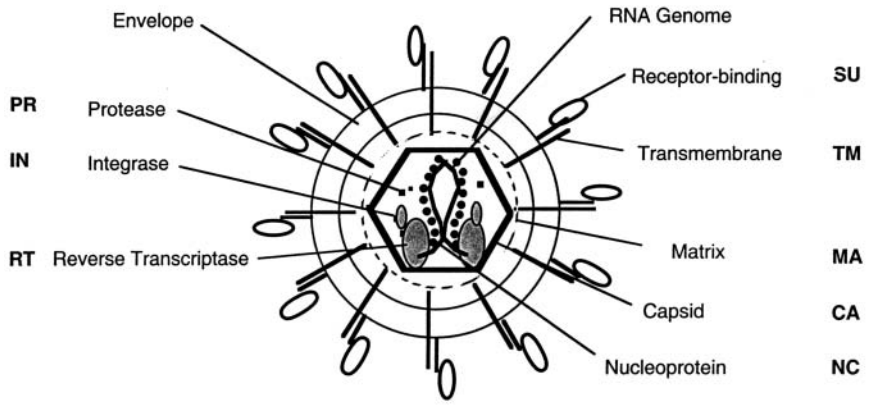

FIG. 4.1. Generalized retrovirion structure and components. A highly schematic view of the arrangement of viral gene products within the virion particle. The two-letter nomenclature for each protein is indicated. From (486) courtesy of Lippincott, Williams and Wilkins.

OVERVIEW OF THE LIFE CYCLE

The retroviruses replicate through a complex life cycle. A short summary of the steps of the cycle is as follows (a schematic view is shown in Fig. 4.2):

- ¥ Binding of the virion particle to the receptor and fusion of the viral and cell membranes
- ¥ Internalization of the virion core and uncoating to allow access of deoxyribonucleotides
- ¥ Reverse transcription of the RNA genome to form double-stranded linear DNA in the cytoplasm
- ¥ Transport of the pre-integration complex (PIC) to the nucleus and import into the nucleus
- ¥ Integration of the linear DNA into the cellular genome to form the provirus

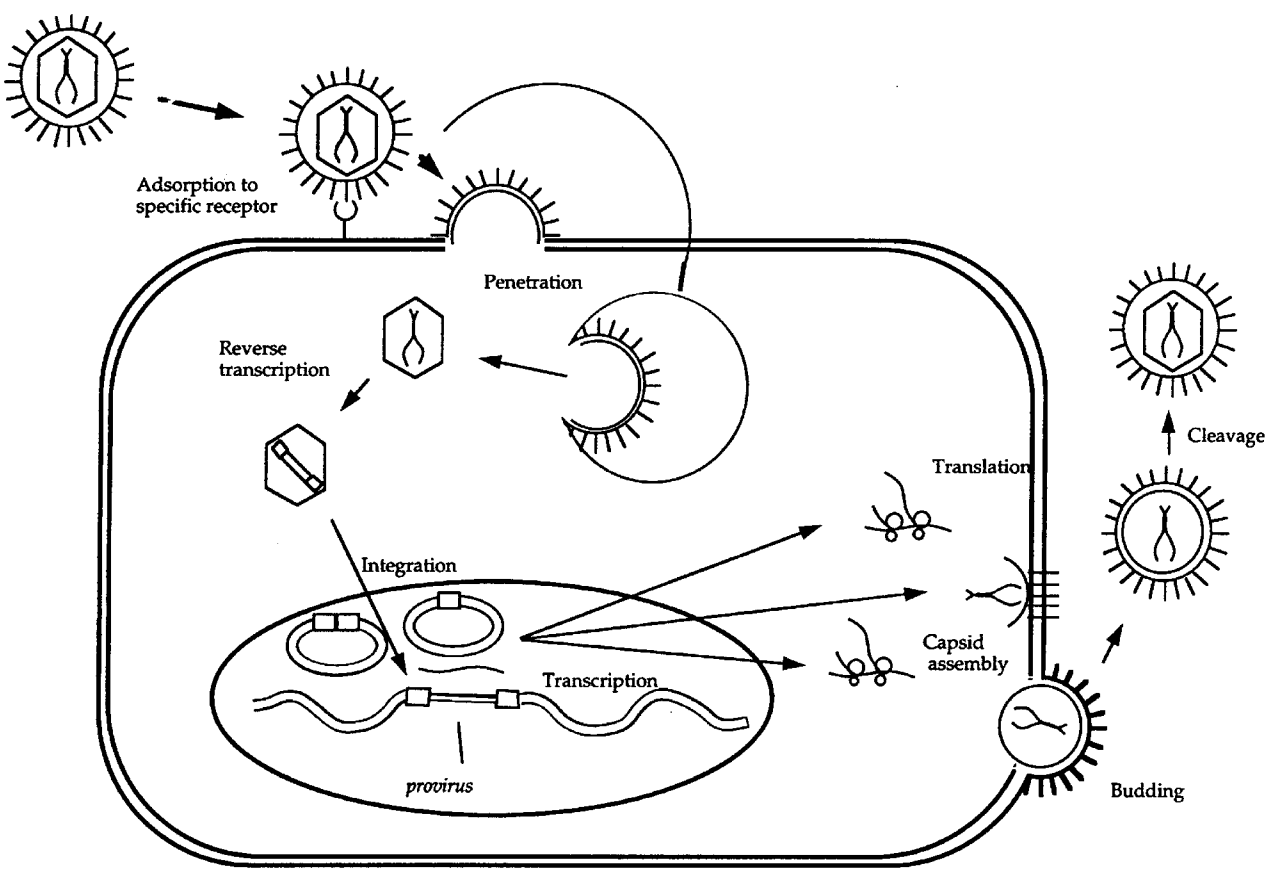

FIG. 4.2. A schematic view of the retrovirus life cycle. The major steps in the replication of a typical retrovirus are indicated, including those in the early phase of the life cycle, extending from the infecting virion (top left) to the formation of the integrated provirus, and those in the late phase of the life cycle, extending from the provirus to the formation of mature progeny virus (right). From (486) courtesy of Lippincott, Williams and Wilkins.

- Transcription of the provirus by RNA Polymerase II to form viral genomic RNA and mRNAs
- Splicing and nuclear export of the RNAs
- Translation of the RNAs to form precursor proteins
- Assembly of the virion particle and packaging of the viral RNA genome
- Budding and release of the virions
- Proteolytic processing of the precursors and maturation of the virions to create an infectious progeny virus.

We will consider each of these steps in detail in the rest of this chapter.

THE VIRUS RECEPTORS

To enter a cell and initiate infection, all retroviruses require an interaction between a cell surface molecule Ða receptor Ðand the envelope protein on the virion surface. The interactions are complex, involving an initial binding, drastic conformational changes in the envelope protein, an induced fusion of the viral and cellular membranes, and the internalization of the virion core into the cytoplasm. The SU subunit of Env is thought to make the major initial contacts with receptor, and the TM subunit is thought to be most important for membrane fusion. The reorganization of the two lipid bilayersÑ one on the virion and one on the cellÑ to join them and evert the core into the cell is a remarkable process. The details of these complex processes are not understood for any retrovirus, and the whole Env protein is likely to be involved in efÞcient entry. However, there is a great deal of information about the identity and structures of the receptors used by various retroviruses. It is apparent that these viruses utilize an extraordinarily diverse set of cell surface molecules as receptors (see (4,5) for reviews).

The Þrst receptor identiÞed for any retrovirus was the CD4 molecule, established as essential for infection by HIV-1 (6Ð8). Soluble forms of CD4 can bind to virions and inhibit infection; in some strains of virus, CD4 induces release or shedding of the SU subunit from the virion. However, other strains are quite resistant to inhibition by soluble CD4. CD4 is an important surface protein on T cells, and with few exceptions serves to deÞne the helper subset of T cells. CD4 is also expressed at signiÞcant levels on dendritic cells, macrophages, and on certain cells in the brain, which may be related to astrocytes rather than cells of neural origin. The limited distribution of expression of CD4 accounts well for the tropism of HIV-1, largely restricted to helper T cells and macrophages. There may be other routes of entry utilized at lower efÞciency: antibody to virus, for example, can promote virus entry into cells by the Fc receptor (9).

Early work established, however, that although CD4 was sufÞcient to mediate virus binding to a cell surface, it was not sufÞcient to mediate virus infection and entry. For example, rodent cells and other cells of non-primate origin could not be successfully infected by HIV-1 even if they were engineered to express human CD4. Searches for genes that would render such cells sensitive to virus infection ultimately led to the identiÞcation of various members of the chemokine receptor family, notably CCR5 and CXCR4, as coreceptors that were needed to mediate the post-binding step of membrane fusion and virus entry (10Ð13). The regions of the human coreceptors that are needed for virus entry have been identiÞed (13Ð16), though, surprisingly, some HIV-1 isolates can utilize the murine CXCR4 (17). Antibodies to the coreceptor as well as the natural ligand for these molecules, the chemokines themselves, can block virus entry. Variants of SIV and HIV-1 have been identiÞed that are CD4-independent, needing only a chemokine receptor for infection; these viruses suggest that the chemokine receptors might have been the primary receptor for a primordial virus. A further proof of the importance of the chemokine receptor is the existence of a mutant allele of the gene encoding CCR5 in the human population, a 32-bp deletion, that confers dramatic virus resistance to homozygous individuals (18Ð20).

REVERSE TRANSCRIPTION

The reverse transcription of the viral RNA genome into a double-stranded DNA form is the deÞning hallmark of the retroviruses, and the step from which these viruses derive their name. The course of reverse transcription is complex and highly ordered, involving the initiation of DNA synthesis at precise positions, and translocations of DNA intermediates that result in duplication of sequence blocks in the Þnal product (for reviews see (21,22)). The major steps in the reaction are relatively well-established, largely through the analysis of reactions carried out *in vitro* in puriÞed virion particles (the so-called Òendogenous reactionÓ).

Reverse transcription normally begins soon after entry of the virion core into the cytoplasm of the infected cell. The reaction takes place in a large complex, roughly resembling the virion core, and containing Gag proteins including NC, RT, IN, and the viral RNA (23). The signal that triggers the onset of DNA synthesis is not known, though it may be as simple as the exposure of the viral core to the relatively high levels of deoxyribonucleotides present in the cytoplasm. This notion is consistent with the observation that simply stripping or permeabilizing the virion membrane with detergents in the presence of deoxyribonucleotides is sufÞcient to induce DNA synthesis. This may also be at least part of the explanation for the difÞculty HIV has in completing reverse transcription and infection in quiescent cells (24). In some cells, notably cells arrested by starvation, triphosphate levels may be low and limiting for reverse transcriptase, so that addition of exogenous nucleosides can stimulate viral DNA synthesis (25).

It has been recently appreciated that DNA synthesis can be initiated "prematurely" during virion assembly and release, such that virion preparations can be shown to contain small amounts of the early DNA intermediates, such as minus strand strong stop DNA (26–29). In most cases the levels of these DNAs are very low, indicating that only a very small minority of the virion particles have carried out any significant synthesis. However, some circumstances affecting the rate of production and release of virions may enhance this synthesis (30). In addition, in some particular retroviruses, notably the spumaviruses, substantial DNA synthesis may occur during assembly such that the major form of the genome found in mature virions is a partially or even completely reverse transcribed DNA molecule (31,32). These viruses thus resemble the hepadnaviruses more closely than the conventional retroviruses in the relative timing of assembly and reverse transcription.

Steps in Reverse Transcription of the Retroviral Genome

The course of reverse transcription is complex. The reaction can be broken down into a series of discrete steps (21), as presented in Fig. 4.3.

- Formation of minus strand strong stop DNA.
  The process of reverse transcription is initiated from the paired 3′ OH of a primer tRNA annealed to the viral RNA genome at a complementary sequence termed the primer binding site (pbs). DNA is first synthesized from this primer, using the plus strand RNA genome as template, to form minus strand DNA sequences. Synthesis occurs toward the 5′ end of the RNA to generate U5 and R sequences. The intermediate formed in this step is termed minus strand strong stop DNA. The primer tRNA remains attached to its 5′ end.
- First translocation.
  The next step involves the translocation, or "jump," of the strong stop DNA from the 5′ to the 3′ end of the genome. This translocation requires the degradation of those 5′ RNA sequences that were placed in RNA:DNA hybrid form by the formation of strong stop DNA. The degradation is mediated by the RNase H activity of RT; mutants with altered RNase H activity do not mediate the translocation (33,34). This step exposes the single stranded DNA and facilitates its annealing to the R sequences at the 3′ end of the genome. Normally a full-length strong stop DNA, synthesized by copying to the 5′ cap of the RNA, performs the translocation (35), though incomplete molecules can jump at low efficiency. Specific sequences or structures in or near the R region are required for efficient jumping (36,37). The NC protein may facilitate the transfer step. Although there have been reports that jumping is always in *trans*, from one RNA template to the other RNA in the virion, the best evidence is that minus strand strong stop jumping goes randomly to either RNA (38).
- Long minus strand DNA synthesis.
  The annealing of minus strand strong stop DNA recreates a suitable primer-template structure for DNA synthesis, and RT can now continue to elongate the minus strand strong stop DNA to form long minus strand products. Synthesis ends in the vicinity of the pbs. As the genome enters RNA:DNA hybrid form, the RNA becomes susceptible to RNase H action and is degraded.
- Initiation of plus strand DNA synthesis.
  The primer for plus strand synthesis is created by the digestion of the genomic RNA by RNase H. A particular short purine-rich sequence near the 3′ end of the genome, the polypurine tract or ppt, is relatively resistant to the activity of RNase H. The oligonucleotide remains hybridized to the minus strand DNA and serves as the primer for synthesis of the plus strand DNA, using

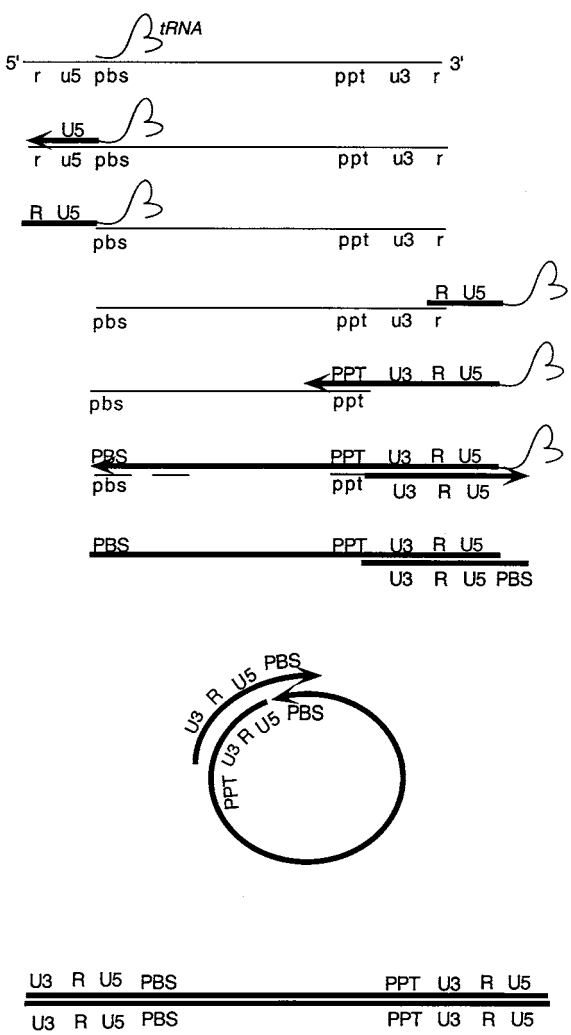

FIG. 4.3. The reverse transcription of the retroviral genome. Thin lines represent RNA; thick lines represent DNA. See text for details. Drawing courtesy of A. Telesnitsky. From (486) courtesy of Lippincott, Williams and Wilkins.

the minus strand DNA as template. Sequences upstream of the polypurine tract, an AT-rich region called the T-box, are also important for proper priming (39,40). The primer, once it has served to initiate DNA synthesis, is quickly removed from the DNA, presumably by RNase H action. Synthesis proceeds toward the 5′ end of the minus strand, Þrst copying the U3, R, and U5 sequences, and then extending to copy a portion of the primer tRNA still present at its 5′ end. Elongation stops at a modiÞed base normally found at position 19 of the tRNA. The resulting intermediate is termed plus strand strong stop DNA.

In some viruses, secondary plus strand initiation sites are used. There may be multiple RNA primers generated from the RNA genome by the nuclease action of RNase H that can initiate DNA synthesis at dispersed heterogeneous sites. In the case of the lentiviruses and spumaviruses, a second copy of the ppt sequence near the center of the genome is used at high efÞciency, and is important for proper completion of reverse transcription (41).

¥ Removal of tRNA

In the next step, the primer tRNA at the 5′ end of the minus strand DNA is removed by RNase H. Its removal may occur in two stages: with an initial cleavage near the RNA-DNA junction, and a second one within the tRNA. The cleavage need not occur exactly at the RNA-DNA junction, and a single ribonucleotide base (A) is normally left on the 5′ terminus of the minus strand (42Ð45) without affecting subsequent processes. The sequence of bases near the junction can signiÞcantly modulate the cleavage by RNase H (46). Recent experiments indicate that the post-transcriptional modiÞcations present in the natural tRNA may be important for proper recognition by RT and for plus strand strong stop translocation (47).

¥ The second translocation

The removal of the tRNA exposes the 3′ end of the plus strand strong stop DNA to permit its pairing with the 3′ end of the minus strand DNA. The sequences anneal via the shared pbs sequences. This annealing forms a circular intermediate, with both 3′ termini in a suitable structure for elongation.

¥ Completion of both strands

Both strands are now elongated. The Þnal extension of the minus strand DNA is coupled to displacement of the plus strand strong stop DNA from the 5′ end of the minus strand; as minus strand elongation occurs, the plus strand strong stop is peeled away and transferred to the 3′ end of the minus strand. At the end of this elongation, the circle is opened up into a linear DNA. The plus strands are all extended, and displacement synthesis may occur to remove short DNAs and make longer plus strand DNAs (48). Any internally primed plus strand DNAs may be prevented from participating in strong stop translocation simply by kinetics; the normal plus strand strong stop DNA may simply form Þrst, and elongation to transfer it to the minus strand may occur before it could be displaced by any plus strand elongation (49). Finally, the plus strand strong stop DNA is extended to complete the plus strand.

When multiple plus strand initiation events occur, the completed plus strand will consist of adjacent fragments and so contain nicks or discontinuities. Displacement synthesis by an upstream fragment can slowly displace downstream RNAs and DNAs, leading to longer plus strands (48). However, some nicks or gaps may persist in the Þnal double stranded product. These breaks may be at heterogeneous positions, though strong sites of plus strand initiation, such as the one at the central ppt of lentiviruses, can lead to speciÞc sites for such discontinuities. Sequences near the central ppt of the lentiviruses cause termination of synthesis during elongation from upstream primers, and thus ensuring the maintenance of a discontinuity at this site (50). This site retains a partially displaced sequence or overlap of a few nucleotidesÑ 99 nt in the case of HIV-1. The structure has been shown to persist even to the time of integration of the DNA into the cell. Host DNA repair processes ultimately correct all such discontinuities.

Biochemistry and Structure of Reverse Transcriptase

The enzyme that mediates the complex series of events outlined above is reverse transcriptase, one of the most famous of the viral polymerases (51); for review, see (52). All RTs contain two separate activities present in two separate domains: a DNA polymerase able to incorporate deoxyribonucleotides on either an RNA or a DNA template, and an RNase H activity able to degrade RNA only in duplex form. These two activities are responsible for the various steps of reverse transcription. Two distinct domains of the enzyme contain these two activities: an aminoterminal domain contains the DNA polymerase, and a carboxyterminal domain contains the RNase H activity (53). While isolated domains can be shown to exhibit either one of the two activities separately, an intact enzyme is required for full activity and speciÞcity. However, the two functions can be provided by two mutant RT molecules so long as they are co-incorporated into a single virion (54).

DNA Polymerase

The DNA polymerase activity is similar to that of all host and viral polymerases in requiring a primer, which can be either RNA or DNA, and a template, which can also be either RNA or DNA. RTs incorporate dXTPs to a growing 3′OH end with release of PPi, and require divalent cations, usually Mg^{++}. The primer must contain a 3′OH end that is paired with the template. RTs are relatively slow DNA polymerases, under standard conditions only incorporating 1Ð100 nucleotides per second,

depending on the template. Further, they exhibit poor processivity, and tend to release primer-template frequently *in vitro*. Secondary structures in RNA templates can strongly enhance the pausing of RT and its tendency to release from the template (55). The enzyme also exhibits low Þdelity, and though the values of the error rate vary widely with the primer (56), template (57,58) and type of assay, the misincorporation rate of most RTs under physiological conditions is on the order of 10^{-4} errors per base incorporated. This rate suggests that during replication there would be approximately one mutation per genome per reverse transcription cycle. Indeed, the mutation rate observed *in vivo* is roughly consistent with this high error rate (59Ð63), though Þdelity *in vivo* may be somewhat better than *in vitro* (64). Drug-resistant variants that do not incorporate chain-terminating analogues are often found to exhibit higher Þdelity, perhaps because they require a more precise Þt for the correct incoming triphosphate to allow for discrimination against the analogue. A wide range of types of mutations are created by RT errors, and both the type and the frequency of appearance of each type of mutation exhibit a complex dependence on sequences and structures in the template (65Ð67).

RTs do not generally exhibit a proofreading nuclease activity (68), and misincorporated bases are not removed efÞciently by most RTs as they are by host DNA polymerases. Very recently, however, mutants of the HIV-1 RT resistant to AZT have been shown to exhibit an enhanced ability to remove the incorporated AZT moiety at the 3′ end through a pyrophosphorolysis reaction (69). Thus, it is possible for RT to remove some such analogues and rescue a terminated chain for continued elongation.

RNase H

The RNase H activity of RT is an endonuclease that releases oligonucleotides with a 3′OH and a 5′PO4. This property allows the products of RNase H action to serve as primers for initiation of DNA synthesis by the DNA polymerase function of RT. There is an obligate requirement that the RNA be in duplex form, normally an RNA-DNA hybrid. However, retroviral RTs are also able to degrade RNA-RNA duplexes (70,71), an activity termed RNaseH* (72). The RNase H enzyme is capable of acting on the RNA of a template in concert with the polymerase as it moves along a nucleic acid, and as it does so its active site is located about 17Ð18 bp behind the growing 3′ end (73). RNase H can also act independently of polymerization.

Crystal Structures

The three-dimensional structure of a number of RTs have been determined by X-ray crystallographic studies. Structures of the unliganded HIV-1 RT (74,75), RT bound to non-nucleoside RT inhibitors (76Ð79), RT bound to an RNA pseudoknot inhibitor (80), and an RT bound to a duplex oligonucleotide (81Ð84), as well as the isolated RNase H domain (85), have all been reported. The two subunits are folded quite differently so that the overall structure is highly asymmetric. The structure of the p66 is similar to that of a right hand, with Þngers, palm, and thumb domains all named on the basis of their position in the structure. The nucleic acid lies in the grip of the hand, held by the Þngers and thumb. The YXDD motif present at the active site for the DNA polymerase lies at the base of the palm. The RNase H domain is attached to the hand at the wrist. The p51 subunit, while made up of the same domains as the aminoterminal part of p66, is folded differently and lies under the hand, not making direct contact with the nucleic acid and so not thought to participate in chemistry. The structure of p66 with and without a liganded nucleic acid is quite different, with the thumb domain ßexing to allow substrate binding. Theoretical considerations suggest the thumb may move during elongation (86). A structure of the Þngers and palm subdomain of the Moloney MuLV RT at very high resolution (87) has also been determined.

Inhibitors

RT is a major target of antiviral drugs useful in the treatment of retroviral diseases such as AIDS. All such drugs used to date are inhibitors of the DNA polymerase activity of RT, and fall into two classes: nucleoside analogue inhibitors (chain terminators), and non-nucleoside RT inhibitors (NNRTIs). The nucleoside analogues are typically precursors, and need to be activated by phosphorylation to the triphosphate form. These are then incorporated by RT into the growing chain, and serve to block further elongation. Examples include AZT, ddC, ddI, d4T, and 3TC. The NNRTIs are a group of compounds that are structurally diverse, but nevertheless interact with a common binding pocket in RT to prevent its normal activity (88). There are indications that the binding may inhibit the enyzmeÕs ßexibility. For both classes of inhibitors, monotherapy with a single drug selects for drug-resistant variants that quickly predominate in the virus population, and for each drug, a pattern of mutations has been identiÞed that serves to indicate the appearance of drug resistance (89). In many cases these mutations alter the binding site for the nucleoside or NNRTI such that the drug cannot bind and so cannot inhibit the enzyme. In the case of AZT, however, the mutations do not prevent the binding and incorporation of AZTTP into the growing chain, but rather seem to activate a reverse reaction in which the AZT nucleotide is removed from the chain, subsequently permitting normal elongation (69). Combination therapy, typically involving the simultaneous treatment with three different drugs, can suppress virus

replication to such an extent that variants resistant to all the drugs do not appear, at least for months or years.

RECOMBINATION

The process of reverse transcription could in principle take place using a single template RNA molecule. In fact, however, retrovirions contain two copies of the RNA genome copackaged into one particle, and the course of reverse transcription typically makes use of both RNAs (90,91). Recombination occurs between homologous sequences in the two RNAs (92,93), and it happens normally at surprisingly high frequencies, perhaps once per replication event per genome on average (94). Normally the two RNAs in a virion are identical, so that homologous recombination events are invisible and without consequence. When the two RNAs are distinct, however, as when they derive from two viruses or viral strains, the result is a very high frequency of recombination between them among the resulting proviral DNAs. Thus, physical markers and genetic markers recombine rapidly whenever the two genomes are copackaged into one virion and so are coextant during a single round of reverse transcription.

INTEGRATION OF THE PROVIRAL DNA

Integration of the linear retroviral DNA, like reverse transcription, is a crucial feature of the retroviral life cycle. Integration is required for efficient replication of most retroviruses; mutants that are unable to integrate do not establish a spreading infection. The orderly and efficient integration of viral DNA is also unique to the retroviruses. Further, the establishment of the integrated provirus is responsible for much of retroviral biology. It accounts for the ability of the viruses to persist in the infected cell; for their ability to enter permanently the germ line; and for the mutagenic and oncogenic activities of the leukemia viruses. Once the provirus is established, the DNA is permanently incorporated into the genome of the infected cell. There is no mechanism by which it can be efficiently eliminated. At very low frequencies, homologous recombination between the two LTRs can delete most of the provirus, but even here a single (ôsoloõ) LTR remains (95).

Entry into the Nucleus

A key step that must take place before integration can occur is entry of the viral DNA into the nucleus. The mechanisms of nuclear entry are largely unknown, but there are probably at least two distinct routes used by different retroviruses. The simple retroviruses show a profound requirement for passage through mitosis for successful establishment of the integrated provirus (96–99), and the block in non-dividing cells is at or close to the step of nuclear entry. Tests of the state of the viral DNA in non-dividing cells are consistent with the notion that the preintegration complex must await the breakdown of the nuclear membrane in order to have access to the cellular DNA. Infection of non-dividing cells results in the accumulation of linear double stranded DNA in the cytoplasm, and no further signs of infection. The viral DNA will persist in the cell for some time, and if the cell is stimulated to undergo division, the viral DNA will integrate and infection will proceed. However, the DNA loses its capacity to become activated in this way fairly rapidly (97,100). The restriction is quantitatively very significant, and it profoundly limits the utility of simple retroviral vectors for gene therapy.

In contrast, lentiviruses and spumaviruses are able to infect successfully non-dividing cells, and thus there must be an active transport of viral DNA through an intact nuclear membrane (101–104). This capability has made lentiviruses very attractive as gene delivery vectors for gene therapy applications. The molecular basis for this capability is a subject of great controversy. The lentiviral MA and Vpr proteins have been argued as essential for the infection of non-dividing cells (105–108), and the phosphorylation of MA has been argued as necessary to promote dissociation from the membrane and allow nuclear import (109–112). These findings have not been universally confirmed (113–115), however, and the role of MA in these steps is uncertain. Similarly, it has been shown that the Vpr protein is present in the preintegration complex, and can bind to nucleoporin components that may mediate nuclear import. However, here too universal support for this model has not been forthcoming. A triple stranded DNA structure, termed a DNA structure, formed by reverse transcriptase at the second internal copy of the polypurine tract have also been suggested as important for infection of non-dividing cells (116). Another attractive model is that the IN protein might be involved in the nuclear import of the complex. IN itself contains nuclear localization signals (107, 117), and these can function to target ectopically expressed IN to the nucleus, but it is not clear whether this is related to preintegration complex nuclear import or nuclear retention. The foamy viruses may have a distinctive route of nuclear entry involving microtubular transport and centrisomal association, but the mechanism is not well understood (104).

Biochemistry of Integration

The actual integration of the viral DNA into a target is mediated *in vivo* by the viral integrase protein, IN (118–120), which is brought into the cell inside the virion, and acts to insert the linear DNA into the host chromosome. Some of IN functions have been studied by analysis of viral DNA formed *in vivo* (121). Most of our

understanding of IN function, however, has been obtained through analysis of *in vitro* integration reactions, Þrst using complexes extracted from infected cells (122, 123), and later using recombinant IN protein. The reaction proceeds in two steps: 3′ end processing and strand transfer. A schematic view of these reactions is shown in Fig. 4.4.

3′ End Processing

In the Þrst step, the two terminal nucleotides at the 3′ ends of the blunt-ended linear DNA are removed by the integrase to produce recessed 3′ ends and correspondingly protruding 5′ ends. This cleavage occurs endonucleolytically at a highly conserved CA sequence, and releases a dinucleotide. For most viruses the terminal sequence is such that a TT dinucleotide is released, though this rule has exceptions. The ends do not remain covalently bound to protein, and the energy of the hydrolyzed phosphodiester bond is not retained.

Strand Transfer

In the second step, the 3′ OH ends created by processing are used in a strand transfer reaction to attack the phosphodiester bonds of the target DNA (123). The attack occurs by an Sn2-type reaction, with inversion of the phosphorus center as detected with chiral labelling of the phosphate (124). The formation of the new phosphodiester bond between the viral end and the host DNA displaces one of the phosphodiester bonds in the host DNA, leaving a nick. The protruding 5′ end of the viral DNA is not joined to the host DNA by IN. The reaction is a direct transesteriÞcation, and so no ATP or other energy source is

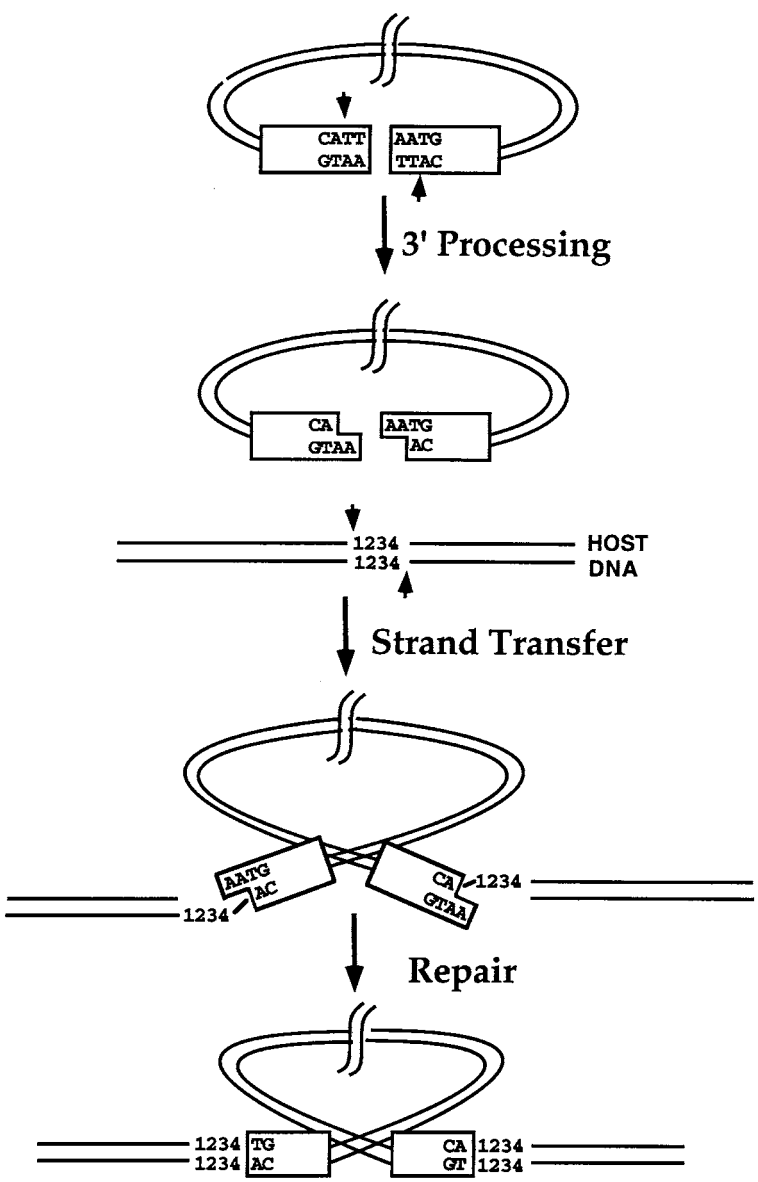

FIG. 4.4. Steps in the integration of the viral DNA. The full-length linear DNA (top) is processed by the viral integrase by the endonucleolytic removal of dinucleotides at the 3′ termini. The resulting DNA is then used in a strand transfer reaction in which the 3′ OH ends make staggered breaks in the two strands of the target site DNA. The resulting gapped intermediate is presumably repaired by host enzymes. From (486) courtesy of Lippincott, Williams and Wilkins.

required. Mutational studies strongly suggest that the two activitiesÑ processing and joiningÑ utilize the same active site residues. In fact the two steps involve similar chemistry: 3′ end processing is an attack on DNA by a hydroxyl residue of water, while joining is an attack on DNA by a 3′ hydroxyl residue of another DNA. It should be noted that other hydroxyl residues can participate; alcohols such as glycerol can be utilized (125), and the 3′ OH of a DNA can even attack a phosphodiester bond on the same DNA, forming a cyclic product (124).

Disintegration

The IN protein exhibits a third enzymatic activity *in vitro*: a reversal of the integration reaction known as disintegration (126). This activity releases DNA from a branched structure and seals the nick at the site of the branch. The signiÞcance of the activity *in vivo* is uncertain.

Target Site Duplication

In a wild-type virus, when the two ends are joined to the two strands of the target DNA, the two sites of attack are staggered by a few base pairs. After the joining, the resulting structure contains short gaps in the host DNA and unpaired bases from each 5′ end of the viral DNA. The 5′ ends of the viral DNA are not joined to host DNA by any known activities of IN. However, the 5′ ends are very quickly repaired *in vivo*, almost as quickly as the initial integration reaction (127). These discontinuities are presumed to be repaired by the host repair enzymes, though it is possible that the viral RT or IN could participate. Indeed, the IN protein has been suggested to manifest a DNA polymerase activity that might Þll in the gaps. The processing and Þlling in the gaps creates a short duplication of sequence that was present only once at the target site; these duplications ßank the integrated provirus. The number of bases duplicated is characteristic of each virus. Thus, the murine and feline viruses cause a 4 bp duplication; HIV-1 causes a 5 bp duplication; and the avian viruses cause a 6 bp duplication.

Viral *att* Sites

The sequences at the termini of the viral DNA, the *att* sites, are recognized by the viral IN protein and are important for end processing and joining (128Ð131). These terminal sequences are imperfect inverted repeats. The most conserved residues are a CA dinucleotide pair that lies near the 3′ terminus and determines the site of 3′ processing. While these bases are absolutely conserved among all the retroviruses, and among many transposable elements, small changes can be tolerated, and mutants can utilize TA or CG relatively well (132). Sequences upstream from the CA for perhaps 10Ð12 bp are needed for efÞcient integration, but these sequences are different for different viruses, with no indication of broadly conserved sequence motifs. Since the two termini of any given virus are somewhat different, they usually show differential efÞciency of utilization in various assays (133). The fact that two distinct ends are bound together in a complex may be important for the concerted integration of these ends into the target (134).

The sequence-speciÞc binding to the att site is probably performed by the core domain of IN (135). The non-speciÞc DNA binding activity of IN has made it difÞcult to detect sequence-speciÞc binding to these regions, though under some conditions preferential binding to the authentic sequences can be demonstrated (136). A demonstration that an IN mutation can compensate for a mutation in the DNA termini provides good evidence for the delicate interaction between IN and the DNA termini (137).

Structure of the Integrase

The IN protein consists of three distinct domains: an N-terminal region containing an HHCC zinc-Þnger motif; a central catalytic core containing the so-called D,D-35-E motif; and a less well-conserved C-terminal region. The IN protein is a multimer: it readily dimerizes, and at high concentration forms tetramers as well. All three regions may be involved in the multimerization of IN (138) and in DNA binding (139,140). Many of the residues important for enzymatic activities have been identiÞed by mutagenesis. The most crucial residues for catalysis are the acidic amino acids in the D,D-35-E motif, a highly conserved array of three residues in the core region of many integrases and transposases (141). Mutants indicate that both the N and C-terminus are also important for function. Surprisingly, pairs of IN mutants with alterations in different regions of the molecule can complement to restore normal function (142,143). The separate N-terminal domain can even complement a non-overlapping fragment, suggesting that these domains can still coassemble into a functional oligomeric complex (144).

The structures of the HIV-1 and avian virus IN cores have been determined by X-ray crystallography (145Ð148), revealing a compact dimer with similarity to prokaryotic recombination enzymes and RNase H (149). The structures of the N- and C-terminal domains have been determined by NMR (150Ð153). The structures can be partially merged into a complete molecule (154), but the models do not yet provide a clear picture of the binding sites for viral or host DNAs or the mechanism of the reaction. Indeed, the likely active sites are not located at positions in the dimeric molecule that could mediate concerted integration of two termini into a single target DNA. Crosslinking studies have helped the development of some models for the interaction of IN with DNA (155).

The avian IN is phosphorylated at a carboxyterminal serine (156), but the signiÞcance of this modiÞcation is not clear.

Preintegration Complex

IN does not normally act alone, and a large complex of proteins and nucleic acid is responsible for mediating the formation of the provirus *in vivo* (23,157,158). The nature and components of the preintegration complex (PIC), or intasome, are not known in any detail for either the simple or the complex viruses. The PIC of the simple viruses seems to contain CA, RT, and IN, but other viral proteins may be present (23). Many of these proteins probably stay with the DNA even after entry into the nucleus (159). The PICs of the complex viruses do not contain detectable CA, but rather contain MA, NC, Vpr, RT and IN (160). Thus, the PICs of these viruses may be very different, consistent with their very distinctive ability to infect non-dividing cells. Normal PICs apparently contain a large structure covering the two ends of the DNA, and perhaps holding them in proximity. The formation of this structure, detected as a footprint in a modiÞed nuclease sensitivity assay (161), requires both IN and the correct sequences at the termini of the DNA (162).

Structure of the Provirus

An important aspect of retroviral integration that distinguishes the process from non-viral or other viral mechanisms of DNA integration is the fact that the insertions create a consistent provirus structure. The integrated provirus is collinear with the product of reverse transcription, and consists of a 5′ LTR, the intervening viral sequences, and a 3′ LTR, inserted cleanly into host sequences. The joints between host and viral DNA are always at the same sites, very near the edges of the viral LTRs. As compared to the unintegrated linear DNA there is a loss of a small number of base pairs, usually two, from each terminus of the viral DNA; and there is a duplication of a small number of base pairs of host DNA initially present once at the site of insertion that ßank the provirus. The number of base pairs duplicated is characteristic of each virus, and ranges from 4 to 6 bp.

EXPRESSION OF VIRAL RNAS

Integration of the provirus signals a dramatic change in the life style of the retrovirus; it marks the end of the early phase of the life cycle and the beginning of the late phase. The early phase is driven by viral enzymes performing abnormal events such as reverse transcription and DNA integration, while the late phase is mediated by host enzymes performing such relatively normal processes as transcription and translation. This late phase of gene expression may begin immediately with the synthesis of viral RNAs and proteins, and the assembly of progeny virions (see Fig. 4.5 for an overview). For many viruses, the transcriptional promoters that drive this expression are constitutively active and cause the production of virions in a relatively unregulated way. In other viruses the activity of the promoter may be regulated, either by viral or host factors. We will review the basic phenomenology of proviral gene expression, and mention brießy the regulation exhibited by the complex retroviruses.

Initiation of Transcription

The efÞciency of initiation of transcription at the 5′ LTR is the major determinant of the levels of viral RNA formed in the cell. The promoter in the LTR is typically a very potent one, and the levels of viral RNA are often constitutively high. However, the cell type, the physiological state, and the integration site (163,164) can all result in substantial variation in the efÞciency of transcription. In some viruses, the promoter is not constitutively active but depends on the activity of speciÞc transcription factors such as the glucocorticoid receptors.

Positive Regulatory Elements in U3

The transcriptional elements in the U3 region of the simple viruses contain both core promoter sequences and enhancers. The core promoters contain a TATA box, bound by TFIIB, a CCAAT box, bound by CEBP (165), and sometimes an initiator sequence near the U3-R border. The U3 regions of even closely related retroviruses are very diverse, and can evolve rapidly during viral replication. The enhancers are similar to those found in many host promoters in containing multiple short sequence motifs, arranged in very close packing; often there are tandemly repeated copies of some of these motifs. These short sequences are the binding sites for a large number of host factors that regulate transcription (e.g. see (166)). Different cells and cell types will make use of distinct arrays of these factors to mediate transcription from a given viral LTR (167Ð170). The factors are not simply additive but may interact in complex ways on particular viral sequences.

Trans-Acting Viral Regulatory Factors

The complex retroviruses encode an array of small regulatory proteins that can activate transcription from the viral LTR in *trans*. Examples of these transactivators include the HTLV-1 Tax protein (171) and the HIV-1 Tat protein (172). The Tax protein acts in concert with a complex of host proteins, the activating transcription

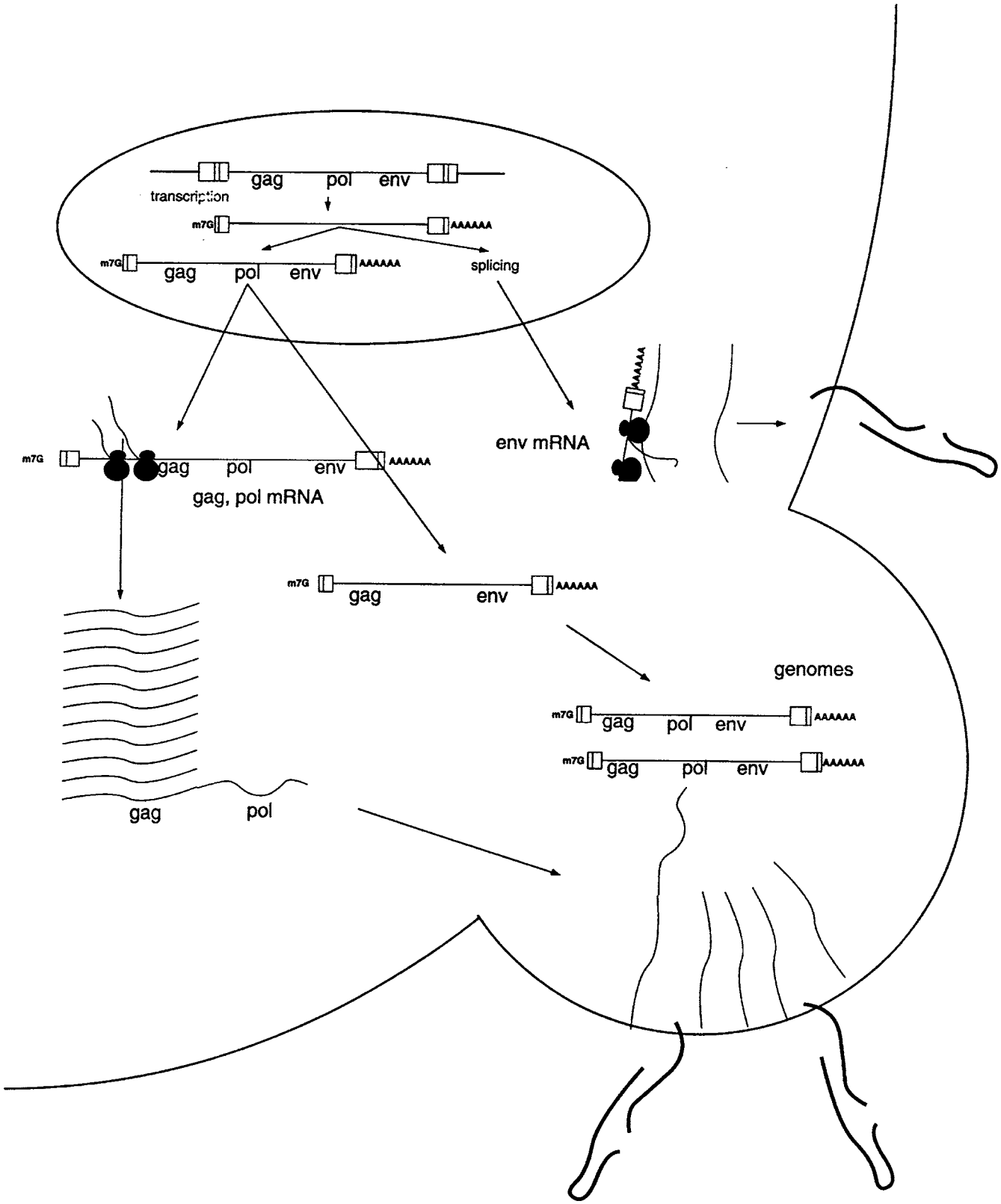

FIG. 4.5. The late stages of the retroviral life cycle. The integrated provirus is used as the template (top) for the expression of viral RNAs. A subset of the transcripts are spliced, and the unspliced and spliced mRNAs are exported to the cytoplasm. The unspliced RNA is used to make Gag and Gag-Pol proteins and also serves as the genome; spliced mRNA is used to make Env proteins. The proteins and RNA associate under the membrane to form the budding progeny virion. From (486) courtesy of Lippincott, Williams and Wilkins.

factor/CRE-binding protein (ATF/CREB), and binds to three cAMP response elements in the viral LTR. Tax thus sets up a positive feedback loop that results in high levels of viral transcripts. The Tat protein is unusual among transcriptional activators in that it binds to a structure in the 5′ end of nascent viral RNA (173, 174), rather than to DNA. Tat binds to a bulged hairpin structure, the TAR element, and recruits a pair of host proteins, cyclinT/cdk9, to the RNA. These proteins enhance the ability of RNA polymerase to elongate beyond the LTR and down the genome with high processivity, probably by phosphorylation of the C-terminal repeat domain (CTD) of the polymerase. Again, the result is a strong positive feedback loop that results in high levels of viral RNA.

RNA Processing

The full-length transcript of the retroviral genome is directed into several pathways. A portion of the transcripts is exported directly from the nucleus and serves as the genome to be packaged into the progeny virion particle, assembling either at the plasma membrane or in the cytoplasm. Another portion with identical structure is also exported and used for translation to form the Gag and Gag-Pol polyproteins. It is not yet clear if these two subsets are truly distinct, whether there can be interchange between the pools, or whether there is a single pool of such molecules used for both purposes. It has been proposed that the translated mRNAs are selectively packaged in cis (175). A third portion is spliced to yield subgenomic mRNAs. For the simple retroviruses, there is a single spliced mRNA encoding the Env glycoprotein. For the complex viruses, there can be multiple alternatively spliced mRNAs, encoding both Env and an array of auxiliary proteins (176,177).

The splicing and subsequent export from the nucleus of only a portion of an initially transcribed RNA is an extraordinary process; normally splicing goes to completion, and only then is the mRNA exported. The export of a precursor mRNA is prevented until splicing is complete. At least three aspects of the retroviral genome may promote the export of unspliced mRNAs. First, the splice sites of the viral RNA may have quite poor overall efficiency of utilization by the splicing machinery in the cell (178). Indeed, the sequences at the splice donor and acceptor regions are often poor matches to the consensus sequences for splice sites, and mutations that make the sites better matches increase splicing and are actually deleterious to virus replication (179,180). These mutations can be suppressed by secondary mutations that reduce splicing efficiency. The overall folding of the RNA may affect the efficiency of splicing, and thus sequences at some distance, as in the *gag* gene, may modulate splicing (181,182).

Secondly, studies of ASLV have identified specific sequences that act as negative regulators of splicing (NRS) through their interaction with host factors (183–186). These elements can be important for the expression of transduced genes in some viruses (187). Similar signals may exist in other viruses; mutations in the Gag region of MuLV can affect RNA processing in complex ways (188).

In addition, unspliced mRNAs contain cis-acting elements that promote the export of the RNA out of the nucleus, the so-called constitutive export elements (CTEs) (189). These sequences are located near the 3′ end of the genomic RNA of MPMV (190), between *env* and the LTR, and possibly in similar regions of ASLV (191). CTE function of the MuLVs may be present in the Gag region (192), or possibly in the R region of the LTR (193). The CTE is recognized by one or more host proteins that assemble a complex onto the RNA to mediate its export, though which proteins are most important is controversial (194–196). In the complex viruses, RNA export is regulated through complex interactions of both trans-acting proteins, the Rex or Rev gene products, and cis-acting sites, the RRE elements that promote RNA export and the CRS elements that prevent it (197–206). A key aspect of this process is the recruitment of Crm1, a cellular nuclear export factor, to the RNA. The Rev/RRE pathways of export are probably distinct from those used by the CTEs, because the two are differentially sensitive to a competitive block acting through Crm1 (207,208).

TRANSLATION AND PROTEIN PROCESSING

All retroviral genomes, at a minimum, contain open reading frames designated the *gag*, *pro*, *pol*, and *env* genes. These genes are expressed by complex mechanisms to form precursor proteins, which are then processed during and after virion assembly to form the mature, infectious virus particle. The expression of the various proteins as large precursors that are subsequently cleaved provides several advantages: it allows for many proteins to be made from one ORF; it ensures that the proteins are made at proper ratios; and it allows for many proteins to be targeted to the virion during assembly as a single entity. The *gag, pro*, and *pol* genes are expressed in a complex way from the full-length unspliced mRNA. The arrangement of these genes, and especially the way *pro* is expressed, are different in different viruses.

Gag Gene Expression

The *gag* gene is present at the 5′ proximal position on all retroviral genomes. A full-length mRNA, identical in sequence to the genomic RNA, is translated in the cytoplasm to form a Gag precursor protein, in the 50–80 kDa range. Translation begins with an AUG initiator codon and proceeds to a terminator codon at the 3′ end of the ORF. The viral RNA typically contains a relatively long 5′

untranslated region, and it has been uncertain whether ribosomes could scan from the 5′ cap to the start codon for Gag translation. These 5′ RNA sequences are predicted to contain stable secondary structures that would inhibit scanning. Furthermore, the long 5′ UTRs often contain AUG codons in contexts that are favorable for translation, that are not in frame with the *gag* ORF, and presumably would inhibit successful translation of Gag (209). Recent experiments suggest that for the MuLVs, an internal ribosome entry site (IRES) is present near the start of the *gag* ORF and is used to initiate translation in a cap-independent mechanism (210,211). Thus, at least in these viruses, ribosomes can bind directly near the *gag* gene and do not need to scan the mRNA. However, other viruses such as HIV-1, may not use such sequences (212).

The major Gag product is often modified by the addition of myristic acid, a relatively rare 14-carbon fatty acid, to the penultimate aminoterminal residue, a glycine (213). The addition is mediated by a myristyl CoA transferase that cotranslationally transfers myristate from a myristyl CoA donor to the amino group of the glycine residue, forming an amide bond. The fatty acid is important for the membrane localization and binding of the Gag precursor, increasing the hydrophobicity of the aminoterminal domain. Mutant Gags in which the glycine is altered are not modified, and these Gags do not associate with membrane properly and do not aggregate to form virions (214–216). It should be noted that although myristate is important, it is not sufficient for membrane targeting; hydrophobic residues in the MA domain are also required. Furthermore, basic residues further downstream in the MA of some viruses form a patch of positive charge that may interact with negatively charged phospholipids in the membrane.

An aminoterminal myristate is not found on the Gags of BIV, EIAV, visna, or ASLV. For the avian retroviruses, the aminoterminus is not myristylated but rather acetylated. The Gag protein of these viruses is apparently sufficiently hydrophobic to be targeted to the membrane without the fatty acid in avian cells, though, curiously, not in mammalian cells. Alteration of the avian Gag to allow its myristylation permits virion assembly in mammalian cells (217) and does not block its function in avian cells (218).

Pro Gene Expression

The relative position of the *pro* gene on retroviral genomes is always similar— in between *gag* and *pol*. However, the *pro* gene is expressed in very different ways in different viruses. Sometimes it is fused in frame onto the 3′ end of *gag*, sometimes it is fused to the 5′ end of *pol*, and sometimes it is present as a separate reading frame. These various patterns have led to considerable confusion in the literature; sometimes *pro* is considered a portion of *gag*, or sometimes of *pol*. Because of these different patterns of expression, it is best to consider this ORF as a separate gene.

The various arrangements of the *pro* gene and its mode of expression are as follows. For the alpharetroviruses, *gag* and *pro* are fused and expressed as a single protein; *pol* is in a different reading frame, and a frameshift is used to express the Gag-Pro-Pol polyprotein. For the beta-retroviruses and deltaretroviruses, *gag*, *pro*, and *pol* are all in different frames and successive frameshifts are used to express Gag-Pro and Gag-Pro-Pol polyproteins. For the gammaretroviruses and epsilonretroviruses, *gag* and a *pro-pol* fusion are in the same reading frame and separated by a stop codon, and translational readthrough is used to make Gag-Pro-Pol. For the lentiviruses, *gag* and a *pro-pol* fusion are in different reading frames, and frameshifting is used to make Gag-Pro-Pol. Finally, for the spumaviruses, *pro* is fused to *pol*, and the Pro-Pol protein is expressed without Gag, from a spliced mRNA. More about these varied mechanisms of expression is in the following section.

Pol Gene Expression

The *pol* gene encodes several proteins needed at lower levels for replication of the virus, including the reverse transcriptase and integrase enzymes. The *pol* ORF is not expressed as a separate protein in most retroviruses, but rather is expressed as a part of a larger Gag-Pro-Pol fusion protein. The Gag-Pro-Pol protein must be made at the correct abundance, in proportion to the amount of Gag protein, for efficient assembly of infectious virus; expression of only Gag-Pro-Pol does not result in virion assembly (219,220). The formation of this protein is mediated by either one of two mechanisms, depending on the virus.

Translational Readthrough

In the gammaretroviruses and epsilonretroviruses, the Gag and Pro-Pol ORFs are in the same reading frame, and are separated by a single UAG stop codon at the boundary between Gag and Pro-Pol. Translation of Gag-Pro-Pol in these viruses occurs by translational readthrough— that is, by suppression of termination— at the UAG stop codon (221). Most of the time translation of the RNA results simply in the formation of the Gag protein. But approximately 5–10% of the time, ribosomes translating the RNA do not terminate at the UAG codon, and instead utilize a normal aminoacyl tRNA, usually a glutamine tRNA, to insert an amino acid at the position of the stop codon. Translation then continues, in frame, through the entire long *pro-pol* ORF, resulting in formation of a long Gag-Pro-Pol precursor protein. The high-level suppression of termination is specified by a specific structure in the RNA immediately downstream of the UAG stop codon (222,223). The precise features of this structure that are required for suppression are not completely known, but

they include a purine-rich sequence immediately downstream of the stop codon, and a pseudoknot formed from the next 60 or so nucleotides (224–229). The structure may slow translation, and it may also in some other way alter the balance between termination, which requires binding of termination factors eRF-1 and eRF-3 by the ribosome, *vs*. incorporation of an amino acid, which requires misreading of the codon by an aminoacyl tRNA. No changes in the tRNA pool occur during infection (230). The signals in the RNA can operate to mediate suppression of both UAA and UGA termination codons as well (231).

Translational Frameshifting

In the alpharetroviruses and lentiviruses, the *gag* and *pol* ORFs lie in different reading frames, and the formation of the Gag-Pro-Pol fusion is mediated by a translational frameshift mechanism (232). Most of the time, translation again results in the simple formation of the Gag protein. But approximately 10% of the time, as the translation approaches a specific site near the end of the *gag* ORF, the ribosome slips back one nucleotide (a-1 frameshift) and proceeds onward in the new reading frame. The ribosome passes through the stop codon out of frame and so continues to synthesize protein using the codons of the *pol* ORF. As for readthrough, the determinants of frameshifting lie in the RNA sequence and structure near the site of the event (233–236). The requirements for frameshifting include a "slippery site", a string of homopolymeric bases where the frameshift occurs; these are oligo U or oligo A in different viruses. In addition, the frameshifting requires either a very large and near-perfect hairpin or stem-loop structure; or a large pseudoknot structure, similar to those used in readthrough, though apparently containing a distinctive bend at the junction of the two paired sequences (237–240). As for readthrough, the proper frameshifting efficiency is crucial for normal virus replication.

In the betaretroviruses (e.g. MMTV) and deltaretroviruses (e.g. BLV, HTLV-1), the *pro* gene is present as a separate ORF, in a different reading frame from that of *gag* or *pol*. Two successive frameshifts are utilized to make the long Gag-Pro-Pol fusion protein (241–243). Near the 3′ end of the *gag* ORF, ribosomes carry out a first (−1) frameshift and continue into the *pro* ORF; near the 3′ end of the *pro* ORF, they perform a second (−1) frameshift and continue on into the *pol* ORF. These two frameshifts occur at extremely high frequencies – as much as 30% of the time that the ribosome transits through each site – so that the overall frequency of formation of the Gag-Pro-Pol protein is perhaps 10% that of formation of Gag.

Separate Pol Expression

The spumaviruses are unique among the retroviruses in that the synthesis of the Pol protein is not mediated by the formation of a Gag-Pol fusion protein. Instead, a separate subgenomic spliced mRNA (244) is translated directly to form a separate Pro-Pol protein (245,246). This protein must be directed to the assembling virion by distinct domains rather than by the Gag portion of a Gag-Pol fusion.

Env Gene Expression

In all retroviruses the *env* gene is expressed from a distinct subgenomic mRNA. The *env* message is a singly spliced mRNA, in which a 5′ leader is joined to the coding region of *env*. Thus, the bulk of the *gag* and *pol* genes are removed as an intron from the mRNA. The resulting message is exported to the cytoplasm and translated from a conventional AUG initiator codon. In the alpharetroviruses, the AUG is actually the same one used for Gag translation; it lies in the leader, and the splicing brings this AUG and the first six codons into frame with the *env* coding region. The first translated amino acids constitute a hydrophobic signal peptide, and direct the nascent protein to the rough endoplasmic reticulum. The leader is removed by a cellular protease (the signal protease) in the ER, and the protein is heavily glycosylated by transfer of oligosaccharide from a dolichol carrier to asparagine residues on Env. These residues lie in the conventional Asn-X-Ser/Thr motifs recognized by the modification enzymes. Near the end of the cotranslational insertion of Env into the ER, a highly hydrophobic sequence acts as a stop transfer signal to anchor the protein in the membrane. The remaining C-terminal portion of the protein stays on the cytoplasmic side of the membrane.

Before the Env proteins are transported to the cell surface, they are folded and oligomerized in the ER. The formation of oligomers is required for stable expression of the protein, and is sensitive to overall conformation; many mutants of Env show defects in oligomerization (247). There is considerable controversy about the oligomeric state of the Env protein in different viruses, and at different times during their transport (248–252). The most studied envelope proteins (ASLV and HIV-1) may pass through dimeric or tetrameric intermediates, but the nature of these intermediates is not clear. Although some laboratories disagree (e.g. (253,250,254,255)), ultimately these envelopes probably form trimers in the mature virus (248,256). The folding of the protein is presumably catalyzed by chaperone proteins in the ER; and the formation of disulfide bonds between various pairs of cysteine residues is similarly catalyzed by disulfide interchange enzymes.

The Env protein is then exported to the Golgi, and cleaved by furin proteases to form the separate SU and TM subunits. This cleavage is essential for the normal function of the Env protein. The cleavage occurs at a dibasic pair of amino acids (257), and produces a hydrophobic N-terminus for the TM protein that is required to mediate fusion of the viral and host membranes during virus entry.

In the Golgi the sugar residues are modiÞed by the sequential removal of mannose residues and addition of N-acetyl glucosamine and other sugars to many of the oligosaccharide. O-linked glycosylation and sulfation of Env glycoproteins have also been documented (258,259). The pathway by which Env is transported to the cell surface is not fully understood, but presumably host vesicular transport systems are utilized. There is evidence that clathrin adaptor complexes interact with the cytoplasmic tail of Env, and direct its movement to the plasma membrane (260). The protein typically becomes a prominent cell surface protein on the infected cell.

In polarized epithelial cells, Env proteins are often restricted to the basolateral surface of the cell (261). This localization is mediated by a tyrosine-based motif, Yxxϕ, present in the cytoplasmic tail of Env (262,263) (x, any amino acid; ϕ, hydrophobic residue). Remarkably, this targetting of Env can redirect the budding of Gag proteins to this surface.

Other Viral Gene Products

The complex retroviruses express a number of small proteins with a range of functions. The proteins are translated from subgenomic mRNAs, usually resulting from multiple splicing events that join a 5′ LTR to a number of small exons encoding the protein. A brief summary of these gene products and their functions is given here (see (176,264,172,265,266) for in-depth reviews).

Deltaretroviruses:

- Tax: The Tax gene product is a positive regulator of transcription from the viral LTR. Tax functions in association with the activating transcription factor/CRE-binding protein (ATF/CREB) by binding to three cAMP response elements in the viral LTR. Tax also plays a role in transformation, perhaps through E2F-1 activation or through effects on the cell cycle.
- Rex: The Rex gene product facilitates the export of unspliced and singly-spliced viral mRNAs from the nucleus. Its action is probably similar to that of the lentiviral rev protein.

Epsilonretroviruses:

- Orf a: The Orf A product of the piscine retroviruses is a cyclin D homologue that functions as a cyclin in yeast (267). The function of the protein in virus replication or tumor formation is uncertain.
- Orfs b,c: The function of these Orfs is unknown.

Lentiviruses:

- Tat: The Tat protein is a potent transactivator of transcription from the viral LTR. The protein acts by binding to a hairpin structure, the TAR element, encoded in the R region of nascent viral RNA, and recruiting host factors cyclinT and Cdk9 to the RNA. Tat does not increase the rate of RNA polymerase II initiation, but seems to enhance its processivity or elongation, perhaps by phosphorylation of the CTD of pol II.
- Rev: The Rev protein mediates the export of the unspliced and singly-spliced viral RNAs from the nucleus, thus permitting the expression of the Gag, Pol, and Env gene products. Rev binds to the Rev-responsive element (RRE) present in the HIV-1 *env* gene and by interacting with the importin Crm1 acts to export the viral RNAs through the nuclear pore.
- Nef: The Nef protein is a multifunctional protein not essential for replication in some cells in culture, but important for replication *in vivo*. Nef-defective viruses do not induce high-level viremia in infected animals, and progression to disease is delayed or prevented (268). Nef downregulates the CD4 receptor from the cell surface, facilitating virus release, probably by bridging CD4 to adapter proteins (APs) (269). Nef also down-regulates MHC class I levels, thereby inhibiting the CTL-mediated lysis of HIV-1 infected cells. Finally, Nef may enhance virion assembly and release through unknown mechanisms.
- Vpr: The Vpr protein, as noted below, is packaged at high levels into virion particles through an interaction with the p6 domain of Gag (270,271). Vpr may facilitate the import of the preintegration complex into the nucleus in non-dividing cells. Vpr also causes a strong cell cycle arrest in the G2 stage of the cell cycle, perhaps through an indirect inhibition of Cdc25 phosphatase activity.
- Vif: The Vif protein is expressed at high levels in the cytoplasm, and is packaged into virion particles of both homologous and heterologous viruses. Vif enhances infectivity of virus produced in certain non-permissive cells, perhaps by blocking the action of a cell-speciÞc inhibitor.
- Vpu: The Vpu gene product, found only in HIV-1, is a membrane protein that enhances virion production and also mediates the degradation of CD4 by the ubiquitin-conjugating pathway (272, 273).

Spumaviruses:

- Tas: The Tas (or Bel1) protein is a transactivator of transcription from the viral LTR, acting at sequences near the 5′ end of the genome. Its mechanism of action may be similar to that of the lentiviral Tat protein.
- Bet, Bel2, and Bel3: The functions of the *bet* (and the overlapping *bel2*) and the *bel3* genes are unknown,

though there is some evidence that the Bel3 protein may be a negative regulator of replication.

VIRION ASSEMBLY

As the Gag, Gag-Pro-Pol, and Env proteins are synthesized, they come together to form progeny virions (for reviews, see (274–278); see Fig. 4.6 for a schematic view of the process). The assembly of the retrovirus particle is driven primarily by the Gag precursor protein. Gag is required for the formation of a virion, and indeed is sufficient on its own to mediate the assembly and release of a "bald" particle—lacking infectivity and the "hair" of the Env protein. The Gag protein that is responsible for assembly is the uncleaved Gag precursor. This form of the protein is thus targeted for assembly and export—the "way out" of the cell. Once the Gag proteins are processed, changes in virion structure can occur to promote entry—the "way in" to the next cell.

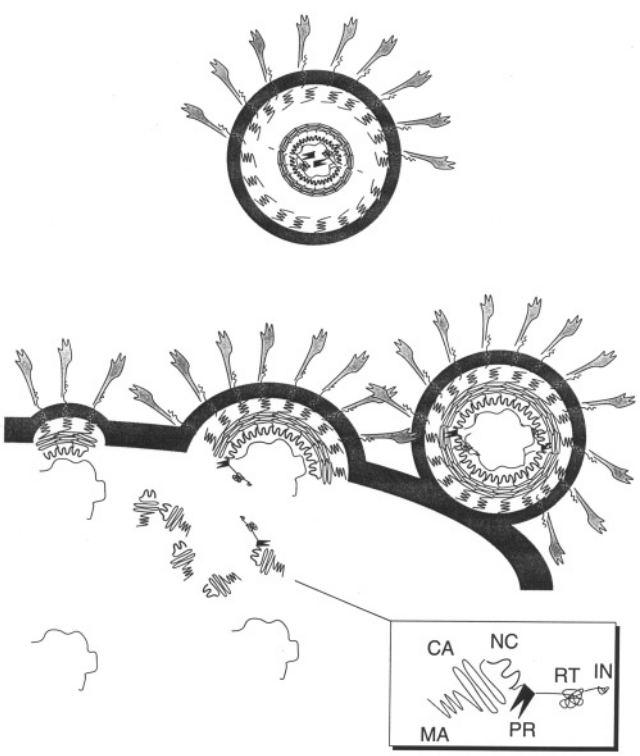

FIG. 4.6. A schematic diagram of the process of virion assembly. The Gag precursor, containing the MA, CA, and NC domains, and the Gag-Pol precursor, containing the MA, CA, NC, PR, RT, and IN domains (see blowup), are transported to the inner leaflet of the plasma membrane. The proteins bind the viral genomic RNA (thin line). Curvature is induced in the membrane as the virion grows, and the roughly spherical particle is finally pinched off and released from the cell. The virion proteins are reorganized upon processing by the viral protease to form the mature, infectious virus (top). From (486) courtesy of Lippincott, Williams and Wilkins.

Gag and Virion Assembly

For most retroviruses, the expression of the Gag precursor is sufficient to mediate virion assembly and release, earning the protein the name of the "particle making machine." (An exception to this rule is the foamy viruses, which also require the presence of the Env glycoprotein for efficient budding (279).) Because of its central role in virion assembly, Gag proteins have been subjected to intense mutational analyses to define the domains required for various steps in the process (280,274). Surprisingly small portions of Gag, containing only a few critical regions, can still assemble virions (281). Three domains seem to be crucial: a membrane-binding (M) domain; an interaction (I) domain; and a late assembly (L) domain. It is important to remember that the form of the Gag protein that is mediating assembly is the precursor; thus, the assembly domains need not lie neatly within any of the cleavage products that form later and can span cleavage sites.

The M Domain

The M domain, or membrane-binding domain, ranging from 30 to 90 residues in length, is located at the amino terminus of Gag, in MA. Mutations affecting this domain abolish assembly, but M mutants retain their ability to interact with other Gags and can be rescued into particles by the coexpression of a wild-type protein. The region seems to contain both hydrophobic and basic residues that are needed for proper interaction with lipid and with the acidic moieties of phospholipids. Structural information for the isolated M domain is consistent with this role (282).

For many retroviruses, myristylation of Gag, along with specific residues in MA, is required for membrane binding. This interaction with membrane, in turn, is important for virion assembly of the C-type viruses, and for their proper subsequent Gag processing (283). Mutational studies have led to the notion of a "myristyl switch," in which the myristic acid is exposed to mediate plasma membrane binding during virion assembly, but then can be sequestered in the compact globular core of MA after Gag processing (284–287).

The I Domain

The I or interaction domain is defined as a major region of Gag-Gag interaction, largely contained in the NC region. Although the major I domain has been suggested to lie in NC, some analyses have suggested that the C-terminal half of CA and NC are equally important for normal assembly (288,289). Mutations in the I domain block or reduce assembly, and those particles produced by these mutants have aberrantly low density, indicating

fewer and poorly packed Gag proteins. The key feature of the I domains is not the zinc binding residues of the cys-his box, but rather basic residues ßanking the boxes (290). The assembly function could involve the binding of RNAÑ either the genomic RNA or other RNAsÑ but it is not completely clear that RNA is necessary. The assembly functions of NC can be replaced by foreign proteins, and the key activity seems to be the formation of protein-protein contacts (291). Mutations in this region can also affect particle size as well as yield (292). Recently the I domain of HIV-1 has been proposed to be involved in the membrane association of the Gag precursor (293), but other studies suggest that NC is not required (294).

The L Domain

The third domain is the L or late assembly domain (295,296). Mutants affected in this function fail to produce and release particles efÞciently, and though the mutant Gag proteins form spherical structures, they accumulate under the membrane and do not progress normally. The buds remain tethered to the cell surface by a membrane stalk, suggesting that the function of the L domain is to mediate virus-cell separation. Recently, the domain has been shown to also be important in determining the size of the virion; the structures formed by L domain mutants are often large and heterogeneous in diameter (297). L domains lie at different locations in the Gag proteins of different viruses. In ASLV, MPMV, and the MuLVs, the L domain lies in the amino terminal third of the protein and its critical residues are PPPY. In HIV-1, the domain lies in p6, at the C terminus, and instead contains the motif PTAP. Remarkably, many of these L domain motifs are interchangeable among the various retroviruses, and show a substantial position independence for their function (298). It is likely that these motifs represent the binding site for some protein important for the late stages of budding, and indeed the PPPY motif has been identiÞed as the recognition sequence for the WW domain family of proteins, including the Yes-associated protein YAP, NEDD45, and many others (299). Recently the late domain of the EIAV Gag has been shown to associate with a cellular adapter protein, AP-2, which could mediate transport or localization steps (300). Which of these or other proteins that may interact with the L domain are most crucial for virion release remains uncertain. A curious additional link between two of these proteins is provided by the observation that both HIV-1 p6 and the MuLV p12 proteins are modiÞed by the addition of ubiquitin at low levels (301). It is possible that a PPPY-binding host protein is responsible for this modiÞcation.

Virion Assembly in Vitro

Recently Gag proteins and fragments of Gag have been shown competent to assemble *in vitro* to form various structures that more or less closely resemble virion cores (302Ð305). The CA-NC portions of ASLV and HIV-1 expressed as recombinant proteins can assemble to form particles or long hollow tubes; the formation of these structures is dramatically enhanced by addition of RNA, and the length of the tubes is determined by the length of the RNA (306). Larger Gag fragments that include more aminoterminal regions can assemble into spherical particles (307), and this assembly is stimulated by RNA and host cell extracts (308). Although HIV-1 Gag interacts with cyclophilin A *in vitro* and *in vivo*, addition of cyclophilin A to assembly reactions has only little effect (309). HIV-1 Gag CA-NC fragments can assemble into conical structures (2), with a pitch that falls into discrete values. Image reconstruction of these cones has allowed the formation of a model for the packing of the protein into hexagonal arrays. Virus-like particles have also been formed with the Gag proteins of MPMV in cell-free protein synthesis systems.

Incorporation of Other Proteins into Assembling Virions

During assembly, other proteins are incorporated into the particle by contacts to Gag; these include Gag-Pol, Env, and auxiliary proteins encoded by the complex viruses. The Gag-Pol precursor is thought to be incorporated into the assembling bud by virtue of the Gag protein present at the aminoterminus. Gag to Gag-Pol contacts can in some cases lead to the incorporation of mutants of Gag-Pol that do not retain the myristate modiÞcation to the aminoterminus (310), suggesting that the interaction is quite strong. Gag fusions to foreign proteins can be similarly incorporated into particles formed by Gag (311); this process can even be used to target antiviral proteins into virions. Consistent with this notion, many mutations that block assembly of Gag, when tested in the context of Gag-Pol, are found have similar effects on the incorporation of Gag-Pol (312,313). However, some mutations in HIV-1 Gag have also been identiÞed that speciÞcally affect the incorporation of Gag-Pol, suggesting that Gag-Pol utilizes some distinctive contacts not important for Gag-Gag interactions (314). Further, in the spumaviruses, Pol is incorporated without an appended Gag region, suggesting that distinct interactions must be utilized for its incorporation (245).

The Env protein is thought to be concentrated at the sites of budding and incorporated into the virions by virtue of contacts between the cytoplasmic tail of Env and the aminoterminal portion of Gag. These interactions have been difÞcult to document directly, though there is some biochemical evidence (315) and crosslinking studies (316) in support of these contacts. Genetics has provided good evidence for this interaction. Selected mutants of MA show defects in Env incorporation (317Ð319), and some mutants of the cytoplasmic tail of TM are not efÞciently

incorporated (320). In addition, Env proteins that are speciÞcally directed to the basolateral surface of polarized epithelial cells can redirect the sites of budding of Gag from a non-speciÞc assembly on both membranes to the exclusive assembly at basolateral membranes (262,263), and can similarly redirect Gag in neurons (321). Finally, mutants and revertants of these mutants with second-site suppressors in the binding partner have provided strong evidence for these interactions (322,319). However, it should be noted that the envelope proteins of viruses very distant from retroviruses, including VSV and inßuenza, can be functionally incorporated into retrovirus particles without any obvious sequence similarity in their cytoplasmic tails. Furthermore, truncating the tail of ASLV Env does not prevent its incorporation or function (323). Thus, there may be mechanisms to direct Env proteins to assembling virions without these speciÞc contacts to GagÑ a default pathway, or a pathway using other interactions. Indeed, other distinct parts of Gag, including the p6 region of HIV-1 Gag, have been implicated in Env incorporation (324).

The HIV-1 protein Vpr is efÞciently incorporated into assembling virions at very high levels, approaching equimolarity with Gag. This incorporation requires the presence of the p6 domain of Gag (271) and may be mediated by a direct interaction (270). The binding can be used to direct foreign proteins into the particle; a fusion between Vpr and a foreign protein will be targeted to virions. Furthermore, Vpr can be used to direct separately expressed versions of RT or IN to particles in a functional form, to complement mutations in the RT or IN domains of the Gag-Pol fusion (325).

Host Proteins in the Virion

A number of host proteins have been shown to be present inside the virion particle; in most cases the signiÞcance of the protein is unknown. Prominent among the virion-associated factors are a number of cytoskeletal proteins. These include actin (326Ð329), and various members of the Ezrin-Radixin-Moesin (ERM) family, speciÞcally including ezrin, moesin, and coÞlin (327). The Gag, and especially the nucleocapsid protein of HIV-1 have been shown to directly bind to actin (330,331,329), perhaps offering a mechanism for its incorportion into the particle. A complication in analyzing virion-associated proteins is that virion preparations tend to be contaminated with substantial amounts of microvesicles, entities released by cells that exhibit a density and size very similar to that of virions, and containing an array of host proteins (332).

The virions of HIV-1 contain substantial levels of cyclophilin A, a protein proline isomerase of uncertain function but implicated in protein folding and signal transduction (333Ð335). The incorporation of cyclophilin A is not required for assembly, but is important for efÞcient entry of HIV-1 into newly infected cells, before the process of reverse transcription is initiated (336). Its exact role is uncertain. Several other proteins have also been found in virions: a translational elongation factor, eIF-1a (337), and a protein known as H03 (338), with similarity to histytdyl tRNA synthetase, are additional examples.

RNA PACKAGING

The RNA genome is incorporated by virtue of interactions between speciÞc RNA sequences near the 5′ end of the genome, termed the packaging or Psi sequences, and speciÞc residues in the NC domain of Gag (see (339,340)). Both partners in this interaction have been intensively studied.

Gag Sequences Important for RNA Packaging

The Gag precursor is probably the form of the protein that is responsible for packaging the RNA (341), and the NC portion of the precursor plays the largest role. Mutations affecting the NC protein often reduce the incorporation of the genomic RNA into the virion particle (see (340) for review). The most crucial sequences are the Cys-His boxes, short sequence blocks resembling zinc Þngers and containing the motif Cys-X2-Cys-X4-His-X4-Cys; but basic residues elsewhere in the NC molecule are also important (342,343). The NC protein of various viruses contains either one or two copies of the Cys-His box. When two copies are present, they are not equivalent or interchangeable, suggesting that they mediate distinct interactions with RNA (344). Some viral cores can cross-package heterologous viral RNAs, suggesting good binding to the heterologous Psi region, and sometimes there is a strong preference for the homologous RNA. Exchanging the NC domains between viruses can sometimes transfer the preferential selectivity of a Gag protein for its cognate RNA, though the speciÞcity of these hybrid Gags is often poor, and in some cases other sequences in Gag can determine the preference for RNA packaging by chimeric Gags.

RNA Sequences Important for Packaging

The packaging or Psi regions on the viral RNA genome that are recognized for incorporation are quite distinct in nucleic acid sequence among the various viruses. The key Psi regions lie near the 5′ end of the RNA, generally between the LTR and Gag. However, other regions of the genome can affect RNA packaging, including sequences upstream in R and in U5, downstream in Gag coding regions, and even near the 3′ end of the genome. In the case of ASLV, a region of 270 nt is necessary and sufÞcient

to mediate the packaging of a foreign RNA (345–347). In the case of the MuLVs, sequences that are at least partially sufficient to mediate packaging have been similarly identified (348). These Psi regions are relatively autonomous; Psi can be moved to ectopic positions in the genome with at least some retention of function (349).

The various Psi sequences have been predicted or shown to form a number of stem-loops, often containing GACG in the loops. Reversion analysis of mutants with alterations in these loops confirms the importance of the stem-loop structure (350). Mutational studies show that several such loops may incrementally contribute to the efficiency of packaging of the RNA, though one or two are often found to be most important (351,352). Recently one of the stem-loop structures of the HIV-1 Psi was replaced by a completely foreign sequence that was selected on the basis of its binding activity with NC; the resulting RNA was efficiently packaged and utilized for replication, strongly suggesting that binding to Gag is the key function of Psi (353). A structure of the HIV-1 NC bound to one such stem-loop has been resolved by NMR, revealing specific contacts between both hydrophobic and basic residues of NC and nucleotides in the stem-loop of the RNA (354).

Dimerization of the Viral Genome

Mature virions contain a dimeric RNA that is highly condensed into a stable, compactly folded structure referred to as the 70S dimer on the basis of its sedimentation rate. Packaging of RNA is associated with the dimerization of the RNA, but it is not completely clear whether the genome is present as a dimer before it is incorporated into virions or is incorporated as a monomer and then dimerized after its incorporation. However, some ASLV mutants suggest that monomeric RNA can be packaged under some circumstances (355), implying that dimerization may follow packaging. There are specific sequences in the RNA, termed dimerization or dimer linkage sequences (DLS), that are required for RNA dimerization *in vitro*, and for the formation of the dimeric virion RNA *in vivo*. These DLS structures are in close proximity or even intermingled with sequences required for packaging of the RNA, often making it difficult to determine their separate contributions to these processes. The dimerization of viral RNAs can be induced *in vitro*, and is stimulated by addition of NC or the Gag precursor. However, it is uncertain to what extent these reactions reflect dimerization *in vivo*.

The viral RNA in newly budded virions is present as an unstable dimer, dissociated by heat at relatively low temperatures, and becomes condensed to a more stable dimer during virion maturation (356). This condensation requires the proteolytic processing of Gag (357) and may be mediated by the free NC upon its release from the precursor. A model for the process of dimerization, the "kissing loop" model, suggests that duplex formation between two RNAs is initiated between loops on the two RNAs and propagates outward through the stems to form a more stable duplex. It is possible, but by no means certain, that such changes in the duplex regions could account for the change in thermal stability upon maturation.

Many cells contain vast arrays of endogenous proviruses and retrovirus-like elements, a subset of which can be expressed constitutively or under various conditions of stress to produce large amounts of genomic RNA. If such a cell is infected by an exogenous virus or has been engineered by expression constructs to produce virions, the particles will incorporate the endogenous RNAs along with the viral RNA (358,359). The endogenous retroviral RNAs, notably the VL30 RNAs of rodents, contain highly efficient Psi sequences (360,361), presumably because they were selected to compete with the homologous genomes of exogenous viruses for packaging.

Virions also contain a number of host RNAs of uncertain significance. There are substantial levels of 7S RNA, a low-molecular weight RNA thought to function in host RNA splicing (362). In addition, there are low levels of host mRNA. Particles released without efficient packaging of the viral genome (as are produced by Psi-mutant genomes) may carry enhanced levels of host RNAs, and various mutants with alterations in NC can show selective enhancement of both endogenous viral and host RNAs (360). A variant avian leukosis virus, SE21Q1b, packages unusually high levels of host RNA (363,364), and is capable of transducing these host sequences into new cells by reverse transcription (365). This phenotype of high-efficiency transduction is associated with an unusually high level of proviral expression and particle production rather than any specific alteration in a viral protein (366).

tRNA Primer Placement

A very small subset of these tRNAs—two per virion—are annealed to the primer binding site (pbs), an 18-nt sequence near the 5′ end of the genome with perfect complementarity to the 3′ sequences of a specific primer tRNA. The pbs sequences are, as one would expect, essential for normal reverse transcription of the virus (367). The sequence of the pbs can determine the primer tRNA that is utilized (368), but changes in the pbs tend to revert back to the wild-type (369), suggesting that alternate tRNAs do not function well. An interesting aspect of reverse transcription provides for an efficient mechanism for this reversion: the use of the original tRNA even once during replication will convert the pbs back to the original sequence, because the tRNA itself is the template for the DNA copy of the pbs. Other sequence blocks of the tRNA are also paired with complementary sequences in R and U5 to form a large, complex structure required for proper tRNA primer placement and utilization (370–374). These other sequences are presumably responsible for the selectivity for the natural tRNA primer. In the

alpharetroviruses, *pol* gene products are required to mediate the placement of the tRNA on the genome; but in the gammaretroviruses, *pol* is not required (375). The Gag precursor, and especially the NC domain, is thought to play a major role in promoting the annealing of the tRNA to the genome. While NC can promote annealing of complementary RNAs and DNAs *in vitro*, its role and the mechanism by which it may act *in vivo* remain uncertain.

PROTEIN PROCESSING AND VIRION MATURATION

As retrovirions are budded from the cell surface, the Gag and Gag-Pro-Pol precursor proteins are proteolytically cleaved to release the smaller proteins present in the infectious virions (for review, see (376)). The cleavage of Gag and Gag-Pro-Pol is mediated by the viral protease PR, which is expressed either in Gag, Gag-Pol, or Gag-Pro-Pol fusion proteins. Thus, PR is responsible for cleaving itself out of a precursor protein, and then making a number of other cleavages in these proteins.

Activation of the Protease

The processing of Gag and Gag-Pro-Pol precursors is intimately linked to assembly and budding, and is controlled so that the precursors are not cleaved until they are assembled. It is not certain how PR is regulated during assembly to begin cleaving its substrates. The structure of PR has revealed that the active enzyme is a homodimer (see below), and thus its activation could be promoted by dimerization of the Gag or Gag-Pro-Pol precursor associated with assembly. As the virions form, one could imagine the high concentrations of the protein generating an active PR that would begin to cleave Gag and Gag-Pro-Pol, and would release the mature PR dimer as well. However, for the betaretroviruses like MPMV, this mechanism cannot explain the delay in processing. For these viruses, assembly occurs in the cytoplasm and should result in the establishment of a high concentration of Gag-Pro-Pol at that time. Yet cleavage does not begin in the cytoplasm, but rather is restrained until budding and export of the preformed virion particle. Thus, other unknown mechanisms, perhaps coupled to membrane association, must be responsible. Similar conclusions were reached from analysis of IAP virions, in which assembly in various intracellular locations did not result in processing; membrane association was required (377).

Various domains of Gag have been suggested to inhibit PR (378), and conformational changes could relieve this inhibition. In the alpharetroviruses, a cleavage at the NC-PR boundary is required to release active PR, so activating this cleavage could serve as a trigger (379). Similar cleavages at the p6*-PR boundary are important for full activation of the HIV-1 PR (380). Another possibility is the activation of the PR by a drop in the pH associated with virion release. It should be noted that the overexpression of PR in many artificial settings, both in bacteria and in animal cells, as a Gag-PR fusion or alone, can result in formation of highly active enzyme (381). The high level expression of PR is often toxic for cells, presumably due to its inappropriate action on many host proteins.

Protease Structure and Function

The retroviral proteases are aspartyl proteases with clear sequence similarity to members of the cellular family of aspartyl proteases (382–384). The three dimensional structure of many proteases, including those from ASLV, HIV-1, HIV-2, SIV, FIV, and EIAV, have been determined by X-ray crystallography (385–392). The viral enzymes are small, typically containing about 100 amino acids, and are homodimers as isolated from virions. Each subunit contributes to the active site a single aspartate residue, lying in a loop near the center of the molecule. There is a long cleft at the interface between the subunits where the substrate lies; there are pockets to interact with each of the side chains of the substrate, conferring specificity to the enzyme. Each subunit has a flap consisting of an antiparallel sheet with a b-turn that covers the cleft, and this flap moves out of the way to permit the binding of the substrate into the active site.

Retroviral proteases have a complex specificity for substrate peptides (393). The enzyme makes contact with approximately seven or eight side chains on the substrate, and thus can select its cleavage sites on the basis of at least these amino acids. The cleavage sites tend to be within hydrophobic sequences, and yet must lie in accessible and extended conformations. Some analyses of the various sites in Gag and Gag-Pol that are recognized by PR suggest that either one of two sequence motifs constitute a consensus site: one set has an aromatic residue or proline flanking the cleavage site, and the other set has aliphatic residues at these positions. Mutational analyses have allowed further definition of the residues on PR that make specific contacts to the substrate.

Protease Inhibitors

Studies of mutant viruses lacking PR demonstrated that the protease is essential for virus replication. Viruses lacking a functional PR can still express Gag and Gag-Pol precursors, and can mediate the assembly and release of immature virion particles. Thus, PR is not required for the process of virion assembly *per se*. However, these particles are non-infectious, and are blocked at an early step prior to the initiation of reverse transcription (394–396). Because of its essential role in virus infectivity, PR was appreciated early in the course of the AIDS epidemic as an attractive target for antiviral therapy. A number of molecules have

been generated that can bind and inhibit PR, including peptide mimetics with uncleavable, non-sessile bonds at the cleavage site (397,398). Some are transition state analogues, and may have inhibition constants (Ki) in the nanomolar or subnanomolar range. These inhibitors have been extremely effective antiviral agents, and because they target a distinct enzyme and distinct step in the life cycle from the RT inhibitors, they have been particularly effective in combination with earlier drugs targeted at RT. The combination of three drugs that include a protease inhibitor is now a standard method of treatment for AIDS, and such highly active antiretroviral therapy (HAART) can keep virus loads below detectable levels in some patients for many years. Ultimately, however, point mutations in PR that confer resistance to the drugs can arise (399), allowing some virus replication in spite of therapy.

Processing of the Gag Precursor

During and after release from the cell, the Gag precursor is cleaved by the protease into a series of products present at equimolar levels in the virion. The number and size of the products vary considerably among the various viruses; the spumaretroviral Gag is exceptional in undergoing the fewest cleavages. Although diverse, these products of most of the retroviruses share many features in common.

The Matrix Protein, MA

Beginning at the amino terminus, most Gags are processed to form a membrane-associated or matrix protein termed MA. The MA protein is thought to remain bound to the inner face of the membrane as a peripheral membrane protein, and can be crosslinked to lipid. MA may make contacts with the cytoplasmic tail of the envelope protein. When the precursor Gag is myristylated at the amino terminus, the corresponding MA protein retains that myristate and so is presumably bound tightly into the membrane. The compact structure of the MPMV MA protein has been elucidated by NMR (400,401). The MA proteins of HIV-1 and SIV have been shown to form trimers in crystallization studies (402,403), and can contribute to the ability of a larger Gag precursor to form trimers in solution (404). The protein can form extended sheets of trimers, with a large opening in the network. If similar structures were to form in a sphere, the surface could have openings into which the envelope tail may Þt.

The Capsid Protein, CA

Gag proteins are cleaved to generate a large product serving as the major capsid protein, CA, in the virion core. The CA protein is relatively well conserved among Gags, and contains the only highly conserved motif among Gags, the so-called Major Homology Region (MHR). The function of this motif remains uncertain; although mutations in the region affect virion assembly in some viruses (405Ð407), it is not absolutely required for this process, since the entire CA domain of ASLV can be deleted without blocking assembly. CA is thought to form the shell of the condensed inner core of the mature virus, and thus makes either a spherical, cylindrical, or conical structure, depending on the virion morphology. The protein has proved difÞcult to crystallize, and the structure of the complete protein is uncertain (408), but the structures of both the N-terminal and C-terminal fragments of the HIV-1 CA have been determined (409,410). Very recently the analogous structures of the RSV capsid (411), and of the EIAV CA protein (412) have been determined and used to model a structure of the virion core. The CA protein can form dimers in solution, and recombinant proteins containing CA, or CA plus NC, can assemble to form higher-order structures consisting of either tubes, spheres, and in the case of HIV-1, cones (2). CA has also been studied after tethering sheets of the protein to membrane (413). Image reconstruction of electron micrographs, coupled to the subdomain structures, have led to models for the packing of CA to form these large assemblies. The major CA-CA contacts must form after processing during the condensation of the virion core, and may be very different from any contacts that exist in the immature virion particle (414).

The Nucleocapsid Protein, NC

All Gag proteins except for those of the spumaviruses are cleaved to produce a nucleocapsid protein, NC, located near the carboxyterminus of the precursor. NC proteins are small, highly basic proteins containing one or two copies of the Cys-His motif, Cys-X2-Cys-X4-His-X4-Cys. These sequences bind a single Zn++ ion avidly, and fold around the ion into a characteristic structure that is smaller and rather different from the better-known zinc Þnger structure. The structures of NC proteins in solution have been studied by NMR, revealing a tightly folded knuckle with disordered ßanking sequences (415,416). The interaction with zinc results in the incorporation of substantial levels of Zn++ into all retrovirus virion particles (417).

The NC protein in virions is closely associated with the viral RNA, probably coating the entire RNA molecule; the stoichiometry of binding is such that each NC molecule can bind to about 6 nucleotides of RNA. NC proteins bind non-speciÞcally to heteropolymeric single-stranded nucleic acid with moderate afÞnity (418). However, NCs also exhibit speciÞcity. Tests of binding to nucleic acids of deÞned sequence have shown that NCs bind poorly to poly(A), and most tightly to nucleic acids containing GT dinucleotides, especially alternating (GT)n polymers (419). In addition, NC has been shown to exhibit sequence-speciÞc binding activity *in vitro* for nucleic acids

containing the Psi region, required for packaging of the viral RNA (420,421). The interaction can also be assayed in reconstructed systems in yeast via the so-called three-hybrid method (422,423). This binding presumably reßects at least some aspect of its role in packaging the genome. A speciÞc complex of the HIV-1 NC with a stem-loop derived from Psi has been studied by NMR, and the resulting structure shows a number of speciÞc contacts between hydrophobic residues of NC and bases in the four-nucleotide loop; and between basic residues and speciÞc phosphates in the stem and loop (354).

NC proteins change the base-pairing properties of nucleic acids, and thus can have profound effects on the kinetics and thermodynamics of annealing (424,425). Under various conditions *in vitro*, NC can stimulate the dimerization of RNAs, and duplex formation between tRNA and its complementary sequences at the primer binding site (426). Thus, NC can help promote primer tRNA placement during virion assembly (427). NC can also help melt out secondary structures, and may facilitate the movement of RT along the template during reverse transcription (428). In addition, it is clear that NC can bind to double-stranded nucleic acid, and thus is probably retained on the viral DNA after its synthesis by reverse transcriptase. NC mutants have been found that affect the course of DNA synthesis or DNA stability during the early stages of virus infection, suggesting a role in the processing of the DNA and protection of DNA from degradation (429,430). Finally, NC has been shown to promote the concerted integration of the two termini of the viral DNA into a target sequence (see above; (431)).

An important class of inhibitors of virus infectivity and replication that act by targeting the NC protein have been identiÞed (432,433). These compounds, disulÞde-substituted benzamides (DIBAs), eject the zinc ion from NC and cross-link the cysteines via disulÞde bonds. Virions treated with these compounds are potently inactivated without disrupting the virion structure, and the course of virion assembly in infected cells is similarly blocked (434–437). Drug-resistant variants are not readily recovered.

Other Gag Products

Some retroviral Gag proteins, including those of the alpharetroviruses, betaretroviruses, and gammaretroviruses, contain one or more poorly conserved domains of 10–24 kDa lying in between MA and CA. The functions of these proteins is unclear. The ASLV p2 protein, the MuLV p12 protein, and the MPMV p24 protein contain a PPPY motif that plays an important role in late stages of virion assembly (see above). The MuLV p12 protein has also been shown to play a role in the early stages of infection (312,438).

In the lentiviruses, a p6 domain is present at the carboxyterminus. The role of p6 is unclear, though it contains the late or L domain and thus may be important in virion release; it also is required to mediate the incorporation of Vpr into virion particles, perhaps by providing a direct docking site. Proteins can be targeted to virions by generating Vpr-X fusions, which are incorporated into lentiviral virions in a p6-dependent manner.

Processing of the Gag-Pro-Pol Precursor

At the same time that the Gag precursors are cleaved during virion maturation, the Pro and Pol region of the Gag-Pro-Pol precursor is also cleaved, giving rise to the PR, RT, and IN products. The Pro and Pol-containing precursors of different viruses are cleaved in diverse patterns (Fig. 4.7). In the gammaretroviruses, the Pol region is processed by complete digestion to form PR, RT, and IN (439,440). In the alpharetroviruses, the Pol region is cleaved to produce a heterodimeric RT with a larger β subunit and a smaller α subunit. The larger β subunit contains both RT and IN domains. It is not clear whether the IN domain in the context of this subunit performs an important function, although it is responsible for a weak nuclease activity associated with RT (441). A portion of the Pol precursor undergoes an additional cleavage to produce the α subunit of RT, an amino-terminal fragment of the β subunit, and the separate IN protein. In the lentiviruses, Pol is processed to give rise to PR, a heterodimeric RT, and IN. However, the RT of these viruses is not identical to the heterodimeric RT of the alpharetroviruses. Here the IN domain is fully removed from RT. One RT subunit remains intact (for HIV-1, this is the p66 subunit), and the other subunit undergoes an additional cleavage to remove a carboxyterminal domain (to form the p51 subunit). The functional signiÞcance of the different subunit structures of these various RTs is unclear, since they all perform a very similar set of reactions during virus replication. The processing of Pol precursors may be associated with the activation of the DNA polymerase of RT. In the alpharetroviruses, the immature Gag-Pol protein has very low DNA polymerase activity, and its maturation results in a large increase in activity (442,443). However, the immature Gag-Pol protein of MuLV and HIV-1 has high DNA polymerase activity, and there is only a very modest increase upon maturation (394).

The Gag-Pro-Pol precursor of the betaretroviruses and the non-primate lentiviruses is also processed to produce the dUTPase protein, DU. In the betaretroviruses, the *pro* ORF encodes both DU and PR; in the non-primate lentiviruses the enzyme is encoded in the *pol* ORF, and DU lies in between RT and IN in the polyprotein. This enzyme acts to reduce the levels of dUTP that could otherwise be incorporated into viral DNA. Mutants of FIV lacking the function indeed show increased rates of mutation during replication (444), and similar mutants of CAEV tend to accumulate G-to-A substitution mutations (445) presumably due to incorporation of dU residues that

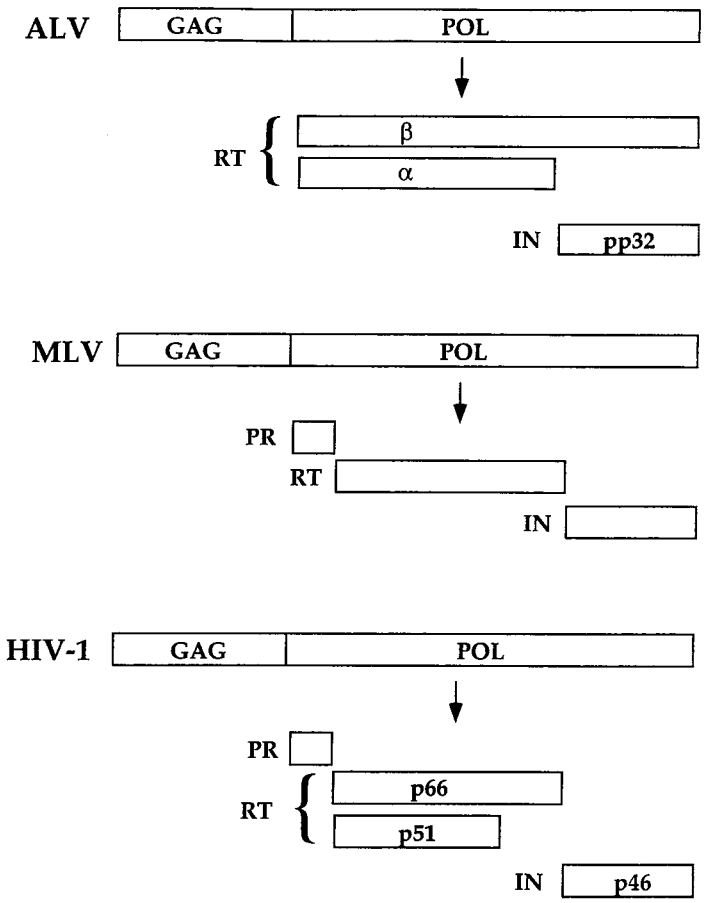

FIG. 4.7. Cleavage patterns during the processing of the Gag-Pol fusion proteins of various retroviruses. The structure of the mature cleavage products found in the virion particles are shown aligned with their location in the precursor. From (486) courtesy of Lippincott, Williams and Wilkins.

are subsequently read as dT. The FIV enzyme has been crystalized, and the structure of the protein has been determined by X-ray diffraction (446).

Processing of the Env Precursor

The major proteolytic cleavage of the Env protein to form the SU and TM subunits is performed during its transport through the ER and golgi by host proteases termed furins. This cleavage is essential for virus infectivity (447,448), and is thought to induce substantial rearrangements of the polypeptide chain. The TM subunit remains embedded in the membrane, and contains an extracellular domain, a membrane-spanning segment, and a cytoplasmic tail. The SU subunit lies wholly outside the cell, and after its incorporation into the virion particle, wholly on the extravirion surface. It is held onto the virion by contacts to TM, and most often by non-covalent bonds (449), though disulÞde links may occur in some viruses (450). SU is heavily glycosylated, and the presence of at least some of these sugars is important for virus infectivity. Perhaps the most important function of this heavy glycosylation is to hide the peptides on the surface of Env from neutralizing antibodies that would otherwise have access to the virion surface.

The Surface Subunit SU

For most viruses the major receptor-binding site is located in hypervariable sequences on the SU subunit, so that SU is a major determinant of host range. Chimeric SU proteins can be generated to demonstrate that the receptor utilization function maps to speciÞc regions of the protein. The key regions of the avian Env proteins have been similarly deÞned by selecting for changes in host range *in vivo*; these studies show that very small changes can result in the use of new receptors (451). The structures of two SU proteins have been recently determined at high resolution: a fragment of murine leukemia virus SU (452), and a fragment of the HIV-1 SU bound to its receptor CD4 (453,454). These structures suggest that the receptors make contacts to the envelope in shallow pockets that may not be readily bound by antibodies.

The Transmembrane Subunit TM

The TM subunit contains the so-called fusion peptide at its aminoterminus, and TM is thought to play the major role in fusion of the virion and host membrane (455Ð459). Many TM mutations are defective for membrane fusion (460,461). However, mutations blocking fusion can lie in SU as well (462). The entire Env protein probably acts as

a unified machine to mediate fusion, with complex interactions between the subunits (463), and with major movements of the subunits during the process of fusion. The fusion peptide of TM may simply insert into the host membrane, or it may make contacts to proteins. The major contacts for oligomer formation of Env are thought to lie in TM (464); isolated TM proteins form trimers in solution (465) and in crystals (466,452,467,468). The trimer is held together by a modified leucine zipper motif that bridges the monomers via hydrophobic interactions. This zipper region is crucial for virus infectivity (469–471).

It is possible to separate the two major functions of the Env protein onto two different molecules that cooperate to mediate these steps. Thus, the receptor binding function can be mediated by one Env protein, and the membrane fusion function can be mediated by another Env. This is apparent in the ability of two Env proteins to complement in mixed oligomers (472,473). It is also demonstrated by the ability of a wild-type Env to provide the membrane fusion function for a chimeric Env that on its own can only mediate cell surface binding.

The TM subunit of the murine leukemia viruses undergoes a second cleavage during virion assembly (474) that is mediated by the viral protease, PR. This step removes a short sequence called p2E, or the R peptide, from the carboxyterminus of TM (475). The cleavage step may require presentation of the tail to the protease, or some conformational change in the tail, that is mediated by Gag proteins; alterations in the MA or p12 Gag proteins can modulate the cleavage of TM (476,438). Astonishingly, the cleavage is necessary to active the fusogenic activity of the envelope protein and thus for virus entry (477,478). Mutants in which the tail is truncated at the site of cleavage are constitutively activated for fusion, and these viruses induce dramatic syncytia in receptor-positive cells. Mutants in which the tail is not removed are inhibited for fusion, and particular residues can be shown to be required (479). How the cytoplasmic tail inhibits the fusogenic activity of Env is very much unclear.

In a similar way, the cytoplasmic tails of the TM of M-PMV (480), and EIAV (481) are processed by the protease. In the case of M-PMV, the presence of the intact tail is necessary for efficient incorporation of Env into the virion. The majority of the retroviruses, however, do not carry out cleavage of TM, and for these viruses an intact tail is needed for infectivity (482). The replication of some viruses in host cells of foreign species can select for alterations and truncations of the TM tails (483,481,484). The selective advantage conferred by this truncation is not well understood, though various aspects of Env function seem to be enhanced by this truncation (485).

Morphological Changes Upon Virion Maturation

The maturation of retrovirus particles is a complex process that is required for the formation of an infectious virus. The particles that are initially assembled either at the plasma membrane (by most retroviruses) or in the cytoplasm (by the betaretroviruses) have a characteristic immature morphology: the particles are round, and stain with an electron dense ring and a relatively electron lucent center. After release from the cell, the morphology changes to a more condensed structure, with a central core largely detached from the surrounding envelope. In the alpharetroviruses, gammaretroviruses, and deltaretroviruses, the core is spherical and concentric with the envelope; in the betaretrovirus the core is spherical but eccentrically placed within the envelope; in the lentiviruses the core is cylindrical or conical, with thin connections to the surrounding shell. In the spumaviruses the morphology does not change dramatically after assembly.

Mutant viruses lacking the protease show little change in morphology. Thus, cleavage of Gag and Gag-Pol is required for the restructuring of the virion into the mature form (395). The changes in morphology visible in electron micrographs are probably associated with major rearrangements of the Gag proteins. Indeed, the physical properties of the virus change dramatically upon maturation. Whereas the immature core is quite stable to non-ionic detergents and harsh conditions, the mature virion core is relatively labile. This change may reflect the inability of the immature virion, and the acquired ability of the mature virion, to uncoat upon infection of new cells and initiate reverse transcription.

PERSPECTIVES

The study of retroviruses has led to a detailed characterization of many steps of virus replication, but also to important fundamental discoveries concerning host physiology and genetics. The viruses have served as entries into such phenomena as cell surface receptors, cell division, DNA synthesis, the cell cycle, mechanisms of gene expression, and intracellular transport. The value of focusing on retrovirus functions in unraveling cellular functions is clear: these agents have evolved over huge periods of time to exploit key aspects of the cell, and we should make use of their success to help identify those aspects. There is every reason to believe that their continued study will yet reveal new aspects of cell physiology.

Beyond the interest that all retroviruses serve as examples of complex biological machines, the primate lentiviruses obviously have particular significance as human pathogens. The AIDS epidemic will be remembered as one of the great pandemics of history, and HIV as its cause has accordingly been an object of both fear and fascination. All of its properties have been subjected to intense scrutiny. An understanding of the basic life cycle of the virus and the functions of its gene products has already allowed for development of potent antiviral drugs, and at great cost the virus can be at least temporarily halted. While it will likely prove difficult to eliminate HIV

from an infected individual, its replication can be controlled for long periods of time. Eventually, vaccination or other manipulations of the immune system may allow for less expensive control of the virus, and furthermore may interrupt its transmission. We strongly suspect that its Þnal control will be accelerated by a deeper understanding of the details of its molecular biology. However, because it has evolved an efÞcient life cycle and a profoundly insidious life style, it is likely that HIV will continue to be the cause of enormous human suffering throughout the world for many years to come.

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Chapter 5

The Neuropathogenesis of HIV-1 Infection

Howard E. Gendelman, Scott Diesing, Harris Gelbard and Susan Swindells

A constellation of cognitive, behavior and motor dysfunction comprise a neurological syndrome, named in its most severe form, human immunodeÞciency virus type one (HIV-1)-associated dementia (HAD), that follows progressive viral infection and immunosuppression in an infected human host. Potent combination antiretroviral therapies (PCAT) (also known as HAART) have diminished the incidence of disease, but have not eliminated it. Usually associated with severe immunosuppression, HAD is linked pathologically to HIV-1 encephalitis. Prior to the use of PCAT, the incidence of disease was 20%, it has now decreased to $<10\%$ (1). Nonetheless, the prevalence of disease may actually increase as patients live longer as a consequence of PCAT. Anti-retroviral treatment failures, viral mutation, a plethora of drug-induced neurological diseases and syndromes are all operative in the post-PCAT era. These demonstrate that HAD will remain a signiÞcant part of the overall disease complex. In regard to viral pathogenesis, HIV-1 invades the CNS soon after infection and many infected individuals develop neurological illness early in the course of disease. For example, aseptic meningitis develops soon after virus exposure and includes headache, fever, malaise and rash, typically resolving within a few days. Alternatively, a signiÞcant number of infected patients in the course of the disease develop subtle cognitive and motor impairments. Termed ÒminorcognitivemotordisorderÓ (MCMD), this may not herald progression to HAD (2). The pathogenesis of this disorder remains unclear, but MCMD has been associated with a diminished overall prognosis (3).

In addition to cognitive dysfunction induced by HIV-1 infection itself, the brain, spinal cord and peripheral nerves also succumb to a variety of cancers, fungal, parasitic and viral infections usually years following viral exposure and associated with CD4+ T lymphocyte depletion. Moreover, HIV-1 infection is also associated with peripheral neuropathies, myopathies, vacuolar and myelopathy.

When Þrst identiÞed more than two decades ago, the constellation of cognitive, behavioral and motor dysfunctions, now called HAD, was termed the AIDS dementia complex. Subsequently, a Þve-part staging system was developed (4). Ten years ago, the World Health Organization and the American Academy of Neurology generated more complex and detailed classiÞcation systems (5). Separate categories for myelopathy and dementia were included, and the new term, HIV-1-associated cognitive motor complex was Þrst introduced. HIV encephalitis is usually the pathological correlate of HAD. HIV encephalopathy remains the preferred term for disease in children (6).

Here we review the epidemiology, clinical manifestations and pathology of HIV-1-associated neurological disease and its associated encephalitis. Particular emphasis will be placed diagnosis, pathogenesis, and treatment options now available or under investigation in our laboratories and elsewhere. This chapter serves to complement other chapters of this book, which address the biology and pathogenesis of HIV, its effects on the immune system, the development of opportunistic infections and cancers, and treatment regimens.

CLINICAL MANIFESTATIONS

Interestingly, for HAD, disease is caused as a direct result of viral infection in brain, usually, but not always secondary to dysfunction and depletion of by CD4+ T

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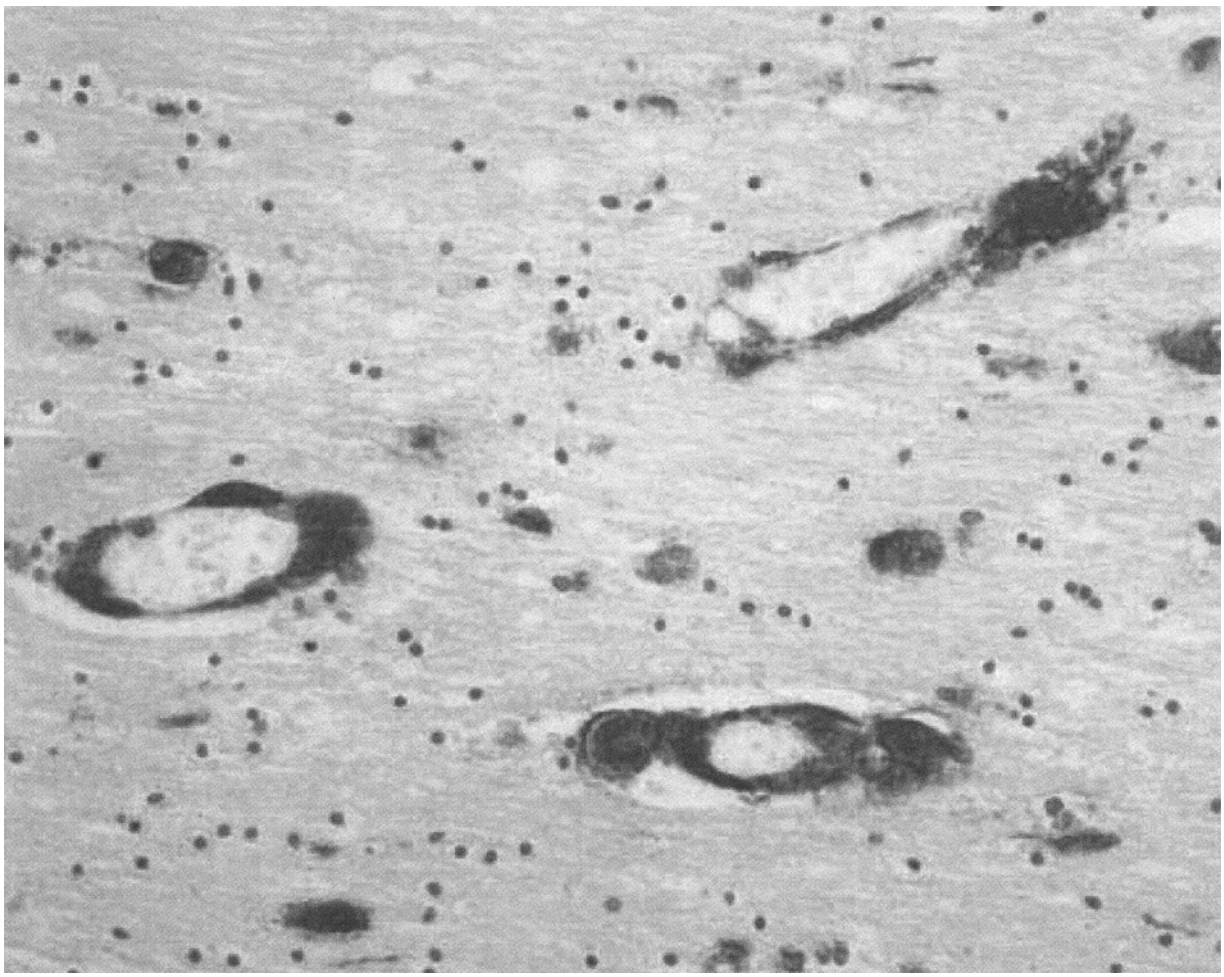

FIG. 5.1. Brain tissue from a subject with HIVE. Five mm sections of formalin- xed paraf n-embedded tissue were prepared for immunocytochemical stains. MP expression of HIV-1 p24 antigens is shown. In addition the typical histopathological features of HIVE are demonstrated including multinucleated giant cells, astrocytosis, microglial nodules, perivascular macrophages and myelin pallor. Original magni cation is x 20.

lymphocytes (1,7). HAD is a subcortical dementia of insidious onset. Cognitive deÞcits, the most frequent symptom complex seen in HAD, emerge early in the course of disease with mental slowing, forgetfulness, and difÞculty in concentration (7). Early motor symptoms include unsteady gait, limb weakness, and tremor. The most common motor symptom, however, is gait disturbance, but difÞculty with hand coordination can often be seen by impaired handwriting or typing skills. Behavioral abnormalities, are frequently observed, and include apathy, anhedonia, and social withdrawal (8). Degrees of impairment may vary between patients, and within patients over time. Symptoms usually progress at variable rates to a global dementia if the patient is not treated. Other late changes indicative of global neurological dysfunction include weakness, prominently of the lower extremities, causing inability to walk unassisted, lack of coordination, hyperreßexia and bladder or bowel dysfunction. Frontal lobe release signs such as the snout reßex may also be detected. In the terminal stages of disease affected individuals are unaware of their surroundings and progress to a spastic quadriparesis that ultimately results in death. HAD may be difÞcult to diagnosis because in its earliest stages, differentiating this syndrome from depression can be difÞcult. Screening for depression, bedside neuropsychological testing and additional information from friends or family close to the patient can be helpful. Other causes of dementia, including toxic/metabolic, vascular and opportunistic infections, must Þrst be ruled out by serum and cerebrospinal ßuid (CSF) analysis and neuroimaging studies examination before HAD is conÞrmed.

NEUROPATHOLOGY OF HAD, HIV-1 ENCEPHALITIS

Neuropathological abnormalities seen in brain tissues of patients with HAD are usually diffuse and predominantly localized to the white and deep grey matter regions (Fig. 5.1). They are referred to as HIV-1 encephalitis (HIVE) and include a characteristic dendritic pruning and vacuolation (9) both of which are indicative of neuronal

dysfunction and subsequent loss (10,11). Myelin pallor and inßammatory inÞltrates composed of macrophages and multinucleated giant cells (MGC) are the hallmarks of this disease process, although a spectrum of lesions has been identiÞed from encephalitis to leukoencephalopathy (12Ð14). A reactive astrocytosis and microgliosis is also typical (15) strongly coincident with the inÞltration into brain of peripheral monocyte-derived macrophages (MDM) (16,17). Microglial nodules form in close proximity to the vasculature (17). Myelin pallor represents a disruption of the blood-brain barrier (BBB) with ßuid accumulation in the brain parenchyma itself rather than frank white matter damage. HIVE is more difÞcult to diagnose in children because of immature myelination. The accumulation of macrophages, MGC, and reactive astrogliosis are common. The presence of MGC is considered a signature pathological Þnding. MGC results from the fusion of HIV-1 infected brain mononuclear phagocytes (MP); (perivascular and brain macrophages and microglia) with uninfected MDM or microglia (18,19). Giant cell formation is found throughout the brain in disease, but is characteristically seen primarily in the deep brain structures and most commonly subcortical white matter. Although pathognomonic of HAD, giant cells are only found in 50% of patients. Although HIV invades the brain early after viral exposure (20,21), functional changes in the CNS occur many years later. These are usually associated with the breakdown of innate and acquired immune mechanisms that serve to control ongoing viral replication, both in the periphery and the brain. The principle mediator of disease in the nervous system is the activated MP (see below). These cells exert their neurotoxic effects primarily through secretory factors (22Ð24). Unlike many other viral infections of the brain where neurons and/or glia are primary targets for persistent infection, HIV-1 predominantly infects MP. These are the cells that serve as a reservoir for persistent viral infection, a vehicle for dissemination of virus throughout the brain and a major source of neurotoxic products that affect neuronal function and lead to deÞcits in cognition. The predominant mechanism for disease is through soluble MP neurotoxins. Viral replication, nonetheless, appears necessary, but not sufÞcient for disease. Interestingly, clinical disease correlates with the number of activated macrophages more so than with viral load in the brain (15). Unlike many other viral infections of the brain which directly infect neurons, HIV-1 mediates neural damage through brain MP by inducing autocrine and paracrine immune ampliÞcation of neurotoxic secretions and affecting subsequent neuropathology. The indirect effect of virus on neural function by glial inßammatory products has led to the term ÒmetabolicencephalopathyÓ for HAD. Although HIV-1 is essential for the development of HAD, many patients never develop HAD, despite persistent high levels of HIV-1 RNA and a rapidly progressive disease course. Likewise, not all patients with HAD have high virus loads. Thus, the association between virus load and neurologic disease is complex, and the onset and progression of HAD are presumably reßective of a multifactorial pathogenesis (discussed below).

The other common histopathological features of HAD are leukoencephalopathy and diffuse polio dystrophy. Leukoencephalopathy is a diffuse neurodegenerative process, often observed without prominent signs of inßammation. Here considerable damage to white matter or myelin attenuation is seen in association with astrogliosis, macrophage accumulation and MGC cells. Diffuse polio dystrophy is reactive astrogliosis and microglia inßammation in gray matter often with associated neuron loss or dendritic damage. Spongiform changes are only seen in the most severe cases (14).

EPIDEMIOLOGY

Taken in the context of other serious illnesses developed as a consequence of HIV-1 infection, HAD may be under diagnosed, so the actual incidence is likely higher than reported. Indeed, autopsy examination of brain tissue of patients who died of HIV disease demonstrates neuropathological changes in as many as 80% of infected patients (25). Changes in antiretroviral therapy over recent years have impacted on the incidence of HAD in developed countries, with a decrease from 20 to less than 10% (26). Data from cohort studies demonstrated that the incidence of HAD was Þrst reduced following the general use of zidovudine and further decreased after the widespread use of potent combination antiretroviral therapy (27Ð29). This is also supported through animal model studies. Rhesus macaques treated with zidovudine had a signiÞcantly decreased viral load within the brain and a delayed onset of CNS dysfunction (30). During recent years, the use of protease inhibitor-based antiretroviral therapies has been associated with dramatic declines in overall morbidity and mortality attributable to HIV-1 disease, including HAD (31). The degree and durability of such improvements remain uncertain, but some estimates of the incidence of HAD are currently nearly 10 cases per 1,000 person years (27). However, such reductions in incidence have not yet been conÞrmed by histopathological examinations. Indeed, a recent neuropathological study from the University of California San Diego obtained over the last 15 years showed that HIVE was found in 25% of HIV-1 infected cases examined (25).

Even with the impact of PCAT on the prevalence of HAD, many neuroepidemiologists believe it remains the most common cause of dementia in 25Ð40 year olds worldwide. Although improved control of viral replication by PCAT has played a major role in the decreasing prevalence, it is not clear how long lasting this effect will be (32,33). Furthermore, the CNS provides a reservoir for virus and the BBB often restricts entry of drugs. Therefore infected persons may continue to be at risk for HAD (34). A number of superimposed biological factors support this

idea. First, resistant viral strains can continuously replicate and evolve (35). Second, as the survival of infected individuals continues to increase, it is likely that there will also be changes in disease prevalence, incidence and manifestations of HIV-1 infection of the CNS as a consequence of drug resistance or drug related evidence effects.

DIAGNOSIS OF HAD

HAD remains a diagnosis of exclusion. Although a number of clinical, laboratory and radiological tests are available to aid in a diagnosis, none as yet is conclusive. Nonetheless, a variety of evaluations may, taken together, permit a diagnosis to be made with near certainly. In addition to a careful history and physical examination including neurological examination, the following tests serve to strengthen the likelihood of HAD.

Neuropsychological Testing

Neuropsychological testing is helpful as an adjunct to neurological examination in deÞning better the type and extent of cognitive and motor impairment. Such tests generally demonstrate deÞcits in attention, memory, mental ßexibility and motor speed. Tests that are helpful in deÞning HAD include Trail Making B, Digit Symbol, Grooved Pegboard, and computerized reaction times (3,36,37). The inßuence of age, education, co-morbid conditions such as systemic illness, previous head trauma or substance abuse must be considered when interpreting any neuropsychological testing results. Moreover, while this testing remains the gold standard for cognitive evaluation, such an evaluation is not always available or practical for the generalist physician. Simpler test batteries, helpful in making a diagnosis of HAD, can be administered by clinicians at the bedside. For example, the HIV Dementia Scale is a reliable and quantiÞable adaptation of the Minimental Status Examination (38). Abbreviated screening tools for depression such as the ProÞle of Mood States can also be helpful in distinguishing depression from HAD.

Neuroimaging

Radiographic and functional imaging tests can delineate the structural and metabolic effects of HIV on the brain and differentiate them from other types of infectious diseases or cancerous lesions (Fig. 5.2). Computerized tomography of the brain characteristically shows cerebral atrophy in the majority of patients with moderate to severe dementia, but atrophic changes may also be present in asymptomatic individuals (39). Magnetic resonance imaging (MRI) is generally more sensitive, and can show white matter abnormalities (40).

More advanced techniques assess functional changes in brain metabolism. These include Single-Photon Emission Computed Tomography (SPECT), Positron-Emission Tomography (PET), functional MRI (fMRI) and magnetic resonance spectroscopy (^{1}H MRS). These techniques have the potential advantage of detecting early functional abnormalities before morphological changes occur. For example, MRS is a non-invasive method of quantitating neuronal loss performed with conventional resonance imagers. Assessment of *in vivo* metabolism gives biochemical information that complements the structural information from the MRI examination, and in a quantitative fashion. Several groups, including our own, have reported a reduction in *N*-acetylaspartate, a marker for neuronal loss, using *in vivo* proton ^{1}H MRS in patients with advanced HIV disease (41Ð44). ^{1}H MRS demonstrated progressive neuronal loss over time in HIV-infected individuals, and the degree of neuronal loss observed correlate with neurological impairment (44,45). Increased choline levels reßect glial membrane turnover indicative of the astrocytosis that follows HIVE. Evidence is also accumulating that 1H MRS may be helpful in differential diagnosis of HAD and other neurological disorders associated with HIV disease, for example other metabolic encephalopathies (46), and in differentiation of CNS toxoplasmosis and lymphoma (39). Moreover, such technologies may also be helpful in monitoring therapeutic responses to anti-retroviral therapies (41). Improving technology is increasing the sensitivity of MRS to detect metabolic alterations and neuronal loss much earlier than conventional neuroimaging and in a quantitative fashion with broad applicability for measuring neuronal loss, brain water content and/or membrane turnover. ACTG 700 was the Þrst multisite *in vivo* ^{1}HMRS study of HIV-related brain injury. Excellent reliability was shown among different sites, and preliminary analyses have revealed signiÞcant differences in regional metabolites between HAD subjects and controls (47).

Cerebrospinal Fluid Test Evaluation

Cerebrospinal ßuid (CSF) abnormalities in HAD are generally nonspeciÞc with mild elevations in protein and/or minimal pleocytosis (48). Generally, HIV-1 RNA levels in CSF correlate better with the presence of cognitive impairment than with plasma levels (49Ð54). Indeed, sensitive quantitative assays of HIV RNA have correlated CSF viral load levels with high levels throughout the brain (55). However, this is not diagnostic for neurological disease. Indeed, the precise relationship between HIV-1 RNA levels in CSF and risk of development or progression of cognitive and/or motor impairments is not established. Although CSF RNA levels have been shown to correlate with the severity of dementia in both children and adults, they also are often increased during other CNS infections (56Ð60). Because the level of HIV in the CNS is usually limited even in patients with neurological disease, CSF

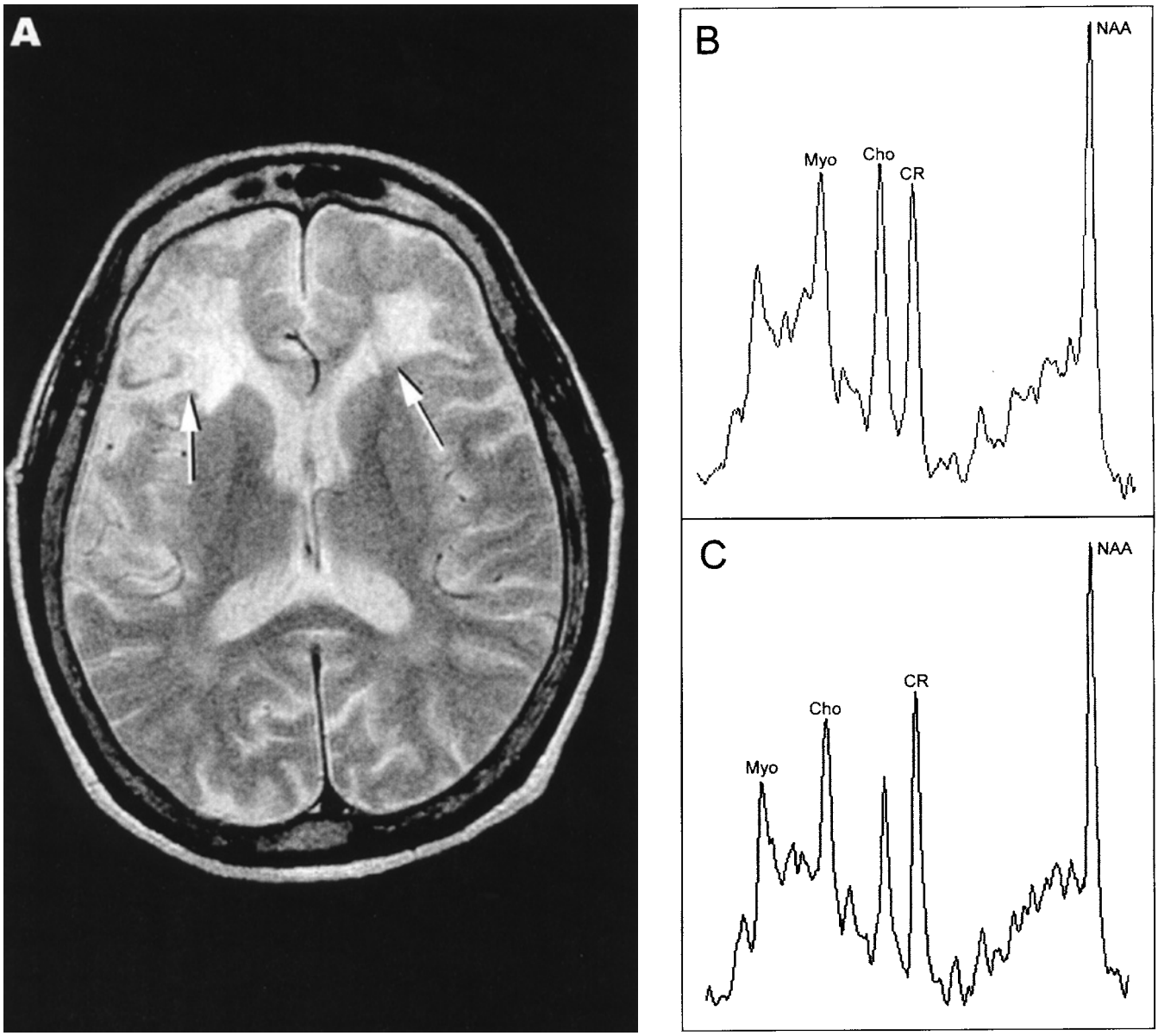

FIG. 5.2. MRI and MRS of HAD. MRI scans of the brain of a 60 year old female patient with moderate HAD (A). The third and lateral ventricles are enlarged and sulcal spaces are prominent, indicating cerebral atrophy. High signal abnormality within the subcortical deep white matter of both frontal lobes is consistent with gliosis (*arrows*). In (B), MRS is shown for Myo, myoinositol; Cho, choline; CR, creatine; NAA, N-acetylaspartate. Single voxel (8 cm^3) MRS within the white matter is abnormal with decreased *N*-acetyl aspartate NAA/Cr ratio of 1.48, elevated Cho/Cr ratio of 1.01, and the MI/Cr ratio is elevated at 0.89. The decreased NAA peak is indicative of neuronal loss. The gray matter spectroscopy shows a NAA/Cr ratio of 1.42, Cho/Cr ratio of 0.65, and a MI/Cr ratio of 0.88 (C). These ndings are consistent with HIVE.

RNA levels are also low (usually about one log lower than in plasma). Regrettably, the false negative rate of CSF RNA values is high as a predictor for disease. Moreover, minor cognitive motor dysfunction (MCMD) is not at all associated with high CSF HIV RNA levels (49,50). Complicating the use of viral load determinations as a diagnostic or predictive marker for disease are issues related to viral kinetics and the relationship between levels of virus in brain and CSF. HIV-1 RNA half-life was longer, the decay rate slower, and the variability of viral strains greater in CSF than in plasma in 15 HIV-infected subjects initiating or changing antiretroviral therapy (61). CSF HIV-1 RNA levels do not always predict viral burden in the brain. Although probably more biologically relevant, HIV RNA levels in brain are difficult to ascertain. Such studies are performed postmortem and provide only descriptive, single point in time determinations (60).

HIV-1 NEUROPATHOGENESIS: AN OVERVIEW

The principle mediator of HIV-1 disease in the nervous system is the activated MP, which includes perivascular

and parenchymal macrophages and microglia. These cells exert their neurotoxic effects primarily through secretory neurotoxins (both cellular and viral toxins) (22–24). In this way, viral replication may be necessary but not sufficient to incite disease. Interestingly, clinical disease correlates better with the number of activated macrophages in the brain than with viral load (16). Unlike many other viral infections of the brain which directly infect neurons, HIV-1 mediates neural damage by inducing an amplification, by autocrine and paracrine immune mechanisms, of neurotoxic secretions from brain MP. Such secretory products as tumor necrosis factor alpha (TNF-α) and IL-1β can induce their own secretion or other neurotoxic products. The indirect effects of HIV on neural function induced by MP inflammatory products has led to the term "metabolic encephalopathy" as it refers to HAD pathogenesis.

VIRAL ENTRY INTO THE CNS

HIV may enter the brain early in primary infection carried into the CNS inside infected cells, macrophages and/or CD4+ T lymphocytes, or alternatively as cell-free viral particles (Fig. 5.3). The movement of virus from the periphery into the brain is facilitated by immune dysfunction and structural BBB compromise. This occurs, in large measure, late in the course of disease and serves to speed the overall pathogenic process. Under normal circumstances, the BBB serves to restrict cell movement between the CNS and the circulation, including movement of ions, polar organic substances, and proteins. Structurally, the BBB consists of a monolayer of specialized non-fenestrated brain microvascular endothelial cells (BMVEC) with a capillary basement membrane on the abluminal side of the monolayer. Tight junctions connect the BMVEC, and there are no transcellular pores. These junctions are dependent on two processes: the first is high concentration of the integral membrane protein occludin and the second is intracellular signaling that determines phosphorylation of the junctional proteins (62). Astrocyte foot processes abut the abluminal surface of the endothelial monolayer. The second process relies on coordination of the BMVEC functions by the surrounding astrocytes (63–67). These together serve to restrict movement of cells and macromolecules including virus throughout much of HIV-1 infection.

Nonetheless, a number of biological situations allow for the trafficking of cells across the BBB. Specifically, monocytes and lymphocytes will induce or respond to signals from the BMVEC, which permits binding to and diapedesis through the BBB. The adhesion molecules, intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), on the luminal surface of the BMEC bind LFA-1 and Vl-4 on the monocyte, resulting in migration of the monocyte between the endothelial cells during the early stages of viral infection. As inflammation proceeds during the later stages of disease, cellular trafficking into the brain is greatly enhanced. ICAM-1, VCAM-1, and E selectin are upregulated on the surface of BMECs and astrocytes after exposure to pro-inflammatory cytokines (for example, TNF-α and IL-1β) secreted from microglial cells and astrocytes and/or activated leukocytes from the periphery. These serve to augment the process of adhesion molecule expression not only on BMVEC but also on astrocytes (12,13,64,67,68). Moreover, pro-inflammatory cytokines induce a transient increase in endothelial permeability by increasing secretion of endothelial vasoactive factors, such as nitric oxide (NO) (14). TNF-α and IL-1β increase the production of other inflammatory mediators including arachidonic acid derived platelet-activating factor (PAF) (69–71). These serve to promote monocyte migration across the BBB and ultimately into the brain parenchyma (65).

Other inflammatory factors and changes in cellular biophysiology induced as a consequence of inflammation also influence transmigration of inflammatory cells into the CNS. For example, chemokines are secreted at sites of inflammation in all tissues including the CNS and guide leukocytes in a concentration-dependent manner. Importantly, specific chemokines will attract specific populations of leukocytes. Macrophage inflammatory protein-one alpha and macrophage chemotactic protein-1 (MIP-1α and MCP-1) are increased during HIVE and are potent chemoattractants for macrophages (65,66). Potassium channels expressed in MDM influence cell migration by altering cell volume and shape and are influenced by inflammatory responses (72). HIV-1 infection alters the BBB itself (73). Numerous functional and structural abnormalities are operative including damage to the basement membrane, morphological alterations of the BMEC, and subsequent protein leakage (73–78). Trafficking is also altered during HIV-1 infection. Macrophages themselves express more LFA-1 and VL-4 (74,75), concurrent with viral infection and/or immune activation, and upregulate adhesion molecule expression by the BMVEC (76). In this pathologic setting, HIV-1 infected monocytes and/or CD4+ T lymphocytes are able to cross the BBB (74). This is thought to be the primary route of entry into the CNS for HIV-1. Nonetheless, cell-free virus may also cross the BBB and subsequently infect MP. The relative proportion of viral entry by either route has not yet been determined.

Late in the disease process, inflammatory cytokines are produced at high levels and allow the BBB to be more easily breached. Increasing damage to the BBB impairs the ability to protect the CNS from the periphery. Cells and toxins are then able to reach the CNS unchecked. Levels of inflammatory factors greatly increase and lead to a cascade of events culminating in further BBB dysfunction. All of these processes contribute to MP-induced neuronal destruction during HAD.

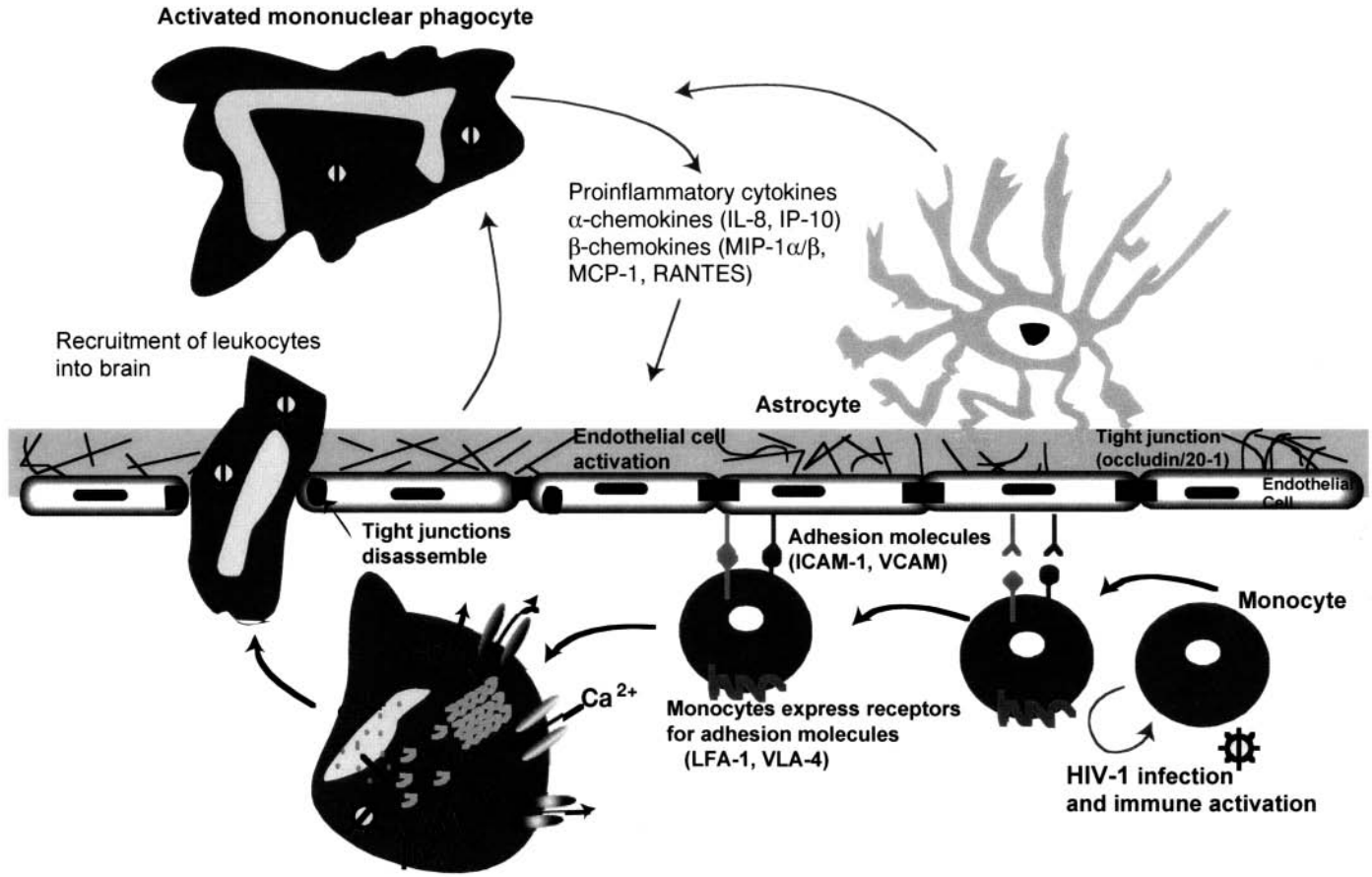

FIG. 5.3. Mechanisms for viral entry into the CNS. Virus is carried into the brain in differentiating macrophages or through lymphocyte-macrophage cell interactions. At the blood-brain interface, circulating monocytes have begun to differentiate into macrophages and acquire the abilities to be productively infected with HIV-1. Many of these cells are already immune competent as speci c CD14 + /CD16 + cells emerge during the onset and progression of cognitive dysfunction in infected people. The immune competent and virus-infected cells secrete a number of pro-in ammatory molecules that induce the upregulation of adhesion molecules (for example, ICAM-1 and VCAM) on the surface of BMVEC. Other factors secreted by macrophages affect tight junctions and cause their disassembly. Tight adhesion of cells occurs, and through expression of ion channels, the macrophages begin to change their shape and size to move freely through the BBB. Once inside the brain the cells represent new cellular targets for HIV-1 and sources of a variety of in ammatory factors including chemokines and cytokines that affect new chemotaxis events for monocyte entry into the brain alone with ongoing neurotoxicity.

HIV REPLICATION AND PERSISTENCE WITHIN THE CNS

HIV-1 is neuroinvasive and enters the CNS early in infection (20,21). Once inside, the CNS brain macrophages and microglia, and to a lesser extent astrocytes, serve as a reservoir for virus. Neuroinvasive viral strains predominantly infect MP (M-tropic), the more prevalent viral subtype early in the course of infection. In contrast, T-lymphocyte-tropic or T-tropic strains predominate later in the course of disease when levels of pro-inßammatory cytokines rise and serve to amplify the entry and replication of the virus through activated brain MP.

HIV-1 is not neurotropic in the sense that it does not infect neurons. Indeed, the virus productively infects only select cell types within the CNS and as noted above, predominantly cells of macrophage lineage (78). Astrocytes may be infected but are highly restricted (56,57,59). Infection of BMVECs has been shown in laboratory cell model systems (58), but there is no evidence that this occurs *in vivo* (79–81).

Tropism of HIV-1 for macrophages is largely determined by the V3 hypervariable region of HIV-1 gp120, the viral surface protein (48). HIV-1 gp120 binds CD4 to the surface of mononuclear phagocytes and T-cells among other CD4+ cell types. CD4 is mandatory for infection of these cells but not sufficient for effective infection by all strains of virus (82). The V3 function is distinct from the HIV-1 gp120–CD4 requirement and is more associated with post-CD4 binding interactions such as proteolytic cleavage and fusion, all of which is critical for productive infection of brain MP (49–53). Although the V3 region is crucial to the tropism of HIV-1, it is not the only factor. The V1/V2 region may also contribute independently or in concert with V3. Chemokine receptors are also associated with viral tropism (54,83). The β-chemokine receptor CCR5 expressed on macrophages is a determinant on m-tropic strains (84,85). T-tropic strains are associated with the α-chemokine receptor CXCR4. T-cells may be infected by either strain of virus.

HIV-1-CELL INTERACTIONS IN BRAIN TISSUE

Mononuclear Phagocytes

The MP is the primary mediator of CNS disease during HIV-1 infection. Included as parts of the MP family are circulating monocytes, meningeal, perivascular and parenchymal macrophages and microglia. Under normal conditions, monocytes become tissue macrophages after cellular differentiation and activation. The majority of monocytes are CD14+/CD16–, however, a subpopulation of monocytes expressing CD14low/CD16+ comprises approximately 8% of the circulating pool. This population is significant because it behaves biologically similar to tissue macrophages (86). The number of CD14+/CD16– cells and CD14low/CD16+ monocytes is increased during inßammation and HIV-1 infection, but the relative proportion of CD14low/CD16+ cells can increase to 40% in patients with advanced HIV disease or AIDS (87). Interestingly, CD4low/CD16+ cells are selectively decreased by systemic glucocorticoid administration (88). In this way these cell populations may serve as an important marker for inßammation and progressive neurological disease during advanced HIV-1 infection (89,90). Their actual role in the neuropathogenesis of HIV-1 infection remains to be determined.

Tissue macrophages throughout the body express low levels of adhesion molecules and MHC II. MP function as phagocytes, as effectors and antigen presenting cells and can kill microbial pathogens through phagolysosomal fusion mechanisms. Macrophages are activated and recruited into tissue during inßammation, and emigrate into the CNS only under like circumstances. However, thus inßux is transient and will revert to a quiescent state after the inßammatory process has subsided (91). For HIVE and HAD the process never subsides as brain inßammation is continuous and induced by ongoing viral replication. One population of MP, meningeal macrophages, is characteristically infected during primary HIV infection paralleling the development of an aseptic meningitis. Later in the course of the disease perivascular macrophages and microglia are infected preferentially (92,93). It is likely that the infected brain macrophage population, the extent of CNS inßammation and the numbers of recruited MDM into the brain predict the course and extent of neurological impairment.

The perivascular macrophages are an actively studied MP cell type involved in HAD pathogenesis. These cells under natural conditions exist between the glia limitans and basement membrane of the choroid plexus and CNS capillaries. They are in close association with the BMVEC, and this position allows them to serve as sentinels for the CNS. They are in fact intermediates between the circulation and the microglia. Since microglia are in contact with these macrophages, signals may be rapidly communicated deep into the CNS from interactions at the perivascular space. These cells are derived from circulating monocytes, but will not become fully active macrophages. Transmission of virus and/or inßammatory responses in the brain may occur between these perivascular macrophages and glial cells within the parenchyma.

Clearly, the most current data strongly suggest that such cells are likely to be responsible for the majority of transmission of virus into the CNS (33). Several observations support this hypothesis, including the presence of often high levels of infected perivascular macrophages. Through such cells virus can be readily transferred to microglia upon microglial activation since there is close contact between the two. Importantly, it is the perivascular macrophage that is the critical CNS resident MP that act as

an antigen-presenting cell to T-cells. Thus, they are at high risk for exposure and contact with infected T-cells and/or inducing T cell protective immunity. Importantly, there is relatively frequent turnover of perivascular macrophages when compared with microglia. Thus, they may bring HIV into the CNS after being infected in the periphery, and release or transmit virus after cell death or through interactions with T cell or microglia.

Parenchymal microglia form a web of opposing dendritic processes throughout the white and gray matter of the CNS (94,95), and extend foot processes to abut the endothelium. They are in signiÞcant numbers in the CNS and may constitute up to 10% of CNS cells. They enter the CNS during gestation and have a very low turnover rate. There are two morphological subtypes of microglia. RamiÞed microglia are resting cells with reduced secretory and phagocytic activity. They compose the web of microglia that spans the CNS. In contrast to perivascular macrophages, they have weak antigen presenting capability, but can be stimulated to do so (96). These cells express few if any adhesion molecules, CD4, or MHC but can be activated to express all three (97Ð99). These cells may convert to fully active macrophages in the CNS. The second type is amoeboid in form. It is a morphological intermediate and transitional cell between the ramiÞed microglia and the brain macrophage. This subtype is not found in the normal adult CNS, but in inßammatory and demyelinating conditions.

Clearly, the pathogenesis of HIV associated CNS disease centers around the macrophage (Fig. 5.4). The

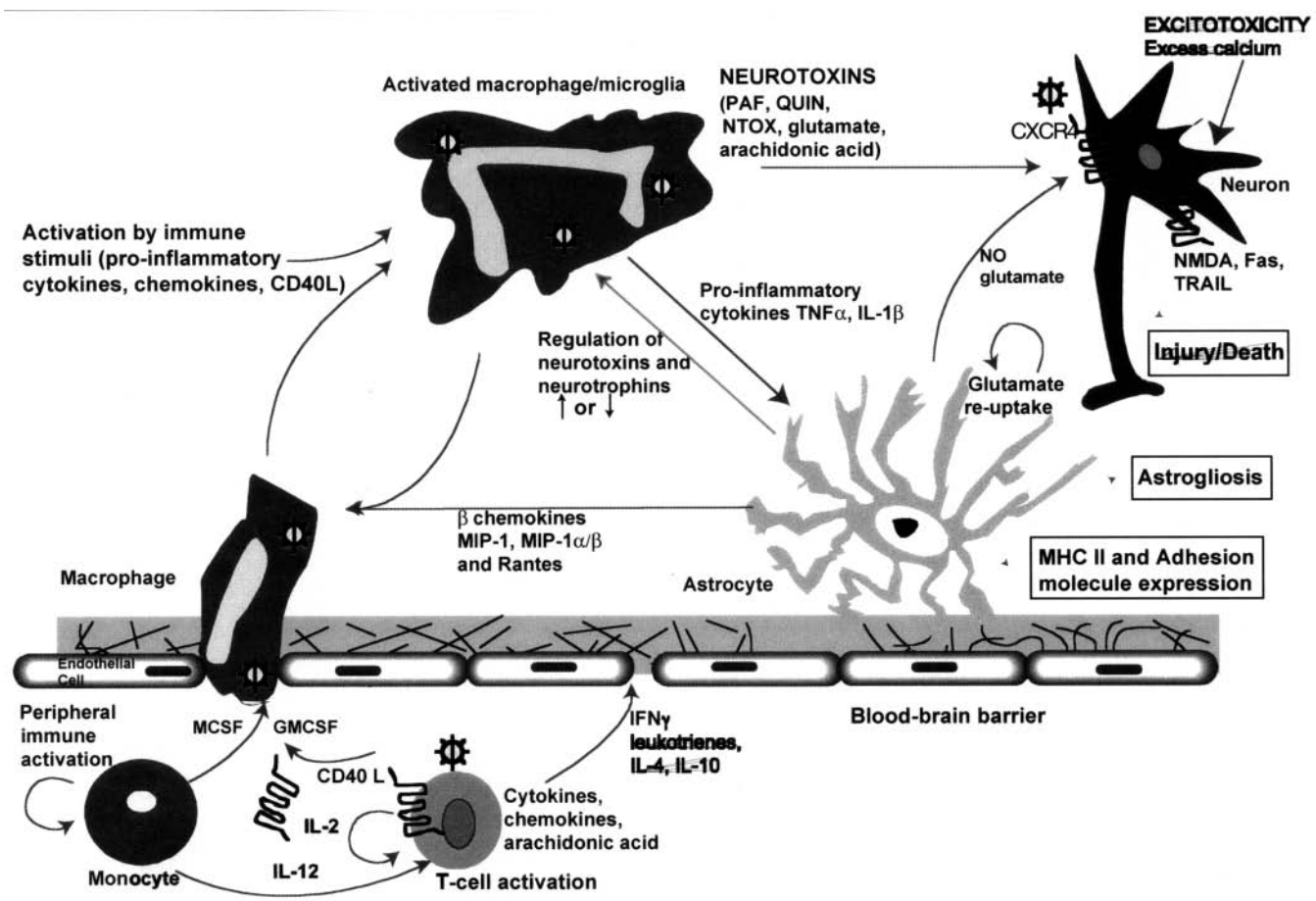

FIG. 5.4. Neuropathogenic mechanisms for HAD and HIVE. HIV-1 infected MP can affect neural function and pathology in a number of distinct ways. Virus-infected MP become immune-activated by a process that remains incompletely understood, but likely involves peripheral pro-in ammatory cytokines and/or cell-cell surface interactions with CD4 + T lymphocytes. CD40L-CD40 represents an example of the latter mechanism. Activated HIV-1-infected brain macrophages secrete a variety of factors (cellular and viral) that affect neuronal function through CNS in ammation. This may occur through excess calcium and excitation of neuronal NMDA receptors or through other mechanisms (for example, Fas and TRAIL). Interactions between MP and astrocytes or each individual cell type can lead to autocrine and paracrine ampli cation of neurotoxins and other in ammatory mediators of neural injury. Glutamate and α- and β- chemokines are secretory factors produced by infected, activated MP. These may affect BBB integrity as well as continuously inciting the recruitment of new monocytes into the brain. Chemokines, HIV-1 gp120, and progeny virions may interact with neuronal receptors to induce intracellular signal transduction alterations, the nal results of which are neuronal compromise and subsequent dementia. Paradoxically, some chemokines act contrary to this paradigm and induce neuronal survival, while others t the model by inducing neuronal apoptosis.

macrophage causes disease in this setting by amplifying the viral load, acting as a reservoir for virus, and secreting toxins that are neurotoxic. The virus replicates in the macrophage and microglia making these cells the target of HIV-1. The virus is able to hide within the macrophage for long periods of time by intracellular budding of progeny viral particles (100). Kolson, et al. speculated that microglia may serve as reservoirs for HIV-1 infection because they have an exceedingly long life-span (101). This in turn might complicate the measurement of viral load in the CSF since it may not adequately reßect viral load in affected brain regions. Moreover, CNS macrophages are, in part, protected from the effects of most antiviral medication because of the BBB. The nature of this viral reservoir and its abilities to harbor and support virus growth remains a key obstacle in eradication of HIV-1 infection from the CNS.

Astrocytes

Astrocytes both proliferate and undergo apoptosis in HIV-1 CNS infection (102Ð104). Astrocytes may be infected by T-tropic strains of HIV-1, and to a much lesser degree by M-tropic virus (105). Infection occurs via a CD4 independent mechanism possibly involving novel astrocyte surface molecules and HIV-1 gp120 (106). Astrocytes may also serve as a reservoir for virus. Latent HIV-1 infection may be maintained for years (105). The actual percentage of restrictively infected astrocytes in brains of patients with HIVE is unknown, but thought to be relatively small. The pathophysiologic signiÞcance of restricted infection in astrocytes remains unknown.

Astrocytes contribute to disease pathogenesis by three major mechanisms. Astrocytes normally regulate extracellular glutamate levels. In HIV-1 infection, astrocyte re-uptake of glutamate is impaired by gp120 (107), and by infection of the astrocyte itself (105). In addition, glutamate release from the astrocyte is induced by activated macrophages (22,108). Stimulation of metabotropic glutamate receptors on the astrocyte may also cause glutamate release (109). All these factors contribute to excitotoxicity. The second pathway involves ampliÞcation of neurotoxic signals. The HIV protein Tat induces expression in astrocytes of MCP-1, a chemoattractant for macrophages (110), and IL-8 and IP-10 which attract multiple leukocyte types (111). Induction of iNOS within astrocytes is promoted by viral proteins and cytokines (112). Fas potentiates inßammation in astrocytes, but induces apoptosis in microglia (113). The third pathway is through Fas ligand (FasL). Here culture supernatants from activated astrocytes and human FasL lead to elevated levels of LDH release and DNA fragmentation in human neurons. Concomitant caspase activation is operative in such neuronal cultures treated with IL-1β-stimulated astrocyte culture ßuids. FasL produced by these activated astrocytes may represent yet another unique pathway for neuronal injury during HAD (114).

CD4+ and CD8+ T Lymphocytes

CD4+ T lymphocytes, as previously discussed, are responsible for the majority of HIV replication in the periphery. HIV-1 may enter the CNS in infected lymphocytes, although this is more frequent in late stage disease. Activated T-cells enter the CNS. During HIV-1 infection, more T-cells are activated to a blast phase, called lymphoblasts (115). Once within the CNS, lymphoblasts search for antigen as they migrate through the parenchyma. If these activated T-cells are infected, they will be shedding virus as they migrate. At the same time, they are inducing CD4 expression on cells susceptible to HIV infection, rendering them even more susceptible. Furthermore, the CNS is normally a hostile environment for the T cell. Because of this, many die by apoptosis within the CNS. Macrophages become activated by way of scavenger receptors as they clear the debris of dead virus-infected cells (116).

INFLAMMATORY NEUROTOXINS

Activated macrophages and microglia are the primary perpetrators of neuronal injury in HIV-1 associated CNS disease (Fig. 5.4). It is widely accepted that these mononuclear phagocytes act to bring about neuronal injury primarily through indirect mechanisms. These indirect mechanisms are alterations in secretory function of chemokines, cytokines, arachidonic acid derivatives and Platelet Activating Factor (PAF), as well as nitric oxide (NO), free radicals, and excitatory amino acids (117). Direct mechanisms bring about neurotoxicity, but probably play a lesser role. These mechanisms consist of soluble viral proteins and glycoproteins that work through neuronal receptors (118,119).

The primary role of the macrophage in HIV neuropathogenesis is neurotoxin secretion (120,121). Among these toxins, excitatory amino acids (EAA) have come to the forefront of the Þeld as of late. Quinolate is one such EAA that acts via the neuronal NMDA glutamate receptor to induce excitotoxicity (60,122). There is a strong correlation between quinolate levels in the CNS and dementia (60). Macrophages have been shown to be the primary source of quinolate in the CNS (123). Other EAA are glutamate, L-cysteine (30), and Ntox.

Secretion of inßammatory mediators also plays a role in HIV-1 associated neurotoxicity. Cytokines and arachidonic acid derivatives, such as platelet activating factor, communicate the inßammatory state, along with a plethora of other functions, to other leukocytes and astrocytes. Macrophages are known to secrete IFN-α, IFN-β, IL-6, IL-1α, IL-1β, TNF-α, and TNF-β. IL-1β deserves special attention, as it is a very potent astrocyte stimulator. Other secretory products produced by the activated macrophage include reactive oxygen species and NO. However, the macrophage does not have the capacity to sustain the high

output production of NO required to cause neurotoxicity. It is more likely that the toxic amounts of NO come from astrocytes stimulated by the macrophage (124).

Macrophage activation during HIV-1 infection may occur by several mechanisms. Pro-inßammatory cytokines such as IFN-γ and TNF-α are potent macrophage activators. TNF-α allows astrocytes and macrophages to amplify the immune activation, resulting in co-activation of other macrophages. T-cells will also activate macrophages by cytokines as well as by direct contact. Activated T-cells will enter the blast phase and enter the CNS. As they migrate through the parenchyma, they secrete the cytokines that serve as a source for macrophage activation (115). As these T-cells die in the brain, their debris is removed by the brain macrophages, also contributing to macrophage activation.

Other mechanisms of macrophage activation involve injury of the axon. Axonal injury results in activation of microglia in the CNS. For example, injury to peripheral processes such as the axon of a motor neuron causes activation of the microglia within the anterior horn. Another theory is that there is exhaustion of T-cells late in the disease state, with compensation by granulocytes and phagocytes that are excessively stimulated. Chemokines are yet another method of macrophage activation. MIP-1β and RANTES act through CCR5 on the cell surface, and SDF-1α through CXCR4. Fractalkine binds CX3CR1 to mediate macrophage recruitment and activation. The mechanisms that involve cytokine and chemokine receptors promote activity of the p38MAPK. This in turn phosphorylates the transcription factorMEF2C. It has been shown that this p38 signal pathway is important in the activation of microglia (125). Inhibition of this pathway prevents the induction of TNF-α and iNOS in microglia (126).

MECHANISMS OF NEURONAL INJURY

Apoptosis

Ultimately, the pathologic processes that occur during progressive HIV-1 infection of the nervous system result in neuronal injury. Histopathological evidence demonstrates neuronal morphological and functional changes during HIVE including a decrease in synaptic density and vacuolation of dendritic spines. Interestingly, however, quantitative analysis of neuronal loss from post-mortem studies of patients with HIV-1 and neurologic dysfunction has not demonstrated a clear correlation between the magnitude of neuronal loss and neurologic disease. A thorough neuropathologic examination of archival post-mortem brain specimens from patients with HIV-1 infection conÞrms that neuronal apoptosis correlates with cerebral atrophy, but not pre-mortem cognitive disorders (127). Nonetheless, apoptosis appears to be the primary mode of neuronal death during progressive HAD and has been found early in the disease state (128,129). Injury and death of neurons by this mechanism is not the result of a single cause (127), but a consequence of multiple mechanisms by the activated MP. Neuronal apoptosis is triggered by calcium inßux. The calcium inßux may result from *N*-methyl *D*-aspartate receptor (NMDAR) dysfunction or other mechanisms. Regardless of the cause, the end cascade is the same. In this context, intracellular calcium triggers p38MAPK to induce chromatin condensation. Mitochondria react to increased calcium by releasing cytochrome-C (cytC) and reactive oxygen species (ROS). CytC subsequently activates caspases that cause chromatin condensation. ROS and free radicals generated in the cascade result in lipid peroxidation and eventual chromatin condensation leading to neuronal death. CXCR4 expressed on neurons may activate p30MAPK and induce apoptosis when bound by SDF-1 of gp120 (122). Indeed, the discovery of HIV-1-binding sites (chemokine receptors) on neurons and glial cells provides fresh insights into the pathways of neural injury during HAD, a rapidly expanding Þeld in NeuroAIDS research (122). Elucidation of the intracellular signaling events that control neuronal apoptosis could provide new approaches for therapeutic interventions.

Chemokines have been found to be inßuential in the signaling involved in neuronal apoptosis (Fig. 5.4). The α-chemokine receptor CXCR4 is expressed by neurons in many areas of the brain (130). The presence of this chemokine receptor may make these neurons susceptible to injury and apoptosis after exposure to gp120 or SDF-1. Soluble HIV-1 gp120 will bind CXCR4 on neurons in a CD4 independent mechanism, and induce neuronal apoptosis (118,131Ð133). SDF-1, the natural ligand for CXCR4, will also stimulate neurotoxicity (134). SDF-1 production is upregulated in astrocytes exposed to soluble factors from activated macrophages (132). SDF-1 is at increased levels in the CNS of demented AIDS patients. CXCR4 is the chemokine receptor preferred by T-tropic virus predominating in late infection and may be an important mechanism for apoptosis in late disease (135). Recent work demonstrates that activation of CXCR4 by SDF-1 induces a signaling cascade that leads to rapid extracellular release of TNF-α from astrocytes and microglia. Autocrine/paracrine TNF-α-dependent signaling inßuences prostaglandin production affecting glutamate release from astrocytes. The interplay between microglia and astrocytes occurs when activated microglia cooperate with astrocytes in the production of pro-inßammatory cytokines (for example, TNF-α) after CXCR4-SDF-1 engagement. This process is thought to lead ultimately to neuronal apoptosis. Thus, there are a variety of ways in which SDF-1-CXCR4 activation may affect neuronal injury during HAD (8).

Pro-inßammatory cytokines from activated or infected MP mediate neurotoxicity by a variety of ways. TNF-α and IL-1β are both major mediators of neuronal injury.

First, both cytokines can stimulate the release of L-cysteine, an excitatory amino acid from macrophages (30). Second, IL-1β inhibits long term potentiation (136). Third, TNF-α can act synergistically with Tat to induce oxidative stress and apoptosis (137), and is involved in the pathway to apoptosis by HIV-1 gp120. Lastly, TNF-α is elevated in the CSF of demented AIDS patients and can directly induce apoptosis (138).

HIV-1 Proteins

The viral proteins implicated in HIV-1 neuropathogenic processes include Tat, Nef, Vpr, gp120, and gp41. Neurotoxicity may be mediated both through direct and indirect mechanisms. HIV-1 gp120 can cause apoptosis by binding CXCR4 on neurons (118,131–133) and is able to elevate intracellular calcium through voltage gated calcium channels and *N*-methyl *D*-aspartate receptor (NMDAR) channels (139–144), as well as by mobilizing intracellular calcium stores (145). The rise in intracellular calcium is an indirect effect mediated by macrophage and microglial release of the excitatory amino acids cysteine and quinolinate. It is thought that HIV-1 gp120 primarily acts by inducing macrophage and microglia to secrete neurotoxins (125). This is potentiated by the ability of HIV-1 gp120 to impair glutamate uptake by astrocytes (119).

The HIV-1 protein Tat is implicated in neuronal injury both indirectly via release of soluble factors from macrophages (78), and by direct neurotoxicity. Tat has been found to potentiate excitotoxicity in neurons (146,147). Moreover, matrix metalloproteinases may be involved in the mechanism of Tat induced neurotoxicity (148). As discussed, there is some synergy with TNF-α to cause neurotoxicity (137). Tat has been shown to interfere with the electrophysiological activity of neurons (139). There is also evidence of signiþcant quantities of Tat measured in CSF of demented patients (149).

In addition to the complex interactions of HIV-1 gp120 and the recently studied effects of Tat on neurons, other viral proteins have neurotoxic properties. The protein HIV-1 gp41 was found to result in elevation of inducible NO synthase (iNOS) (112). Also, Vpr can have direct neurotoxic activity *in vitro*. The mechanism of this effect is by formation of cation permeable channels (150).

Other Mechanisms for Neuronal Injury in HAD: Oxidative Stress

Oxidative stress plays a signiþcant role in the neuropathogenesis of HIV-1. Free radical formation combined with impaired antioxidant capabilities brings about this stress (151). The free radical species in this situation are superoxide anions, nitric oxide, and peroxynitrite. NO is produced by neurons in response to excitation and changes in intracellular calcium. Neurons can be exposed to additional NO released from astrocytes, macrophages, and microglia which have been stimulated with pro-inßammatory cytokines such as TNF-α and IL-1β, HIV-1 gp120 (152,153), or HIV-1 gp41 (112). These stimulants upregulate the inducible isoform iNOS (154,155).

The major effector of oxidative stress is peroxynitrite. NO alone is also neurotoxic. However, when it combines with superoxide to form peroxynitrite, the effect is much more signiþcant. NO as discussed is produced by neurons, BMVEC, astrocytes, and MP. Superoxide anion comes from activated infected MP (154). One of the ways in which peroxynitrite damages neurons is by targeting neuroþlament, a structural protein (156).

HIV-1 infected patients lack the antioxidant glutathione and have reduced levels of the hydrogen peroxide scavenger, catalase, in CD8+ T-cells. These factors contribute to an impaired ability to clear free radicals. In addition, the levels of iNOS in HIV-infected patients are elevated. This elevation is even more signiþcant in demented AIDS patients (154). These factors all contribute to a chronic state of oxidative stress in HIV-1 infected patients.

Excitotoxicity is brought about by repeated and excessive stimulation of NMDA receptors on neurons. Overactivation of this receptor allows calcium inßux into the neurons, which in turn leads to apoptosis (122). If, however, the overstimulation is rapid, necrosis may ensue due to ionic imbalances (157). Stimulation of the NMDAR can be accomplished by glutamate, L-cysteine (30), quinolate (60), and Ntox (122). Other mediators can cause excitotoxicity indirectly. Platelet activating factor (PAF) is secreted by activated macrophages in response to TNF-α (Perry, 1998 #6443. PAF is a neurotoxin (24) causing glutamate release in the CNS (158,159) and increased calcium inßux in neurons (160). Other factors including HIV-1 gp120 have also been found to increase calcium inßux in vulnerable neurons (161).

Neuroprotective pathways are also operative. Activation of CCR5 on the neuronal surface by RANTES or MIP-1α interferes with HIV-1 gp120 and NMDAR associated toxicity (125,162,163). Fractalkine binding to CX3CR1 is neuroprotective for apoptosis induced by HIV-1 gp120, Tat or PAF (133,164). This mechanism is dependent on Akt activation, a protein kinase signaling pathway in neurons (160).

ANTIRETROVIRAL THERAPIES

Productive viral replication is a necessary component of the neuropathogenesis of HAD supporting the pivotal importance of antiretroviral agents in the prevention, treatment and reversal of dementia. This approach is both logical and well founded in disease mechanisms and is

supported by a multitude of basic and applied research studies. Nonetheless, despite remarkable recent advances made in the treatment of HIV disease, effective therapies for HAD remain incomplete. Remarkably, zidovudine is the only drug to date with clinical trial data demonstrating efficacy in the treatment of dementia (165–169). BBB penetrance, optimal dosing, the interface between peripheral and CNS viral load and the potential role of antiretroviral therapies in prevention of dementia remain unclear.

Potent antiretroviral combination therapies have been shown to suppress plasma viremia below the limit of detection of currently available assays, increase CD4+ T lymphocyte counts, and improve morbidity and mortality (31,170). Encouragingly, reports already suggest improvement in neuropsychological test performance and radiological abnormalities after initiation of therapy with protease inhibitors (26,28,29,171,172). Because neurologic disease does not correlate with neuronal loss, but rather the relative burden of MP in vulnerable brain areas, the question arises whether HAD is due to, in part, a reversible metabolic encephalopathy. Profoundly demented patients treated with triple anti-retroviral therapy at our center have evidenced dramatic reversal of cognitive impairment, concomitant with a reduction of viral load in plasma and CSF (173). Significantly, this was accompanied by a more profound reduction in levels of potential neurotoxins in plasma and in CSF. In further support of this concept, Fox et al. (173a) showed that when SIV-infected rhesus macaques were treated with antiretrovirals, neuropsychological deficits were ameliorated. Sensory evoked potentials (SEP), a measure of the integrity of brainstem polysynaptic pathways in response to an event-related stimulus (such as light, sound, or recognition of a singular event in a stimulation pattern), were also improved without reversal of motor deficits. Cessation of treatment led to a return of neuropsychological deficits and abnormal SEP. Because the brain-resident MP is the productively infected cell type in the CNS, there is ample evidence to demonstrate that they are the major source of candidate HIV-1 neurotoxins. Taken together these data strongly support the notion that HAD is a metabolic encephalopathy, and can be reversed, at least in the earliest stages of disease, by potent combination antiretroviral therapies.

Many of the available antiretroviral compounds are highly plasma protein bound, and therefore do not have good penetration of the blood-brain barrier (174). The degree to which some newer drugs in development penetrate the CSF is not known, and CSF penetration of available protease inhibitors is negligible (175–180).

Whether or not CNS penetration is critical, or whether suppression of plasma viremia may be adequate for prevention and treatment for CNS HIV-related diseases, is not known. Some experts believe that inclusion of a drug with good CSF penetration in antiretroviral combinations is an important component, although there are few data to guide the practicing physician. Combination antiretroviral therapy has been shown to decrease CSF HIV-1 RNA levels despite poor drug penetration (176,181,182). However, some investigators are concerned that the CNS may continue to present a potential viral sanctuary despite antiretroviral drugs (32,78). Reports of patients receiving and responding to antiretroviral therapy as evidenced by undetectable plasma viremia, while still developing CNS dysfunction, are disquieting (183). Preliminary findings support compartmentalization of virus with independent evolution in CSF and plasma (61,184,185). Autopsy studies suggest that tissues in which the main target for HIV-1 is the macrophage, predominantly the CNS, harbor viral sequences that cluster separately (186).

Moreover, 40–60% of patients in clinical practice will have detectable viremia after more than one year on combination therapy (187–190). High baseline viral load, low CD4+ T cell count, previous antiretroviral therapy, and non-adherance all contribute to risk of virologic failure (191). Drug exposure, as determined by adherence, absorption and metabolism is also an important factor. Although definitive evidence is lacking, limited drug penetration into tissues may allow for ongoing viral replication and further contribute to failure of antiretroviral therapy. Experts in the field believe that it is premature to conclude that control of viral replication in the periphery will prevent HIV-1-related CNS disease (32).

ADJUNCTIVE THERAPIES

As described above, the dementia produced by CNS infection with HIV elicits a cascade of events involving both resident and invading cell types and ultimately the devastating loss of neuronal function and cognitive deterioration. As the disease process occurs in a stepwise fashion it provides an opportunity for development of therapeutics directed at discrete pathogenic mechanisms (Fig. 5.5). Based on a clearer understanding of the neuropathogenesis of HIVE and HAD, the selection of rational adjunctive therapies for HAD has now been developed. There are several reasons for demand for such therapies. First, PCAT, while successful in decreasing viral burden and delaying the onset of HAD, does not prevent it. Second, combination antiretroviral therapies are not always well tolerated. Third, only a very limited fraction of the global population of HIV-infected persons has access to PCAT. This is not likely to change in the immediately foreseeable future. Fourth, because HIV-1 has an evolutionary advantage in its ability to adapt to most of the PCAT agents, and since most PCAT agents do not freely cross the blood-brain barrier, the need for adjunctive neuroprotective agents is likely to increase in future years. Fifth, overlapping pathogenetic mechanisms

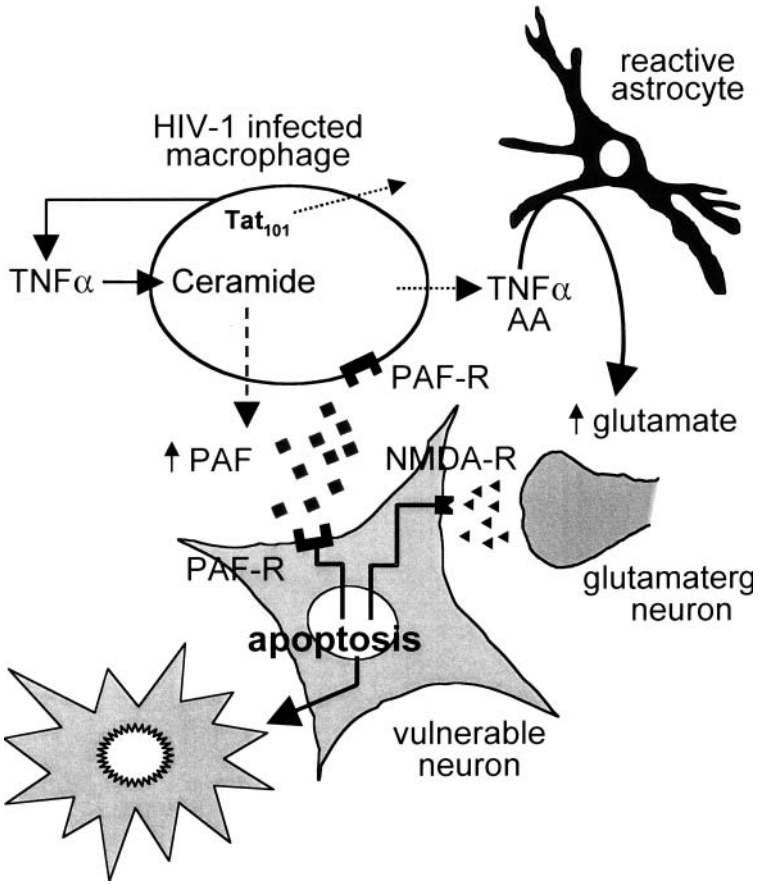

FIG. 5.5. MP neurotoxins including Tat, TNF-α, and PAF may also have direct effects on vulnerable neurons. Tat has been shown to induce hippocampal CA1 neuron depolarization, probably via a non-NMDA receptor mechanism, and induce neuronal apoptosis via a mechanism independent of nuclear factor kappa B (NFκB) activation. TNF-α can induce neuronal cell death in part by activation of non-NMDA (for example, AMPA) receptors. This TNF-α-induced neuronal death has the biochemical and morphologic features of apoptosis, involves oxidative stress, and is independent of NFκB activation. PAF can also induce neuronal cell death through apoptosis via a mechanism that involves activation of NMDA receptor channels. Because these three neurotoxins appear to work in part through activation of non-NMDA and NMDA receptors, it is important to note that NMDA receptor activation and production of nitric oxide (potentially through HIV-1 gp120) can also induce neuronal apoptosis.

are operative for other neurodegenerative diseases containing an inßammatory component (for example, AlzheimerÕs disease and stroke), making it likely that agents will have Ò cross-overÓ potential for a treatment of a wide range of neuro-destructive processes.

Based on these observations and despite diverse mechanisms of actions, a number of promising small molecules have already been identiÞed and are currently being used or soon will be employed for therapeutic trials in man. Outlined below are several adjunctive therapies which are being developed for treatment of HAD. Each act independently both of the viral life cycle and of conventional antiretroviral agents and affect secondary neurodegenerative processes elicited by MP secretory products. They include anti-inßammatory, neuroprotective and neuroregenerative compounds, which either target speciÞc neurotoxins, a wide range of toxins or protect neurons against their Ò down streamÓ effects. Because of the numbers of such drugs now developed, we have selected a few to review and describe their proposed mechanisms of action and possible clinical utility.

In AIDS Clinical Trial Group protocol 162, 41 HIV-1 infected patients with varying degrees of dementia were examined for whether nimodipine, a calcium channel blocker (192), could affect disease outcome. The results showed that nimodipine was safe, but the study was not sufÞciently powered for efÞcacy, and only a trend toward improvement in neuropsychological performance was seen. Clinical trials of other compounds are under way and described below.

GSK-3 Inhibitors

Some of the adjunctive drugs now being developed for treatment of HAD have had indications for other inßammatory-based diseases or are in general use for other secondary disease processes associated with progressive HIV-1 infection. One example is sodium valproate (VPA). Interest in VPA for treatment of HAD has increased recently as it is known that the drug possesses neuroprotective properties because it inhibits GSK-3 beta activity (193), and because of its cytoprotective effect on neurons exposed to HIV-1 neurotoxins. VPA has already been used in HIV-1 infected individuals to manage psychiatric disorders, and the drug appears to be generally well tolerated. Although the compound has never been systematically examined, it has no known adverse interactions with anti-retroviral agents. Thus, VPA represents an excellent potential candidate for neuroprotection in patients with or at risk for HAD. VPA is preferable to the other presently available inhibitor of GSK-3 beta, lithium. One conceptual concern for the use of VPA in persons with

HIV-1 infection is that it has been shown to enhance HIV-1 replication in certain cultured cell lines (194). This observation has not been reproduced, however, in biologically relevant primary host cells including macrophages and CD4+ T lymphocytes.

Dopaminergic Agents

A second class of therapies is dopaminergic agents. The utility of such compounds is based on data obtained in cell culture model systems, which have shown that HIV-1 gp120 is toxic for dopaminergic neurons (195). Studies have also shown that HIV-1 Tat can inhibit the activity of tyrosine hydroxylase, the major rate-limiting enzyme in the dopamine biosynthetic pathway. Furthermore, *in vivo* injection of HIV-1 Tat into the striatum of healthy rats induces a subclinical Parkinson's-like disease. Moreover, in HIV-1 infected humans, neurologic disease has been linked to changes in dopamine metabolism, and dopamine levels within the CSF are reduced. Based on these findings, it has been proposed that alterations in dopamine metabolism may contribute to motor deficits in persons with HAD. Pramipexole is a dopamine agonist with antioxidant and neuroprotective properties in small animal model systems and has been used particularly in the context of MPTP-induced injury, and ischemia (196–199). It has been shown to upregulate the expression of anti-apoptotic proteins such as Bcl-2, and to protect cultured neurons from pro-apoptotic insults (200). Bcl-2 and other anti-apoptotic gene products may represent the final effector proteins, which are responsible for NFκB-mediated neuroprotection. Furthermore, Bcl-2 can mediate neuroprotection against candidate HIV-1 neurotoxins, including HIV-1 Tat and TNF-α. Pramipexole has also been shown to have a beneficial effect in the context of Parkinson's disease, based on its dopaminergic properties. This drug has not yet been studied to any significant degree in the context of HIV-1 infection.

PAF Receptor Antagonists

PAF is a phospholipid mediator that may play a key role in NMDA receptor activation in HAD, as described above in the section on HIV-1 induced neurotoxins. Intriguingly, *in vivo* experiments using the SCID mouse model of HIVE demonstrate that PAF receptor antagonists are neuroprotective. A recent Phase I trial of the PAF receptor antagonist, lexipafant, in a cohort of patients with varying stages of HAD, demonstrated trends in improvement of neuropsychological parameters including verbal memory (201). Ongoing strokes are investigating the use of this compound in the setting of PCAT and other adjunctive therapies with distinct targets of action.

Glutamate Receptor Antagonists

It is well accepted that the neuronal dysfunction and death that occurs in HIVE and is associated with HAD may ultimately be mediated by pathologic activation of excitatory subtypes of glutamate receptors, in particular the NMDA receptor. Numerous examples in the literature demonstrate involvement of NMDA and non-NMDA receptor activation in vulnerable neurons after exposure to HIV-1 associated neurotoxins. One problem in designing a practical therapeutic approach to ameliorating excitotoxic neuronal damage is that most of the available small molecule agents that block ionotropic receptor function are uncompetitive or noncompetitive channel antagonists. Thus, administration of such an agent at concentrations that might be neuroprotective in the CNS might also significantly interfere with fast excitatory neurotransmission and thus have untoward clinical effects. Thus far the most promising NMDA antagonist currently in clinical trials for HIV-1 related neurologic disease is memantine, which appears to lack the side effects of dizocilpine. Recent clinical trials have not, however, demonstrated a significant positive effect on HAD.

SUMMARY

This chapter demonstrates both how far we have come and how far we still need to go in beginning to understand fully how HIV-1 infection of the brain results in neuronal injury and death. Aside from the significant morbidity induced by HIV-1 for cognitive, motor and behavioral function, the CNS represents an important reservoir for continuous virus production and the emergence of viral strain variance. Moreover, the mechanisms of how MP secretory products may damage neurons have potential wide applicability for other neurodegenerative disorders where inflammation is an important component of disease pathogenesis (for example, Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis). Finding potential strategies to retard the expression and effects of such neurotoxins remain one important primary goal for biomedical research efforts. Adjunctive therapies such as NMDAR blockers, chemokine and cytokine effectors, antioxidants, caspase inhibitors, and p38MAPK inhibitors, are all potential interventions and are being developed by many laboratories. If successful, these drugs may find utility in a broad range of neurodestructive processes.

Over, the past 18 years, since the first description of the neurological complications of HIV-1, surprising changes in the way we think about virus persistence and its abilities to elicit CNS inflammation and concomitant neurotoxicity have emerged. The frequency of and propensity for HIV-1 induced neurological disease represent some of the more significant realizations in AIDS research. Such information is essential for vaccine design, in understanding viral resistance and in utilizing pharmacological approaches to

reduce or perhaps eradicate viral infection in the CNS. Moreover, the uncertainty of the long-term efficacy of PCAT therapies suggests that neurological disease could increase in the future and other therapeutic modalities need be developed. Such adjunctive therapies should focus on brain inflammation brought about through MP neurotoxins. It is, after all, these inflammatory toxins that contribute in significant measure to a reversible state of neuronal dysfunction (a metabolic encephalopathy) that includes impairment of neuronal function and synaptic transmission. Importantly, many of the hallmarks of HIV-1 neuropathology are seen in chronic inflammation, have parallels to other neurodegenerative processes, and follow defective MP secretory function. Such observations provide an impetus for development of adjunctive therapies and support the hypothesis that rational therapeutic approaches for treatment should include compounds that intercede in critical steps of the pathogenic sequence and ultimately lead to reduced CNS inflammation. As can be seen by the information given in this chapter, the accomplishments made in basic and applied research into the neurological aspects of HIV-1 infection are significant and have brought about advances in the understanding of the disease process, its relatedness to other neurodegenerative disorders and therapeutic directions.

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Chapter 6

Viral Cofactors in the Immune Pathogenesis and Clinical Manifestations of HIV Infection

Jeffrey Laurence

"PathogenicityÓ refers to the ability of a microorganism to cause disease; ÒvirulenceÓ deÞnes variations in the magnitude of this effect (1). All replication competent isolates of HIV-1, -2 disrupt the immune systems of their human hosts. The intriguing differences are ones of degree.

But can exogenous factorsÑfor the purposes of this chapter, other virusesÑalter HIV virulence, shifting the balance between populations of quiescent, uninfected and latently infected T cells vs. virally-activated T cells, which may die either as a consequence of activation or via HIV-mediated cytolysis? There is *in vitro* evidence, and mounting clinical data, that this may be the case.

Virulence is a property of host-parasite interactions, but host Þtness is exceedingly difÞcult to assess in nature. Investigations of the extremes of this interplay in HIV infectionÑthe rapid progressors to AIDS versus long-term delayed (Ònon-)Ó-progressors (2)Ñsuggest models by which to sort out protective vs. pathologic mechanisms in HIV infection. These observations also provide an opportunity to assess the role of cofactors in the progression of HIV disease.

BACKGROUND: HIV KINETICS AND DISEASE PROGRESSION

To understand the impact of exogenous viral infections on HIV, we need to review the kinetics of HIV growth *in vivo*, and its transcriptional regulation, as deÞned *in vitro*. The marked differences in average viral load at various immunologic and clinical stages of HIV disease, coupled with the extraordinarily high turnover of HIV virions ($\sim 10^9$/day) and the rapid death of productively infected CD4+ T cells *in vivo* ($t_{1/2} = 2.0 + 0.9$ day), attest to the complex regulatory control of HIV replication (3Ð5). Some 99% of circulating HIV appears to derive from cells with a half-life of one to two days, suggesting that repetitive cycles of *de novo* infection, virus production, and cell destruction clearly occur. But the fact that CD4+ and CD8+ T cell turnover are virtually identical in HIV disease, while CD4+ T cells alone are HIV infected, argue for the prominence of indirect mechanisms of T cell death, and perhaps interference with precursor cell production or maturation in the thymus gland, bone marrow and elsewhere (3,4).

The 1% of HIV-infected cells that harbor latent virus, as well as ongoing viral replication in T cells at levels that are not detectable by standard measures of viral load, may play critical roles in the later stages of HIV disease. These and other reservoirs, existing in a milieu complicated by rapid development of drug resistant strains, and little affected by current highly active antiretroviral therapies (HAART) (5), may underlie the perplexing failure of our best anti-retroviral agents, alone or in combination, to effect more than a 2Ð3 log decrease in viral burden, along with the failure to normalize CD4:CD8 T cell ratios (4).

HIV also may be transiently rescued from latently infected cells by modulators of cell or HIV activation, providing another avenue by which viral cofactors may accelerate or, in certain cases, mitigate HIV replication. Indeed, studies begun over 30 years ago by the co-discoverer of reverse transcriptase, Dr. Howard Temin, demonstrated that the state of host cell activation at the time of a retroviral infection inßuences reverse transcription and retroviral expression (6). Either direct cofactor virus infections, or cytokines induced by such infections, could provide such cell stimulatory signals. These activation signals appear to be key factors in the T cell depletion characteristic of HIV/AIDS.

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Dr. William Paul, an early director of the federal Office of AIDS Research, and his associates summarized our current under-standing of T cell dynamics as affected by HIV stating, ÒThe intuitively appealing but simplistic vision of an ongoing competition between massive destruction and massive replacement of CD4+ cells (the Òtap and drainÓ model) is still energetically advocated by some. Others envision a classical confrontation between protective immune responses and an evasive virus. But a growing body of evidence and reasoning point to infection-induced immune activation both as the driving force behind virus replication and as a facilitator of CD4+ cell depletionÓ(7).

HIV infection results in high levels of activation and turnover of both CD4+ and CD8+ T lymphocytes. These cells then undergo several rapid rounds of cell division, finally dying by apoptosis, a phenomenon referred to as activation-induced cell death (AICD). Those few activated cells which escape such a fate enter, or re-enter, the population of resting, memory T lymphocytes (7). Large numbers of T cells may also be stimulated by antigens from HIV or co-infecting microorganisms, and the pro-inflammatory cytokines they induce.

It might be anticipated that each new cycle of immune activation in HIV infection would add to the memory pool. But, in the presence of persistent antigen and inflammation, as often occurs even in the most stable patients on HAART (8), the net change in memory cells appears to be a negative one, and a shifting steady state is realized. Normally, production and elimination of T cells is perfectly balanced, with Òelimination Ó including entry of naive and effector T cells into the memory compartment. But this homeostasis is disrupted in HIV infection as, with time and further increases in immune activation, the total T cell number is progressively shifted downwards.

This is particularly bad news when one considers that HIV appears to specifically infect the very cells that respond to it (9). In one study, HIV-specific vs. cytomegalovirus (CMV)-specific CD4+ memory T cells producing interferon-γ were obtained from 12 HIV-infected individuals (9 on HAART), sorted by flow cytometry, and compared with their unstimulated CD45RO+ general memory pool (9). Quantitative DNA PCR was used to determine viral copy number in these cells. There was no significant difference in terms of HIV load in CMV-specific vs. pooled memory CD4+ T cells; most of the HIV sat in the HIV-specific T cell pool. The total fraction of HIV-specific CD4+ T cells was relatively lowÑ 1.01% for those off therapy and 0.89% for those on HAARTÑ but the ratio for HIV DNA in HIV specific vs. other CD4 cells was high, varying from 2.1 to 5.3 (9). The authors concluded that, Òthis preferential but low-frequency infection of HIV-specific and other activated T cells may represent an evolutionary adaptation that enables persistent infection in an otherwise immunocompetent host, and prolonged host-parasite coexistenceÓ(9).

This also suggests that the salutary effects of HAART therapy may be based on something more than just suppressing viral replication. For example, Nobel laureate Dr. Rolf Zinkernagel has long argued, prior to any HIV kinetic studies or the clinical use of HAART, that immune activation, particularly of cytolytic CD8+ T lymphocytes (CTL), contributes greatly to the pathology of certain viral diseases, including AIDS (10). His group noted that certain protease inhibitors (PIs) markedly inhibit antiviral CTL activity and impair MHC class I-restricted viral epitope presentation in a mouse model of a persistent viral infection, lymphocytic choriomeningitis virus (LCM). They do so by selectively inhibiting the chymotrypsin-like activity of the 20S proteasome, an activity critical to viral epitope presentation (11).

Dr. Zinkernagel and associates proposed that while anti-HIV CTL contribute to initial control of viral replication, ÒAIDS may partially result from immunopathological damage by anti-HIV CTLs, (and) the direct effects of ritonavir on class I-restricted Ag (antigen) presentation may reduce the immunopathological destruction of Ag-presenting cells, CD4+ lymphocytes, and other HIV-infected cellsÓ(11). The concept of immune activation, and possibly related autoimmune phenomena, as important to immune pathogenesis in AIDS received a boost from clinical trials of immune suppressants in acute HIV infections, as detailed at the end of this chapter.

BACKGROUND: A MOLECULAR APPROACH TO VIRAL CO-FACTORS FOR HIV TARGET CELL ACTIVATION

Viral persistence may be viewed at three distinct levels: persistence in the population as a whole, in the individual host, and within a cell or group of cells (12). In terms of the latter, viruses may survive, despite an intact immune system, by limited growth in a cell population (carrier state); continuous multipli-cation without eliciting cytopathic effects (steady-state infection); true latency (complete absence of viral transcripts and protein); or cell-to-cell passage without maturation (intracyto-plasmic persistence, characteristic of infection of cells which normally remain in Go, such as monocytes) (12).

As noted above, 99% of HIV is produced by actively infected cells, but the small pool of latently infected cells appears to be a critical source for reactivation of virus. HIV has adopted a transcriptional strategy in both T cells and monocytes which resembles that used by cellular genes regulating T cell signalling and growth (13), with HIV replication modulated by a variety of signals to cell differentiation, activation, and/or proliferation (14). HIV growth can be dissociated from cellular proliferative responses, with both mitogenic and non-mitogenic activation signals provided by cofactor virusesÑ through antigen and superantigen stimulation, receptor cross-linking, and promotion of cytokine releaseÑ sufficient to induce HIV transcription (15).

Initiation of HIV reverse transcription occurs simultaneously in both activated and quiescent T lymphocytes, monocytes, and probably other cell types, but it terminates prematurely and proceeds more slowly in quiescent cells. Stimulation of such cells may reinitiate DNA synthesis off of partial reverse transcripts, but this mode of viral rescue is highly inefficient (16). And proviral DNA in quiescent cells is labile, degrading with a $t_{1/2}$ of <24 hrs *in vitro*. This may partially account for the low number of infected cells in the peripheral blood of most asymptomatic HIV-1 seropositive individuals (17).

VIRAL ANTIGENS AND SUPERANTIGENS

Cofactor viruses implicated in AIDS tend to be highly antigenic. For example, during antigen-specific immune activation, 100-fold less HIV-1 is required to initiate a productive infection in cultures which attempt to mimic the lymphoid microenvironment: CD4+ T cells admixed with HIV-pulsed dendritic cells (18). Optimal stimulation of CD4+ T cells requires engagement not only of their T cell receptor (TCR)/CD3 complex by antigenic peptides presented in the context of MHC-II, a process often referred to as "signal 1", but also critical co-stimulatory ("signal 2") events provided by cross-linking of accessory receptors on the T cell surface with ligands on antigen presenting cells (APCs), including dendritic cells, monocyte/macrophages, B lymphocytes, endothelial cells, and possibly CD4+ T cells themselves (19). These latter ligands include the β_2 integrin LFA-I (CD 11a), CD2, and CD28. Their engagement leads to translocation of protein kinase C, required for expression of previously silent cellular and viral genes, changes in cell phenotype, and entry of cells into G1 of the cell cycle. While signal 2 alone has no observable effects on APC or T cell phenotype (19), in the form of a B7-CD28 interaction it is sufficient to induce transcription and replication of HIV in naturally infected T lymphocytes (20). Subsequent progression through the cell cycle, as opposed to simple T cell activation, is dependent upon three main factors: local IL-2 concentration, IL-2 receptor (R) density on the cell, and the duration of the IL-2/IL-2R interaction. These aspects may be modulated, positively or negatively, by cofactor virus infections.

Viral cofactors may also activate HIV cell targets via superantigen (SAg) phenomena (21), blockade of factors linked to suppression of viral and cell growth, or do both. For example, mycoplasmas can enhance HIV replication, at least *in vitro*, both by inhibiting production of the antiretroviral product α-interferon and by cross-linking CD4+ T cells of specific TCR V_β subytpes, a SAg phenomenon leading to cell activation (22). Cytomegalovirus (CMV) is another potent superantigen in the stimulation of HIV replication (23,24). But, despite some early reports (25), it is unlikely that HIV itself serves as a SAg.

All these processes set two basic cellular events into motion: induction of sequence-specific cellular transcriptional factors, and chromatin reorganization to permit effective interaction of the induced factors with their proviral targets. Both can enhance uptake and integration of newly acquired HIV, or promote viral induction in latently infected hosts.

MECHANISMS FOR UPREGULATION OF HIV IN ACTIVATED HOST CELLS

One general pathway by which viral cofactors, in activating normal host cells, can in turn promote HIV replication involves NF-κB, a member of the *rel* family of transcriptional enhancers. It is found in nearly all eukaryotic cells, coupled to an inhibitor, IκB, in an inactive form. Cell activation, with or without cell proliferation, leads to upregulation of a variety of protein kinases one of which, IKK, in phosphorylating IκB, permits translocation of NFB to the nucleus, its binding to transcriptional enhancer sequences of the HIV LTR, and facilitation of RNA polymerase II activity (22). This induction of NF-κB transforms the HIV LTR into a type of housekeeping gene promoter, increasing the assembly of general transcription factors on the LTR to form pre-initiation complexes. It also proscribes transcriptional inhibition through cellular factors such as p53. A variety of exogeneous viruses, including all of the herpesviruses, share the NF-κB pathway with cellular and HIV genes, and thus may activate both the cell, and HIV within that cell (23). Viruses may also modulate transcription of normal cellular proto-oncogenes and tumor suppressor genes, influencing cell activation through increases in production of growth factors related to c-*myc*, c-*myb* and c-*fos*, and perhaps factors from other proto-oncogenes. Finally, under certain conditions, indirect suppression of HIV replication may occur, as discussed later in this section.

CYTOKINES AND CHEMOKINES

Cytokines and the cytokine chemoattractants, or chemokines, are an important means by which viral cofactors impact both HIV replication and manifestations of HIV disease. One might have anticipated that HAART would abolish many cytokine-mediated phenomena, as there is a sharp decline in proliferating T cells following such therapy (26,27), in parallel with decreased production of IL-2 (27). But this decline may be accompanied by a significant rise in T cell secretion of TNF-α, a key factor in the stimulation of HIV replication. Whether this rise occurs in the majority of infected patients (27), or just a subgroup (8) is controversial. A list of the most prominent molecules linked to HIV and viral cofactors for HIV induction, acting by direct viral infections or mediated by soluble viral products, is presented in Table 6.1 (28).

The character and quantity of the cytokines and chemokines involved may differ depending on the stage of

TABLE 6.1. *Cytokines and chemokines induced by viral infections and viral proteins**

Virus	Viral Protein	Induced Molecules
HSV	gD	TNF-α, IFN-α/β
CMV	gB	IL-1, IL-6
EBV	gp350	IL-1, IL-6, IL-8, TNF-α, MIP-1α, GM-CSF
	LMP-1	IL-1, IL-6, IL-10
	EBNA2	IFN-α/β
HBV	HBx	IL-6, IL-8, TNF-α
	HBsAg	IL-2, IL-10, IFN-γ
	HBc/eAg	IL-10, TNF-α, IFN-γ
HTLV-I	Tax	IL-1, IL-2, IL-3, IL-5, IL-8, L-15, TGF-β, TNF-α, IFN-γ, MIP-1α/β, GM-CSF
HIV	gp120	IL-1, IL-4, IL-6, IL-8, IL-10, IL-13, TNF-α, IFN-α/β, IFN-γ
	Tat	IL-1, IL-2, IL-6, IL-8, TGF-β, TNF-α, MCP-1

*Table adapted from (28).

HIV infection. In acute HIV infection, serum concentrations of IL-2, as well as soluble IL-2 receptor (R), IL-1, IL-4, and the pro-inßammatory cytokines tumor necrosis factor (TNF)-α and IL-6, may be equivalent to healthy controls (29). In contrast, levels of most of these factors are markedly elevated in the setting of acute EBV (Epstein-Barr virus) or CMV infection (29).

Later in the course of HIV infection, levels of these cytokines become chronically elevated, but virus-mediated shifts in plasma concentrations may occur at any stage. Of particular interest is the ability of the T_H2 type of CD4+ T cell, in secreting IL-4 and IL-6, to blunt the differentiation of TH1 CD4+ T cells, required for mounting an effective cytolytic anti-HIV response. These T_H2 responses can be induced by infection with measles, vaccinia, herpes simplex viruses (HSV), and human T cell lymphotropic virus (HTLV)-I,II (24,28), along with many other microorganisms. That such changes may have a clinical impact is suggested by therapeutic trials of some of these agents. For example, high-dose IL-2 infusions in HIV-1+ patients leads to transient increases in HIV viremia, even in the presence of effective anti-retroviral therapy (30).

Apart from activating HIV target cells and rescuing HIV from latently infected cells, immunocyte secretory products induced by viral cofactors may also inßuence the type of AIDS pathology expressed. For example, many viral infections can upregulate production of chemokines which recruit inßammatory cells, HIV infected or not, into the central nervous system, and have been postulated to mediate neuropathology in AIDS (31).

VIRAL CO-FACTORS FOR DIRECT HIV TRANS-ACTIVATION

Viral co-pathogens may also serve to regulate HIV replication directly through several transcriptional and post-transcriptional events. The former effects are linked to speciÞc and promiscuous transcriptional factors encoded by all of the herpesvirusesÑ CMV, EBV, HSV-1,2, and human herpesvirus type 6 (HHV-6)Ñ as well as by hepatitis B (HBV), HTLV-I and -II, and papovaviruses (32).

NF-κB, proto-oncogenes, and tumor suppressor genes were mentioned earlier in the context of viral activation of cellular replication. Similarly, tumor suppressor genes may directly impact on HIV transcription. For example, the wild type p53 tumor suppressor gene product inhibits HIV-LTR-directed transcription through bridging of certain gene promoter sites, known as Sp1 and TFIID, in the LTR (33). This is consistent with the Þnding of high rates of HIV replication in metastatic colon carcinoma cells from HIV+ individuals (34), as such tumors typically contain mutant p53 incapable of complexing with the HIV-LTR. Certain viruses which commonly infect HIV+ individuals also encode proteins which can complex with and inactivate p53, thereby promoting HIV transcription. These include the HBV X protein, E6 of human papillomavirus (HPV), and EBV nuclear antigen (EBNA)-V. Immediate early proteins produced by CMV can *trans*-activate the HIV LTR, a phenomenon also documented with other viruses that frequently co-infect HIV+ individuals, including HTLV-I,II, EBV, HSV-1,2, and JC papovavirus (32).

Apart from direct *trans*-activation of the HIV-LTR, or suppression of p53 activity and thus indirect promotion of transcription, viruses common in HIV infected individuals may also facilitate HIV replication by other mechanisms. Unlike the DNA tumor viruses, for which the order of gene expression is transcrip-tionally regulated, this process is controlled entirely at the post-transcriptional level in HIV (35). EBV may act on HIV through its latent membrane protein (36), and parvovirus via an early non-

structural gene product (37). HSV-1 encodes a late gene product, Us11, which can post-transcriptionally activate the expression of HIV-1 envelope proteins via interaction with a regulatory protein of HIV, Rev, which regulates the nuclear export of certain HIV transcripts (38). Many of the herpesviruses and other viruses also induce cellular heat shock stress proteins, which can modulate HIV transcription (32).

It should be emphasized that the clinical signiÞcance of *in vitro* phenomena dependent upon co-infection of single cells with two viruses is of much less impact than the broad inßuences on HIV replication that might be elicited by indirect mechanisms such as activation of cell stimulation and cytokine production. Indeed, some viruses implicated as cofactors in HIV/AIDS grow preferentially not in typical HIV host cellsÑ CD4+ T lymphocytes, monocytes and microgliaÑ but in neuronal and epithelial cells, and they often inhibit host cell gene expression soon after infection (35). This would be expected to down-regulate HIV as it is dependent upon host cells to express its genetic material.

CLINICAL EVIDENCE FOR VIRUSES AS CO-FACTORS IN AIDS

Cytomegalovirus

Selection of an appropriate *in vitro* system to predict *in vivo* effects of any virus as a cofactor for HIV disease is important, but not yet established. For example, transient activation of HIV-1 by CMV products can occur in cells in which HIV gene expression is limited, but under conditions in which both HIV and CMV undergo fully permissive infection, CMV may *repress* HIV expression (39). A similar problem arises in experiments with other herpesviruses.

Clinically, the risk of developing AIDS among hemophiliacs in the 1.3 to 9 years from time of Þrst recognition of HIV seroconversion was 2.5 times greater in individuals who acquired CMV than in those who remained CMV seronegative ($P = 0.020$) (40). This correlation was supported by the Þnding that the mean decline in CD4+ T cells in HIV-seropositive/CMV seronegative asymptomatic individuals was 85 cells/mm^3/year, but 170 cells/mm^3/year in the CMV seropositive (41). The presence of multiple CMV strains in HIV-1+ homosexual men is also associated with progression to AIDS (42). The extent of peripheral HIV replication and the rapidity of CD4+ T cell decline is highly correlated with CMV antigenemia (43,44).

Findings from those early studies were recently conÞrmed in a French cohort of 290 subjects who were HIV+ but CMV seronegative at the time of enrollment (45). After a median follow-up of 85 months, there were 61 CMV seroconversions. Although the risk of progression to a CD4+ T cell count below 200/mm^3 was not increased in the seroconverters, the risk of progression to AIDS was increased 2.1-fold ($p = 0.01$) (45).

One might anticipate that immune reconstitution associated with HAART might mitigate the pathogenic effects of a latent or newly acuired CMV infection. However, antigen-speciÞc CD4+ and CD8+ T cell defects often persist despite HAART, and have been reported in association with recurrent CMV disease (46).

Human Herpesvirus Type 6

HHV-6 can infect myriad types of hematopoietic cells, including CD4+ T lymphocytes (32). Nearly all people in the U.S. show evidence of infection by age two, but only a few healthy individuals continuously secrete HHV-6 in their saliva and blood. HHV-6 can trans-activate the HIV-LTR and promote the release of cytokines which stimulate both cell and HIV proliferation. But, despite the ability of HIV and HHV-6 to co-infect cells, HHV-6 does not appear to predispose to or affect the course of HIV infection (47,48). Indeed, some studies indicate that HHV-6 can inhibit, rather than activate, HIV *in vitro* (49), and HHV-6 genomes are found at higher frequency in individuals with higher CD4+ T cell counts (50).

Regardless of the actual HIV/HHV-6 interactions, if any, which occur *in vivo*, HHV-6 pneumonitis may be a cause of signiÞcant pathology in the setting of HIV (51). There also may be a link between HHV-6 and the promotion of B cell lymphomas in HIV+ individuals (52).

Human Herpesvirus Type 7

HHV-7 infection is almost universal among people in the U.S. by age Þve. In contrast to HHV-6, up to 80% of adults actively shed HHV-7 in saliva. Clinically, it has been associated with a roseola-like infection, as has HHV-6. HHV-7 is also a CD4+ T cell-tropic herpesvirus, using CD4 as its high-afÞnity receptor (53). This suggests the possibility that competition between HIV and HHV-7 for CD4 sites might be used to inhibit HIV infection. *In vitro*, HHV-7 is a potent inhibitor of HIV infection in monocytes, despite the inability of HHV-7 to replicate actively in such cells (54).

Human Herpesvirus Type 8

HHV-8, or KaposiÕs sarcoma (KS) associated herpesvirus (KSHV), was originally discovered in KS tissue from HIV+ patients through the application of representational difference analysis to isolate DNA sequences in KS that were absent from DNA of normal tissue (55). It has a high degree of homology to two γ-herpesviruses, EBV in humans, and *Herpes saimiri* in monkeys (56). It is also

present in non-HIV associated KS, and in two disorders increased in incidence in the setting of HIV: a rare form of B cell lymphoma known as body cavity-based or primary effusion lymphoma, and Castleman's disease, a multicentric angiolymphoproliferative hyperplasia (57).

The exact role of HHV-8 in causation of these disorders, and in the progression of HIV disease in general, is unclear. Identification of HHV-8 genes with homology to cyclin D and G-protein-coupled receptors, factors which could confer oncogenic potential (57), support its clinical relevance.

Herpes Simplex Virus, Types 1 and 2

Coinfection of keratinocytes and macrophages by HSV-1 and HIV-1 has been noted within cutaneous HSV lesions, with cyclical enhancement of proliferation of both viruses in single cells (58). In addition, transient but marked increases in HIV viremia may occur during clinically apparent HSV-1 reactivation (59). Serologic studies suggest that HSV-2 infection is a risk factor for subsequent or concurrent HIV infection (60), but these data give no indication of mechanism. HSV-2 may facilitate transmission of HIV, as it causes blistering, erosion, and bleeding of penile, ano-rectal and vulvovaginal mucosa, and HIV-1 has been isolated from such genital ulcers (61). Reciprocally, HSV-2 appears to be shed from the genital tract more commonly in individuals infected with HIV (62). This appears to be a common finding for all herpesviruses examined in HIV disease.

In one recent study of HSV/HIV coinfected women, 70% and 79% of subjects shed HSV from the oral cavity and anogenital area, respectively (63). Shedding of HSV occurred for a mean of 3.2 days for oral and 5.4 days for anogenital regions, but plasma HIV viral loads did not fluctuate with such shedding (63).

Human T Cell Lymphotropic Virus, Types I and II

Several independent reports (64–66) have implicated HTLV-I in the progression of HIV-1 disease. HIV/HTLV-I co-infection is a serious problem worldwide. Prevalence of HTLV-I is high in many developing countries where HIV is also common, including Haiti (13% coinfection) and northeast Brazil (19%) (66). Such co-infection is associated with much higher CD4+ lymphocyte counts and percentages at all clinical stages than is typical for HIV infection in the absence of HTLV-I (66). These higher mean CD4 counts do not offer immunologic benefit, however, suggesting that the T cells in such individuals are functionally effete. This has led to speculation that CD4+ T cell guidelines for initiation of prophylactic antibiotics and antiretroviral therapy may be inadequate for HIV+ individuals co-infected with HTLV-I (66).

In the U.S., anti-HTLV-I,II seropositivity has been reported in 8–19% of HIV+ individuals who were injection drug abusers or attended sexually transmitted disease clinics (66); however, most of these infections involve HTLV-II, not HTLV-I. The distinction between HTLV-I and HTLV-II infection is critical, and cannot be made on the basis of simple ELISA serology screens; competitive peptide ELISAs or PCR technology must be used. HTLV-II has not been linked to acceleration of HIV disease (66,67). This may relate to the fact that HTLV-I is associated with higher rates of spontaneous lymphocyte proliferation than HTLV-II (68).

Hepatitis Viruses

Five hepatitis viruses, A through E, cause more than 80% of cases of viral hepatitis (69). Extensive efforts are ongoing to identify etiologic agents for the remaining one-fifth of cases, which translates into some 68,000 annual instances of post-transfusion hepatitis of unknown etiology in the United States alone (70). Complicating this issue is the fact that definitive evidence for the involvement of many of the newer parenterally transmitted agents in acute or chronic liver damage is lacking. However, some interesting and clinically important interactions between the hepatotoxic or perhaps just hepatotropic viruses and HIV have recently been documented.

Hepatitis A

In a series of 9 HIV+ patients who developed acute hepatitis, all patients spontaneously recovered and there was no significant change in CD4 count or viral load in the six months following hepatitis diagnosis (71). Although HAART was suspended for the 7 of 9 patients who were receiving it, there is no evidence that HAART has a detrimental effect on the course of hepatitis A disease (72).

Hepatitis B and C

The HBV X gene can *trans*-activate HIV *in vitro* (73), but HBV infection appears to be unrelated to progression of immune deficiency or clinical symptoms of HIV infection in most studies (74,75). The reciprocal situation may be important, however. Hepatitis viruses A through D are prevalent among patients at risk for HIV, and the course of HBV, HCV, and delta virus/HBV disease does appear to be accelerated by HIV (75).

Increased carriage rates and replication of HBV, decline in anti-HBsAg titers, reactivation of latent HBV, and reinfection with another subtype of HBV have all been reported in HIV+ patients (75). HBV is a factor in the development of hepatocellular carcinoma in these individuals, as in HIV seronegative patients, a correlation similar to that between cervical and anal carcinoma and HPV (76),

TABLE 6.2. *Viral co-factors for neoplasia in HIV infection*

I. **Common associations, in the presence or absence of HIV**	
Malignancy	Viral Co-factor
Cervical carcinoma	HPV-16,18
Anal squamous cell carcinoma	HPV
Hepatocellular carcinoma	HBV
Adult T cell leukemia/lymphoma	HTLV-I
II. **Associations particularly important in HIV infection**	
High-grade B cell lymphomas	EBV
Kaposi's sarcoma	HHV-8
Body cavity based lymphoma	HHV-8

and EBV and malignant lymphomas (77). These associations are listed in Table 6.2.

HCV infects an estimated 200–400 million people, or up to 3% of the global population (78). HCV-related cirrhosis is poised to become the leading cause of death in patients coinfected with HIV (79), presently about one-half of the HIV+ population in resource-rich nations (80,81). Acknowledging that HIV markedly increases the 20% twenty year risk for cirrhosis found in HIV negative, HCV+ cohorts, in 1999 the U.S. Public Health Service added progressive chronic HCV infection to its list of AIDS-deÞning opportunistic infections (82).

Apart from virus-speciÞc cofactors, synergy among hepatitis viruses and HIV with anti-HIV therapies is an additional means by which infectious cofactors could augment the pathogenicity of HIV. This may be most evident with HCV. For example, six large clinical studies found that between 2% and 18% of HIV+ patients suffered severe transaminase elevations following HAART; most of this liver injury was related to pre-existing HCV infection (83).

A retrospective analysis of all HIV+, antiretroviral-naive patients seen in a single hospital in Madrid, Spain from January 1997 to January 2000 was conducted to examine HIV-hepatitis virus interactions (83). Mean follow-up was 245 days. Of the 222 individuals identiÞed, 38% were coinfected with HCV, 5% with HBV, and 2% with hepatitis D. Transaminase elevations of any grade were seen within a median of six months of HAART initiation in 31%. SigniÞcant independent variables for this occurrence included alcohol abuse, HCV infection, and didanosine therapy. Use of the nucleoside reverse transcriptase inhibitor lamivudine was linked to a highly signiÞcant protection against liver damage ($p = 0.001$). Lamivudine is an inhibitor of HIV as well as HBV replication (84), as both require reverse transcriptase at some point in their life cycle. However, speciÞc note of the subsets of patients showing such protection with lamivudine was not provided, and only 5% of the cohort were HBV infected.

Severe transaminitis was seen in only 5% of HCV negative study subjects, but in over triple this fraction, 16%, of individuals with HCV coinfection (83). Similar enzyme elevations were recorded in 27% of HBsAg+ subjects and in 8.5% of HBsAg negative subjects. There was no relationship between classes of drugs used in the various HAART regimens and either incidence or severity of ALT/AST elevations. In a multivariate analysis, alcohol abuse, anti-HCV antibody, and older age (greater than the median of 35 years) were the only signiÞcant variables for development of grade 3 or 4 liver injury.

HBV infection was not an important factor in this group. Indeed, it was noted almost a decade ago, pre-HAART, that the overall prevalence of HBV markers is greater in HIV+ patients who progressed to AIDS (85). But this difference, though signiÞcant, was small (89% vs. 80%). Although the prevalence of HBsAg, a marker of persistent infection, was twice as great in those who progressed to AIDS (10.7% vs. 4.7%), a survival difference attributable to HBV was not seen (85). However, HBV reactivation of various degrees of severity, including fulminant hepatitis, does develop in 20–50% of HIV negative patients undergoing immune suppressive treatments or cancer chemotherapy (84). The frequency of such reactivation may be reduced by lamivudine prophylaxis (84), something which might be considered in the HBV/HIV co-infected population in late stages of immune suppression.

HCV is currently the more pressing issue. It is unclear why the profound interaction between HAART and HCV noted in the Madrid cohort and several prior, smaller studies occurred. The mitochondrial toxicity typical of all antiretroviral drugs is one possibility. Immune-mediated damage, elicited by the partial immune recovery typical of HAART and involving HCV antigen targets on hepatocytes, is an intriguing mechanism. This might be part of the immune reactivation syndromes, involving cytomegalovirus, Mycobacterium avium complex, and possibly other opportunistic infections, documented within days to weeks after initiating HAART. The authors of the Madrid analysis concluded that a more aggressive stance against chronic HCV infection should be taken, including pre-treatment with α-interferon and ribavirin. ÒThe risk of

developing severe hepatic injury on HAART should be lower on a ÔhealedÕ liver,Ó they felt (84).

A recent pathologic study (86) of 58 HIV/HCV coinfected patients showed advanced Þbrosis in 45% of those with CD4 counts <200, compared with only 10% in HCV+/HIV negative controls. Care was taken to match groups by other risk factors for advancing liver disease, including age at infection, duration of infection, sex, and alcohol use. As Þbrosis was more severe in those with CD4 counts <200 (45%) vs. those >200 (17%; $p=0.04$), enhanced HCV replication in the face of increased HIV-related immune suppression was suggested (86).

In conclusion, up to 18% of HIV-infected patients who start HAART have a severe rise in transaminases. Most of this rise is attributable to alcohol use and HCV infection. Attempts to limit the former, and perhaps to treat the later prior to initiating HAART, may be warranted. Some of the increases in ALT/AST on HAART will resolve spontaneously. But, as patients with HIV disease live longer, mortality from HCV and HBV liver disease is expected to account for over one-third of all AIDS-related deaths in the industrial world (87).

TT Viruses

TT virus (TTV) is a non-enveloped, circular, single (minus)-stranded DNA virus of 3.8kb Þrst isolated from a patient with post-transfusion hepatitis in Japan (88). It has been provisionally classiÞed in the Circoviridiae family and is transmitted parenterally. It is also shed via the bile into feces, making fecal-oral transmission a potential route (88). Persistent infection with TTV of genotypes 1Ð16 is common among healthy individuals (5%), but markedly increased in the HIV+ population (23%) (88).

The high titer viremia observed in AIDS patients and those with high HIV load or low CD4 counts suggests that TTV replication is facilitated in the immune compromised (88). Whether TTV itself mediates any aspect of HIV-related disease is unclear.

Hepatitis virus GB-C

There is some potential good news from the hepatitis-HIV coinfection saga. GBV-C, also referred to as hepatitis G (HGV), is a common, usually non-hepatotoxic RNA virus of the Flaviridae family related genetically to hepatitis C. But, unlike virtually every viral agent discussed thus far, it appears to *decrease* HIV mortality (89,90).

The pedigree of the GB viruses A through C is confusing, but well outlined in one review (91). Brießy, it was discovered in 1967 that acute phase plasma from surgeon GB, who acquired hepatitis within four weeks of becoming icteric, caused transaminitis when inoculated into tamarin monkeys. Sera from these animals were used in serial passage experiments with additional tamarins. Then, using representational difference analysis, previously mentioned in the context of the discovery of HHV-8, two novel clones of virus were obtained from the 11th monkey passage. One of these, GBV-A, is almost certainly a monkey virus contaminant. The other, GBV-B, may have been the etiologic agent of the surgeon GBÕs hepatitis.

Based on recombinant proteins prepared from GBV-A and -B genetic material, ELISAs were developed and used to screen blood obtained from West African natives. One positive sample yielded GBV-C. HGV appears to be an independent isolate of a GBV-C virus.

Although GBV-C is clearly hepatotropic, it is an uncommon cause of liver disease. But it has grown in prominence because of its salutary effects on HIV mortality.

One recent study involved 197 HIV+ patients, 33 with detectable GBV-C RNA, 112 anti-GBV-C antibody positive, and 52 GBV-C negative, that have been followed since 1993 (90). Survival and progression to AIDS were signiÞcantly prolonged in those with GBV-C antibody. GBV-C positivity also correlated with a lower HIV viral load, but not with higher CD4 counts.

A second study (89) involved 362 HIV+ patients, 144 with GBV-C viremia. Again, the mortality rate for those coinfected was signiÞcantly decreased. 28.5% of the GBV-C group had died over the mean 4.1 year follow-up, compared with 56.4% of the controls ($p<0.001$).

As with other infectious cofactors for HIV, it is unclear whether GBV-C is directly involved, or simply a marker for something else. Nor is it clear, should this virus prove protective against HIV progression, if it needs to coinfect the same cell to reduce HIV replication. Given the paucity of cells typically infected with HIV, this would be unlikely. Peripheral blood cells infected with HIV *in vitro* produce lower levels of HIV Gag core antigen (p24) when GBV-C is added to the cultures, despite the fact that membrane levels of the high afÞnity HIV receptor, CD4, and its chemokine co-receptors, CXCR4 and CCR5, were unchanged (89).

Approximately 2% of voluntary blood donors, and up to 20% of injection drug users in the U.S. have detectable levels of plasma GBV-C RNA (89,92). It is not screened for in blood collections. Whether this epidemiologic correlation can translate into new forms of HIV therapy is unclear, but clearly in the investigatorsÕ minds.

ADDITIONAL CONSIDERATIONS

Viral Suppression of HIV Infection

Apart from the apparent suppression of HIV replication *in vivo* suggested by epidemiologic and *in vitro* studies of GBV-C, at least two other viruses and one bacterium have been linked to similar interactions. All may provide leads

to new avenues of anti-HIV therapy. *In vitro*, HHV-6, described above, as well as inßuenza virus (93) may inhibit HIV replication. *In vivo*, *Orientia tsutsugamushi*, the etiologic agent of scrub typhis, has a similar effect (94). Finally, plasma levels of two potential HIV suppressive factors, RANTES and IL-10, were elevated during acute measles virus infection, and acute measles infection was associated with marked decreases in plasma HIV load (95). Measles virus is highly immune suppressive *in vivo* (96); dampening of certain types of immune activation which facilitate HIV replication and augment activation induced cell death (AICD) by measles may account for its salutary role in HIV coinfection.

Viral Immunizations

It is reasonable to speculate that any stimulus to the immune system, whether by intercurrent infection, exposure to novel or recall antigens, injury accompanied by inßammation, or immunizations might enhance HIV expression, at least transiently. This is consistent with the fact that primary and secondary effector cells and memory cells express a variety of activation antigens and accessory molecules not found on naive lymphocytes (97). An elevation in numbers of infected PBMC, ranging from 2- to 25-fold, was seen within one to two weeks of administration of inactivated vaccinia or HBV vaccines to HIV-1 infected chimpanzees (98). This parallels the increase in HIV transcripts (>10-fold) and viral titers (>5-fold) seen in plasma following inßuenza immunization of HIV+ individuals (99). A peak in virus replication was noted at one to two weeks after immunization, falling to baseline values only after several months. This raises the issue as to the risk-beneÞts of certain vaccinations in HIV+ individuals, particularly for those diseases, such as inßuenza, which do not cause increased mortality in the setting of HIV.

However, recent records from the Adult and Adolescent Spectrum of HIV Disease Surveillance Project, involving more than 25,000 HIV+ patients between 1990 and 1999, showed that there was a slight decrease in the progression to AIDS-deÞning opportunistic disease among patients who were vaccinated against inßuenza (100). Thus there appears to be no clinical risk to such vaccinations. The basis for the ostensible improvement in mortality could not be determined, and may have been related to increased preventive care or overall interest in physical health (100).

Superantigen Effects And Host Cell Activation

In vivo animal data support the concept of a clinically relevant viral SAg effect, similar to the *in vitro* effects described earlier in this chapter. Pre-activation of uninfected CD4+ T lymphocytes may also lead to enhanced viral replication and accelerated T cell death following viral exposure. This has been demonstrated in animals inoculated with PBj-14, an unusual isolate of the simian immunodeÞciency virus (SIV) which, in pig-tailed macaques and cynomolgus monkeys, leads to immediate skewing of CD4+ T cells towards an activated phenotype and death of all animals within six to ten days (101). In contrast, rhesus macaques, with fewer circulating activated T cells pre- and post-viral exposure, have a much more prolonged course following PB_j-14 inoculation (101). Unlike HIV, for which a direct SAg effect has not been demonstrated, this simian isolate has a SAg-like effect, *in vitro* and *in vivo*, inducing T cell proliferation in a V- restricted manner.

Pre-existing target cell activation has also been linked epidemiologically to enhanced susceptibility to acquisition of HIV infection (102). This may account, at least in part, for the facility with which HIV appears to be acquired heterosexually in Haiti, Africa, India, and parts of Southeast Asia, where multiple parasitic and venereal infections among HIV at-risk populations are associated with activated T cells, both in the lymphoid network and in mucosal tissues (18,102).

Expansion of Cellular Targets for HIV

It has been suggested that the AIDS epidemic in Africa is distinct in comparison with other areas of the world in terms of clinical manifestations, if not rate of disease progression (103). Immune activation linked to viral, bacterial and protozoal cofactors have been implicated in this difference (103). For example, HIV co-receptor expression may be modulated by environmentally-driven infectious co-factors. CCR5-speciÞc transcripts and protein were signiÞcantly increased in HIV negative individuals of Italian or African descent living in sub-Saharan Africa, but not in African subjects living in Italy (104).

Other receptor-based phenomena may link HIV and viral cofactors. CMV can code for or induce expression of Fc receptors on CD4 negative cells, enabling HIV to infect such targets via antibody-HIV complexes taken up by Fc (32). HHV-6 can induce *de novo* expression of the high-afÞnity receptor for HIV, CD4, on mature CD8+ T cells, again potentially broadening the range of HIV susceptible lymphocytes (47). It may also form viral pseudotypes with HIV, as can several of the herpesviruses. This would expand the host range for HIV, at least through one life-cycle, as the outer coat and receptor afÞnity of the herpesvirus, packages and transports the genetic material of HIV (32). But these receptor strategies may go both ways, depending upon the cofactor virus involved. For example, as discussed earlier, HHV-7 can compete with HIV for CD4 binding, blocking HIV infection of CD4+ T cells *in vitro*.

The chemokine co-receptors may also be inßuenced by viral co-factors, either directly or via cytokine-mediated

processes (28). Such interactions have been extensively studied with a probable bacterial co-factor for HIV disease, *Mycobacterium tuberculosis* (105). Expression of HIV co-receptors CXCR4 and CCR5 is elevated on CD4+ T cells from patients with active tuberculosis as well as T cells obtained from healthy donors and exposed to mycobacterial lipoarabinomannan *in vitro* (106).

VIRAL COFACTOR-BASED THERAPEUTIC STRATEGIES IN HIV/AIDS

Careful consideration of viral cofactors implicated in the clinical progression of HIV disease should have a direct bearing on the design of new forms of therapy for HIV infection (Table 6.3). Apart from preventionÑ the use of CMV negative blood in CMV seronegative patients, for exampleÑ anti-viral drugs would be the most direct means to suppress cofactor viruses. The probable clinical impact of anti-herpes drugs in HIV/AIDS has been shown in retrospective analysis of HHV-8 linked Kaposi sarcoma (107).

Agents that block virally-mediated HIV target cell activation should inhibit induction of HIV, regardless of their impact on cell or viral proliferation (15). Cell activation with induction of protein kinase C is essential for HIV-infected T cells to become fusogenic and form multinucleated giant cells. Cholera toxin, an inhibitor of protein kinase C through its effect on inositol triphosphate and diacylglycerol production, and H7, an isoquinoline and non-competitive inhibitor of protein kinase C, can both prevent unstimulated CD4+ T cells from forming syncytia (108). While these drugs are too toxic for *in vivo* use, suppression of inappropriately activated T lymphocyte and APC populations has been accomplished clinically in HIV/AIDS utilizing cyclosporin A (CsA) (109) and glucocorticoids (110).

CsA and glucocorticoids may also directly suppress HIV replication. CsA disrupts the interaction between Gag core protein and cellular cyclophilins A and B, associations essential in the life cycle of HIV (111). Glucocorticoids are of particular interest, as various disorders frequently found in HIV disease are treated with short-term or prolonged courses of steroids, including severe *Pneumocystis carinii* pnemonia, immune thrombocytopenic purpura, and nephropathies. Surprisingly few adverse reactions have been reported with their use (110,112). Glucocorticoids can suppress HIV replication *in vitro*, at least in T cells, through speciÞc interactions with the HIV LTR (113). They can also block HIV-associated induction of apoptosis or accelerated programmed cell death (110). Apoptosis is initiated in activated T cells by several viruses, including EBV, vaccinia (114), and HIV (115), and may be pathophysiologically relevant in HIV disease (116).

CsA has yielded the most intriguing results, though in speciÞc types of HIV+ patients. For example, nine adults with primary HIV-1 infection were treated with kidney transplant-level doses of CsA, 0.3Ð0.6mg/kg, orally every 12 hours, along with HAART (117). CsA was discontinued at week 8. Viral replication was suppressed to equivalent levels in these patients and 29 contemporary controls receiving HAART alone, with approximately a 4 log decrease in viral load maintained through the 64 week study. CsA had no adverse effect on virus-speciÞc CD8 or CD4 responses. But CsA was associated with a much greater rise in CD4 counts, returning them to normal levels by week 8, a change which persisted and increased throughout 64 weeks of observation. CD4+ naive and memory cells were elevated to equivalent degrees.

The authors of this trial noted that Òthis is the Þrst study that provides proof that the beneÞts achieved with HAART during primary HIV-1 infection may be extended via the use of an immune-modulating strategy that interferes with early pathogenic eventsÓ (117). However, these results may not be generalizable to the vast majority of cases of HIV, which are not detected within the seroconversion period.

For example, a randomized, double-blind, placebo-controlled trial of a higher dose of CsA (2mg/kg, orally twice daily) for 12 weeks in 15 individuals on no therapy and 13 on HAART showed no beneÞt in these patients with stable, early HIV disease (118). It was also associated with a small but signiÞcant rise in viral load, concerns that led the investigators to advise caution in monitoring organ transplant patients receiving CsA (118). Given the recent interest in immune activation states as major contributors

TABLE 6.3. *Summary of mechanisms by which viruses may serve as co-factors in HIV pathogenesis*

- Activation of cellular targets for HIV infection
- Induction of proliferation in resting, latently infected cells via mitogenic, antigenic, or superantigen stimulation
- Induction of cytokines or stress molecules (e.g. heat shock proteins) which act to transcriptionally activate HIV replication, or cellular proliferation, or both
- Induction of chemokines which modify disease manifestations through altering the traf cking of HIV+ and HIV uninfected cells
- Upregulation or suppression of HIV receptors
- Formation of viral pseudotypes with altered host tropism
- Coinfection of single cells, either directly stimulating or suppressing HIV replication, by both transcriptional and post-transcriptional mechanisms togenic, antigenic, or superantigen stimulation

to T cell death in AIDS, I suspect that many more immune suppressants will be tried in combination with HAART.

Novel applications of agents such as hydroxyurea (119), which can affect the size of intracellular deoxynucleoside pools essential for HIV replication, are also being pursued, along with certain antiretroviral drugs, in the combination therapy of HIV disease. Finally, speciÞc cytokine inhibitors, including pentoxyÞlline (120) and thalidomide (N-pthalimido-glutarimide) (121), which block TNF-α effects, can inhibit HIV replication, *in vitro* and *in vivo*. The challenge is to deÞne how one might take advantage of interactions between HIV and viral and other cofactors for HIV progression to anticipate the need for pharmacologic intervention, and have a panoply of therapeutics available (122).

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Chapter 7

Virologic and Biologic Features of Human Immunode ciency V irus Type 2 (HIV-2)

Jean-Louis Sankalé and Phyllis J. Kanki

Human immunodeÞciency virus type 2 (HIV-2) is the second human immunodeÞciency virus in the class of human retroviruses, it constitutes the closest known virus related to the prototype AIDS virus, HIV-1. HIV-2 shares many virologic and biologic features with HIV-1, both being transmitted by the same routes, both infecting the same cells, and both exhibiting considerable genetic variation in the outer envelope gene. As with other retroviruses, both HIVs induce lifelong infection, with permanent integration of viral genetic material into the host cellÕ DNA. Based on these initial similarities, some believed that HIV-2 might cause a second worldwide AIDS epidemic. However, over 17 years since its discovery, research studies conducted both in the laboratory and in HIV-2 infected people have highlighted distinct biological differences between these related viruses (1,2). Some of these unique properties include a distinct global distribution of the virus with limited spread, signiÞcantly reduced perinatal and sexual transmission, slower rates of progression to AIDS and the potential protective effect of HIV-2 infection on subsequent HIV-1 infection (2). A complete review and update of all aspects of HIV-2 infection are beyond the scope of this chapter, rather, the purpose is to highlight those biological aspects of HIV-2 which have been of most interest from a comparative perspective. Based on current understanding, the distinct biological differences between these related viruses suggest that viral properties may be more responsible than host determinants for the unique pathogenic mechanisms employed by HIV viruses in general. It is hoped that the further characterization of such properties will be useful for the design of effective HIV interventions.

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GENETICS OF HIV-2

The antigenic relatedness of both SIV and HIV-2 to the prototype HIV-1 virus prompted both the discovery and further classiÞcation of these related viruses (3Ð5). When sera from West African female sex workers were screened for antibodies to HIV-1 antigens, testing revealed extensive cross-reactivity for the virus core antigens but minimal antibody binding reactivity for the HIV-1 envelope (3). Yet, when the same West African human sera were assayed on SIV antigens, they reacted strongly with the envelope proteins as well as the core antigens, suggesting infection with a virus that was more closely related to SIV than HIV-1. As more sequence data have become available from various HIV-2 and SIV strains, it has also become apparent that no branching order of divergence can be speciÞed and that these virus types may in fact share a common ancestor (6,7)

It has been estimated that the HIV-1 and HIV-2/SIV groups of viruses might have diverged from each other as recently as 50Ð60 years ago (8,9). The SIVs of mangabeys and macaques are the closest relatives of HIV-2s (10Ð12). As a group, the SIVs apparently vary more between strains recovered from between different species and subspecies of monkeys than different HIVs vary from each other (10,12Ð14). As HIV-2s appear to be largely limited to West Africa, it is not surprising that they are most closely related to SIVs originating from monkeys in that region (10Ð12).

By comparison to HIV-1, the genetic diversity of HIV-2 is less extensive and only two subtypes (A, B) have been well characterized; other studies have reported the existence of four additional subtypes (C, D, E and F), but subsequent attempts to isolate viruses or obtain additional samples to sequence from these identiÞed subtypes have been unsuccessful (15). An unusual HIV-2 isolate Abt96 from an asymptomatic blood donor from Ivory Coast has

been sequenced and phylogenetic data suggest a novel HIV-2 subtype, preliminarily designated as subtype G. Thus far, HIV-2 subtype A is the most well characterized subtype and appears to be the major variant circulating in West Africa (16–20) The Ghanaian isolates, subtype B (21,22) are believed to be most distant from the prototype HIV-2/-mangabey/macaque virus (10). A recent study from Ivory Coast, however, suggests a predominance of HIV-2 subtype B in that country (23). Similar to the situation with HIV-1 subtypes, the potential impact of subtype differences on the epidemiology, pathogenicity and transmission of HIV-2 is not yet well appreciated.

HIV-2 VIROLOGY

HIV-2s are spherical, 100–120nm virions, morphologically similar to HIV-1s. The length of the entire genome of HIV-2 is about 9 kilobases similar to HIV-1. The open reading frames of HIV-2 are also similar to those of HIV-1. For different HIV-1, HIV-2, and SIVs, the genes may be in different reading frames, but the level of conservation within each gene is high. The gag and pol messages are unspliced, the env singly spliced, and the major regulatory genes doubly spliced and made from multiple messages. The regulatory gene sequences appear to vary more among HIV-2s when compared with HIV-1s (6,24,25). HIV-1 has one gene, vpu, which is not found in HIV-2 (26–28). However, the function of the HIV-1 vpu, namely the ability to increase viral particle release, may be provided by HIV-2 envelope (29). HIV-2 has one gene, vpx, which is not found in HIV-1 (30–33). While a complete review of HIV-2 virology is beyond the scope of this chapter, speciÞc updates are highlighted herein.

HIV-2 infects the same range of cells as HIV-1, including CD4 bearing helper T-lymphocytes, monocytes, and macrophages, and microglia cells in the central nervous system. Although HIV-2 infects through the same CD4 receptor, it apparently does so with a 10- to 100-fold greater afÞnity than HIV-1 (34–36). Although the HIV-2 envelope protein appears to be slightly less glycosylated than in HIV-1, this does not appear to account for the differences in binding efÞciency (36). It is believed that infection by HIV-2 is inhibited more efÞciently by soluble CD4 than is HIV-1 (37).

Several members of the chemokine receptor family have been shown to function in association with CD4 to permit HIV-1 entry and infection in various types of cells (37–39). Similar to HIV-1, certain HIV-2s have been shown to utilize Fusin/CXCR4, as both a coreceptor (in the presence of CD4) and as an alternative receptor, in the absence of CD4 (38,40). HIV-2 viruses appear to be more promiscuous in their utilization of various coreceptors, compared with HIV-1, including CXCR4, CCR15, and the coreceptors designated BONZO/STRL33 and BOB/GPR15 (38,40,41). Most CCR5-requiring isolates are non-syncytium inducing, whereas isolates utilizing multiple coreceptors were syncytium inducing. However, the efÞciency with which the coreceptors are utilized by HIV-2, appears to vary greatly among various isolates (42).

In vitro studies of HIV-2 isolates by a number of laboratories have described differences in cytopathicity of HIV-2 as compared with HIV-1 (43–46). In comparison with HIV-1, HIV-2 isolates demonstrate decreased cell killing, less syncytial cell formation, reduced virus replication, and differences in interaction with CD4, in some cases related to the clinical stage of the HIV-2-infected individual (46). The V3 loop of rapid/high HIV-2 isolates is signiÞcantly more heterogeneous and has a higher net charge than the V3 sequence from slow/low isolates. Mutations at amino acid positions 313 and 314, to positively charged amino acids, has also been signiÞcantly associated with the rapid/high phenotype; similar to that of HIV-1 (46). These amino acid changes in the V3 loop of HIV-2 also appear to determine the co-receptor usage for CXCR4 and CCR5. The distinct co-receptor usage of two reference HIV-2 molecular clones allowed identiÞcation of the region of gp120 important for co-receptor speciÞcity. By constructing a series of chimeric viruses between GH-1 and ROD, the C-terminal half of the V3 loop region of gp120 was found to determine the differential co-receptor usage between GH-1 and ROD. Notably, the shift in the co-receptor usage from CCR5 to CXCR4 was associated with an increase in the net positive charge in the V3 region (47).

One of the most important characteristics of HIVs that makes them different from other retroviruses is the ability to regulate their own expression (48). The long terminal repeat (LTR) regions at the ends of the viral genome contain sequences necessary for activation of transcription and for termination of the transcripts, but an important element of control for these viruses occurs through RNA processing or splicing. The LTR of HIV-1 is more responsive to cellular activation signals than the HIV-2 LTR, indicating that HIV-2 has different response elements (49,50). Whereas HIV-1 has two NF-kB enhancer binding sites, only one can be identiÞed for HIV-2 or most SIVs (51). Studies also suggest that activation of the HIV-2 enhancer may require four or more cis-acting elements, most of which are not in HIV-1 (52). This may be of biologic signiÞcance, as studies of two different strains of SIV with dramatic difference in virulence *in vivo* have demonstrated the duplication of the NF-kB binding sites in the virulent strain (53).

The gag gene products are essentially the same for HIV-1 and HIV-2 (54) and are highly conserved. A 55-kD precursor is myristylated and cleaved by the viral protease to form p17 (MA), p24 (CA), p2, p7 (NC), pl, and p6. The matrix protein (MA) p17 is at the amino terminus, and in its cleaved form surrounds the nucleocapsid. The capsid (CA) p24 is slightly larger for HIV-2 or SIV and is sometimes described as p27 (3,55). It provides the structure for the core that contains the genome and the polymerase and integrase enzymes (56). Workers have identiÞed NC p7–8 as a nucleic acid binding protein (57),

and both zinc Þnger motifs are involved with the speciÞcity of packaging the genomic viral RNA into the virions (58).

The envelope glycoprotein precursor is slightly smaller for HIV-2, as are the external glycoprotein and the transmembrane protein. Although usually described using the terminology for HIV-1 (gp160, gp120, gp41, respectively), the HIV-2 glycoproteins are sometimes designated with lower sizes (e.g. gp140Ð145, gp105Ð110, gp32Ð40). By analysis of the primary amino acid sequence for N-linked glycosylation sites, the HIV-2 and SIV gp120s have signiÞcantly fewer conserved sites. This could presumably account for the lower molecular weight of the HIV-2 gp120, as carbohydrates make up 50% or more of the HIV-1 gp120 (59Ð61).

HIV-2 envelope sequences vary to approximately the same degree as HIV-1 sequences 124 30, 62Ð68). Although the V3 regions correspond in both virus types, HIV-2s do not have the conserved GPGR amino acid sequence at the tip of the V3 loop (30,67). External glycosylation sites appear to be conserved between HIV-1 and HIV-2 as are the sites for CD4 binding (6,69), precursor processing (70), fusion (70), principal neutralizing domain at V3 (67,71,72), cytolytic T cell activity (73,74), and perhaps for antibody dependent cell cytotoxicity (72,75,76). With HIV-1, much of the envelope glycoprotein appears to generate binding antibodies. Antibodies from people infected with HIV2, demonstrate binding activity concentrated in the middle of gp120, including the V3 loop (77,78), as well as the amino terminus of the transmembrane protein (77,79). Another region, at the carboxy terminus of gp120, may be slightly less immunogenic, but it appears to be the most cross-reactive between HIV-1 and HIV-2 (79).

The most type-speciÞc region of HIV-2 g120, the V3 loop region, is also the principal neutralizing domain, as it is for HIV-1 (67,71,72). Two distinct antigenic sites in the third variable region (V3) of HIV-2 have been identiÞed that appear to correspond to the principal neutralizing determinant (PND) of HIV-1; the conserved Phe-His-Ser-Gln and Trp-Cys-Arg motifs (positions 315Ð318 and 329Ð331) possibly interact to form a discontinuous antigenic site based on computer simulation modeling (80). However, other neutralizing regions of lower activity have been described, including a linear epitope in V2 and one conformational epitope outside V1, V2 and V3 (72,81). Some investigators have described cross-neutralization between HIV-1 and HIV-2 with human antibodies (82,83), although weakly bidirectional, they appear stronger for serum from HIV-2-infected people for cross-neutralization of HIV-1 (82,83).

As with HIV-1, the polymerase activity of HIV-2 is error prone, and as a result extensive variation occurs between isolates (84). This is primarily exhibited in the envelope gene against which much of the selective pressure of the immune system is exerted. This results in differences in env of as much as 1% per year for evolutionary selection (48,67). Limited studies on the inter-patient variability of HIV-2 have shown that the range of variability in the envelope V3 sequence is similar to the inter-patient variability of HIV-1 (16,67). Tissue-speciÞc quasi species has been identiÞed in HIV-1 infection *in vivo*, and this has also been demonstrated in the analysis of blood and brain viral sequences from an HIV-2 infected individual (68,85). Evaluation of intra-patient variation in the V3 envelope region of HIV-2 in asymptomatic and symptomatic individuals followed over time has shown a lower variation when compared to HIV-1 (16). This lower intra-patient variation appears to be a distinct feature of HIV-2 infection that may result from decreased viral burden and also contribute to lower rates of transmission and disease development.

HIV-2 DIAGNOSIS

Studies of HIV-2 epidemiology and natural history are heavily dependent on accurate HIV-2 viral diagnosis. The same procedures for serologic testing, virus culture, and detection at viral nucleic acids such as polymerase chain reaction (PCR), that were developed for HIV-1 have been modiÞed for HIV-2 diagnosis and improved over the years. The close relationship of HIV-2 to HIV-1 on a genetic and antigenic level has necessitated the development and implementation of type-speciÞc diagnostic assays. Because most of the tests were developed for HIV-1 using HIV-1 antigens, the degree of cross-reactivity and speciÞcity for HIV-2 is variable. Most of the Þrst generation tests used whole virus antigens, where core antigens such as p24 and pol p66/p51 are well represented. These antigens are more strongly cross-reactive than envelope antigens, especially gp120 (3,4,86,87).

Blood bank screening for HIV-2 was instituted in the United States in 1990, following the lead of many European nations. As a result, the vast majority of commercial HIV ELISA assays utilize a combined antigen source that incorporates HIV-2 speciÞc antigens. ConÞrmation of HIV-2 serostatus requires HIV-2 speciÞc immunoblot assay, or speciÞc peptide assays. Immunoblots demonstrating a proÞle of major structural gene products are typically used to conÞrm HIV-1 and HIV-2 diagnosis using standard criteria. HIV-2 speciÞc diagnosis by immunoblot requires antibody reactivity to env ± gag ± pol antigens. In the absence of reactivity to gag or pol antigens, the presence of reactivity to two envelope antigens is required (gpl2, and gp32 transmembrane protein) (88).

Various investigations have focused on identifying type-speciÞc antigens to allow conÞrmatory tests that will distinguish between HIV-1 and HIV-2. Most have been made as synthetic peptides (89Ð92), but some are larger bacterially expressed peptides (93,94); these peptides vary in sensitivity and speciÞcity. As most were selected for speciÞcity, one might expect that sensitivity could be

compromised. Thus, although appropriate for type-speciÞc conÞrmation, they may not be as useful as larger HIV-2-speciÞc antigens for initial screening.

The HIV dual antibody proÞle is characterized by antibodies with strong reactivity to the env antigens of both HIV-1 and HIV-2 by immunoblot and/or radioimmunoprecipitation analysis (RIPA) (3,87,93,95Ð97). Several possible biologic explanations for this phenomenon can be entertained including: extensive cross reactivity by either of the HIVÕs, dual infection or infection with a recombinant virus. When human serum samples have been tested in places such as Ivory Coast, Senegal, and Burkina Faso, a disproportionately large fraction of the samples often test as Òdual positiveÓ because they appeared reactive on both HIV-1 and HIV-2 conÞrmatory tests (4,94,98,99). These geographic sites have substantial rates of both HIV-1 and HIV-2 infection, and distinction of viruses and designation of dual reactivity remains a diagnostic challenge for the typical HIV laboratory.

Isolation of both HIV-1 and HIV-2 has been reported from selected HIV-dual positive case, and PCR evidence of both viruses has ranged from 30Ð80% in serologically deÞned dual reactives reported from similar populations (100Ð102). It is unclear whether the low and variable rate of concordance between serology and PCR is due to extensive HIV-1 cross reactivity as suggested, misclassiÞcation of samples based on serodiagnosis or insensitivity of the PCR assays. Improvement of PCR assays with Southern blot detection of ampliÞed product has maximized the sensitivity and speciÞcity for HIV-2 provirus detection (103,104). The further development of such assays will be critical to studies that seek to characterize HIV-2 biology and its interaction with HIV-1 *in vivo*. Largely due to such problems, it has been difÞcult to predict from the published literature what the interactions of these two distinct HIV type viruses would be in human populations. It seems clear that reports of dual infection in certain parts of Africa as well as the rest of the world, in the absence of HIV-2 infection alone, were more likely misinterpreted laboratory data than a true biological entity.

EPIDEMIOLOGY AND GLOBAL DISTRIBUTION

In 1985, the discovery of HIV-2 in West Africa prompted numerous serologic studies to determine the geographic distribution. Since that time, HIV-2 infection has been well documented in most West African countries, a distinctly different worldwide distribution compared to HIV-1 (2). Largely due to relatively low prevalence rates in most places outside of West Africa, it has been difÞcult to compare the prevalence rates in different countries due to differences in study design and diagnostic methodologies. Often times serosurveys were conducted with methods that failed to distinguish HIV-1 from HIV-2, and thus erroneously reported type speciÞc prevalence rates; in addition, there were difÞculties with diagnosis and distinction of HIV-dual infections resultant from the lack of HIV speciÞcity in the serologic or nucleic-acid based assays.

Isolation of a second retrovirus, HIV-2, led to fears that a second AIDS pandemic would occur, similar in scope and magnitude to that caused by HIV-1. However, the peculiar biologic properties of HIV-2, namely the lower transmissibility of this virus through both sexual and vertical routes, has contributed to a more regionalized endemic distribution of the virus. Studies conducted shortly after the discovery of HIV-2 found substantial rates of HIV-2 in a number of West African countries, frequently with low to absent rates of HIV-1 infection (4,105). In the late 1980s, in countries such as Guinea Bissau, Gambia, Cape Verde and Senegal, the prevalence of HIV-2 infection exceeded that of infection with HIV-1, but in most instances, HIV-1 infection has now increased and has exceeded rates of HIV-2 infection (4,98,106,107). Rates of HIV-2 infection are highest in sexually active populations such as commercial sex workers, sexually transmitted disease patients, prisoners and people hospitalized with infectious diseases (4,98,108,109).

In most other countries of West Africa such as Burkina Faso, Ghana, Ivory Coast, Nigeria, and Mali, infection with HIV-1 is also more prevalent than with HIV-2, ranging from a 3Ð24 fold rate ratio (HIV-1 versus HIV-2) (2,110Ð114). For example, in a study from Bamako Mali, HIV-2 infection was found in 3.9% of 176 commercial sex workers, in contrast to a 20.4% rate of HIV-1 infection. Comparison of previous HIV-1 and HIV-2 seroprevalence data from Mali shows a signiÞcant rise in HIV-1 prevalence and a signiÞcant decrease in HIV-2 prevalence and conÞrms similar trends observed in neighboring countries (19).

The existence of substantial rates of both HIV-1 and HIV-2 infections in many of these countries raised the question of what outcome would result from the interaction of these viruses at a population level. Anderson and May analyzed the available biological and epidemiological data on the pathogenicity, transmissibility and antigenic similarity of HIV-1 and HIV-2, and used simple mathematical models to study the competition between the two viral types (115). The mathematical model of the concomitant transmission of the two viruses transmitted within the same sexually active population, suggested a positive association between pathogenicity and reproductive success, suggesting that HIV-1 would competitively displace HIV-2 in the long term. This is supported by studies that measure and compare the rates of sexual and perinatal transmission of the two viruses, as well as the temporal reduction in HIV-2 prevalence throughout the region (116Ð119). Thus, the current data suggest that HIV-2 has been present in certain populations for a long time in order to establish endemic infection and its spread outside of these endemic areas is limited by a low transmission potential. It therefore seems unlikely that this virus will cause a global pandemic similar to that of HIV-1.

A second epidemiologic pattern of HIV-2 infection has been suggested from reports of HIV-2 in Portugal, Mozambique, Angola, southwestern India and Brazil, all areas with former ties to Portugal (120) which appear to have low but stable rates of HIV-2 in the population (111). These countries once shared common historical-political ties, with economic relationships existing even today. HIV-2 has also been detected in the large cities of southwestern India (121), perhaps because of interactions with the former Portuguese colonies of Africa. Goa, a former Portuguese colony, situated south of Bombay on the western coast, has reported 4.9% HIV-2 and 9.8% HIV-1 infection in STD patients (122), whereas, substantial rates of HIV-2 infection have not been reported in other parts of Asia to date.

TRANSMISSION OF HIV-2

Although HIV-2 is transmitted by sex and blood, as is HIV-1, the rate of infection in West Africa appears more stable than for HIV-1. In Senegal, during an eight-year period of follow-up, there was a 26-fold increase in the rate of HIV-1 infection, whereas the rate for HIV-2 infection remained relatively constant (116,123). This implies that HIV-2 may have been in the human population in Africa at least as long as HIV-1, and in West Africa HIV-2 has apparently been present considerably longer. The relative poveits of HIV-2 infections in Europe, North America and Asia in the face of HIV-1 expansion also supports the general observation that HIV-2 is spread less efficiently than HIV-1 (4,115,116,120, 124–130).

Case reports of HIV-2 infections transmitted by blood and blood products have been published, but the common practice of HIV testing in blood bank settings has limited the risk of this mode of transmission (131,132). The most frequent routes of HIV transmission in HIV-2 endemic areas are perinatal and heterosexual transmission. Since most West African countries have been afflicted with both HIV-1 and HIV-2 infections, direct comparison of transmission rates between the two viruses has been possible. In Senegalese female commercial sex workers followed over an 11 year period, the annual incidence of HIV-l infection dramatically increased, with a 1.18-fold increased risk per year and a 13-fold increase in risk over the entire study period. The incidence of HIV-2 infection remained stable, despite higher HIV-2 prevalence (116,133). In this high risk group the heterosexual transmission of HIV-2 was significantly lower than that of HIV-1, which strongly suggests differences in the infectivity potential of these two related immunodeficiency viruses (116,133).

In followup study of 1948 women followed from 1985 to 1999, we have compared sexual transmission probability of HIV-1 and HIV-2. New nonparametric competing risks failure time methods were used, which minimized modeling assumptions and controlled for risk factors for HIV infection. The HIV-1 versus HIV-2 infectivity ratio over time was estimated by nonparametric kernel smoothing of the HIV-1/HIV-2 infection hazard ratio in sex workers adjusted by an estimation of the relative HIV-1 versus HIV-2 prevalence in the partner population. HIV-1 was found to be significantly more infectious than HIV-2 throughout the follow-up period ($p < 0.0001$). The HIV-1/HIV-2 infectivity ratio was inferred to be approximately constant over time, with an estimated ratio of 3.55 (134).

Maternal (vertical) transmission of HIV-2 also occurs (135), but this rate appears to be less efficient than for HIV-1 (136–138). Prospective studies of HIV-2 perinatal transmission have been conducted in Guinea Bissau, Ivory Coast, France and Senegal, all demonstrating extremely low rates of perinatal transmission of HIV-2 (0–3.7% transmission) in contrast to that of HIV-1 (15–45% transmission) (117–119,139,140). In studies that measured perinatal transmission of both viruses, the rate of HIV-1 transmission was 10–20-fold higher than that of HIV-2.

PATHOGENESIS OF HIV-2

Early reports included descriptions of cases of AIDS in HIV-2-infected people (108,141,142). Disease characteristics, including tuberculosis, chronic diarrhea, and *Candida* infections, seemed similar to that seen in HIV-1-associated AIDS (108,143,144). Central nervous system involvement was also occasionally seen in HIV-2-infected people (145,146). However, classical African AIDS-associated diseases such as tuberculosis often had only a weak epidemiological association with HIV-2 infection, even in HIV-2 endemic areas (2,147,148).

Our prospective studies conducted in a registered female sex worker cohort in Dakar, Senegal, has provided the unique opportunity of measuring disease progression rates of both HIV-1 and HIV-2 infections (147–149). Importantly, these prospective studies have compared disease progression in individuals with known time of infection, and the cohort has been now observed for over 17 years, representing one of the longest HIV natural history studies conducted. The Kaplan-Meier analysis of HIV-2 incident infected individuals indicate that 85% (95%CI = 50–96%) remain AIDS-free after eight years of HIV-2 infection which was significantly less then that observed in HIV-1 infected patients (99) (Fig. 7.1). These differences in probabilities between HIV-2 and HIV-1 were also seen for development of CDC IV disease and for reduction of CD4+ lymphocyte counts to 400 and below 200 cells/mm^3, as outcomes.

In our prospective study of HIV-2 infected individuals, we have also identified individuals that fit a definition of long-term non-progression and can determine a rate of this phenotype in the study population (148,149, unpublished data). Using a definition of long-term non-progression of ≥ 8 years infection in the absence of AIDS or related symptoms, and stable CD4+ lymphocytes > 500 cells/

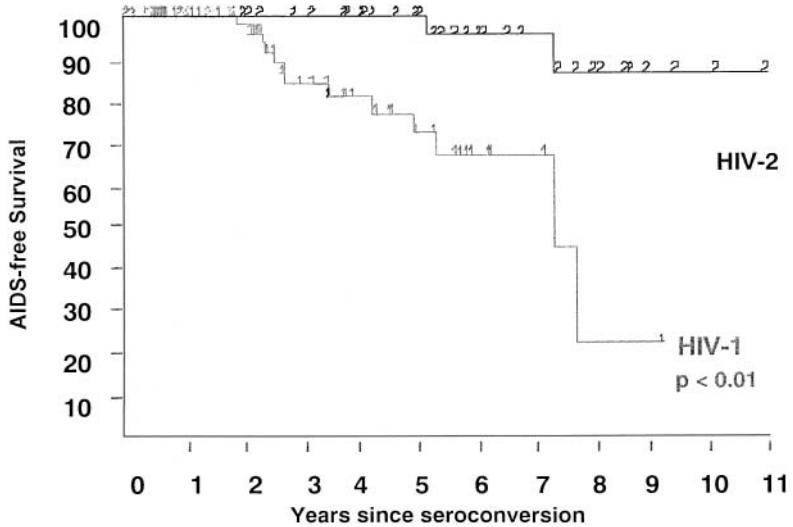

FIG. 7.1. Kaplan-Meier analysis of AIDS-free survival in HIV-1 and HIV-2 infected commercial sex workers from the Dakar cohort with known time of infection.

mm^3, we have found that 39 of 41 (95%) of our women would be classiÞed as long-term non-progressors.

In cross-sectional studies, T4 lymphocyte counts and T4 : T8 ratios appear reduced in HIV-2-infected healthy carriers, but less dramatically than for HIV-1-infected carriers (148,150Ð152). Alterations in T-cell subsets evaluated prospectively have shown similar results, where immunosuppression in HIV-2 infected people was signiÞcantly slower than HIV-1-infected patients and could not be demonstrated in all followed subjects (149,153). Skin test anergy to various antigens is also less pronounced in HIV-2 infection (148,152).

This dramatic difference in pathogenicity provides a unique opportunity to identify viral and host immune mechanisms involved in a closely related and relevant virus system that has been observed to have a signiÞcantly slower course of progression. Evidence for a lower viral burden in HIV-2 infected individuals has been reported from both virus isolation and PCR studies (103,104, 154Ð157). The isolation rate of HIV-2 from peripheral blood mononuclear cells or plasma of asymptomatic HIV-2 infected individuals was lower than the isolation rate for HIV-1 (154). At lower CD4+ lymphocyte counts, however, virus isolation was equally efÞcient in both infections. Studies in the Gambia and Senegal, suggest that proviral HIV-2 copies increase with disease development and with a drop in CD4+ lymphocyte count (104,155).

HIV-2 is less pathogenic than HIV-1, but the mechanisms underlying this difference have not been deÞned. We developed an internally controlled quantitative reverse transcriptase-polymerase chain reaction to measure HIV-2 viral load and determined levels of plasma virus in a cohort of registered commercial sex workers in Dakar, Senegal (156). The assay has a lower limit of detection of 100 copies/mL and is linear over 4 logs. HIV-2 viral RNA was detectable in 56% of all samples tested; the median load was 141 copies/mL. Levels of viral RNA in plasma were inversely related to CD4+ cell counts. HIV-2 and HIV-1 viral loads were compared among the seroincident women in the cohort; the median viral load was 30-fold lower in the HIV-2-infected women ($P < 0.001$, Wilcoxon rank sum test), irrespective of the length of time infected. This suggests that plasma viremia is linked to the differences in pathogenicity of the two viruses (156). Similar Þndings have been described in the Gambia, and Guinea Bissau (157,158).

Levels of virus in plasma are closely related to pathogenicity of HIV-1 and infection with HIV-2 leads to a signiÞcantly lower plasma viral load. To identify further the source of this difference, we measured both viral RNA and proviral DNA in matched samples from 34 HIV-2 infected individuals. The median level of HIV-2 RNA for the group was 189 copies/ml. Levels of HIV-2 RNA were below the limit of detection in nearly half the women, consistent with what we have previously reported in this population (159). Levels of HIV-2 proviral DNA failed to correlate with levels of viral RNA. Thus, it appears that signiÞcant differences occur upon expression, release and/or maintenance of virions in the bloodstream. It is formally possible that shifts in splicing patterns could be responsible for differences in virion production between HIV-1 and HIV-2, but further studies would be required to verify such an explanation. Comparative studies of both viral and host factors that may affect expression will be useful for understanding differences between HIV-1 and HIV-2 pathogenesis.

Since slower disease course appears to be common in HIV-2 infection, we reasoned that certain subsets of the population would possess host characteristics that might predispose to a more rapid disease course. We conducted a case-control study investigating possible associations between HLA and the risk of disease progression in persons with HIV-2 infection (160). The HLA class I status was molecularly typed in 62 female sex workers from the Dakar, Senegal, cohort; lack of antibodies to

HIV-2 antigen p26 was used as a surrogate marker for risk of disease progression (161). Statistical analysis showed that HLA B35 was associated with lack of p26 antibodies ($p < 0.05$), and higher risk of disease progression. The same association was found for the class I haplotypes B35-Cw4 and A23-Cw7 ($p < 0.05$), similar to the association with HIV-1 (162). Our data shows that certain HLA molecules are associated with risk of disease progression in HIV-2 infections and that some of the alleles and haplotypes involved in susceptibility to disease are similar for both HIV-1 and HIV-2.

HIV-2 Immunity

The attenuated phenotype of HIV-2 infection *in vivo* has sparked considerable interest in understanding the immunopathogenesis of this particular HIV infection. Although certain viral determinants appear to be central to the lower replicative capacity *in vivo*, the virus also appears to differ from HIV-1 in its effects on the immune system. The degree of immune activation and apoptosis in lymphocytes were compared in healthy West African patients infected with HIV-1 or -2. The lower decline of CD4 T cells in HIV-2 compared with HIV-1-infected donors was associated with lower levels of immune activation, evaluated by HLA-DR expression on lymphocytes and serum concentrations of IgC and beta2 microglobulin (151,152,163). *Ex vivo* apoptosis was found in both infections in all lymphocyte subsets, including CD4 and CD8 T cells, as well as B cells, but was less in HIV-2 than in HIV-1 infection (164). These observations support the hypothesis that long-term activation of the immune system, weaker in HIV-2 infection, signiÞcantly contributes to T cell deletion and disease evolution (164).

As with HIV-1, the antibody response to most viral structural proteins occurs shortly after infection and is thought to persist. In HIV-2 infection, over 90% of seropositives evaluated in Senegal demonstrated strong antibodies to gag, pol and env-encoded antigens (165,166). As already noted, the cross-reactive response to analogous HIV-1 antigens is signiÞcant in highly conserved gene products such as gag and pol and less with envelope antigens.

Previously, the presence of circulating p24 antigen of HIV-1 was a useful marker of virus replication *in vivo*, although this testing has been largely replaced with more direct measurements of virion particles using PCR technology. Analogous studies with HIV-2 have not been described, perhaps because antibodies to p26 are found in the majority of HIV-2 infected sera and therefore it has been assumed that complexing by free circulating p26 is less frequent. In our studies, we have found that the lack of antibodies to p26 is a Þxed phenotype, not a result of complexing of free p26, and predictive of a more rapid disease course (160,161).

HIV-2 neutralizing antibodies have been described (71,82,83,167,168), and in studies with fresh isolates, the broadness of the HIV-2 neutralizing response has been unusual and distinct from the neutralizing antibody response to HIV-1 (169). Virus neutralization studies have shown that a proportion of HIV-2 sera are also capable of cross neutralizing HIV-1 isolates in addition to HIV-2 isolates (82,86). The degree to which HIV-1 positive sera can cross-neutralize HIV-2 isolates remains controversial. It is still not known whether some of the conserved domains of env will be capable of eliciting a cross-protective response to both viruses.

Studies of HIV-2 humoral immunity have now been extended to evaluate the highly related mucosal immune response. Evaluation of HIV-2 antibody responses in cervicovaginal secretions have shown that only a third of infected women generate lgA responses to HIV-2 envelope antigens in this compartment, suggesting lower levels of viral replication compared to HIV-1. Of interest, cross-reactivity by IgG and IgA to heterologous envelope antigens was more frequent with HIV-2 infection (170). Cervicovaginal cross-reactivity was more pronounced for HIV-2-speciÞc antibodies to HIV-1 epitopes than conversely. Such features could be relevant to a differential heterosexual transmission of one type of HIV in an individual infected by the other type, in accordance with epidemiologic studies showing that HIV-2 infection protects from HIV-1 infection, and that HIV-1 infection does not appear to protect signiÞcantly from HIV-2 infection.

Knowledge of immune mechanisms responsible for cross-protection between highly divergent viruses such as HIV-1 and HIV-2 may contribute to an understanding of whether virus variability may be overcome in the design of vaccine candidates, which are broadly protective across HIV subtypes. In early CTL studies of HIV-2, responses against HIV-2 gag, pol and nef proteins were described; HLA-B53 restricted, HIV-2 gag-speciÞc CTLs did not recognize target cells expressing HIV-1 gag proteins suggesting the absence of a cross-protective cellular response (171). More recently, however, Bertoletti and colleagues also working in the Gambia, have shown that the majority of HIV-2-infected individuals regardless of HLA haplotype possess a dominant cytotoxic T-cell response which is able to recognize HIV-1 Gag protein (172). Furthermore, HLA-B5801-positive subjects have shown broad cross-recognition of HIV-1 subtypes since they mounted a T-cell response that tolerated extensive amino acid substitutions within HLA-B5801-restricted HIV-1 and HIV-2 epitopes. These results suggests that HLA-B5801-positive HIV-2-infected individuals have an enhanced ability to react with HIV-1 that could play a role in cross-protection (172).

We have applied the sensitive Elispot technique to quantify further the HIV-2 speciÞc CTL response in HIV-2 infected individuals (173). To assess the antigen delivery system using the modiÞed non-toxic form of the anthrax toxin, we fused the HIV-2 gag (P-26) to the terminal domain of Lethal Factor (LFn; 255 aa). The LFn-HIV-2 recombinant protein was expressed and used as antigen to

stimulate CTL in a ELISPOT assay in comparison to the classic delivery system using recombinant vaccinia Virus (rVV) expressing HIV-2 gag. We found that 87.5% of individuals in our study showed a speciÞc gag CTL response. Our results have shown that priming cells with the LFn-P-26 gave better sensitivity and resolution when subjects had a low frequency of CTL precursors. Interestingly, we found that the group with strong cellular immune response had no detectable HIV-2 plasma load.

CORRELATES OF IMMUNITY

The developing world, particularly regions of Africa, continue to bear a signiÞcant portion of the global HIV burden, and they are unlikely to beneÞt from recent advances in therapeutic regimes in the foreseeable future. Important to vaccine design is the understanding of pathogenic mechanisms of HIV infection and the potential immunologic responses or correlates necessary for HIV containment. IdentiÞcation of such correlates has been hampered by relatively rare instances of natural immunity that have withstood the challenge of viral exposure.

Demonstrated differences in the infectivity and disease potential of HIV-2 compared to HIV-1 support the notion that the mechanism for such protection might be analogous to the attenuated virus vaccine model. In our studies of the female sex worker cohort in Dakar, Senegal, we tested the hypothesis that the attenuated phenotype of HIV-2 infection might protect from subsequent HIV-1 infection (174). HIV-1 infection was documented over the study period with both serology and PCR assays. A Poisson regression model was used to estimate the independent effect of demographic, behavioral, and biologic variables on the risk of HIV-1 infection. Despite a higher incidence of other sexually transmitted diseases (STDs), HIV-2 infected women had a lower incidence of HIV-1 infection than HIV-2 seronegatives, with an incidence rate ratio (IRR) of 0.32 ($p = 0.008$). This analysis led to the conclusion that HIV-9 infection conferred a signiÞcant reduction in the subsequent risk of HIV-1 infection. Continued analysis of the Dakar cohort has extended the observation period from the Þrst published report to over 13 years, and HIV-2 protection ranges from 52Ð74% depending on the method of analysis (2,174,175).

The generalizability of these Þndings has been questioned by studies from other West African sites. In Ivory Coast, Guinea Bissau and the Gambia, studies originally designed as cross-sectional surveys were analyzed for short periods of longitudinal observation (176Ð178). As a result of their design, these studies failed to possess sufÞcient statistical power, capable only of detecting an extremely high protected fraction ($> 99\%$) of HIV-1 infection due to HIV-2 infection (175). In addition, a mixture of serologic methods were employed by these studies, failing to meet the PCR based, gold standard for diagnosis of dual infection, critical to the objective evaluation of HIV-2 protection *in vivo* (176Ð178). In a longitudinal study conducted in police ofÞcers in Guinea Bissau, Norrgren et al. failed to report a statistic signiÞcant result; however, only a portion of the samples was diagnosed by PCR technology (179). Unbiased, powerful studies, using sensitive and speciÞc classiÞcation methods, will be needed to address the generalizability of the observation of HIV-2õ protective efÞcacy against subsequent HIV-1 infection.

Studies from research groups including our own have described *in vitro* interactions of HIV-1 and HIV-2 that support a protective role for HIV-2: these range from virus-virus interactions to potential immune mediated mechanisms for HIV-2 protection. Arya et al. have reported that HIV-2 inhibits the replication of HIV-1 at the molecular level. This inhibition was selective, dose-dependent, and nonreciprocal. Though the exact mechanism remains to be deÞned, the inhibition appeared to be mainly due to an intracellular molecular event because it could not be explained solely on the basis of cell surfuce receptor mediated interference. The results support the notion that the inhibition likely occurred at the level of viral RNA, possibly involving competition between viral RNAs for some transcriptional factor essential for virus replication (180,181).

One might hypothesize that HIV-2 would differentially protect against certain HIV-1 subtypes better than others. To investigate the HIV-1 subtypes involved in dual HIV-1 and HIV-2 infections, we sequenced the env region from 29 dually infected female commercial sex workers from Senegal (182,183). The majority of women (23 of 29) were infected by HIV-l subtype A; within the HIV-1 subtype A sequences, 14 of 23 (60.8%) clustered with the West African associated A/G recombinant form (IbNG), and 9 of 23 (39.2%) formed a separate cluster distinct from the A/G IbNG. In contrast, in HIV-1 singly infected individuals, non-IbNG subtype A was found in only 13 of 98 (13.3%). Therefore, the lack of protection and/or interaction with HIV-2 was associated with a distinct HIV-1 A genotype. These results suggest differences in the biological properties of HIV-1 genotypes and their *in vivo* interaction with HIV-2 (183).

Beta chemokines have now been identiÞed as potent soluble suppressors of macrophagetropic HIV infection *in vitro*. Studies of multiply exposed uninfected individuals have implicated the role of elevated beta-chemokines in HIV resistance, in many cases, independent of genetic mutations in the chemokine receptor (184Ð186). Macaque studies have also suggested a role for beta-chemokines in vaccine induced protective immunity using a variety of vaccine candidates and live virus challenge (187). We used an HIV-1 *in vitro* challenge system to determine if PBMCs from HIV-2 infected individuals showed altered susceptibility to HIV-1 infection (188). Peripheral blood mononuclear cells were stimulated and infected with either R5 or X4 HIV-1 viruses. 14 of 28 (50%) HIV-2

PBMCs demonstrated over 90% inhibition of R5/HIV-1 infection compared to 0 of 19 HIV negative controls (Fisher exact test, p value = 0.0002). In contrast, HIV-2 positive and HIV negative control cells were equally susceptible to HIV-1/X4 infection. RANTES, MIP1α, MIP1β, the natural ligands of the CCR5 receptor, were measured in culture supernatant by ELISA, and supernatant levels of MIP-1α (r = −0.56, p = 0.03) and MIP-1p (r = −0.69, p = 0.004) were inversely correlated with HIV-1 replication. Using polyclonal antibodies to RANTES, MIP1a and MIP1b, resistance was neutralized. A significant proportion of HIV-2 infected PBMCs demonstrate HIV-1 resistance *in vitro*; this resistance was beta-chemokine dependent (188). Studies are further needed to characterize this potent anti-viral activity and determine its potential contribution to *in vivo* protection.

HIV-2 infection might dramatically influence beta-chemokine production by enhancing, it in magnitude and duration, thus enabling HIV-2-infected individuals to cope favorably with subsequent exposure to HIV-1. This is supported by studies demonstrating that binding of the HIV-2 envelope to the alpha chain of CD8 stimulates much higher levels of beta-chemokine production in comparison to HIV-1 gp120 activity (189). Not only does this implicate a novel viral suppressive mechanism but one that may be adapted for immunoprophylaxis. Antiretroviral vaccine strategies that incorporate beta-chemokine induction or other receptor-blocking functions raise some encouraging possibilities for vaccine design and development.

HIV-2 infected baboons (*Papio cynocephalus*) provide a valuable animal model for the study of HIV pathogenesis since many features of disease progression resemble HIV-1 infection of humans. In some HIV-2-infected baboons that are clinically healthy, a CD8+ cell antiviral response, that was partly mediated by a soluble factor, appeared to control viral replication *in vitro*. A soluble factor was found to be active against the chemokine-resistant, syncytium-inducing HIV-1 isolates and was relatively heat stable. Therefore, the soluble suppressing activity of CD8+ cells in HIV-2-infected baboons may be analogous to the CD8+ cell antiviral factor described in HIV-infected asymptomatic people (190).

HIV-2 is primarily in West Africa where it infects an estimated 1–2 million people. It is spread less efficiently than HIV-1, making projections for future infections much lower than for HIV-1. For these reasons, development of an HIV-2 vaccine has not been a high research priority. However, HIV-2 infects monkeys whereas HIV-1 does not, and the HIV vaccine model uses viruses that are closer to HIV-2 than HIV-1. The development and testing of an HIV-2 vaccine might therefore be simpler than the development of an HIV-1 vaccine. A few reports with HIV-2 have described experimental studies with limited vaccine protection (191,192), but immune correlates of protection have not been identified (193–195). Nonetheless, the data from human studies which suggest that HIV-2 may afford protection from HIV-1, is suggestive that a candidate vaccine based on HIV-2 might provide necessary cross-immunity for HIV-1 protection, and is worthy of further consideration.

THERAPY

Drugs that have efficacy against HIV-1 are generally assumed to be effective against HIV-2. However, with a few exceptions (196), most HIV-1 drugs have not been tested for activity against HIV-2. In addition, experience with antiretroviral therapy (ARV) has been largely confined to the developed world where HIV-1 non-B subtypes and HIV-2 are rare. Hence, it is still not clear that current ARV therapeutic regimes will be as efficacious in the developing world given the difference in virus subtype or type. Further, the natural history of HIV/AIDS in Africa differs from the disease in the developed world, since in Africa there are distinct endemic opportunistic infections, multiple common infectious disease agents, as well as different standards of clinical care. This suggests that certain aspects of HIV clinical management in African settings may be relatively unique. ARV of HIV-1 is directed at lowering *in vivo* virus replication, but this is problematic for managing HIV-2-infected patients because of the absence of a commercially available HIV-2 plasma viral load assay. We have had limited experience in the therapy of HIV-2-infected patients in the U.S., where it appears that standard HAART can readily reduce viral load levels below detection (197,198).

The reverse transcriptase (RT) and protease genes from twelve HIV-2-infected individuals who had been exposed to antiretroviral drugs were examined for the presence of mutations, which could be involved in drug resistance. Four individuals carried virus genotypes with amino acid substitutions potentially associated with resistance to nucleoside analogues: two at codon 70 (K → R) and two at codon 184 (M → V); the latter two patients harbored a codon Q151M mutation which has been associated with multidrug resistance in HIV-1. Substitutions associated with resistance to protease inhibitors at codon 46 were observed in all individuals. Moreover, minor resistance mutations, as well as new ones, were often seen in the protease gene. Thus, amino acid changes in the HIV-2 RT and protease genes, which could be associated with drug resistance, seem to occur at positions identical to those for HIV-1 (199).

Smith and colleagues have reported on the clinical management of seven HIV-2 infected patients followed in the U.K. on antiretroviral therapy (9). HIV-2 viremia was detectable only at CD4 counts $< 300 \times 106$/ml. Two patients with high viral loads (> 50,000 copies/ml) failed to respond to therapy, although these patients were given sub-optimal therapy. One of these patients had a viral load > 50,000 copies/ml and a CD4 count of 66; his initial regimen was dual therapy followed by sequential single

drug switches. Drug resistance data on this patient's virus demonstrated a 20-fold higher IC_{50} to 3TC (after six months of therapy) and a 10-fold higher IC_{50} for SQV after nine months of therapy. In four patients, ARV therapy led to a fall in viral load and a rise in CD4 cell count, although all had initial viral load levels of < 50,000 copies/ml. From the few published studies including our own, we are not able to adequately assess the clinical utility of the NNRTI class of antivirals as a treatment option for HIV-2, although, *in vitro* data suggest that this class of drugs may be less effacious for HIV-2 compared with HIV-1. Similar to our observations, it appears that HIV-2 differs from HIV-1 in the risk of disease progression at any given CD4 cell count and may therefore have different implications for the timing and management of ARV therapy (200).

SUMMARY

HIV-2 was first described in West Africa in 1985, since that time there has been considerable progress in understanding the virology and epidemiology of HIV-2. HIV-2 and HIV-1 have unique and striking differences in their geographic distribution, epidemic trends, perinatal and heterosexual transmission rates and incubation periods to the development of AIDS. The virologic determinants and mechanisms for these apparent biological differences are still unknown. The comparative approach to understanding the biologic differences between HIV-2 and HIV-1 may prove informative to understanding HIV pathogenesis in general. This is best exemplified in studies that indicate that HIV-2 infection via viral, host and immune mechanisms may confer significant protection from subsequent HIV-1 infection. A further understanding and characterization of HIV-2 immunity and cross-immunity may be useful for HIV vaccine design and development. Finally, our appreciation of the biologic diversity found in closely related HIV viruses may better help us anticipate the future consequences of HIV infections at the host and population level.

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Chapter 8

Laboratory Detection of Human Retroviral Infection

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The characterization in 1984 of a new human retrovirus, the human immunodeÞciency virus (HIV), is the foundation for understanding the acquired immunodeÞciency syndrome (AIDS) epidemic (1Ð3). The evidence is overwhelming that HIV infection is necessary for a person to develop AIDS (4Ð6). The development of disease in otherwise healthy workers who have occupationally acquired HIV indicates HIV is sufÞcient, as well (7). The spectrum and natural history of retroviral infections have been well-deÞned (5,6,8).

In 1988, a nomenclature committee suggested that the term human immunodeÞciency virus be used for this generic class of viruses (9), rather than the earlier terms Òlymphadenopathy associated virusÓ (LAV) or ÒhumanT-cell lymphotropic virus type IIIÓ (HTLV-III) (1,10). SigniÞcant genetic variations occur among HIV strains (11Ð14). Nearly all HIV infection in the United States is due to HIV type 1 (HIV-1), but in other parts of the world, such as West Africa, HIV type 2 (HIV-2) predominates (15,16). In light of the many differences in the serological, immunological, clinical and therapeutic correlates of different HIV strains (17), biologicals derived from speciÞc isolates should specify the viral strain of origin (e.g. HTLV-IIIB), and tests based upon HIV reagents should reference the precise sources.

This chapter reviews the methods and limitations of assays to detect evidence of HIV exposure, and provides an approach to the rational use and interpretation of these tests. In order to interpret past literature, an historical context is provided.

The period from initial exposure to HIV until tests can rule infection in or out is of great relevance in terms of maximizing the safety of the blood supply as well as to the follow-up and counseling of persons who have had a speciÞc exposure. These issues, including the early dynamics of infection and the meaning of the Òwindow Ó period in the context of our current test armamentarium, are reviewed in detail.

Given that the human T-cell lymphotropic family of viruses (HTLV) (18) are sometimes confused with HIV (particularly given historical changes in nomenclature), that the U.S. blood supply is routinely screened for HTLV-I and HTLV-II, that HTLV-I is the etiologic agent of adult T-cell leukemia-lymphoma syndrome (ATL), HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and other diseases, and the general similarity in testing methodologies, a brief separate section discusses tests for HTLV.

Whereas Arabic numerals are usually used to differentiate the HIV species (HIV-1, HIV-2), for historical reasons Roman numerals are usually used for HTLV (HTLV-I, HTLV-II).

HIV testing in the United States makes use of several different types of tests. For example, testing historically usually began with an enzyme immunoassay (EIA) to detect antibody to HIV. Rapid tests have become available in recent years to provide a test result to the patient in a single visit. Direct testing for the virus includes assays that detect antigen or nucleic acids. These and other tests are described in detail below, including the variations in testing approaches as they pertain to testing patients, persons believed at risk, and screening of blood donors.

A current, complete listing of FDA licensed or approved assays can be found at *http://www.fda.gov/cber/products/*

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testkits.htm. There are also unlicensed assays sold for "research use only" and there are tests that are used only "in house" by commercial or research laboratories. (These assays are not approved or licensed by the FDA for clinical use in the U.S.) Each individual assay has its own associated special characteristics and is not interchangeable with other assays, even within a given class of test.

GENERAL APPROACHES TO THE DETECTION OF INFECTIOUS AGENTS

Infectious agents can be detected either directly, by detecting the agent itself (or a component), or indirectly through what are referred to as surrogate markers of infection. Examples of surrogate markers are the immune response to the agent or a clinical consequence of the infection. A laboratory measure that directly detects the presence of an infectious agent is highly desirable since there is usually a delay between the time that the host is infected and when a host response is generated (discussed in detail later in this chapter). For some agents, such as hepatitis B virus, large quantities of viral antigen are produced during active infection and tests that effectively measure viral antigen are available. However, for many other viral agents, antigen may not be directly detectable even during active infection (19).

The presence or absence of antibodies (a humoral or serological response) is one way to detect whether or not a response to the agent by the host's immune system has occurred. In general, antibodies can be generated in response to either a live or a killed infectious agent. The presence of antibodies can thus mean:

a. there was exposure to antigen from the infectious agent, but no live infection existed;
b. there was active infection in the past that has resolved;
c. there is a latent infection; or
d. there is an active infection.

It is important to remember that many viruses, including HIV, are characterized by persistence; i.e. the presence of antibodies does not indicate resolution of infection. Exogenous retroviruses, such as HTLV and HIV, can integrate within the genome (DNA) of the host cell. It is uncertain whether continual viral expression must occur to sustain antibody production over a prolonged time, e.g. by periodically or continuously re-challenging the immune system. In this vein, prospective epidemiologic serologic studies indicate that once an adult produces antibodies to HIV ("seroconversion" to a "seropositive" status), complete loss of antibodies ("seroreversion") is rare (20); virus can usually be recovered from seropositive persons. The presence of anti-HIV antibodies is therefore generally interpreted as evidence of persistent infection, not resolution of past infection.

An important exception is the infant less than 18 months of age whose mother was HIV seropositive at delivery (21). Since maternal IgG antibodies can cross the placenta to high titer, it is not surprising that it can take many half-lives of decay for these to reach an undetectable level. Serological studies based on cord blood thus give useful data on maternal, but not newborn, infection. Only a fraction of initially seropositive newborns are actually HIV-infected.

PERFORMANCE MEASURES FOR TESTS TO DETECT HIV INFECTION: SENSITIVITY, SPECIFICITY, AND PREDICTIVE VALUE

When considering the performance of a test to detect HIV infection, we are concerned about the ability of the test to identify correctly individuals who are either infected or uninfected. These performance criteria are most commonly addressed by the sensitivity and specificity of the test (see Table 8.1). For the "perfect" test, sensitivity and specificity would both be 100%. However, real-world technical limitations dictate a balance between sensitivity and specificity. For example, a test that simply identified everyone as "infected" would be 100% sensitive by definition, but the test result, in fact, would be meaningless.

The balance between sensitivity and specificity will vary depending on the application of the test. For example, although the intended purposes of HIV testing in the blood bank and clinical/diagnostic settings are similar, the practical applications of tests to detect HIV infection are quite different. In the former situation, the intent is to identify a blood donor who is infected with HIV. Therefore, it is critical to minimize non-reactive results on specimens from infected individuals (known as false negative results), in order to maximally assure the safety of the blood supply and blood products. In the latter clinical instance, the goal is to counsel correctly persons concerning whether or not they have been infected, with implications for behavioral modification, medical evaluation and appropriate therapy.

Medical and non-medical considerations concerning AIDS and the HIV epidemic have elevated the impact of HIV testing to a level that is far greater than for most other laboratory tests (3). HIV testing is perceived as an invasive procedure in our society. Fears concerning the medical implications of reactive tests results and concerning potential social risk in the event of a break in confidentiality have foundation (3). False positive results similarly raise special concern. To address these issues, a written informed consent procedure in advance of testing was initially recommended in 1985, prior to FDA licensure of the first test to detect HIV infection (3), and is now used by many to document pre-test discussions.

Receiver operator characteristic ("ROC") curve analysis is a useful method to compare the performance of assays when congruent definitions of sample groups have been provided, and to assess claims of vendor improvement (a

TABLE 8.1. *Epidemologic assessment of tests: DeÞnitions*

Sensitivity*—The probability that a test result will be positive if infection is present, which is the ratio of (true positives)/(all those with infection).

Specificity*—The probability that a test result will be negative if infection is not present, which is the ratio of (true negatives)/(all those without infection).

Positive Predictive Value—The probability of infection being present given a positive test result, which is the ratio of (true positives)/(true positives + false positives) (equivalently: (true positives)/(all those with a positive test result)).

Negative Predictive Value—The probability of infection not being present given a negative test result, which is the ratio of (true negatives)/(true negatives + false negatives) (equivalently: (true negatives)/(all those with a negatives test result)).

Gold Standard (Reference Standard)—A de nitive means of categorization, widely accepted by experts in the eld, for absolutely de ning the presence or absence of a condition (such as HIV infection).

Silver Standard (Criterion Standard)—The best currently available (or the accepted) standard, which is expected to be superseded as technology advances; used as an interim reference standard when a gold standard either does not exist or is otherwise unavailable.

Supplementary Test—An additional test that further clari es the result of a screening assay . Does not necessarily have truly independent** qualities. Usually used as part of a sequence of tests, which may be referred to as a "testing algorithm."

Confirmatory Test—A supplementary test that is maximally independent** from any other tests that have been utilized. A well-performing con rmatory test will be part of a "con rmatory algorithm," the results of which would serve as the basis for optimally de nitive test result categorization.

* Laboratory personnel frequently use these terms differently, with the terms applied to detection and strength of a "test signal" (e.g. titer) rather than the epidemiologic, statistical de nitions above.

** For example, if a specimen were to give a false positive result on multiple assays, those assays are not maximally independent. For HIV, if the antigens used in both an EIA and WB were derived from whole viral lysate, then anti-HLA antibodies to the cell line used to grow the virus might cause false positive reactions for both, so that the assays would not be truly independent (51).

ROC curve is a plot of sensitivity vs. (1 Ð speciÞcity)). Such analysis, for example, might show that a purportedly improved prototype EIA (22) apparently had, in fact, poorer performance than a predecessor assay (23).

One should note that sensitivity and speciÞcity as deÞned by the epidemiologist for laboratory tests (Table 8.1) reßect aspects of the test conÞguration as deÞned by the manufacturer, and remain constant for all approved uses. Alternative interpretive criteria or cut-off values will affect the sensitivity and speciÞcity (23). It is also important to consider the statistical limitations to these estimates, which are generally reßected by 95% conÞdence intervals and are a function of the number of specimens used to derive point estimates in clinical trials.

Much more important to the users, in practice, are the positive and negative predictive values. These will vary depending upon the (estimated) ÒtrueÓ positivity proportion in the population being tested; ideally, a gold standard would be employed to determine this proportion (Table 8.1). For example, for a hypothetical test with 99.5% sensitivity and 99.5% speciÞcity, the predictive values vary with the HIV prevalence in the population.

In each of these settings, except for the case of very high prevalence (last row in above table), the negative predictive value would be quite high. Likewise, in the very low prevalence setting (Þrst row of above table), the positive predictive value of this particular test would be low.

When dealing with an individual patient, the predictive values depend upon the Òprior probability of infectionÓ or (analogously) the Òprior probability of diseaseÓ (24), which represents an estimate of the relevant population proportion. A *pre*-test probability assessment is required whenever test results are to be meaningfully interpreted. Thus, in the setting where a patient ÒclearlyÓ has AIDS (e.g. assume 99.9% assurance based on clinical and epidemiologic grounds pre-test), a negative result is very likely to be falsely negative (as can be seen in the last row of Table 8.1). CDC has developed a series of guidelines for counseling, testing, and referral to assist clinicians in the proper interpretation and reporting of test results to patients (25Ð29).

Although health care professionals who order HIV tests have become increasingly familiar with these tests, it is important for the laboratory report to provide the clinician with considerable guidance concerning the implications and limitations of the test results. Further consultation with the laboratory (or blood bank) director, infectious disease specialist, or other expert may be particularly important in circumstances of an ÒunexpectedÓ result. Such experts should be able to assist the clinician in employing standard principles of decision theory to apply the battery of tests appropriately and efÞciently to a given situation. For example, blood donor screening assays have been developed to maximize sensitivity to meet the specialized needs of safety for the blood supply. This results in decreased speciÞcity, identifying the need for conÞrmatory testing of reactive samples.

ConÞdentiality concerns may severely limit the information that is routinely provided to the testing laboratory by the referring professional. Since clinical data impacts on the Òpre-test probabilityÓ assessment and thus on the predictive value of a test, such direct discussions may help resolve the clinical interpretation of putatively Òindeterminate Ó results. The types of tests and considerations regarding them are discussed in depth below.

In newborns, the presence of anti-HIV antibodies indicates passive acquisition of antibodies from the mother

and/or active HIV infection. Tests that detect the virus itself can thus be particularly informative in this clinical setting. These specialized tests are also important to detect virus in a person who is infected, but antibody negative (Òseronegative)Ó This would occur in special circumstances such as in the peri-seroconversion period, and in the case of certain strains of HIV not well detected by some standard assays (e.g. HIV Group O).

ANTIBODY DETECTION

The enzyme-linked immunosorbent assay (ELISA), or enzyme immunoassay (EIA), was originally successfully employed to detect antibodies to HTLV (30), and led to the development of a prototype HIV EIA that was reproducible, sensitive and speciÞc (23,31,32); the methodology is described in these studies. The research breakthrough enabling production of puriÞed HIV type HTLV-IIIB in large quantities was integral to the development of these assays (2).

The FDA Þrst licensed anti-HIV-1 antibody EIA kits for commercial distribution to screen donated blood products in the U.S. in 1985. ÒAlternative test sites,Ówhere persons who considered themselves at increased risk for HIV infection could be tested voluntarily, were also developed (33). In the ensuing years, testing for HIV became more widely available, and in 1996 home specimen collection with samples sent to a central laboratory that uses standard assays became an FDA-approved option. The Centers for Disease Control and Prevention recommends that all states and territories conduct surveillance for cases of HIV infection (34). States vary as to whether personal identiÞers are required as part of the conÞdential reporting of positive HIV test results to their health department.

Tests that detect anti-HIV antibodies can be divided into two broad categories, screening tests and supplemental tests (Table 8.1). The latter are usually more speciÞc tests that are used to conÞrm a screening test result as part of a testing algorithm. The FDA has licensed and approved anti-HIV antibody screening tests and supplemental tests from multiple vendors and for use with a variety of specimen types. For example, screening and supplemental tests are available for use with whole blood, serum, plasma, dried blood spots, urine, and/or oral ßuid.

In the absence of gold standards, the true sensitivity and speciÞcity for the detection of HIV antibodies remain somewhat imprecise. Initial generation HIV EIAs primarily detected IgG, not IgM or IgA, antibodies. Current EIAs may also detect IgM. HIV p24 antigenemia and/or IgM antibodies can precede the expression of IgG antibodies. Thus, even if an IgG assay were perfectly sensitive for such antibodies, some persons truly infected with HIV could not and would not be detected by serologic tests for HIV IgG antibody. The frequency, duration, and importance of the seronegative window period remain the subject of investigation (35,36). Concern about narrowing the window led the FDA to license and the U.S. blood banking industry to implement sequentially revised and additional screening methodologies for the U.S. blood supply (see below).

SCREENING TESTS

There are currently two types of screening tests in use in the U.S. to detect anti-HIV antibodies, EIAs and rapid HIV tests.

EIA

The EIA (ELISA) was the Þrst type of test to be licensed in the U.S. to detect infection with HIV. The Þrst generation of commercial EIAs had sensitivities and speciÞcities (see Table 8.1 and reference (23) for deÞnitions and formulae) of approximately 98% (37,38). However, comparative data on the EIAs were limited and complicated by the absence of a generally accepted gold (or even silver) standard (see Table 8.1). Licensure required correct identiÞcation of sera in an FDA test panel, but the panel was limited in extent and was designed as but one of several essential minimum requirements. Different manufacturers have used different panels of sera in their evaluations. Therefore, apparent observed differences sometimes merely reßect differences in patient selection and not true differences among the tests.

Lacking an established reference laboratory standard, in 1984 a prototype HIV EIA was assessed on the basis of clinical-epidemiologic considerations, which included a panel of sera drawn from healthy blood donors prior to the AIDS epidemic who were considered Òtrue negativesÓon epidemiologic grounds (23). The subsequent FDA assessments of vendor products rely upon current blood donors to determine how a given product performs, with assessments in both high and low risk populations and individuals known to be infected with HIV, complemented by specialized historical collections such as seroconversion panels to assess not only the clinical sensitivity and speciÞcity, but also the analytical sensitivity of a given test (24,39,40).

Screening of a person for HIV as of this writing most often begins with an initial screening EIA (23,41), and may begin with a rapid test in some other situations (see below). If the EIA is reactive, the EIA is repeated in duplicate on the same specimen. If repeatedly reactive, the specimen is tested with a more speciÞc supplemental test to validate the EIA results and to prevent notiÞcation based on false-positive results that might occur with the screening tests. Supplemental tests include the Western blot (WB) or the indirect immunoßuorescence assay (IFA)

(see below). This algorithm is used with serum, plasma, dried whole-blood spots, cadaveric and oral ßuid specimens (42Ð50).

To perform an EIA, the specimen is diluted and added either to a well that is coated with HIV antigens (whole virus lysates, recombinant HIV proteins, or HIV peptides) or to a well containing an antigen coated bead. Antibodies present in the solution bind to the HIV antigens. Following an incubation and wash step to remove unbound material, a conjugate is added that binds to the anti-HIV antibodies that are present. For example, anti-human IgG conjugated to an enzyme, such as horseradish peroxidase, is used in some systems. Bound conjugate is detected by adding a substrate that changes color in the presence of the enzyme. The result is read by a spectrophotometer. Negative or positive controls (or both) are also run and used to calculate a cutoff value for the assay. Results are typically reported in terms of the signal to cutoff ratio, or S/CO. S/CO values > 1.00 are considered to be reactive. Similar to the EIA is the chemiluminescent immunoassay (ChLIA). In this system, the readout is photons that are read by a luminometer.

The Þrst generation HIV EIA kits used puriÞed disrupted whole virus, which included steps that attempted to remove the cellular contaminants during the manufacturing process. However, simply removing cellular debris from HIV preparations produced in human cell lines, such as H9/HTLV-IIIB, did not remove residual reactivity to high-titer HLA-antibody sera due to the physical association of HIV virions with HLA class II molecules. Although it turned out that there was very little such reactivity with the prototype assay itself, reßecting high antigen purity (23,51), problems associated with false HLA reactivity were noted subsequent to licensure for at least two other Þrst generation commercial assays (52,53). Methods to reduce HLA reactivity were by necessity subsequently developed (54) and the second generation of HIV EIAs had improved speciÞcity. Another opportunity for problems with speciÞcity came about with the advent of viral components produced through recombinant methods in bacteria such as *Escherichia coli* (55). Injection drug users use non-sterile works (paraphernalia involved in injections) and are frequently repetitively exposed to bacterial pathogens, including the ubiquitous *E. coli*. It is suspected that this may account for why such persons had particularly high rates of false reactivity on these recombinant assays.

EIA currently remains the method of choice to screen blood donors for antibodies to HIV (Table 8.2). Whereas these tests are technically complex to perform and require dedicated equipment in a centralized testing laboratory, they are well suited to processing large numbers of specimens efÞciently. There are currently several licensed or approved EIAs that detect antibodies to only HIV-1, one that detects antibodies to HIV-2, and others that detect antibodies to both anti-HIV-1 and HIV-2 (referred to as ÒcombiÓ tests). In the U.S., the latest generation of licensed EIA screening tests typically has sensitivities of ³99.99% and speciÞcities of ³99.9% when using serum, plasma, or dried blood spot specimens. These performance values are based on clinical trial information provided by the manufacturer is support of licensure or approval.

The reference body ßuid for antibody testing is blood, utilizing either serum or plasma. Antibodies may also be reliably detected in urine, both with research assays and with minor modiÞcation of the recommendations by the vendor of commercial assays (56Ð65). The relatively acellular nature of a clean-catch urine in the absence of genital or bacterial urinary tract infections may balance the apparently lower titer of antibody, to enable the attainment of a reasonable sensitivity/speciÞcity trade-off. The original reports used tests designed for blood with modiÞcation, whereas newer assays are designed speciÞcally for testing urine. There was a controversial report in 1993 claiming to Þnd HIV antibodies in urine despite absence of antibodies in blood (66). In licensing urine assays in 1996 (67), the FDA required as the criterion standard demonstration of HIV infection based on blood tests.

Detection of HIV antibody in oral ßuids has been somewhat more problematic (68Ð70). One possibility is that proteins or other substances may bind to HIV antibodies, impairing detection. False positives clearly can occur (71). Results on paired serum and saliva samples have been reported (72). There may be differences between testing saliva and oral ßuid.

Assays licensed for use with other specimen types such as urine or oral ßuid have performance characteristics that are generally lower. As of this writing, according to clinical data supplied by the manufacturer and listed in the package insert of the test kit, a urine EIA (Calypte® HIV-1 Urine EIA) had sensitivity of 99.0% and its speciÞcity varied in studies from 82% to 99.14%. An oral ßuid EIA (Oral Fluid Vironostika® HIV-1 Microelisa System) had sensitivity of ³98.6% and speciÞcity of ³97.7%. Tests that use these alternative specimen types are useful when collection of a blood specimen is not possible, but it must be recognized that sensitivity and speciÞcity are lower.

The ability to test dried blood spot specimens by EIA and by supplemental testing led to the development and approval of a test system for self-use by people who wish to obtain anonymous HIV testing (Home Access HIV-1 Test System, Home Access Health Corp., Hoffman Estates, IL). In this case, the test subject is provided with materials to self-collect a dried blood spot specimen that is shipped to a central testing center. The testing center performs EIA on the specimen and supplemental testing as necessary. The test subject is instructed to call the center within one week, using a unique identiÞer to receive the test results. Results are provided by an appropriately trained individual who explains the test result and its interpretation. Counseling and referral are also provided as part of the service. Such a system has been useful for

individuals who, for whatever reason, wish to maintain anonymity in the testing process.

The Public Health Service recommends that repeatedly reactive EIA results not be reported to the test subjects until they are conÞrmed by supplemental testing as discussed in detail below (73).

Rapid HIV Tests

Tests used to screen blood donors for HIV infection were developed for efÞciently testing large numbers of specimens. As such, these tests are technically complex, requiring multiple timed, temperature-sensitive steps and, in most cases, dedicated equipment is necessary to perform the test effectively. Therefore, these tests are performed in centralized laboratories. This testing has served and continues to serve blood donor screening well with tests that are highly sensitive and speciÞc. However, the needs in the blood donor screening arena are very different from those in the diagnostic arena. Whereas the intent of blood donor screening is primarily to determine the suitability of a donated unit of blood or plasma for medical use (i.e. product-oriented), the intent of diagnostic testing is to provide the patient and the professional caregiver with information that will be used to allow for counseling to change behavior and reduce the risk of transmission, to notify partners of the infected individual, and to make medical decisions. Therefore, diagnostic testing is patient-oriented. Using a blood donor screening test for diagnostic purposes requires two visits to the testing site, one to collect the specimen and another to receive the test results, often one to two weeks later. Such

TABLE 8.2. *Evaluation of potential sources of error with HIV EIA screening tests*

I. *Dichotomous Interpretation of Results*
The test is actually a continuous measurement: the chance of misclassi cation varies with the relative test reactivity strength of the sample. Thus, if the test result is merely classi ed as negative/positive, information is lost. As an alternative, results may be classi ed as:
a. Non-reactive
b. "Gray zone positive," weakly reactive, or borderline
c. Reactive
d. Strongly reactive

II. *Procedural Error*
a. Physical mixing up of specimens during testing (retesting correctly labeled sera would reveal a discrepancy).
b. Subsequent incorrect linkage of test results (testing of a newly acquired specimen from the same subject would reveal a discrepancy).

III. *Technical Error During Testing*
a. Erroneous dilution (or omission) of sera or reagents
b. Splashing during test sera or reagent addition, or pipette contamination
c. Washing errors
d. Incorrect absorbance measurement (e.g. wrong wavelength; bubbles on wet plate or scratches on plate; equipment or electronic malfunction).

IV. *Test Kit Error*
a. Variability in kit reactivity (related to vendor quality control)
b. Instability or deterioration of reagents

V. *False Positive Results*
Although some occur in association with certain clinical conditions, speci c con rmatory tests are important.
a. Recognized Problems
1. HLA-antibodies: (particularly related to the viral puri cation process of some early test kits; poses a diagnostic question for multiparous women and others with repeated HLA exposures)
2. Repetitive freeze/thaws (e.g. some stored sera)
3. Other retroviruses
4. Heating of sera
5. Autoantibodies (e.g. ? antinuclear antibodies as in systemic lupus erythematosus, or anti-mitochondrial antibodies) (419)
6. Hypergammaglobulinemia, “sticky sera” (e.g. specimens from Africa)
7. Cross-reactive proteins (e.g. 25–30 Kd) (420)
8. Non-speci c IgM binding (e.g. after vaccination; possibly also related to acute or in ammatory phase responses) (421–425)

VI. *False Negative Results*
a. Group-O or other variants (see text) (94)
b. HIV-speci c antibodies may decline as a function of severity of HIV related immune dysfunction
c. Viremic patients without (IgG) antibodies—including those persons recently exposed/infected.
d. Hypogammaglobulinemia (including congenital conditions) as a variant of (B) above.
e. States of antigen excess.
f. Heating of sera.
g. Transient seronegativity (? selected pediatric infection)

a system can result in considerable anxiety for the patient. In addition, CDC estimates that 8,000 HIV-infected people who come into public clinics for HIV testing do not return to receive their test results. Even in hospital settings it can take at least 24 hours to receive an HIV test result. One possible way to remedy this situation is through the use of point-of-care tests that permit test results to be provided to patients at the time that their specimen is taken, commonly referred to as rapid HIV tests.

Alternative screening formats besides EIA had been developed; for example, dot-blots and latex agglutination formats. The latter can yield results in seconds (74), analogous to some home pregnancy kits. Early developmental work indicated frequent pro-zone phenomena, in which sera with high levels of HIV antibody gave false negative results. Dilution of sera both identified and resolved this problem, but the sensitivity and specificity have remained below that of the EIAs. Thus, these kits were recommended only where sophisticated laboratories were impractical or rapid preliminary results were very valuable.

Rapid HIV tests are, at present, immunoassays that can be in a few different formats. In the flow-through immunoconcentration system, a specimen is mixed with HIV-1 antigen-coated latex particles. If anti-HIV antibodies are present, they will bind to the antigen on microparticles. This mixture is applied to a membrane housed in a cartridge. Absorbent material below the membrane facilitates flow through the membrane. Antibody bound to the latex beads is detected by means of an anti-human antibody conjugated to a readout system, such as an enzyme and a substrate. In this type of system, a series of wash steps are needed following the addition of each reagent.

A second type of immunoassay used for rapid HIV tests is the lateral flow immunochromatographic system. In this case, a specimen is added to a dilution buffer, and the test device containing a test strip is inserted into that solution. The solution flows up the device by capillary action and rehydrates a colored conjugate that binds to antibodies in the solution. The mixture then passes through a region of the test strip containing immobilized HIV antigens. If antibodies to HIV are present, the anti-HIV-conjugate complex binds to that portion of the membrane and a line appears. This type of test may also include a second line that captures conjugate complexed with any human IgG, thus serving as a procedural control. (Some home pregnancy test kits have used similar methodologies.)

A third type of device is a cross between the first two, a flow-through device containing a membrane on which antigens are bound. All tests are typically manually performed and visually read qualitative tests.

The first rapid HIV test available for use in the U.S. was Recombigen, a latex agglutination test licensed by the FDA in 1989. However, it was not widely used because it was not as accurate as other licensed HIV tests, and it is no longer available.

The Single Use Diagnostic System (SUDS) manufactured by Abbott/Murex was licensed in 1992 for the detection of antibodies to HIV-1 in serum and plasma. The test requires refrigerated storage of the test kit reagents and takes approximately 30–45 minutes to perform with multiple washing and incubation steps.

Rapid HIV tests should ideally have several characteristics to be useful in point-of-care settings as an aid in the diagnosis of HIV infection. They should (1) provide a test result within about 20 minutes, (2) not require the use of specialized equipment, (3) not require special storage conditions, (4) be simple to run with few manipulations, and (5) be nearly as sensitive and specific as licensed blood donor screening tests.

One such test is the OraQuick Rapid HIV-1 Antibody Test (OraSure Technologies, Inc., Bethlehem, PA), which was approved by the FDA in November 2002 (26). This visually read immunoassay provides a test result on a whole blood specimen obtained via fingerstick within 20–60 minutes, with a stated sensitivity of 99.6% (95% C.I = 98.5%–99.9%) and specificity of 100% (95% C.I = 99.7%–100%), according to data provided by the manufacturer in support of FDA approval.

According to guidelines set by CDC (25), reactive rapid test results should be confirmed by more specific testing, such as WB, requiring a return visit by the test subject to obtain the confirmatory test result. On the other hand, recognizing that highly accurate tests have a very high likelihood of a correct result and that there is a clear benefit to the test subject and the test subject's contacts, a reactive result may be reported to the test subject as a preliminary positive result, with appropriate information on the meaning of that result in the context of the test subject's risk factors, if any. A non-reactive test result is reported as negative, but careful explanation of the interpretation of that test result is needed, especially in the case of a test subject who may have had a recent exposure placing that subject in the window period before seroconversion (see below).

As additional rapid HIV tests become available in the U.S., the appropriateness of using different combinations of such tests will be evaluated. A multitest algorithm could conceivably allow a test subject to receive a highly accurate test result in a single, relatively short visit to a testing site.

SPECIAL SCREENING ISSUES

HIV-2 and HIV-1

The initial serologic studies in West Africa that found reactivity to a second, related HIV were based on cross-reactivity to simian retroviruses. Isolation and characterization of LAV-2 led to the acceptance of what became known as HIV-2 as a virus distinct from HIV-1, but controversy existed initially regarding pathogenicity

since most persons infected with HIV-2 appeared to be healthy in several of the initial serosurveys in Africa.

In 1988, the first instance of AIDS in an HIV-2 infected individual in the United States was confirmed (75). This case helped to establish the fact that HIV-2, at least in some persons, can cause clinical AIDS (76). Importantly, this case of HIV-2 infection highlighted the limitation of some HIV-1 screening tests for detection of HIV-2, indicating the limitations involved in depending solely upon cross-reactivity between antibodies to HIV-2 and HIV-1 antigens (77). As a result, screening tests for HIV-2 were developed, incorporating HIV-2 viral lysates. In addition, tests were developed that detect antibodies to HIV-1 and HIV-2. These screening tests are quite useful in countries with a very low prevalence of HIV-2, such as the United States (78–80). Contrary to some theoretic expectations, the sensitivity of the early combined assays was comparable to many of the individual HIV assays. Since implementation of testing for HIV-2 in the United States, additional cases of HIV-2 infection have been documented (81). There are currently one licensed EIA to detect antibodies to HIV-2 and two licensed EIAs to detect antibodies to both HIV-1 and HIV-2.

Serologic discrimination of HIV-1 and HIV-2 can be difficult (82), so that confirmation by nucleic acid testing is helpful (77,83,84) and has been utilized to demonstrate apparent co-infection with both HIV-1 and HIV-2. Other possible approaches include competitive EIAs (85) or assays containing type-specific synthetic peptides. The size of the HIV-1 and HIV-2 antigens also differ somewhat (confer Table 8.3), so that WB and radioimmunoprecipitation assays prepared with the respective viruses (see below) also can provide some assistance (77,86–88). Because of the serological cross reactivities between HIV-1 and HIV-2 (Fig. 8.1), in certain populations it is difficult to ascertain whether an individual is infected with HIV-1,

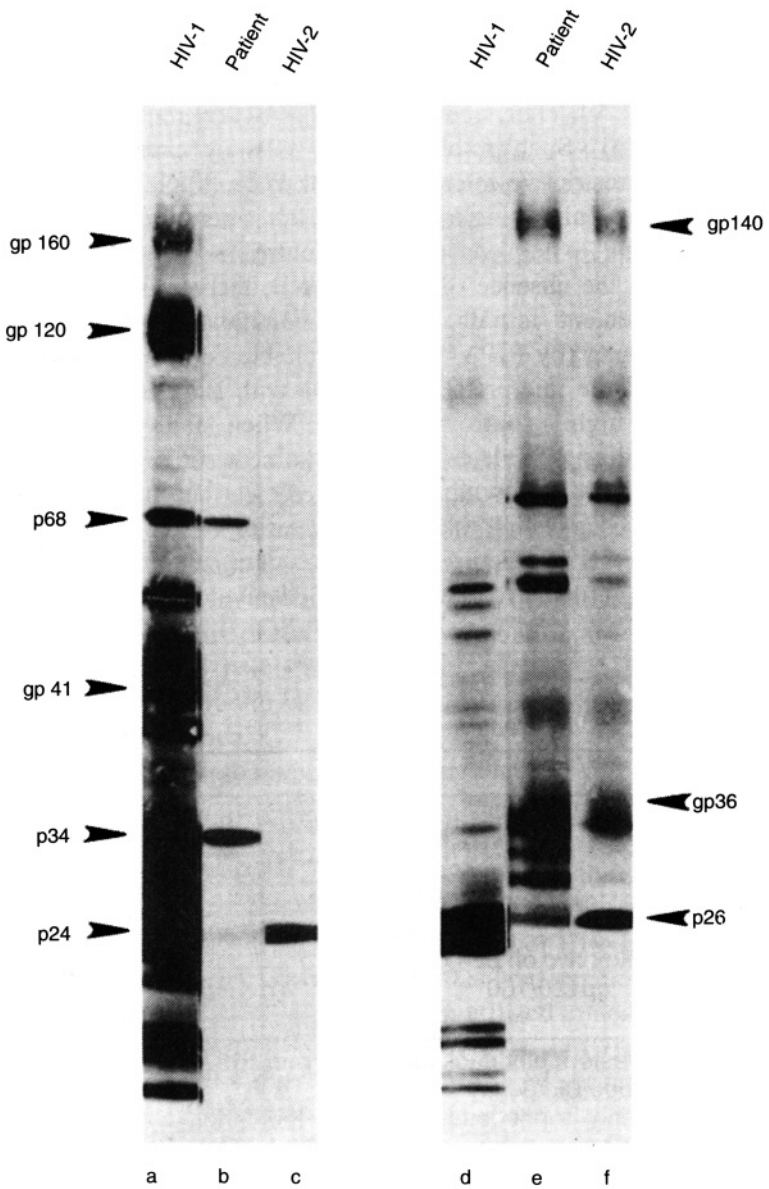


FIG. 8.1. Western blot analysis of sera samples from a HIV-1 control, an AIDS patient with PCR-confirmed HIV-2 infection (77), and a HIV-2 control on HIV-1 antigens (lanes a through c) and on HIV-2 antigens (lanes d through f). Reactivity and cross-reactivity of HIV-1 and HIV-2 are demonstrated in the *gag* (p24/p26), *pol* (p31/p34 and p66/p68) and *env* (gp41/gp36 and gp160/gp140) gene products. Figure courtesy of Joseph M. Lombardo, M.D., Ph.D. and Stanley H. Weiss, M.D.

HIV-2, a new viral type, or whether the individual is infected simultaneously with multiple viruses (88). More speciÞc tests such as viral isolation or molecular probes (e.g. nucleic acid ampliÞcation) remain necessary to distinguish between infections with these viruses in some populations (89).

Dual EIA Assays to Detect Recent Infections

An interesting use of EIAs has been described that is effective in identifying recently infected individuals (90,91). This strategy is based on the knowledge that during the Þrst three to four weeks following exposure to HIV, circulating antibody levels progressively increase (see Window Period section below). At this time, conventional EIAs are effective at detecting these relatively low levels of antibodies. On the other hand, an EIA that is run using a modiÞed protocol to make it less sensitive (a so-called ÒdetunedÓ assay) cannot detect anti-HIV antibodies at this stage of the infection. Therefore, a specimen that is repeatedly reactive using the conventional assay but is negative using the detuned assay most likely represents a recent infection. This strategy is being employed by CDC to distinguish recently infected individuals from those who have established infections, in an effort to better understand the spread of HIV.

HIV Group O

In classifying HIV-1 strains, most are classiÞed under Group M (Òmain)Ó, but some are classiÞed as Group (O) (ÒotherÓ). These Group O strains were initially noted on rare occasions in some African sera that had screened negative for HIV antibodies with some commonly employed tests. Tests using recombinant products (with increased speciÞcity) instead of cultured viral products (with potential broader range of detection) appear to be more likely to give false negative results with Group O infections. With the detection of sera from a Group O infected person in the U.S., increased emphasis has been placed on monitoring for these strains and the potential need to modify current screening procedures (92Ð94). As also discussed further below, in persons in whom HIV infection is strongly suspected, additional testing may be necessary even if the initial screening test is negative (77,94). Although there are currently no serological assays licensed or approved to detect antibodies to HIV-1 Group O, and these strains currently still remain very rare in the U.S.

IgM and IgA Assays

Research serological tests for IgM and for IgA antibodies have been developed (95). Since IgM and IgA do not cross the placenta, particular attention was placed on the potential for use in neonatal diagnosis. Some negative HIV IgM tests during the Þrst month of life, however, occurred in children who were later shown to be HIV-infected. These misclassiÞcations, due to biologic phenomena (the child having been infected pre-partum) rather than technical issues, led to alternative methodologies to resolve whether a newborn is infected. Neither IgM nor IgA assays have been commercialized.

ANTIBODY CONFIRMATION ASSAYS

Due to the imperfect speciÞcity of screening tests, reactive EIA results are not sufÞcient to make the absolute determination that an individual is infected with HIV. Given the low prevalence of HIV infection in the U.S. and the high speciÞcity of HIV screening tests, the negative predictive value for EIA screening tests in blood donors is high (see above). CDC has recommended that repeatedly reactive EIA results and reactive rapid test results be followed up with supplemental testing, sometimes referred to colloquially as Òconfirmatory testingÓ (see Table 8.1). There are two types of supplemental tests in common use today, the WB and the indirect immunoßuorescence assay.

Spectrum of HIV Antibodies

While the disrupted whole virus EIA detects HIV antibodies, the precise spectrum of component antibodies remains undetermined. Several confirmation assays enable the visualization of speciÞc antibody reactivities. With the sequencing of the nucleic acid structure of several isolates of HIV, the functional structure of this RNA virus was rapidly unveiled. The HIV genome includes several Òopen reading framesÓ related to essential viral components, including:

1. *gag* Ðthe viral core,
2. *pol* Ð the enzymes reverse transcriptase (which is essential in creating a DNA copy of the viral RNA) and endonuclease,
3. *env* Ðthe envelope of the virus, including the glycoprotein (gp) membrane,
4. *tat* Ða trans-acting protein related to the control of viral replication.

Serologic work based on cloned monoclonal reagents (96Ð98) along with concordance to predictions based on the viral genome have provided an evolving picture of the relationship of various component viral proteins to HIV-1 (Table 8.3). (The gene product sizes for HIV-2 differ somewhat; confer (77,83,84,86,99) and Table 8.3). These components vary in inherent immunogenicity, and may be produced to varying degrees during the course of infection. Antibodies to some viral components, e.g. the HIV-1

TABLE 8.3. *Major genes and gene products of HIV-1, HIV-2, and HTLV*

GENES	GENE PRODUCTS*		
	HIV-1	HIV-2	HTLV
Group-speci c antigen/core (*gag*)	p17, p24, p40, p55	p16, p26, p56	p19, p24, p28, p53
Polymerase** (pol)	p31, p51, p66	p34, p53, p68	
Envelope (env)	gp41, gp120, gp160	gp36/41, gp80, gp105/125, gp140	gp46, gp62/68, p21***
Miscellaneous			p38 tax, p42****

* Represented by: p = protein or gp = glycoprotein; plus the approximate molecular weight of the antigen in kilodaltons (kd).
** Endonuclease and reverse transcriptase.
*** Can be produced in high titer by recombinant methods.
**** p42 is of uncertain origin; some believe it may be *gag* related, but it is not included in current standard genome maps.

regulatory gene tat-III (14 KD), are not detected by standard techniques.

Western Blot

The Western blot (WB), also known as immunoblot, procedure has become a mainstay in supplemental testing since it readily permits visualization of the component antibody reactivities and, in essence, reveals the reason behind the reactivity in the screening assay (31,32) (Fig. 8.1).

Preparation of the WB begins with puri Þcation of HIV virions from cultured cells, followed by dissociation of the concentrated HIV with detergent. The viral proteins in this lysate are then resolved by polyacrylamide gel electrophoresis, separated by their molecular weights, after which they are electrophoretically transferred to a solid support, such as nitrocellulose paper, and then cut into strips. Individual strips are reacted with either test or control sera; bands where the serum antibodies have bound are normally detected by anti-human-IgG conjugated either directly or indirectly (through biotin-avidin) to an enzyme (e.g. horseradish peroxidase or alkaline phosphatase) and reagents that react with the conjugate to produce a band. Whereas the viral lysate puri Þcation steps in early assays resulted in the loss of gp120 and gp160, second generation WBs use puri Þcation techniques that allow gp120 and gp160 to be saved. WB results are given as a series of intensity readings for each band.

Several alternative criteria had been proposed for HIV-1 WB interpretation (Table 8.4). As discussed further below, since the CDC recommends the ASTPHLD criteria based on CDC evaluations, the other criteria are rarely now applied. The Cambridge Biotech HIV-1 Urine Western Blot Kit, which is manufactured by Calypte Biomedical, has different criteria; only a gp160 is required for positivity.

Radioimmunoprecipitation

The radioimmunoprecipitation assay (RIPA) requires HIV to be grown in cell culture in the presence of a radioactive label, puri Þed, and disrupted (100Ð102). Standard techniques lead to poor labeling of gp41, but lentil-lectin enhancement or alternative radiolabels may be utilized if gp41 detection by RIPA is desired. In this technically complex procedure, the radiolabeled lysate is reacted with the test serum, the immune complexes

TABLE 8.4. *Criteria for Òconfirmed positiveÓ HIV-1 Western Blot result on serum, plasma or dried blood spot*[1]

Organization	Minimum Band Pattern Required
1. Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) (42)[2]	Any *two* of: p24, gp41, OR gp120/160
2. DuPont-Biotech[3]	p24 *AND* p31 *AND* (gp41 OR gp120/160)
3. Consortium for Retrovirus Serology Standardization (426)	(p24 OR p31) *AND* (gp41 OR gp120/160)
4. American Red Cross (148)	At least one band from *each* gene product group: *gag AND pol AND env*

[1] The “Cambridge Biotech HIV-1 Urine Western Blot Kit” which is manufactured by Calypte Biomedical has different criteria; only a gp160 is required for positivity.
[2] Also supported by the Centers for Disease Control and Prevention (42,427), and utilized in the current package inserts by Bio-Rad Laboratories and Calypte Biomedical Corp.
[3] Food and Drug Administration-licensed DuPont Western blot package insert from 1987. However, the criteria for this blot, which is now called the “Cambridge Biotech HIV-1 Western Blot Kit” which is manufactured by Calypte Biomedical Corp., per the current package insert now conforms to the ASTPHLD criteria.

isolated by precipitation, washed, resolved electrophoretically, and exposed to Þlm to reveal the viral components bound by the patient specimen. The entire process takes several days to over a week. Since RIPA reliably detects gp120 and gp160 antibodies, it was the reference standard for these large envelope antibodies until WBs were improved. RIPA analysis routinely includes a comparison against control material from a radiolabeled uninfected cell culture, to differentiate cellular bands from viral bands.

Utilization and Interpretation of WB and RIPA

WB and RIPA are said to conÞrm a repeatedly reactive EIA screening test result because they can readily reveal the speciÞc anti-HIV antibodies responsible for the reactive result. When classic patterns are present, positivity is a virtual certainty (Table 8.4). When several related antibodies are detected, the pattern still has high predictive value. Thus, the WBÕs that detect gp120 and gp160 are helpful in classifying potentially ambiguous sera. In some lots of WB reagents, the gp120 and gp160 bands may not be sufÞciently separated to appear as clearly independent bands.

A broad gp41 WB band was initially accepted as adequate conÞrmation of sera from a person at high risk of HIV. It was also clearly necessary to have further conÞrmatory evidence for sera from very low risk group sera. Since a solitary sharp (thin) WB band at p40 was likely to indicate reactivity unrelated to HIV, an experienced virologist and laboratory were crucial in making this critical but subjective distinction during the Þrst years of HIV testing. As in other areas of medicine, adequate training in subtle differences can be essential.

Greater experience and the extension of testing to many labs revealed that signiÞcant variations in WB reactivity were observed in practice, with results frequently discordant compared to those from reference WB laboratories (103). Thus, aberrant Þndings clearly required follow-up (104). Such problems led to calls for more formalized and restrictive interpretation of WBs (Table 8.4). In some states, only a single centralized lab is permitted to perform testing to help ensure quality control.

In the absence of gp41 on WB, there initially was widespread agreement that the combination of p24 and pr53gag (by RIPA) provided reliable conÞrmation. However, the interpretation of WBs with isolated reactivities was highly controversial (105). When single bands were noted as the sole reactivity, in some instances subsequent testing of follow-up sera showed clear-cut broad reactivitiesÑ suggesting that the initial result was consistent with early HIV infection (106). Some data suggested that p24 core antibody frequently preceded envelope antibodies.

However, persons with solitary p24 bands may be found among historic blood donor sera (23) and instances of prolonged persistent p24 reactivity in low risk subjects have been noted. This persistent reactivity pattern can be associated with a relatively sharp (i.e. atypical) p24 region. Whereas the initial CDC WB interpretative criteria accepted p24 alone as sufÞcient for WB conÞrmation of sera that were repeatedly reactive by EIA, retrovirologists now routinely treat such WB patterns as equivocal. Thus, in some early epidemiologic studies some persons may have been misclassiÞed. Incorrect classiÞcation by WB can occur (107).

The observed sequence of development of HIV antibodies is dependent upon the inherent technical sensitivities of the assays employed as well as the biology of infection. Thus, although gp41 antibody may be absent on WB, other *env* antibodies such as gp120/160 may be detected. Some sera that have appeared to have antibodies solely to the p24 core antigen can be shown to also have *env* reactivity with RIPA and other newer assays. Commercial HIV antibody assays have tended to Þrst detect p24 antibody in seroconverters.

The interpretation of other WB and RIPA patterns in terms of speciÞcity and sensitivity for HIV infection remains uncertain (39,87,108). Persons without evidence of HIV antibodies or with unusual WB reactivity patterns have sometimes been found to be HIV-infected by culture (39,109) or had positive PCR results (110,111), as discussed below.

Several alternative criteria have been proposed for HIV-1 WB interpretation (e.g. see (112,113)), and all are in agreement that the lack of any bands on the WB is considered to be a negative result. CDC has recommended that a positive result when testing serum, plasma or dried blood spots is deÞned as at least two of the following: p24, gp41 and gp120/160 and it is this interpretive scheme that is generally followed (Table 8.4). (42). When testing urine, the Cambridge Biotech HIV-1 Urine Western Blot Kit has different criteria; only a gp160 is required for positivity. Any other pattern on these assays is considered to be an indeterminate result.

No WB is currently licensed in the U.S. for conÞrmation of HIV-2; although the long-term follow-up of indeterminate results from research assays has been examined (114), limited data exist.

Indirect Immunoßuorescence Assays

The indirect immunoßuorescence assay (IFA) relies on the ability to detect anti-HIV antibodies from patient serum or plasma that bind to the surface of infected test cells. Acetone-Þxed HIV-infected and uninfected (control) cells are typically applied to slides or isolated wells, incubated with test sera, and counter-stained (e.g. with ßuorescein-conjugated anti-human IgG). A dilution series of a given serum is usually run to assess strength (titer) of reactivity. The reactions are viewed microscopically. The ßuorescent staining pattern, when read by a skilled observer, is quite informative. Anti-nuclear antibody and

anti-mitochondrial antibody reactivity patterns may be causes of non-speciÞc reactivity. Furthermore, the titration-endpoints of the reactions on the infected and uninfected cells can be compared. This information is then synthesized as a positive or negative result. There is signiÞcant potential for observer bias in these subjective assessments. Reactions against both infected and uninfected cells may suggest non-speciÞc reactivity. However, such an IFA pattern might also occur concomitantly with true HIV-speciÞc antibody, such as in high-risk persons with high anti-HLA titers (51). There is currently one FDA-licensed IFA available for clinical use.

OTHER ANTIBODY TESTS

Neutralizing Antibodies

Some HIV antibodies have neutralizing properties *in vitro*, detected by inhibition of syncytial formation (115Ð118). The protective role, if any, of antibodies detected by these methods remains to be determined (119,120). These research assays have found particular application in the HIV vaccine developmental efforts (120). Antibodies to puriÞed thymosin alpha-1 also show some evidence of neutralizing capability *in vitro*, which may be related to its similarity to HIV p17 (121). SpeciÞc antibody may sometimes reduce mother-to-child transmission (122Ð124).

The principal neutralizing domain of HIV appears to be in the V3 hypervariable region of the envelope (the ÒV3 loopÓ) (125Ð130), although other viral regions may also prove to be important. Limited variation, either in the V3 loop (131) or outside of it, can affect neutralization. The reactivity pattern to various neutralizing antibodies were useful in classifying the viral strain (132Ð138), but this approach has been supplanted by newer techniques.

Neutralizing antibodies appear to be in higher titer in HIV-2 infection than in HIV-1 infection, and they may be more important in the clinical epidemiology of HIV-2 as compared with HIV-1 (139). In this context, it is interesting to note that the natural history of HIV-2 is associated with slower progression to disease than is seen with HIV-1. These Þndings carry possible implications for vaccine development. Unless tests for neutralizing antibody to HIV prove to be important clinically, diagnostic assays will likely remain in the province of research laboratories.

Control Assays

HIV antibody detection tests generally involve the use of preparations containing one or more HIV antigens. An analogous assay may be run in parallel with comparable reagents but excluding HIV antigen, providing a comparison (control) assay for the evaluation of reactivity. Absence of reactivity on the control assay in the face of reactivity on the primary assay implies the reactivity was related to HIV itself, i.e. a true positive. This technique was used historically in research evaluation studies of EIA (e.g. the proprietary H9 control plate), immunoßuorescence, and WB methods, and it was a critical parallel control in radioimmunoprecipitation and in early direct viral detection methods. While reactivity in the control assay indicates the presence of some reactivity to non-viral constituents (e.g. related to HLA antibodies or antinuclear antibodies), it cannot rule out the possibility of concomitant true reactivity. Thus, false positivity cannot be presumed. With the advent today of many alternative HIV assays, this type of control assay (which could not deÞnitively discriminate between true and false positivity, and as tests that directly detect conditions associated with false reactivity (e.g. HLA antibodies) do not rule out true positivity (51,53)) has few uses today. In summary, although control assays assisted in data interpretation, they could not be relied upon as conÞrmation tools.

ISSUES PRIMARILY RELEVANT HISTORICALLY OR DURING DEVELOPMENT OF TESTS

Repository or Other Stored Specimens

Specimen integrity can have a signiÞcant impact on the performance of any assay. Manufacturers generally recommend that their kits be used on fresh sera.

Repetitive freezing and thawing of sera increases the probability of false positive reactions (reßecting decreased speciÞcity, usually manifested as weak reactives), and may potentially also decrease sensitivity. Likewise, if sera are heated in an attempt to inactivate virus as a laboratory biosafety measure, both assay sensitivity and speciÞcity may be impaired.

An analysis of speciÞc clinical conditions that have given difÞculties for certain tests in practice and knowledge concerning the design of these tests (and thus the theoretical problems) can help guide the discriminating laboratory manager in judiciously evaluating the results from a commercial assay (Table 8.2).

Reactivity Strength

Reactivity in the whole virus EIA, which detects reactivity to any of a variety of viral components, can vary depending upon the clinical status of the patient. For example, AIDS patients with opportunistic infections have been shown to be signiÞcantly less reactive by EIA than AIDS patients with KaposiÕs sarcoma (23). This probably reßects the observation that during the course of HIV infection, not only cellular immunity but also humoral immunity is impaired resulting in decreased levels of speciÞc antibodies. In contrast, persons with HIV-associated lymphadenopathy tend to be very strongly reactive

by EIA, as well as by immunoßuorescence (140), suggesting an enhanced humoral response.

The relative titers of viral component-speciÞc antibodies vary over time in individuals, leading to systematic differences in EIA reactivity (and, consequently, detection rates) among various populations or patient groups. Furthermore, in some persons HIV antigen may be produced in sufÞcient quantity to form immune complexes with corresponding HIV antibodies, potentially reducing the ability of those antibodies to bind to viral antigens in the assay and giving a false negative test result. As discussed above, in several panels of sequential sera from seroconverters, antibody to p24 was the Þrst to be routinely detected by WBs or monoclonal reagents.

Serologic evidence of anti-HIV antibodies reactive with live HIV-infected cells have been described recently, and called Òearly antibodies.Ó These may mark a relatively early immune response to HIV (141). The speciÞcities of these antibodies are characterized by the recognition of type-speciÞc conformational epitopes of gp160 and gp41 (see Table 8.3). It has been claimed that they may occur earlier than many other markers of HIV infection (141).

In late stages of HIV infection, given the existence of a spectrum of viral antigens, immunologic reactivity usually persists to at least some components. In early infection, the phenomenon of immune complex formation probably contributes to the Òseronegative windowÓ (see below). AcidiÞcation techniques, which disrupt immune complexes, were brießy used historically to improve the sensitivity of some early HIV antigen assays.

Special Problems for Tests that Detect Anti-HIV Antibodies

Studies undertaken in Africa suggested the occurrence of non-speciÞc, weak HIV EIA reactivity, particularly among persons with high antibody titers against species of malaria (142). Additionally, assays may be changed continually during their development, as investigators vary reagents and conditions, and it should be assumed that the performance characteristics will also change. Indeed, the non-speciÞc association seen with malaria in some early studies in Africa (142) may have been partly related to an insufÞcient accounting for this source of variation (143). This again highlights the issue of interpreting low level (Òweakly reactive result)Óreactivity on a screening test, in the absence of conÞrmatory assays.

Subsequent early investigators also found African sera to be unusually falsely reactive on certain EIAs (144). This would be consistent with the hypothesis that the higher immunoglobulin levels observed in this population may, in effect, systematically shift the standardization curve for African sera as compared to U.S. and European sera. Since a competitive EIA out-performed other screening methodologies in some research studies of African sera (145), there may be selected circumstances for specialized approaches. Exposure to other retroviruses related to HIV, human and/or non-human, may explain some of the low level reactivities in regions such as Africa. The early Þndings of very low titer (or low reactive) antibodies to HIV in stored sera from Uganda is consistent with such a possibility (146). Infrequently, high titer HTLV-I antibodies can lead to falsely reactive HIV screening results (23), perhaps relating to p24 *gag* protein cross-reactivity (147). With the continuing discovery of additional retroviruses, ongoing research is likely to clarify gradually these cross-reactivity issues.

Transient Issues

The ability to conÞrm a serological screening test result for HIV in the U.S. depends upon the availability of tests approved or licensed for that purpose. Early on, this was a problem as no conÞrmatory test was licensed when the Þrst screening tests became available. This became an issue again, transiently, in 2002 when the Public Health Service became aware of a potential shortage of licensed WBs (41).

INTERPRETATION OF HIV SEROLOGIC TEST RESULTS: DETAILED ISSUES

An essential part of the testing process takes place even before testing done; that is, the estimation of the probability of infection (the Òpre-testÓprobability). This is necessary in order to interpret a test result appropriately, whatever the purposeÑ whether it is clinical, counseling or researchÑ and can dramatically impact the predictive value after testing (or Òpost-testÓprobability).

In the absence of any information to assess the pre-test probability, as in the blinded laboratory, the positive predictive value can be maximized (and false positives minimized) by using extremely conservative classiÞcation criteria. This is done at the expense of classifying many samples Òindeterminate.ÓIn part, this explains the rationale for conservative WB criteria (148) (Table 8.4). The use of such criteria is analogous to the assumption that the pre-test probability is very low.

Gray Zone

Licensed clinical laboratories are required to adhere strictly to the package insert of licensed products, which represents the vendorÕs recommendations as approved by regulatory agencies. Historically and for research purposes, some laboratories have utilized additional cut-off points compared to the vendorÕs recommendation, since simple dichotomization (reactive vs. non-reactive) may be misleading (Table 8.2). Indeed, during a development process (such as clinical trials) or post-licensure surveillance of a productÕs efÞcacy, vendors may bring

"in-house" both reactive specimens as well as those with somewhat lesser reactivity. A threshold of 10%, and occasionally even 20%, below the standard cut-off is chosen; the gray zone (weakly reactive results) is defined as those between this threshold and the standard cut-off.

Epidemiologic assessment of HIV tests confirmed early on that HIV EIA reactivity was monotonically associated with positivity (23). A lower EIA cut-off value provides higher sensitivity but lower specificity for a given assay than obtained with a higher cut-off (23).

The nature and extent of further evaluation, and the ultimate clinical interpretation, vary as a function of the strength of EIA reactivity and the pre-test assessment.

Persons at High Risk

When the prior probability is high (as for persons at high risk from hyper-endemic regions or high risk groups), the positive predictive value of a reactive or strongly reactive EIA is extremely high (Table 8.2). Although a confirmatory assay, such as a WB, may give an "indeterminate" result based on simple application of some generic criteria (Table 8.4), the use of alternative interpretative criteria or additional tests can often be utilized to confirm the diagnostic impression.

A weakly reactive screening result, still likely to be truly positive based on risk factors, requires further testing to obtain confirmation if the WB is not clearly positive. Thus, it is important to evaluate the actual absorbance values, and not simply a reactive/non-reactive result, and these values should be routinely obtained from the testing laboratory in such circumstances.

A non-reactive result from a high-risk patient has only a moderate negative predictive value in this situation. Therefore, it is important to rule out technical or procedural error (Table 8.2), recheck the clinical history, and possibly employ alternative and/or more sensitive assays. Thus, an unexpected negative result should not be accepted as final without further evaluation, since it is essential to minimize false negative results. An historical example is the case of a patient in the United States with clinical AIDS who had multiple negative HIV-1 screening tests. Epidemiologic investigation found that this person had recently entered the U.S. from West Africa, where one research study had just reported finding a high prevalence of HIV-2. Thus, specific evaluation for HIV-2 was warranted on both epidemiological and decision theory grounds (75,77). In the U.S., HIV screening now routinely includes HIV-2.

Persons at Low Risk

For an asymptomatic person at very low HIV risk, such as a blood donor, the negative predictive value of a non-reactive EIA result is extremely high. In the event of a reactive result, procedural or technical error should be ruled out (Table 8.2), since most will be false positives. Thus, specimens with initially reactive results are retested in duplicate. Many will be clearly negative on both repeat tests. Given the very low pre-test estimate, the overwhelming likelihood is an erroneous first result. However, if the person's history raises any suspicion of risk, which would revise the pre-test estimate upwards, additional testing by another methodology would be warranted. A repeatedly reactive result is likely to be a true positive, but confirmatory tests may be necessary to attain an acceptable positive predictive value. (Regulatory practices may mandate that a confirmatory test be run in any event.) For example, an indeterminate WB may be clarified by other tests or by clinical follow-up. The related finding, that most persons with repeatedly reactive screening test results and persistently indeterminate WB appear to be uninfected with HIV (149,150), is consistent with these expectations based upon decision analysis considerations.

Care must be taken, however, when interpreting the results from a sequence of tests. Assays are generally not strictly independent, since one source of bias may simultaneously affect multiple laboratory techniques. For example, EIA, WB and IFA are all techniques that detect antibodies to HIV, as opposed to techniques that detect viral antigens of viral RNA directly (see below). Pure Bayesian analysis, which assumes strictly independent tests, will typically therefore lead to overestimation of predictive values with most "supplementary" tests (see Table 8.1).

A weakly reactive result often suggests the need for clinical reassessment, and thus further discussion with the patient. If there is a possibility that the result reflects an early seroconversion, repeat antibody testing several weeks later might clarify an equivocal result. Alternatively, other diagnostic tests may be done immediately, e.g. WB, antigen assays or other direct viral detection methods, or screening assays from other vendors. If the subject has a condition associated with false positive reactions, a negative confirmatory test (e.g. WB) is strong evidence that the patient is not infected with HIV.

Among low risk persons, the repeat clinical history may continue to indicate no risk behaviors or exposures, which means that the pre-test probability remains extremely low. In this case, even an initial positive confirmatory test implies only a low positive predictive value and further evaluation may be appropriate, e.g. by reference research laboratories.

Additional Considerations in HIV Testing

Although the intended use in the blood bank and in clinical/diagnostic settings is similar, the practical applications of tests to detect antibodies to HIV are quite different. In the former situation, the intent is to identify a blood donor who is infected with HIV. Therefore, it is

critical to minimize non-reactive results on specimens from infected individuals (known as false negative results), in order to assure maximally the safety of the blood supply and blood products. In the latter clinical instance, the goal is to counsel persons correctly concerning whether or not they have been infected, with implications for behavioral counseling, medical evaluation and appropriate therapy.

Since some current donors are HIV-infected, the operative assumption that Òall blood donors are true negativesÓ is false. This would lead to a paradoxical situation that perfect speciÞcity (no false positives) would be attained only for a test that detected absolutely *no* positives among current blood donors. A test that was never positive would have perfect speciÞcity (but zero sensitivity). This paradox might tempt manufacturers to adjust assays to take advantage of this speciÞcity loophole, leading to undesirable results. Furthermore, the inclusion of true positives that get tabulated as false positives would wrongly underestimate the assay characteristics. Thus, all repeat reactives that come up in the low prevalence population (assumed zero prevalence) are tested further in current clinical trials, and, if shown to be infected by other methodologies, are permitted to be excluded from the speciÞcity calculations. In essence, the control (very low prevalence) group is redeÞned post-facto to avert the preceding paradox. If the reclassiÞcation as true positive were erroneousÑ as could occur if there was a condition leading to false reactivity on the screening assay which also led to falsely positive conÞrmatory assay(s)Ñ there would be a serious problem and circulatory in deÞnition. For this reason, the reclassiÞcation needs to be done using methodologies as disparate as possible.

CELLULAR IMMUNOLOGIC DETECTION OF INFECTION

Before the discovery of HIV, the diagnosis of AIDS relied upon clinical criteria (151Ð158). Early studies also characterized immunologic impairment, particularly low CD4 counts, comparatively elevated CD8 counts, and a low CD4/CD8 ratio. Persons without AIDS, but who had similar risk behaviors as those with AIDS, frequently also had similar abnormalities. These and other immunologic tests were useful in epidemiologic studies to examine risk factors associated with the epidemic (159Ð166). The advent of HIV serology indicated that tests of immune function remained useful for staging patients, but conÞrmed the expectation that they were not sufÞciently accurate to be used to predict if a given individual were infected with HIV (167Ð174).

Helper T-cell defects occur early in HIV infection (175Ð178). Lymphokine production (such as interleukin-2) can be used to detect antigenic peptide recognition by T-helper lymphocytes, in symptomatic and asymptomatic persons known to be infected with HIV (179Ð182). These tests are also useful to monitor the response of persons receiving prototype vaccinations against HIV (183Ð185). HIV-1-speciÞc T-helper cell responses have been reported in HIV seronegative individuals at high risk of HIV exposure, including several studies in which extensive virologic studies were negative (186Ð192). At least two people with this immunologic proÞle subsequently seroconverted while under prospective study (193) (and SH Weiss et al., unpublished data), indicating that these responses do not indicate complete, if indeed any, protection. Although a correlation between these responses and a novel HLA antibody was reported (187), so far the possibility of a serologic marker for this has not come to fruition. Further studies and follow-up remain of interest to interpret these immunologic Þndings, which

a. may be non-speciÞc,
b. may represent an immunologic response following exposure to non-viable virus,
c. may represent an immune response to a HIV infection that is cleared, without residual latent infection, and/or
d. may indicate early evidence of HIV infection in seronegative persons.

DIRECT HIV DETECTION METHODS

Several methods of direct detection, including tests that measure viral load (typically, the level of ribonucleic acid), are summarized in Table 8.5 with a note of some key limitations of each method, and are reviewed in detail below.

Antigen Assays

Current tests designed to detect antibodies to HIV are able to detect infection following a window period of at least 22 days (see Figs. 8.2Ð8.5). Identifying infected individuals earlier in the infection (closing the window period) is the goal of a number of other methods that can directly detect the virus, as opposed to detecting a host immune response to it.

One of these methods is the detection of HIV-1 p24 antigen, which can detect HIV-1 approximately 16Ð17 days following infection (see Fig. 8.5). These assays typically make use of a monoclonal antibody to HIV-1 p24 antigen coated on a solid support. Antigen in the specimen is captured by the antibody and detected by a second antibody and a system to allow a colorimetric readout. The speciÞcity of these assays can be as high as 99.92% and the sensitivity up to 99.0%. However, note that p24 antigen may only be detectable until approximately 45 days following onset of infectivity (see Figs. 8.4 and 8.5). Therefore, HIV-1 p24 antigen testing should be used as an adjunct to a non-reactive serological screening test for anti-HIV antibody in an individual at risk for HIV infection, and not as a stand-alone screening test.

TABLE 8.5. *Direct viral detection methods and limitations method*

	METHOD	LIMITATIONS
1.	Viral p24 (core) antigen	Limited by detection by latency and immune complex formation. Latter may be partly overcome with acidi cation techniques (see text). See Figs. 8.4 and 8.5.
2.	Nucleic acid ampli cation tests	Prone to false positives due to reaction contamination. False negative results possible with viral variants (232), if a variable region is ampli ed or limited number of cells or organisms (see text).
3.	*In vitro* propagation (viral culture)	Variable success rates. Generally qualitative. Viral strains cultured may be highly selected.
4.	Southern blot or *in situ* hybridization for viral nucleic acid	Sensitivity limited by the number of infected cells.

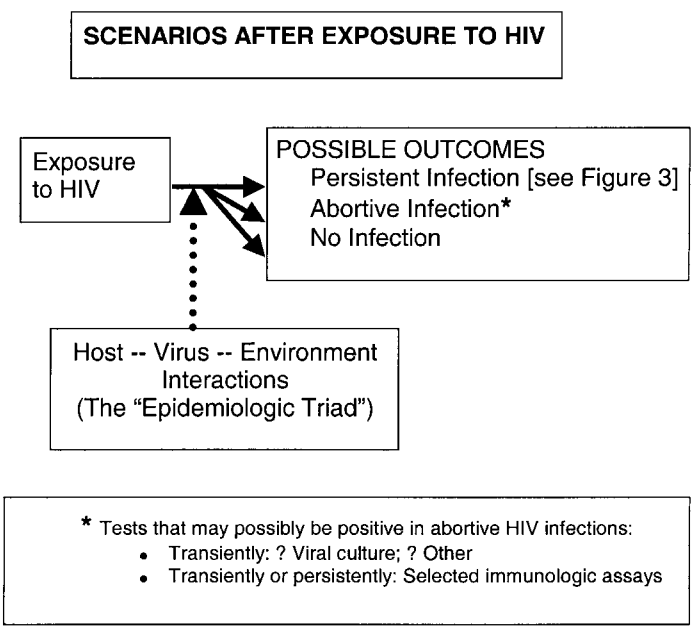


FIG. 8.2. Outcomes after exposure to HIV.

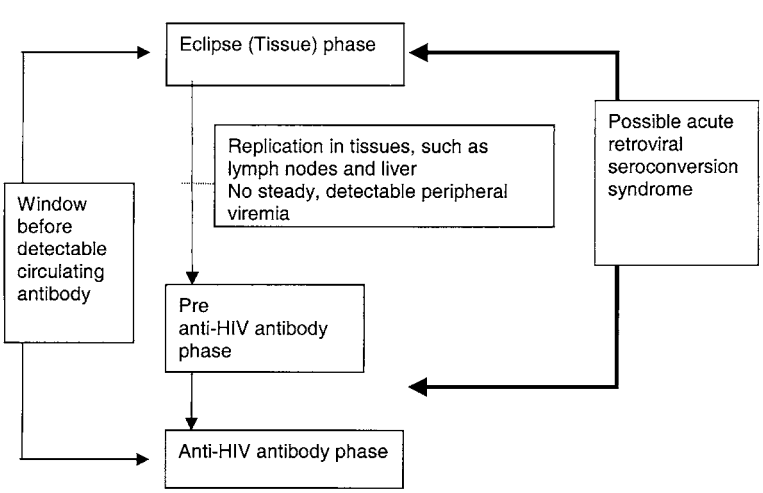


FIG. 8.3. Phases in persistent HIV infection.

SCHEMATIC REPRESENTATION OF KEY EVENTS IN HIV TRANSMISSION DYNAMICS

	E →	T →	I →	X_i →	A →	M
Event	**E**xposure to HIV	Beginning of **T**issue-only period	Onset of high **I**nfectivity	Time test *i* (NAT, Ag, culture) first becomes positive	**A**ntibody detectable	**M**orbidity and mortality
Relative infectivity risk	Low		Very high		Variable	

CHARACTERISTICS OF THE ABOVE INTERVAL PERIODS:

Interval Period	Characteristics
E-T	This interval is probably short
T-I	Intermittent low-level viremia hypothesized
I-X_{NAT}	HIV still not reliably detected
X_{NAT}-X_{Ag}	NAT, but not Ag or Ab, detectable (see Figure 5)
X_{Ag}-A	NAT and Ag, but not Ab, detectable
I-A	Infectious, Ab negative, period
A-M	Ab detectable*; Ag test may turn negative for an extended period after the appearance of Ab

NAT – Nucleic acid amplification tests
Ag – p24 antigen
Ab – anti-HIV antibody
* Some newly infected persons may have a reactive EIA, and a negative or an indeterminate WB, before they develop a positive WB (205;428;429).

FIG. 8.4. Schematic representation of key events in HIV transmission dynamics.

Parenteral sharps injuries among health care and laboratory workers exposed to HIV have led to HIV transmission only infrequently (5,194Ð196). This suggested that the usual titer of viable HIV in peripheral blood was much lower than typical hepatitis B virus levels. *In vitro* hybridization experiments, which indicated that HIV infected cells are rare in blood, brain and lung (197Ð199), suggested that detection of HIV antigen in tissue homogenates would also likely require sensitive assays. Sensitive *in vitro* HIV antigen detection systems have been developed (200Ð204) and are currently in use in the U.S.

Demonstration that an assay speciÞcally measures viral antigen was a challenging task, insofar as there are no independent laboratory criteria. The speciÞcity of a given assay is likely to prove greater in an acellular sterile body ßuid such as cerebrospinal ßuid. Furthermore, antigen that is detected need not represent viable virus, so care in clinical and epidemiologic interpretation may be necessary. Circulating HIV p24 antigen may precede detectable HIV antibodies and thereafter become non-detectable (see Fig. 8.4), only to reappear in some persons with severe clinical immunosuppression (204Ð206). Antigen has some value in evaluating response to therapy (207). Viral load measures (see below) are alternative surrogate markers for assessing response, and appear to be better for this purpose (208). In some circumstances such as measurements in ßuids other than blood, antigen tests have been claimed as more useful than viral load (209).

In regions where HIV transmission and seroconversion rates are high, and conÞdential self-exclusion programs are too non-speciÞc or otherwise of limited value, screening of blood for HIV antigen is warranted (210). The beneÞts of various screening algorithms is, in part, a function of seroprevalence and policy decisions concerning screening will vary (112,113,211,212). In 1995Ð1996, there was a controversy in the U.S. regarding whether the U.S. blood supply should be routinely screened for HIV antigen. Some studies had suggested that screening HIV seronegative blood donors for antigen might detect many false positives (213,214) in a setting of limited residual infection (215Ð217). In 1996, the FDA decided that the additional safety margin was warranted on public policy grounds. Two new HIV p24 antigen tests were licensed and U.S. blood banks implemented p24 antigen as part of their screening.

The initial p24 antigen assay licensed in 1996 in the U.S., the Coulter HIV-1 p24 Antigen Assay, did not include performance criteria. The Abbott HIVAG-1 Monoclonal Kit, which was licensed the next month, claimed a 99.92% speciÞcity and sensitivity of 99.0% amongst a panel of HIV-1 EIA seropositive subjects. This was a marked improvement to a sensitivity of only 79% for the prior Abbott p24 antigen assay that had been licensed in August of 1989.

Historically, acidiÞcation in the p24 antigen assay was used in some settings, since it can lead to detection of

antigen in a higher percentage of HIV seropositive persons (218). This acidiÞcation step disrupts immune complexes that exist between p24 antibody and antigen, but it also reduces total detectable p24 antigen as evidenced by neutralization. Some investigators also used alkalinization successfully. Innovations in testing by various vendors also widened the scope of application for antigen assays (218,219).

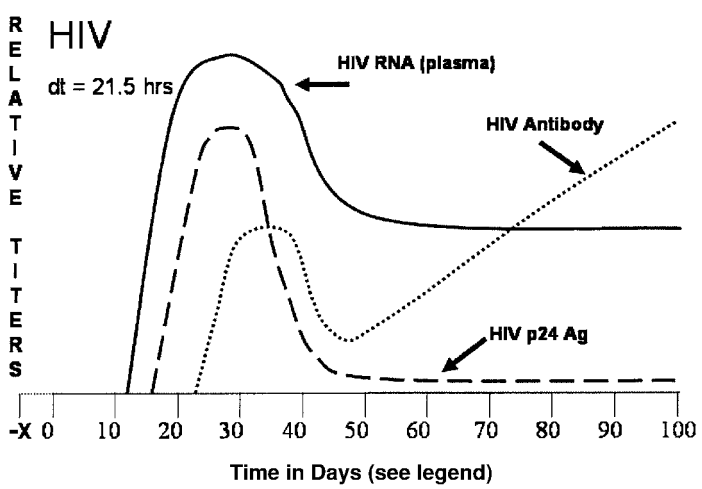
Nucleic Acid AmpliÞcation Tests (NAT) and Viral Load Measures

Techniques

Another approach to the direct detection of HIV is through the detection of viral nucleic acid through what is commonly referred to as nucleic acid testing (NAT). The challenge of NAT is to detect a minimal amount of material in a relatively small sample. Several technologies have met this challenge through novel chemistries that amplify either the nucleic acid itself or the signal that detects it.

Gene AmpliÞcation Techniques

The methodology can be used to amplify either DNA or RNA and are referred to as Ònucleic acid testsÓ(NAT). The principles are outside the scope of this chapter and have been reviewed by others (220,221). These methods have led to the routine assessment of viral titer in plasma (Òviral loadÓ which for HIV-1, an RNA virus, is a measure of RNA level) (28,222Ð224) as well as in lymphoid tissue (225). Two gene ampliÞcation techniques are presently available, that rely on ampliÞcation of either DNA or RNA copies. Gene ampliÞcation, utilizing the polymerase chain reaction (PCR) technology (originally developed by Cetus) as well as other methodologies, has opened a new window on the detection of infectious agents. In polymerase chain reaction-based assays, commonly known as PCR, HIV RNA is reverse transcribed, and DNA copies are ampliÞed exponentially through a series of repetitive reactions. The signal is then detected colorimetrically. Transcription-mediated ampliÞcation (TMA) (226,227) and the related nucleic acid-based signal ampliÞcation (NASBA) (228) are also based on reverse transcribed RNA to DNA, but in this case RNA copies are made. Rather than amplify the nucleic acid, the branched DNA signal ampliÞcation (bDNA) assay (229) relies on the ampliÞcation of a signal when it binds to the HIV-1 target RNA.



LEGEND: Virologic events during primary human immunodeficiency virus (HIV) infection: a schematic summary of window period dynamics. Time is number of days subsequent to initiation of infectivity (on Day 0), which is a time point occurring X days consequent to a causative HIV exposure (on Day – X).

After a person is exposed to HIV on Day **-X**, several biologic steps including propagation of HIV in tissues such as lymph nodes may occur (Figs. 8.2, 8.3 and 8.4). A person is presumed to be infectious to others beginning sometime later, on Day 0. (Fig. 5 does not depict low-level intermittent viremia that may precede the ramp-up viremia described above.) See text for details.

(Figure adapted from M.P. Busch (316), with permission.)

dt = mean doubling time
CI = confidence interval

FIG. 8.5. Virologic events during primary human immunode ciency virus infection.

TABLE 8.6. *Degrees of polymerase chain reaction contamination**

	METHOD	LIMITATIONS
CLASSIFICATION	VOLUME OF CONTAMINANT	APPROXIMATE NUMBER OF COPIES OF PCR PRODUCT PRODUCED AFTER 30 CYCLES
1. Spill or gross contamination	1 to 100 μl	10^7 to 10^9
2. Smudge	10^{-4} to 0.1 μl	10^3 to 10^6
3. Aerosol	10^{-9} to 10^{-5} μl	0.01 to 100

* Adapted from McCreedy et al. (234).

Each of these techniques has been approved for different applications) (222,227,228,230).

For DNA PCR amplification, synthetic primers complementary to the region bordering each side of the area of interest are used. Efficiency considerations lead to the design of primers of 15 to 35 bases in length. The region chosen for detection and amplification requires care; for example, sequences where hairpin turns or loops can form need to be avoided.

Theoretically, the nucleotide area of interest will double with each cycle; in practice, 80 to 90% efficiency is often achieved. This exponential doubling leads to sufficient amounts of DNA to be amenable for many forms of analysis. Probes for the amplified region aid in detection. Amplifying pro-viral integrated HIV within the human genome is analogous to finding successfully the proverbial needle-in-a-haystack. Examples of HIV primer-probes have been reviewed (220).

False negative PCR results can occur for technical reasons. Heparin, for example, can inhibit PCR (231). Acid citrate dextran (ACD) solution is the preferred anticoagulant for specimen collection, especially when cells will not be processed and extensively washed, to reduce false negatives. Specimens collected with heparin that are then tested quantitatively (e.g. "viral load" measurements) will have systematically, artifactually reduced viral titers. (Such systematically reduced load measurements can be internally consistent within a given, standardized research study. Although the trends in such studies have clinical relevance, the specific values will not translate directly and consistently with measurements made on ACD specimens.)

Biologic reasons for false negatives include (a) genomic variation in HIV (232): the nucleotides flanking a presumed constant region may vary (primer failure) or the amplified region may vary (probe failure); and (b) virus below the detectable level.

False positive PCR results have raised even greater concern (233). The exponential amplification carries great risk of inadvertent contamination, which may vary from gross contamination to amounts consistent with aerosol passage, as may occur with simply flicking open a specimen tube cap near an adjacent specimen (Table 8.6).

When the number of expected copies produced by PCR approaches one (or less), then statistical considerations (which can be modeled as a Poisson distribution) predict the likelihood of PCR positivity. With very low original copy number in a sample, PCR is predicted to be positive only some of the time. When a positive result is not consistently confirmed, it can be difficult to determine whether this represents a true positive with low copy number (e.g. few infected cells in a terminal, severely CD4 depleted AIDS patient or perhaps a seronegative person at high exposure risk) versus aerosol contamination. In viral load assays with "undetectable" results or numeric results below the standard "positive" threshold cut-off value, these issues must be carefully considered when interpreting the result. Limits of detection for plasma viral HIV RNA are the subject of ongoing certification programs and have been recently reviewed (222).

In devising a commercial PCR diagnostic center, Roche built a facility in North Carolina to minimize the risk of contamination (234,235). Since carryover has been identified as a major cause of contamination, separate rooms for reagent preparation, specimen preparation, PCR set-up, and analysis exist. Movement of materials only proceeds forward to the next self-contained room, never backwards. Manufacturers have developed closed-type systems to reduce contamination in laboratory settings (236). Furthermore, multiple different PCR reactions ("multiplex PCR") can be carried out in a single tube with retention of specificity and sensitivity (237–239).

False positive results may be further minimized by requiring the PCR be positive on two of more gene products. PCR reactivity on only one gene would be classified as indeterminate, and require further investigation to clarify its meaning. Viral variation or defective viruses, or insufficient sample material may contribute to indeterminate results. In preliminary HIV PCR trials by the Roche facility, indeterminate HIV PCR results have been rare (234).

PCR may be performed on fresh or cultured lymphocytes. Obviously, in either case virus siblings (variants) that differ in the primer region will not be amplified. In addition, conformational or related issues may lead to varying efficiency in replication for different sibling variants, which may give rise to large differences in relative copy number over many PCR cycles. Subject to the above caveats, on fresh material the results will reflect the underlying HIV sibling distribution. Since defective

retrovirus variants may be among those amplified, and at least in theory might be the only HIV detected, a positive PCR result (particularly in a seronegative individual) does not necessarily indicate active infection. If the cells are cultured first, then only viruses capable of *in vitro* replication will be detected, and the relative ability to replicate in culture will heavily influence the distribution of viral variants that are detected. This latter methodology is thus inferior when attempting to understand molecular epidemiologic events and sibling evolution.

Applications of NAT

NAT offers the possibility to close the window period for HIV detection further than can be achieved with assays that detect an immune response to HIV or viral antigen (240). Studies have shown, for example, that NAT can reduce the window period (see below) to approximately 11 days following the onset of infectivity. At this writing, two NAT assays have been licensed to screen blood donors for HIV-1 infection (241). The PCR-based UltraQual HIV-1 RT-PCR Assay (National Genetics Institute, Los Angeles, CA) is licensed to detect HIV-1 in pooled source plasma. The TMA-based Procleix assay from Gen-Probe (San Diego, CA) is licensed to detect HIV-1 RNA in human plasma from donations of whole blood and blood components for transfusion. Both of these tests are qualitative assays, but have the ability to detect less than 40 copies of HIV-1 RNA per milliliter.

Beyond screening, NAT has an application in the prognostic assessment of HIV-1 infected patients, and in monitoring the effects of anti-retroviral therapy through the determination of viral load. The Roche Amplicor HIV-1 Monitor Test (Roche Molecular Systems, Branchburg Township, NJ) was the first test approved for this use, in 1996. Two additional assays have recently been approved, NASBA-based NucliSens HIV-1 QT (bioMerieux, Durham, NC) and bDNA-based VERSANT™ HIV-1 RNA 3.0 Assay for the quantitation of HIV-1 RNA in plasma.

NAT also has the ability to provide useful information that can be applied to specific therapeutic modalities for an infected patient. The TRUGENE HIV-1 Genotyping Kit and OpenGene DNA Sequencing System (Visible Genetics, Toronto, Ontario, Canada) identifies HIV genomic mutations in regions of the virus known to confer drug resistance to particular anti-retroviral medications. PCR-amplified material is subjected to nucleic acid sequencing to provide this information. This test has been cleared as an aid in the therapeutic management of HIV-1 infected patients.

NAT when used qualitatively (absent/present) have very high specificity (nearing 100%), but cannot demonstrate whether or not the detected agent is in an infectious state. False negative results can commonly occur in a number of circumstances, limiting the sensitivity of these approaches. Thus, despite the great power to amplify by NAT (see below), it is still true that viral load below given thresholds can be "undetectable" (i.e. limitation due to a limited number of organisms or viral particles). This issue can lead to diagnostic difficulty in ruling HIV infection in or out by these methods, exemplified by the problems in diagnosing neonatal infection (at a time when maternal antibodies limit the utility of other standard approaches such as antibody assays). Furthermore, substances can inhibit the reactions (e.g. heparin interferes with some assays) and lead to false negative results.

The quantitative application of nucleic acid amplification tests in both epidemiologic studies and clinical trials has become increasingly common. However, the reproducibility of a specific titer result is limited. In 1996, it was proposed that at least a three-fold change in titer (approximately an 0.5 log change) should be considered the minimum "significant" change. As these assays continue to evolve and the minimum detectable threshold value decreases, the epidemiologic and clinical value of testing is likely to further rise. Differences in interpretation of some clinical trials has, in fact, been attributable to different testing algorithms for viral load and the associated limitations of those algorithms, related to varying vendor assays, sensitivities, and modes of data analysis (including varying categorical cut-offs). When these laboratory characteristics are taken into account, contrasting results of different studies can be understood in context and the clinical implications may be seen to be convergent rather than disparate.

The potential uses of NAT are considerable, including

a. confirmation of serologic results (242–246), examination of other bodily fluids (247,248) or stored material (249), and staging (250)
b. resolution of indeterminate WB (150)
c. resolution of infective status in infants (251–257)
d. reassurance of persons at low HIV risk (258,259)
e. screening for retroviral infection risk (240,260,261)
f. identification of latent and primary infections in seronegative persons at HIV risk (110,240,262–275)
g. quantitation of viral load (218,222–225,244,276)
h. detection of defective or aberrant retroviruses (277)
i. evaluation of viral variation (232,278–283).

These applications are still being refined (275,284). Possible interpretations of positive NAT results in seronegative persons range from:

- false positive, to
- transient infection with clearing, to
- window period of infection (e.g. primary HIV infection), to
- latent infection.

In part, this controversy reflects our still emerging understanding of the biology of retroviral infection (see below).

NAT and viral culture have been used to generate sufficient substrate for genetic sequencing. In conjunction

with statistical techniques such as phylogenetic analysis, these molecular epidemiologic analyses have helped delineate some major macroscopic variations in HIV strains around the world and assisted in mapping geographic transmission patterns (285–293). However, the picture is greatly complicated when trying to extend these techniques to the assessment of "epidemiologic" linkage (280,294–302). HIV variation is influenced by many factors, including the biology of HIV and immunologic pressures, and there are issues related to possible recombination between HIV clades and coinfection or exposure to multiple strains (303–306). Molecular epidemiologic analyses thus remain a research tool, with many theoretical and practical limitations to more generalized use.

OTHER DIRECT DETECTION METHODS

In Situ Hybridization

The technique of *in situ* hybridization (direct detection of the viral nucleic acid in individual infected cells) offers a direct approach for localization of HIV nucleic acid sequences in cellular preparations (197–199). The limited proportion of infected cells *in vivo*, the low viral sequence copy number per cell, and the need to examine slide preparations by hand with light microscopy limit its application outside highly sophisticated research laboratories. Recent studies demonstrate higher proportions of infected cells than reported in the early studies. It is uncertain whether these differences reflect improved technique versus an artifact related to stage of HIV illness in the early reports.

Tissue Culture Of Virus

HIV virus isolation by cell culture involves the use of carefully selected permissive cell lines (such as some malignant T-cell lines) or co-cultivation with fresh, normal lymphocytes which have been stimulated with mitogens and maintained with T-cell growth factor (and sometimes alpha-interferon) (1,2,307). HIV isolates with a propensity for growth in specific lines, e.g. monocyte/macrophage lines, have been described, and may reflect differences in cellular biology among HIV isolates (300,308–310). Culture enhances viral titer and permits detection by other assays (311). Retroviruses can be detected by testing the culture at periodic intervals for specific Mg^{++}-dependent reverse transcriptase activity in the medium and/or viral antigen detection (as described above). These findings are then further confirmed by detection of viral antigens within the cultured cells, as by electron microscopy and/or with in situ use of monoclonal reagents.

Positive cultures may indicate either active or latent HIV infective states. These culture methods require advanced technology within the context of adequate provisions for biosafety and are not feasible beyond the research setting. The cost is great, and the laboratory equipment and skilled research technicians are extremely limited. The limited sensitivity and difficulty of HIV cultures does not allow widespread use for the determination of HIV carrier states.

Ligase Chain Reaction

The ligase chain reaction (LCR) originally seemed a potentially promising technique for amplifying DNA (312,313), but in light of HIV variation, LCR has so far had limited application in terms of HIV, and LCR holds greater promise with regard to other infectious agents.. LCR, like PCR, uses oligonucleotides that anneal to specific, complementary sequences on the target DNA to be amplified. In contrast to PCR, LCR will only amplify stretches of DNA that have the entire exact sequence utilized, essentially combining both an amplification and a detection step. Specific DNA alleles can be selectively amplified (314).

TEMPORAL DYNAMICS OF HIV ACQUISITION AND TRANSMISSION: SEROCONVERSION AND "WINDOW" ISSUES

Exposure to an infectious agent does not invariably lead to active infection: the classic epidemiologic triad involving interaction amongst the agent, the host and the environment govern initial dynamics and response (Fig. 8.2). If the agent is defective or disrupted, introduced into an inhospitable body compartment, necessary receptors not present, etc., no infection may occur at all. Even if it begins to multiply, an abortive infection may occur, e.g. if host immunological defenses are effective.

There will be a period until the concentration is sufficient (high enough titer) to be detected by a given methodology (Figs 8.3 and 8.4). Furthermore, shedding may only be intermittent, so samples from a given time point may be true negatives, which fail to reflect the full biologic picture given the underlying biologic complexities. There will also be a period until antibodies begin to be produced in highest titer to be detectable, and the host generates a series of multiple different antibodies.

The "window" period refers to the time from acquisition of infection by the host until a test can detect the specific presence of that infection. The window will vary with the infectious agent and test methodology, and likely varies as well with route and dose of the original exposure, and host immunologic factors. In terms of screening of blood donors, the interval I-A in Fig. 8.4 reflects a subset of this overall window period, in which a seronegative (anti-HIV antibody negative) donor is infectious.

The HIV Window Period for Seroconversion

Much of what we know about the HIV infectious window period comes from analyses of serially collected specimens from seroconverting subjects such as plasma donors, hemodialysis patients, or persons enrolled in high-risk cohort studies. Statistical analysis of the data has resulted in the model shown in Fig. 8.5. Some of the clearest data has come from epidemiologic studies concerning recipients of blood transfusions (315), but the high dose of exposure involved in those cases likely leads to more rapid and more limited variation than other types of exposures.

For those persons who become persistently infected with HIV, Busch and colleagues have estimated the mean time points of initial HIV detection (Fig. 8.5):

¥ HIV RNA becoming detectable in plasma on days 10 through 12,
¥ HIV-1 antigen (p24) on day 17, and
¥ HIV antibody on day 22 (using current generation EIAs) (316Ð319).

Subsequently, HIV p24 antigen often becomes undetectable, and HIV antibody level may decrease temporarily owing to complexing with HIV-1 antigen (antigen-antibody complexes may form). After that transient decline, HIV antibody levels continue to increase during the initial three to six months of infection and persist indeÞnitely. Antibody may be lost or non-detectable in the pre-terminal stage of HIV disease, paralleled by a surge in viral burden that heralds collapse of the immune system. Plasma levels of HIV RNA generally remain detectable throughout the course of infection, but may decrease to undetectable levels during treatment with highly active antiretroviral therapy (HAART).

Mathematical modeling, in a study of blood donors that utilized earlier generation antibody EIAs (1985Ð1990), estimated the mean interval from onset of infectiousness until HIV antibody detection (the Òseronegative windowÓ) as 45 days (95% CI, 34Ð55 days) (320). The upper 95% CI estimate of the point by which 90% of seroconversions occur was 141 days from the onset of infectiousness. The upper 95% CI estimate of the point by which 95% of seroconversions occur was 214 days. Thus, few donors appear to remain infectious and seronegative for more than six months.

The interval (X days) is potentially quite variable, and will be a function of mode of acquisition, exposure dose, and other factors of the Òepidemiologic triadÓ (Fig. 8.2). This interval, from the original HIV exposure until onset of infectiousness, was not estimated in the above models. However, this additional time period is highly relevant when considering for how long a person who was exposed to HIV (e.g. a health care worker who has a sharps exposureÑ see Chapter (NOTE TO EDITOR: insert right chapter # by SHW Weiss & Leschek from this book) should be monitored to rule-out acquisition of HIV infection.

Data from Other Persons at Risk

Horsburgh and colleagues examined 27 homosexual and 12 hemophiliac males with stored blood samples collected before and after HIV seroconversion (321). By statistical modeling, they estimated that the time from infection with HIV (assessed by detection of HIV DNA) to Þrst detection of HIV antibody was 2.4 months for the median individual, with a standard deviation of 2.1 months (321). This observation of a much longer interval than that suggested by Busch and colleagues (315) may reßect the marked differences between their respective patient groups and suggests that mode and dose of exposure may be important parameters that affect the interval. Not addressed in these studies is whether prophylaxis with antiretroviral drugs might also affect the dynamics.

Modeling of 45 published cases of HIV infection with known exposure gave a median estimate of 2.1 months from exposure to antibody detection, with 95% of cases expected to seroconvert within 5.8 months (321). They concluded that HIV infection for longer than six months without detectable antibody (assuming no false negative tests) seemed to be uncommon (321). Given the apparent limited residual risk after six months, the standard recommendation for one year of observation is likely sufÞcient in the absence of chemoprophylaxis. Furthermore, these data suggest that testing at three and six months will implicitly diagnose most of the ultimate seroconversions.

Delayed HIV Seroconversion

There are limitations to these data. For example, with increasing time between an exposure and documentation of infection, the task of ruling out potential competing exposure risks deÞnitively grows more difÞcult, with increased reluctance to ascribe a seroconversion to an occupational incident. Indeed, in France the assumption is made that a potentially contaminating accident must be before the sixth month, *a priori* dismissing delayed seroconversion from consideration (322). If more than one (potential) exposure to HIV occurs, it may be difÞcult to ascertain which one led to the acquisition of HIV infection.

Complicating the picture is that some instances of delayed seroconversions might not be detected. There is also the possibility that the seroconversion might not be attributed to the correct event, as more proximal events within the anticipated window might systematically be considered as more likely, a form of circular logic. Thus, arbitrary selection of the incident based on the assumptions above may further bias our estimates. Some false

negative screening tests do occur (35,323). Finally, even if delayed seroconversions occur, the initial temporal data distributions will be biased from the early occurring cases. (This phenomenon led to progressive lengthening of the median Òtime to AIDSÓ estimates, since the short interval cases are the Þrst to be observed.) The modeling of Horsburgh relied upon cases published in the literature only up until 1989, and they excluded some cases the CDC considered controversial or which were complicated by the above factors. In summary, the current distribution curves are likely somewhat biased in terms of inclusion of shorter seroconversion intervals.

Acute Retroviral Seroconversion Syndrome

These issues pertain as well to acute viral syndromes in temporal association with HIV seroconversion (see Fig. 8.3) (324,325). Persons with such symptoms, even though non-speciÞc, are more likely to be carefully evaluated for seroconversion (325). (It is also important to note that such persons should be carefully monitored, in order to render appropriate care and prevention advice in a timely manner.) If occurrence of the retroviral seroconversion syndrome were associated with the magnitude of viral replication, it seems reasonable that persons with delayed seroconversion might exhibit such symptoms less frequently. Thus, there are multiple reasons why persons without such a syndrome or with delayed seroconversion might be more likely to be missed.

However, only a limited number of cases of delayed seroconversion have been reported (322,326). A woman accidentally pricked by a needle at a clinic where she worked as a cleaner had negative HIV serology at the third and sixth months after the accident. She did not receive any antiretroviral chemoprophylaxis. HIV antibodies were detectable at the eighth month and thereafter, with conÞrmation by detection of HIV-1 RNA (see below) and WB. (Interestingly, HIV could not be isolated by culture on successive samples (322).) The authors noted that the possibility of a serologically silent window of more than six months has public health consequences. ÒThe underestimation of the diagnosis of HIV infection may wrongly lead to the reassurance of individuals remaining negative 6 months after the potentially infectious accident and thus may contribute to further transmissionÓ (322).

A speculative issue is whether some people treated with anti-retroviral therapy might experience delayed seroconversion. For research purposes, it would be worthwhile to extend the follow-up monitoring period to help resolve these issues. In practice, outside the realm of research studies, duration should be individualized based on magnitude of exposure risk. The decision concerning length of monitoring also needs to balance the psychological pressures attendant to extended follow-up. In general, it still appears to be reasonable to use negative tests at six months in an asymptomatic worker as strong reassurance, while still counseling concerning the need for further follow-up. These issues pertain to the monitoring of persons after an at-risk exposure to sharps as well as other occupational exposures (see the chapter in this text on ÒOccupational Risk in the HIV Era.Ó) Additional factors at play in the Òepidemiologic triadÓ (Fig. 8.2), such as post-exposure treatment with anti-retroviral therapy, may potentially signiÞcantly alter the dynamics from that observed in the seroconversion modeling data.

DIAGNOSIS OF HTLV

HTLV-I was the Þrst human retrovirus to be discovered (327;328), followed shortly by HTLV-II (329,330). HTLV-I infects 15Ð20 million people worldwide, with endemic foci in southeastern Japan, the Caribbean, Melanesia, sub-Saharan Africa, and some areas of Central and South America (331). The worldwide prevalence of HTLV-II is less certain, but HTLV-II prevalence is of similar magnitude within the U.S. to that of HTLV-I, with high prevalences in some regionsÑ especially among at-risk persons such as injection drug users and some Amerindian tribes in the Americas (332Ð339).

HTLV-I is causally associated with an aggressive leukemia and lymphoma syndrome (340Ð345), as well as neurologic disease. Both HTLV-I and human HTLV-II are associated with immunologic abnormalities (346Ð349). It remains uncertain whether HTLV-II is linked to an increased risk for cancer (350,350Ð352). There have also been claims of association of HTLV-II with neurologic disease and some infectious diseases (332,353,354).

The development of the HIV EIA methodology was aided by the developmental work that had been done for the HTLV-I EIA (23,30). The discovery of the second instance of HTLV-II infection, in an intravenous drug user who died of *Pneumocystis carinii* pneumonia prior to the discovery of HIV, was prompted by atypical reactivity on the existing HTLV-I diagnostic tests and required viral isolation to prove its near identity to the HTLV-II Mo prototype (330). It is not surprising that there are many similarities in the diagnostic modalities for HTLV and HIV. This section highlights the differences.

In 1988, a blood screening program for HTLV-I was instituted in the United States utilizing EIAs licensed by the FDA (355,356). Due to some antigenic cross-reactivity HTLV-I and HTLV-II, the HTLV-I based assays were able to detect some of the instances of HLTV-II infection. (330,332,333), but considerable numbers of HLTLV-II infection were missed (357Ð362).

Subsequently, in 1997 and 1998, two EIAs were licensed to detect antibodies to both HTLV-I and HTLV-II. These assays are based on HTLV-I and HTLV-II infected cell lysates as the source of capture antigen, along with a recombinant form of the HTLV-I p21 envelope protein (p21e) in one of the assays; these screening assays do not differentiate between HTLV-I and HTLV-II.

HIV-1 can be cultured to much higher titers than HTLV-I, so that the antigen preparation for the whole virus EIA can be puriÞed more easily for HIV than for HTLV. Cellular contamination of the viral preparation leads to cross-reactivity and non-speciÞc (false positive) reactions more frequently for HTLV than HIV. Serologic assays for HTLV antigen have been used to monitor HTLV cell cultures, but have not found clinical application since measurable cell-free HTLV concentration have not been detected in peripheral blood. This absence of signiÞcant cell-free virus is in contrast to HIV, and may help explain some of the differences in transmission patterns.

Further complicating the picture, many persons infected with HTLV have only low titers of antibody. In contrast, the titers for HIV antibody tend to be relatively high. Some HTLV samples have low titer antibodies, as occur particularly in some asymptomatic HTLV-I carriers as well as persons co-infected with both HTLV-II and HIV (333,363). Such specimens are particularly difÞcult for some assays to detect (363,364), leading to limited sensitivity. In Japan, a particle agglutination test has been used for HTLV-I screening with good success (365Ð367).

If a conÞrmatory test cannot detect antibody titers as weak as the screening test can detect, some true positives will be unconÞrmed and categorized erroneously as negative. This situation has been noted with HTLV diagnostics (and, more rarely, with HIV; see footnote in Fig. 8.4). Thus, care must be taken in interpreting HTLV results, both when counseling patients and when critically reading the literature.

A research use HTLV-I/II WB makes use of not only an HTLV-I viral lysates, but also recombinant forms of HTLV-I p21e, HTLV-I gp46, and HTLV-II gp46 (Genelabs Diagnostics, Singapore). Thus, this WB is able to distinguish between antibodies to HTLV-I and HTLV-II. The U.S. Public Health Service has recommended that a positive HTLV WB have bands for both gp46 and p24, though there is evidence that p21e reactivity may be sufÞcient for the env component in place of gp46.

If both *gag* and *env* reactivity are required for conÞrmation, a WB alone will conÞrm only a limited proportion of the true-positive sera (368). WBs used for conÞrmation of EIA reactive specimens among injecting drug users within the U.S. may be indeterminate or negative in a majority of individuals (337,369Ð371). In addition, the relative titers of HTLV speciÞc proteins vary greatly depending upon both the cell line and HTLV variant used to produce HTLV for the test kit (SH Weiss, unpublished observations).

An algorithm for HTLV testing for diagnostic purposes in a clinical laboratory is outlined in Fig. 8.6 (372). If the initial screening EIA is reactive, a repeat assay on the same specimen is performed in duplicate. If one or both of the repeat tests are reactive, the specimen is classiÞed as repeatedly reactive. The repeatedly reactive specimens are

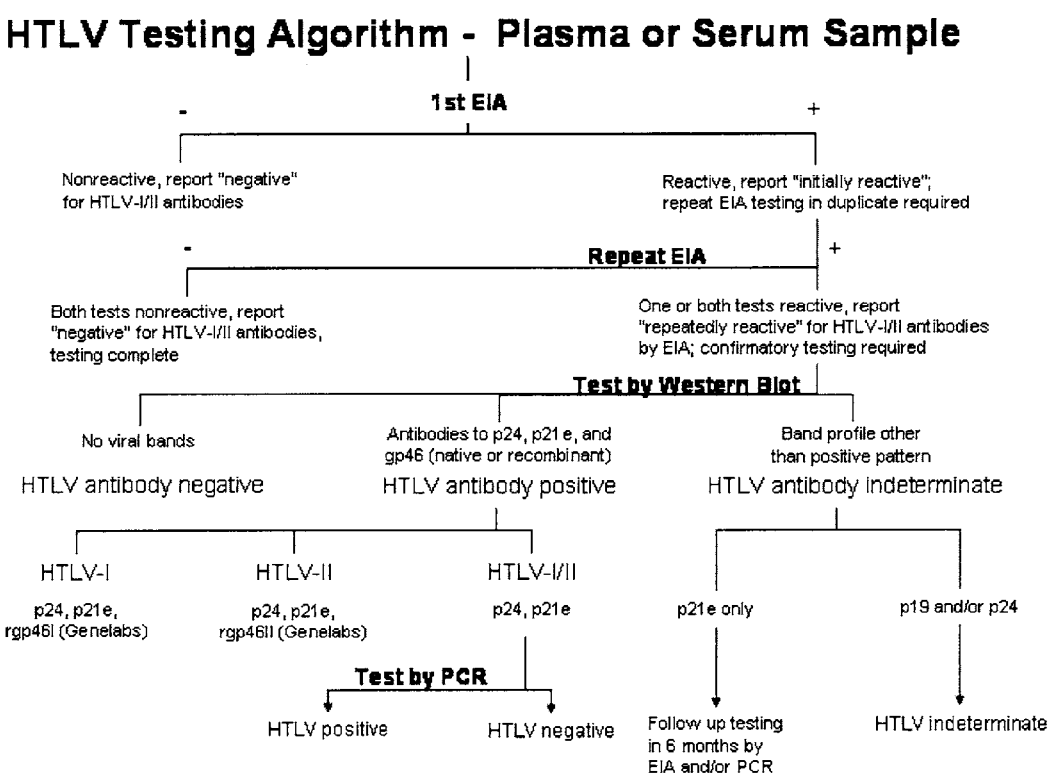


FIG. 8.6. HTLV testing algorithm—plasma or serum sample. Figure courtesy of Charlene S. Dezzutti, Ph.D., Elliot P. Cowan, Ph.D. and Renu B. Lal, Ph.D.

subjected to conÞrmatory supplemental testing which is typically done by WB.

Some investigators have routinely used RIPA to conÞrm the indeterminate HTLV WB patterns. There are some limitations to their implementation, as in theory the WB negative sera should also be tested by RIPA. However, RIPA frequently cannot detect low titer antibody, so even the WB plus RIPA conÞrmation schema will miss some true positives. RIPA is available only in select research laboratories, further limiting its application. In summary, standard WBs have limited utility, and cannot play the same role as they do in HIV diagnostics. Neither RIPA nor the research use WB is available for clinical use in the U.S.

The current PHS diagnostic criteria for supplemental testing require that a specimen demonstrating antibodies to p24gag and to native gp46env or gp61/68env are considered seropositive for HTLV-I or -II (372). Specimens reacting with any of the bands, but not satisfying the above criteria, are designated indeterminate. In the majority of cases, indeterminate specimens do not represent true HTLV infection, with one exception. Specimens with p21e reactivity may represent an early seroconverter, and repeat testing should be performed within six months. Persons who have clinical neurological symptoms and HTLV-I and -II indeterminate reactivity should be further investigated for possible retroviral infection. Further testing could include PCR in conjunction with serologic testing. Specimens with no immunoreactivity to any bands upon repeat testing are considered negative for antibodies to HTLV-I and -II (false-positive EIA specimen). Persons with HTLV-I or -II positive or indeterminate test results are counseled according to the guidelines established by the PHS Working Group (373). These guidelines state that persons should be informed that HTLV is not the acquired immunodeÞciency syndrome (AIDS) virus and their risk of developing HTLV-related diseases is low. HTLV-I- or -II-infected persons are also asked not to donate blood, semen, body organs, or other tissues as well as not to share needles or syringes with anyone. To prevent transmission of HTLV, the infected person is asked to use protective measures during sexual relations and women are asked to refrain from breastfeeding. Persons who have indeterminate results on two separate occasions at least three months apart should be advised that their specimens were reactive in screening for HTLV-I/II, but these results could not be conÞrmed by tests that are more speciÞc. Further, they should be reassured that indeterminate results are rarely caused by HTLV-I or HTLV-II infection. Repeat testing should be offered (372).

Historically, competitive EIAs were used to conÞrm HTLV screening results, and to categorize whether the infection is due to HTLV-I or HTLV-II (332). Although extraordinarily time consuming, this approach was the Þrst to demonstrate high rates of HTLV infection in a cohort of U.S. intravenous drug abusers, and also the Þrst to show HTLV-II seroprevalence rates that exceeded HTLV-I (332,374).

Immunoßuorescence was also used in many early HTLV studies either for screening or for conÞrmation of EIA results (364,375,376). When HTLV-II infected cells, in addition to an assay with HTLV-I infected cells, are used for immunoßuorescence, in light of the existence of high HTLV-II seroprevalence in the U.S., only a few additional sera were actually conÞrmed (333,377). Many HTLV-II sera, including the Mo HTLV-II prototype, react well on HTLV-I assays, including immunoßuorescence. Immunoßuorescence patterns consistent with anti-nuclear antibodies (ANA) were also seen frequently among the intravenous drug users. Since true infection simultaneous with ANA is possible, interpretation in this high-risk group is difÞcult.

HTLV-II p24 antibodies can strongly cross-react with HTLV-I p24 antigen, so that a radioimmunoassay (RIA) utilizing puriÞed HTLV-I p24 has been suggested as an alternative HTLV conÞrmatory test (378). However, HTLV-II may tend to give systematically lower reactivity than HTLV-I on this HTLV-I based assay (S. H. Weiss, unpublished data). Given all these problems with the standard assays, an alternative approach was suggested in 1987 utilizing concomitant reactivity on an HTLV-I EIA, an HTLV-I p24 RIA, and on either an HTLV-I or HTLV-II immunoßuorescence assay (333). The rates of HTLV infection reported in that study were quite high. Subsequent work nevertheless indicate that these criteria were too strict, and underestimated true seroprevalence (379,380).

A signiÞcant proportion of HTLV-II was missed with HTLV-I-only assays, with the problem particularly acute in regions or among groups at high risk for HTLV-II (363,364,381Ð386). Transmission of HTLV-II by HTLV-I screened blood has been documented (387). HTLV-I based diagnostics have been shown to have limited sensitivity and speciÞcity for HTLV-II (358). The FDA Blood Products Advisory Committee reviewed HTLV-II test kit criteria, leading to the revised recommendation that HLTV-II antigen(s) needed to be part of a kit designed to detect antibodies to HTLV-II. The advent of WB spiked with recombinant proteins, including p21e and HTLV-I and HTLV-II speciÞc proteins, have been demonstrated to have roles as alternative conÞrmatory tests, with differentiation of HTLV-I from HTLV-II (370,380,388) (Fig. 8.7). Other serologic approaches have also been used (389Ð396). The currently available EIAs contain antigens from both HTLV-I and HTLV-II, and these antibody tests have Þlled the prior gap. However, some assays may still give false-negative results on HTLV-II-positive specimens (362,397).

PCR has proven extremely useful in conÞrming and delineating the type of HTLV infection, but remains unlicensed (380,398Ð402). The multiplex PCR approaches discussed above also hold promise for simplifying the approach to testing for multiple agents. It is reasonable to

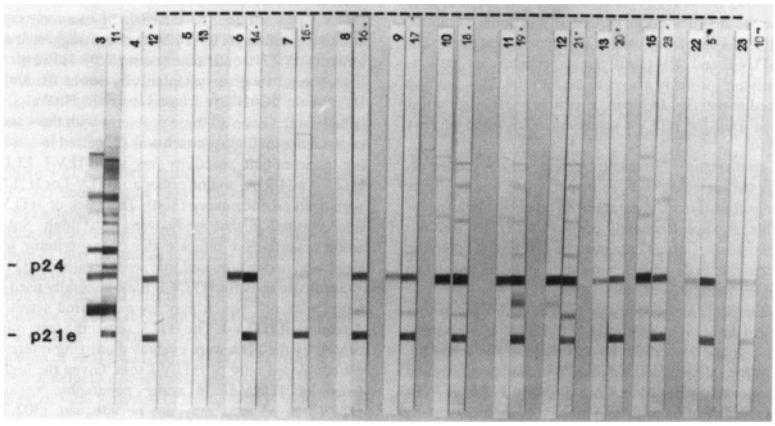


FIG. 8.7. Paired Western Blot results comparing standard Biotech/DuPont strips with the Cambridge Biotech strips containing added recombinant p21e protein The major bands represented are p24 and p21e. Strips 3 and 11 were incubated with HTLV-I positive control serum, strips 4 and 12 with HTLV-II postive control serum, and strips 5 and 13 with control serum negative for HTLV-I and HTLV-II. Al remaining pairs represent individuals from a study of intravenous drug users in New Jersey who were both HTLV EIA (Abbott Laboratories) reactive and con rmed by PCR to represent HTLV-II infection (380). Figure courtesy of Paul E. Palumbo, M.D., Steven S. Alexander, Ph.D., and Stanley H. Weiss, M.D.

expect that different primer pairs will have different sensitivities concerning identiÞcation of HTLV-I and HTLV-II within given populations (380). Differences in PCR ampliÞcation efÞciency have been noted for HTLV primer pairs SK 43/44 and SK 110/111 (B. McCreedy, unpublished observations). Primer set SK 43/44 and probe SK 45 appear to yield better results following ampliÞcation of low copy number HTLV DNA than does the primer pair SK 110/111 and probes SK 112 and SK 188. Further research is this area is necessary (380).

In addition, nucleotide sequence diversity and the issue of defective or incomplete retroviral elements may need to be considered. Sequence diversity among HTLV-II isolates has received recent attention (403Ð409). However, deleted HTLV-I proviruses have been shown to exist in fresh leukemic cells of patients with adult T-cell leukemia-lymphoma syndrome (410,411). Hall and colleagues found deleted HTLV-I provirus in HTLV EIA seronegative patients with mycosis fungoides (277). Some analyses of HTLV EIA reactive cohorts by means of PCR have found 12% to 24% of the HTLV infection non-typeable (401,412), although others found almost all specimens to be typeable with the newer primers and probes (380). The ability to detect all HTLV-II infected individuals by EIA screening assays, or to conÞrm infection by WB or PCR, may therefore be limited by sequence diversity and possibly proviral deletions. Further characterization of optimal PCR primer pairs, EIA and WB capture antigens (388), and possible newer serological assays (such as the use of synthetic peptides (395,396)) should be a target of future research.

One study has linked sexual transmission of HTLV-I with the presence of anti-HTLV-I-tax antibody (413). Thus, selective assays may be important in further deÞning epidemiologic transmission characteristics. For HIV, studies of speciÞc antibody have been controversial with respect to prediction of transmission (122). More work in this area for HTLV is also necessary.

Currently, multiple assays are necessary to deÞne accurately the prevalence of HTLV-I and -II in a given population. Further work is required for the development of assays that are highly sensitive and speciÞc. Recent studies suggest that HTLV-II may have immunologic and health consequences (346,379,414Ð417), as does HTLV-I (18,418). SpeciÞc laboratory assays will be important tools in further clarifying the epidemiology of HTLV-II. These newer approaches, in combination with an EIA that includes HTLV-II antigens, hold promise for improving HTLV diagnostics.

The initial commercial HTLV WB that was commonly used by blood banks to conÞrm repeat HTLV EIA reactivity was inadequate for conÞrmation of HTLV-II infection. Since counseling of donors by blood banks is done when EIA results are conÞrmed, the sensitivity and speciÞcity of the conÞrmatory tests are critical. As discussed above, the conventional WB needs to be replaced or supplemented by other easily performed assays, particularly in geographic regions where the prevalence of HTLV-II represents a signiÞcant proportion of the HTLV serologic reactivity (358,359,363,382).

CONCLUSIONS

The armamentarium of HIV and HTLV detection systems has continued to grow rapidly. Testing alternatives will continue to move from the research bench to the clinical laboratory. The judicious choice of these tools will depend upon an increased understanding of the dynamics of retroviral infection, critical head-to-head comparison testing, and cost-beneÞt decision analyses. Further development and evaluation are warranted for direct HIV detection methods as well as other immunologic tests of HIV exposure, and serologic detection and conÞrmation of HTLV infection. The context within which any test is used is of critical importance to its interpretation. No test, per se, should be the basis for diagnosis on its own, but rather a test is merely an aid in correct diagnosis. The practitioner must use test results in the context of a clinical picture to reach an accurate diagnosis.

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Chapter 9

Molecular Diagnostic Techniques and Other Tests for Direct Detection of HIV

Joseph DeSimone and Roger J. Pomerantz

Since the discovery that human immunodeÞciency virus type 1 (HIV-1) is the causative agent of the acquired immunodeÞciency syndrome (AIDS) in 1984, enormous progress has been made in our ability to detect the presence of both HIV-1 as well as human immunodeÞciency virus type 2 (HIV-2). Serologic testing has been greatly reÞned, vastly improving the sensitivity and speciÞcity of such testing. HIV antigen testing, as well as HIV culture techniques, have become useful in certain clinical situations. Finally, polymerase chain reaction (PCR) testing has allowed direct detection of both HIV complementary DNA (cDNA) as well as RNA. Plasma viral RNA measurement, by PCR or other methodologies, has become an essential component of HIV clinical care, particularly when implemented as a prognostic tool.

P24 ANTIGEN DETECTION

p24 is an HIV-1 core protein encoded by the *gag* gene. It is expressed soon after acquisition of HIV-1, and antibody to p24 forms shortly thereafter (3,18). p24 antigen can be detected in serum, plasma, and cerebrospinal ßuid (19). Assays to detect p24 antigen utilize an antigen capture with ELISA technique. The patientÕs serum is added to a plate or beads coated with anti-p24 antibody, allowing the p24 antigen to be captured. An enzyme-linked anti-HIV-1 IgG is then bound to the complex, and is subsequently measured colorimetrically. Results can be conÞrmed with a neutralization step thereafter. The level of detection of p24 antigen with current assays is 10 pg/mL. Since antigen-antibody complexes can limit the level of detection of p24 antigen, a dissociation step to separate these immune complexes has been added to the assay. This dissociation step has increased the sensitivity of the assay signiÞcantly (20, 21). Though uncommon, false positive p24 antigen results, presumably due to cross-reacting proteins, have occurred in uninfected patients (22).

Prior to the advent of plasma HIV-1 viral RNA measurement, the p24 antigen assay was used as a prognostic tool. Levels of p24 antigen were found to reappear or increase in HIV-1-infected patients shortly before or during the development of AIDS (23Ð25). A decline in HIV-1 core antibodies is the postulated source of the p24 antigenemia during this transition to AIDS (26). Similarly, this assay was useful in measuring the antiretroviral effect of medical therapy (21,27,28). Again, use of the p24 antigen assay in these roles has been mostly supplanted by quantitation of plasma HIV-1 RNA assays.

Measurement of p24 antigen in serum may be clinically useful in HIV-1-infected patients prior to seroconversion. Detectable p24 antigen levels have been noted in both serum as well as cerebrospinal ßuid in acutely HIV-1-infected individuals (18,19). Use of the p24 antigen assay in the setting of acute HIV-1 infection is discussed in detail below. Finally, measurement of p24 antigen may also be useful in detecting HIV-1 in children born to women with HIV-1 infection. Due to passive transfer of maternal antibody, serologic diagnosis of an infant born to an HIV-1-infected mother is not helpful, and p24 measurement in such children has assisted with the diagnosis (29,30). Sensitivity in this setting, however, varies with age, and is signiÞcantly less if the infant is younger than one month old (31,32). The presence of p24 antigenemia at birth, however, has been associated with development of early and severe HIV-1-related disease (33).

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CULTURES OF HIV-1

HIV-1 can be cultured from plasma, serum, peripheral blood mononuclear cells (PBMCs), cerebrospinal ßuid (CSF), saliva, semen, cervical specimens, and breast milk (5). Standardized culture methods for recovery from blood usually utilize the patientÕs plasma or PBMCs, which are incubated with uninfected donor PBMCs. Interleukin-2 (IL-2) is present to activate and stimulate cell growth. The culture supernatant, which now contains progeny HIV-1 virions, can then be tested qualitatively or quantitatively for the presence of HIV-1 with assays for reverse transcriptase or p24 antigen. Most cultures from HIV-1-infected patients, excluding those on virally-suppressive highly active antiretroviral therapy (HAART), become positive within 21 days (34). Sensitivity of these culture methods in patients who are HIV-1 seropositive has been reported at greater than 97%, with a speciÞcity of 100% (35,36).

In the age of plasma viral RNA testing, the utility of HIV-1 culture in clinical management is minimal. The *in vitro* rate of replication when culturing HIV-1 has been shown to correlate with the patientÕs clinical status and may serve as a means of measuring response to antiretroviral therapy (37Ð40). However, when compared with plasma viral RNA testing, culture methods for HIV-1 are far more laborious, time-consuming, and less sensitive. Similarly, although culture may be useful in diagnosing HIV infection in infants born to women who are HIV-1-infected, the sensitivity of this test is far lower when compared with a diagnostic method such as proviral DNA polymerase chain reaction (PCR) testing (33,41). For these reasons, culture of HIV-1 has mainly been relegated to the research and clinical trial realm.

PCR AMPLIFICATION OF PROVIROL DNA

Upon entering human cells, HIV-1 RNA is converted into a complementary strand of DNA by reverse transcriptase. These linear DNA molecules then integrate into the host genome, becoming the proviral form of HIV-1. By using the PCR, the presence of proviral DNA can be detected. In the HIV-1 DNA PCR assay, PCR using oligonucleotide primers ampliÞes a segment of the highly-conserved HIV-1 *gag* gene. This is followed by hybridization of an identifying DNA probe, with a subsequent qualitative enzymatic colorimetric assay. The sensitivity of this technique has been reported as greater than 95%, with a speciÞcity of greater than 98% (42,43).

Failure to detect group O isolates and non-clade B isolates with the HIV-1 DNA-PCR method has led to the recommendation that this method not be used as a general screening measure for diagnosis of HIV-1 infection (44Ð46). Because of the somewhat high inaccuracy rate, this test can not be recommended as a routine screening measure (47). The DNA-PCR assay has been used in diagnosing infants born to women who are HIV-1-infected. The sensitivity in this setting, ranging between 75Ð97%, is better than that of the p24 antigen and HIV-1 culture assays. Nevertheless, as with other diagnostic methods, the sensitivity of DNA-PCR is signiÞcantly less for infants who are less than six months of age (41,42).

HIV-1 RNA QUANTITATION

Quantitation of circulating virion-associated HIV-1 RNA in plasma, commonly referred to as the plasma viral load, has had an enormous impact on management of HIV-1 infection. This measurement has allowed greater understanding of HIV-1 viral dynamics, and the continuous, high-level rate at which viral replication occurs (48,49). The advent of the plasma viral load allowed investigators to understand that a basal level of high viremia is continuously present, regardless of the patientÕs clinical stage (50,51). With this information, the natural history of HIV-1 infection was more clearly deÞned, and the expected clinical course is now well-established (Fig. 9.1). Furthermore, knowledge of the extraordinary rate at which HIV-1 replication occurs has helped explain the development of antiretroviral-resistant quasipecies, and the reasons behind antiretroviral drug failure (48).

Perhaps most importantly, though, the advent of assays to quantitate plasma HIV-1 RNA in the early 1990s gave clinicians a powerful new prognostic tool. Prior to the use of plasma viral load, clinicians caring for patients with HIV-1 infection relied on numerous, albeit often inadequate, surrogate markers for disease activity and prognosis. For example, assays for serum p24 antigen (an HIV-1 core antigen), B_2-microglobulin (a component of the class I major histocompatability complex), and neopterin (a marker for activated macrophages) had some predictive value, but lacked the sensitivity and speciÞcity necessary as optimal prognosticators (52Ð54). The CD4+ T-lymphocyte count, an adequate marker to gauge risk for development of opportunistic infection, did not necessarily determine disease activity and long-term prognosis. Day-to-day variability in the CD4+ T-lymphocyte count made interpretation even more difÞcult. Furthermore, the CD4+ T-lymphocyte count was not adequate in evaluating response to antiretroviral therapy. Quantitative culture of HIV-1 in plasma or PBMCs had been utilized, but was laborious and marked by a high degree of variability (55,56). Thus, a reliable and sensitive prognostic assay was clearly in need, and the plasma HIV-1 RNA quantitation assays Þlled this role.

Several studies performed during the mid-1990s consistently conÞrmed that the risk of progression to AIDS and death from HIV-1 infection was directly related to the plasma viral load (57Ð61). In addition, the plasma viral load was found to predict strongly the decline of CD4+ T-lymphocytes (62,63). Subsequent studies revealed the superiority of the plasma HIV-1 RNA level over the CD4+

Figure 9.1 to be supplied by author . . .

FIG. 9.1. The natural history of HIV disease.
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T-lymphocyte count in predicting disease progression, but importantly the combined measurement of both plasma HIV-1 RNA and the CD4+ T-lymphocyte count was found to be a better prognosticator of disease progression than either single test (62,64,65). New, simpler, and more rapid techniques of quantitating plasma HIV-1 RNA have allowed the plasma viral load to become the most clinically useful and meaningful prognostic tool.

Of equal importance has been the impact of plasma viral load on the use and understanding of antiretroviral therapy. With the advent of the plasma viral RNA assays, investigators had a reliable means of measuring both the short and long-term impact of antiretroviral therapy (59,66Ð70). Viral load reduction has now become the standard measure for determining response to antiretroviral therapy, as well as when comparing efÞcacy of different antiretroviral medications in clinical trials. Indeed, it was the response in plasma viral load that allowed investigators to propose that use of dual or triple antiretroviral drugs is of greater beneÞt than monotherapy (71). Finally, the plasma viral load has proven useful in predicting disease progression in the setting of antiretroviral therapeutic response, and the progressive change in this level has been shown to be even more predictive of disease progression than the pre-therapy level (64,72).

The two most common methods of quantitating plasma HIV-1 RNA in the clinical setting are the reverse transcriptase polymerase chain reaction (RT-PCR) assay and the branched DNA (bDNA) assay (73Ð77). In the RT-PCR assay, HIV-1 RNA from the patient is converted to complementary DNA (cDNA) by adding reverse transcriptase. A well-preserved portion of the *gag* gene is ampliÞed by PCR and hybridized to an enzyme-linked DNA probe. Simultaneously, a competitive RNA template, with a known standard copy number, is used in competitive titration (Fig. 9.2). The ratio of detected signal is compared to the signal of the known standard, thus determining the amount of HIV-1 RNA in the patientÕs plasma.

The bDNA technique for measuring viral RNA differs in concept from the RT-PCR method (Fig. 9.3). In this assay, HIV-1 RNA is captured by probes on to a microplate. Multiple DNA probes are hybridized to speciÞc *pol* (or *gag*) gene segments of the bound RNA, thereby amplifying the signal. Alkaline phosphatase is then added in the presence of a substrate to generate a chemiluminescent reaction. The chemical light units are then compared to a standard to determine the amount of RNA in the sample. Thus the bDNA method is based on signal ampliÞcation rather than target ampliÞcation.

A third method for quantitating plasma HIV-1 RNA, the nucleic acid sequence-based ampliÞcation assay

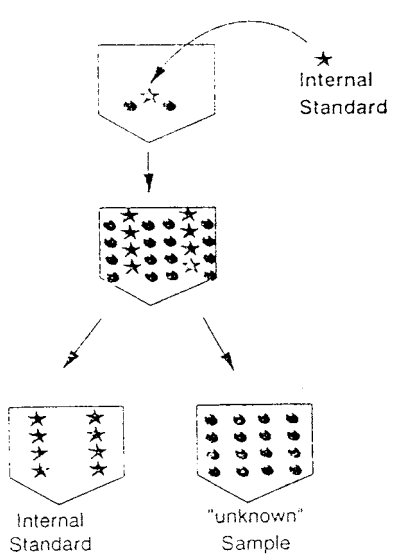

FIG. 9.2. Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) for measurement of HIV-1 plasma RNA. After HIV-1 RNA from the patient is converted to complementary DNA (cDNA), a portion of the *gag* gene is ampli ed by PCR. Competitive titration with a known standard copy number allows for quantitation of patient HIV-1 RNA. Harrigan J. *AIDS* 1995;10:139. Permission for use pending.

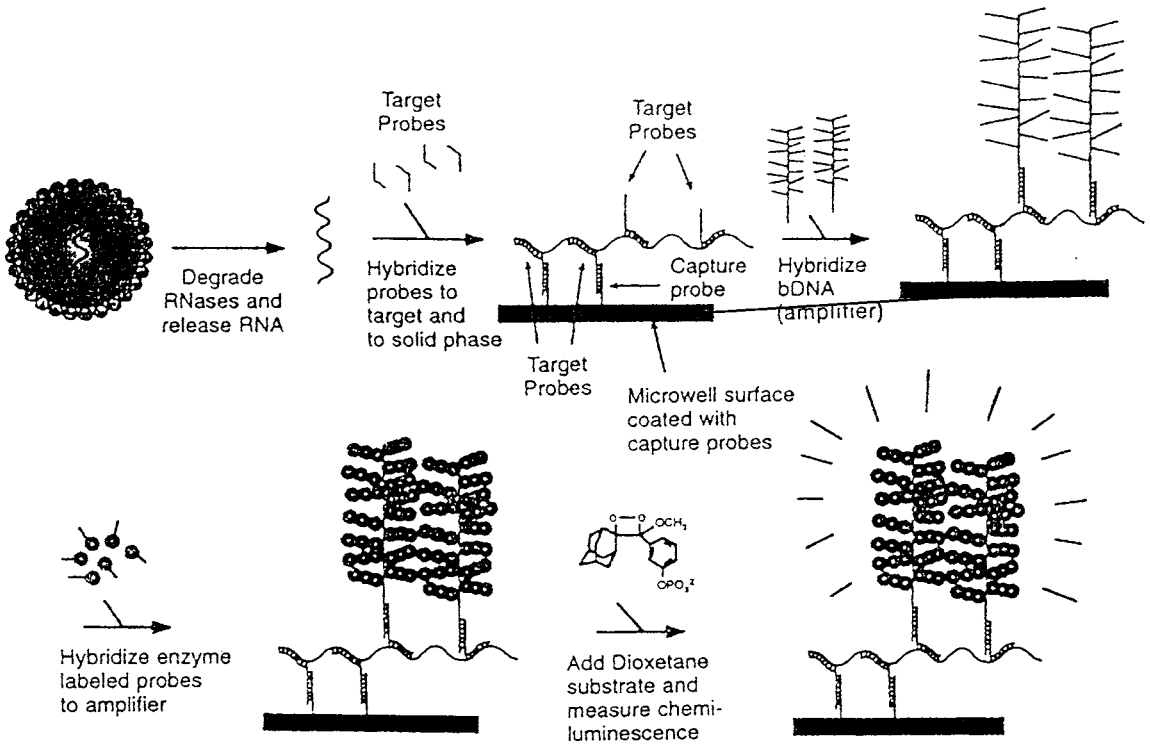

FIG. 9.3. The branched DNA (bDNA) assay for measurement of HIV-1 plasma RNA. After HIV-1 RNA from the patient is captured on to a microplate, DNA probes are hybridized to speci c gene segments of the baound RNA, thereby amplifying the signal. A chemiluminescent reaction is then used to quantitate the amount of RNA in the sample. Harrigan J. *AIDS* 1995;10:139. Permission for use pending.

(NASBA), is also commercially available, but less frequently utilized at this time. This assay, like the RT-PCR method, involves repetitive rounds of target amplification (78). Unlike the RT-PCR method, the NASBA method amplifies viral RNA as opposed to cDNA.

These three assay methods appear to be equivalent in sensitivity and specificity, particularly when measuring high or moderately high levels of viremia (79,80). Variability among the assays, however, may be more pronounced when evaluating low or undetectable viral loads (81). Currently, only the RT-PCR viral load test has been approved by the FDA for determining prognosis and for monitoring the response to therapy. Also, absolute levels of nucleic acid can vary among the assays. For example, the levels of viremia when determined by RT-PCR are typically two times higher than when measured by bDNA (62). For these reasons, direct comparisons of viral load should occur only if the same methodology is utilized, and the same assay should be used when serially testing viral loads.

The issue regarding HIV-1 viral load differences in men compared with women is not yet resolved. Some studies have noted that women tend to have lower HIV-1 RNA levels than men at seroconversion and early in infection, although other studies revealed no such differences (82–86). Even when viral load was found to be lower in women than men at seroconversion, the plasma HIV-1 RNA levels appeared to equalize with time (87). More recent data suggest that because viral loads are lower at seroconversion in women, and since the rate of progression to AIDS is similar in both sexes, guidelines recommending when to initiate therapy should be different for men and women (88). Until the issue of gender differences on plasma HIV-1 viral load is clarified, it is unlikely that different treatment recommendations for males and females will be made.

A number of factors can lead to variation in viral load for an individual patient. This variation can be related to assay methodology or can be biological in nature. For example, use of heparin as an anticoagulant can result in lower quantities of HIV-1 RNA than with use of EDTA as the anticoagulant (62,89). Similarly, time to processing of the specimen can alter levels of RNA (90).

In addition, plasma viral load can be transiently affected by a number of host or biologic factors. Vaccination against tetanus, the pneumococcus, and influenza have all been shown to cause a moderate, but transient, increase in plasma HIV-1 RNA levels (91–94). The cellular activation that results from vaccination may explain the increased HIV-1 viral expression and replication. Interestingly, those patients with higher CD4+ T-lymphocyte counts, and those who mounted the best response to the vaccine, typically developed a higher increase in plasma HIV-1 RNA.

Several infectious diseases, such as tuberculosis, herpes simplex virus infection, and bacterial pneumonia have also been shown to cause a transient increase in HIV-1 viral load (95–97). In fact, a transient increase in viral load has been associated with the development of any of several opportunistic infections, such as *Candida* esophagitis or *Pneumocystis carinii* pneumonia (98). Again, this may be related to cellular activation in response to infection.

Transient changes in plasma viral load may occur for less obvious reasons. A decrease in plasma viral load had been demonstrated in ovulating women, particularly during the early follicular phase to the mid-luteal phase, perhaps as a result of hormonal regulation of lymphocytes or cytokines (99). Also, missing even a few doses of antiretroviral therapy prior to measurement of viral load can result in an increased RNA level (65). Given the above variability in viral load, it is strongly recommended that decisions regarding therapy be based on at least two plasma viral RNA determinations separated over time (100,101).

Recommendations regarding use of plasma HIV-1 RNA levels in guiding therapeutic decisions have been made by the Department of Health and Human Services, as well as by the International AIDS Society (102,103). For patients who are naïve to antiretroviral therapy, determination of baseline plasma viral load, in addition to the CD4+ T-lymphocyte count, is essential. The plasma HIV-1 RNA threshold values used in determining when to initiate therapy differ slightly between the two sets of recommendations. The guidelines by the Department of Health and Human Services also consider different threshold values based on which assay method for plasma viral load is used.

Both sets of guidlelines state that if combination antiretroviral therapy is instituted, the goal of therapy is to obtain and maintain a plasma viral load below the level of detection. The level of detection, however, will depend on the sensitivity of the assay. Both the RT-PCR and bDNA methods for plasma HIV-1 RNA can detect viremia as low as 50 copies/mL. Currently, the guidelines suggest that a viral load less than 50 copies/mL is optimal, and there are some data to suggest that a more durable virologic response is obtained if such a viral load can be achieved (104–106).

It is expected that a 1- to 2-log reduction of plasma HIV-1 RNA should occur within four to eight weeks after starting therapy, and that the plasma viral load should be undetectable within sixteen to twenty-four weeks. One factor that can affect the time it takes for the viral load to become undetectable after beginning therapy is the level of viremia at baseline. A viral load that is greater than 100,000 copies/mL has been associated with a poorer chance of attaining an undetectable viral load with highly active antiretroviral therapy (102,107,108). It has also been demonstrated that the less time it takes to obtain an undetectable viral load after initiating therapy, the more likely the viral load will remain undetectable while on therapy (107). Thus, the lower the nadir, and the less time it takes to achieve an undetectable viral load, the greater the chance of successful therapy.

The optimal frequency for performing plasma viral load measurements once therapy has begun is not clear. Current guidelines recommend measuring the plasma viral load within one month after initiating or changing therapy, monthly until the goal of therapy is reached, and every two to three months thereafter (102). The guidelines also make recommendations regarding a change in antiretroviral therapy if viral rebound or failure to attain an undetectable viral load occurs. This issue is somewhat less clear, since the exact plasma viral load threshold for changing therapy is unknown, and other factors such as immune status as well as adherence and side effects of medications should be considered before altering therapy.

THE DIAGNOSIS OF ACUTE HIV-1 INFECTION

Although development of antibodies to HIV-1 can occur within weeks after exposure to the virus, routine serologic testing for HIV-1 using ELISA and Western Blot assays may initially be negative or indeterminate, particularly during the first few days to weeks after exposure. Diagnosis and treatment of HIV-1 infection shortly after exposure may be beneficial. Potential benefits of identifying and treating HIV-1 infection during the acute phase include preserving immune function, reducing the risk of transmission, and potentially altering the natural history and progression of disease (109). Initial viremia is thought to occur approximately four to eleven days after mucosal exposure (110). Quantitation of HIV-1 and p24 antigen during this period has revealed extraordinarily high levels of HIV-1 in the plasma (19,50,111,112). Control of viremia and a subsequent decrease in these levels occur shortly thereafter, reflecting the development of antibody and a cytotoxic T-lymphocyte (CTL) response (113,114). Presumably, at this time a plasma viral set-point is established. It is felt that antiretroviral therapy during acute HIV-1 infection may limit viral replication and thus lower the viral set-point, improving overall prognosis (115,116). Therapy at this time may also result in an enhanced CTL and T-helper lymphocyte response, thereby also potentially affecting outcome (117,118). Thus, making the diagnosis of acute HIV-1 infection, combined with early intervention, may be very beneficial.

Unfortunately, making this diagnosis can also be difficult. Although a well-described mononucleosis-like syndrome occurs in at least 50% of patients with acute HIV-1 infection, the findings are variable and non-specific, and can be easily attributed to other viral illnesses. In fact, less than 10% of cases of acute HIV-1 infection are diagnosed by clinicians (119). These symptoms usually occur two to six weeks after exposure and last for approximately one to two weeks (120). Since HIV-1 serologic tests become positive an average of at least 22

days after exposure, other methods of detection of primary HIV-1 infection are necessary (121).

Measurement of serum p24 antigen, HIV-1 DNA in plasma or PBMCs, or plasma HIV-1 RNA may be useful during this time period. As mentioned above, p24 antigen levels are often initially high, but may wane after one to two weeks (112,122). One recent study determined that the sensitivity of the p24 antigen assay during acute HIV-1 infection is approximately 89%, with a speciÞcity of 100% (123). A more sensitive method of diagnosing acute HIV-1 infection may be the plasma HIV-1 RNA level. Studies evaluating the use of this marker in diagnosing acute HIV-1 infection have found that the plasma viral load is nearly always greater than 50,000 copies/mL, and often greater than 100,000 copies/mL (115,123). The sensitivity of this assay during acute HIV-1 infection was 100% in a recent study, with a speciÞcity of 97% (123). It should be noted, however, that false positive plasma viral loads (usually with less than 5,000 copies/mL) have occurred and can lead to misdiagnosis (70,124). Although the above tests are more easily performed, a third option for diagnosis of acute HIV-1 infection is PCR for HIV-1 DNA in plasma or PBMCs. The sensitivity of this assay in this situation is not known. Finally, if a diagnosis of acute HIV-1 infection is made based on the above tests, standard serologic testing should still be performed at a later date to conÞrm the diagnosis.

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Chapter 10

Simian Retroviruses

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Animal retroviruses are classiÞed within seven different genera (Fig. 10.1), of which members of Þve genera (Table 10.1) are present in primates: (1) C-type virus; (2) D-type virus; (3) Human T-cell leukemia virus (HTLV), Simian T-cell lymphotropic virus (STLV), Bovine leukemia virus (BLV); (4) Lentiviruses; and (5) spuma Ð or foamy viruses (1). This chapter will review the salient biology of each of these Þve viral groups, in the order given with relevant information available up to the present time (2003). Emphasis will be placed on the D-type simian retroviruses (SRVs) and lentiviruses (SIVs) because of their etiologic association with simian AIDS (SAIDS). In the last 20 years it has become evident that humans also harbor retroviruses: two strains of human T-lymphotropic virus (HTLV-1 and HTLV-2), with simian counterparts (STLV-1 and STLV-2); and two major strains of lentivirus, human immunodeÞciency virus (HIV-1 and HIV-2), which also have simian counterparts (SIVs). Humans apparently are not infected with any exogenous D-type or C-type viruses nor do they carry any indigenous spumaviruses. Of all the animal lentiviruses, SIV is genetically most closely related to HIV and apparently was the source of HIV by cross-species spread from infected chimpanzees and sooty mangabeys in Africa. SIV is a heterogeneous group indigenous to many species of African non-human primates, including chimpanzees. In the natural African simian hosts, SIV causes no disease but experimental infection of captive macaques with certain SIV strains, especially from the sooty mangabey, produces a progressive and fatal immunodeÞciency syndrome similar to AIDS in humans, making this primate lentivirus model very useful for research into AIDS pathogenesis, vaccines and antiviral therapy. In recent years much headway has been made using the SIV macaque model for these purposes (2Ð5). This animal model is also covered by FultzÕs chapter in this volume.

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MAMMALIAN C-TYPE VIRUSES

This genus of C-type retroviruses, formerly called RNA tumor viruses, oncoretroviruses or oncornaviruses, was the target of extensive research in the 1970s seeking to identify cancer-causing retroviruses in animals and man. Several prior reviews (6,7) have thoroughly covered this material; this section will only highlight the major features of this viral group. C-type viruses are further divided into two major groups depending on their endogenous or exogenous route of transmission under natural conditions.

Endogenous C-Type Primate Viruses

Evidence became available in the early 1970s that C-type viral-related genes were present in the genomic DNA of many mammalian species, including primates (8). Most of these endogenous elements Þrst appeared in the primate germline prior to the New World/Old World divergence over 40 million years ago, but then underwent major ampliÞcation in Old World primates (9). All known human endogenous retroviruses (HERVs) were thus integrated into the germline prior to the speciation of Homo Sapiens and higher apes. Although all of these endogenous retroviral sequences are defective in humans and unable therefore to synthesize infectious viruses, a few endogenous viral isolates were obtained in the 1970s from non-human primates (baboon, macaque, owl monkey) by cocultivation of their cells with permissive cells of heterologous host species. These endogenous virus isolates are not infectious for cells of their species of origin,

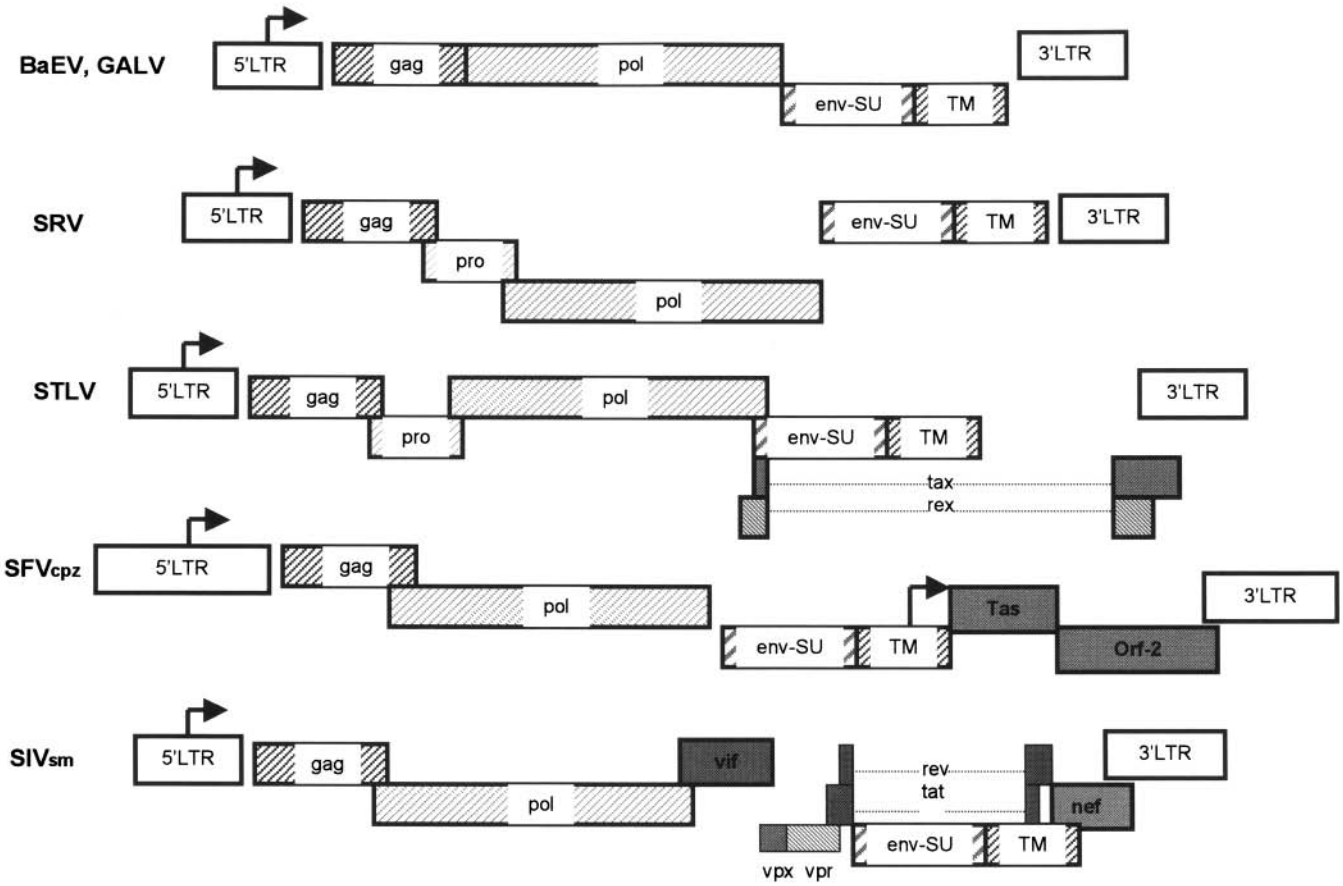

FIG. 10.1. Genomic organization of primate retroviruses. The genetic structure of the proviral form of several primate retroviruses is shown, Abbreviations for viruses are given in Table 10.1. Long terminal repeats (LTRs) ank the provirus. Genes for structural proteins incorporated into virions are crosshatched. Accessory or auxiliary genes are shaded. Table 10.3 contains a description of retroviral gene functions for the primate lentiviruses. The arrows indicate the transcriptional promoter in the LTR and the internal promoter for the foamy viruses (e.g. SFV_{cpz}).

i.e. they have a xenotropic host range. The endogenous viral genes appear to be well conserved in evolution and to represent part of the natural inheritance of the species. It is assumed that the endogenous viral genes are evolutionarily conserved relics of ancient infections by exogenous retroviruses, primarily of the same animal species, but occasionally across species barriers. This phenomenon of retroviral integration into the germ line can be accounted for by the presence of the viral-encoded reverse transcriptase enzyme, which provides the mechanism for converting the complete RNA genome of exogenous retroviruses into double-stranded DNA (10,11). Another viral enzyme, the integrase, functions to insert this proviral information, more or less at random, into the chromosomal DNA of somatic and, on rarer occasions, into germ cells. There is evidence from *in situ* hybridization that endogenous retroviruses may show speciÞc integration site preferences (12). It was discovered that: (a) not only all chickens and mice contain such C- type information in their cellular genomes, but that this holds true for all mammals; (b) endogenous C-type viral genes coding for core proteins, envelope glycoproteins and the enzyme reverse transcriptase, are expressed under independent regulation; and (c) the endogenous C-type viral genes are expressed in many instances in the absence of virus production. In certain inbred strains of chickens and mice, the inherited endogenous C-type viral genes become activated spontaneously to form complete viruses, which are infectious for their own speciesÕcells (i.e. ecotropic host range). In the inbred line AK at Rockefeller Institute, NY (AKR) and related leukemia-prone strains of inbred mice (but not in outbred feral mice) (13), such endogenous ecotropic viruses (i.e. murine leukemia virus) may recombine with other endogenous defective C-type-related viral genes, and eventually cause thymic lymphomas (14).

The viral oncogene hypothesis that guided much of cancer virus research in the 1970s was based on the similar idea that activation of endogenous virogenes might also include viral oncogenes and lead to cancer (15). However, this generalization, although of tremendous heuristic value, did not prove entirely accurate. Pioneering studies of animal retroviruses, primarily in chickens, mice and cats, did indeed lead to the discovery of oncogenes, but in the mid-1970s it was realized that these sequences were

TABLE 10.1. *Simian retroviruses*

Virus Genera	Host	Exogenous	Endogenous	Pathogenic
C Type	Tree shrew		TRV-1	No
	Baboons		BaEV	No
	Baboons		SERV*	No
	Baboons		PcEV*	No
	Rhesus Macaques		Mac-1	No
	Stumptail Macaques		MMC-1	No
	Colobus		CPC-1	No
	Owl Monkey		OMC-1	No
	Gibbon Apes	GaLV		Lymphoma and myelogenous leukemia
	Wooly Monkey	SSAV/SSV		Sarcoma
HTLV/STLV/BLV	African and Asian Monkeys and Apes		STLV-1	
	Lymphoma			
	Macaques	STLV-1		No
	Spider Monkeys	STLV-2		No
	Pygmy Chimpanzees	STLV-2		No
D-Type				
	Macaques	SRV 1–5 (MPMV)		
	SAIDS			
	Hanuman Langur	SRV-6		No
	Spectacle Langur		Po 1-Lu	No
	Squirrel Monkey		SMRV	No
Lentivirus	African Monkeys (natural host)		SIV	No
	Macaques (experimental host)	SIV		SAIDS
Spumavirus	African and Asian Monkeys, Apes and Chimpanzees; Squirrel, Spider, and Capuchin Monkeys	SFV		No

* Complete endogenous proviral DNA based on PCR ampli cation and DNA sequence analysis, but not detectable as virus particles

not viral but instead represented normal cellular growth promoting genes present in all eukaryotes (16). In animals, such oncogenes could be activated by nearby insertion of C-type viral DNA, and thus lead to leukemia. Rarely, such oncogenes were transduced, i.e. Òpicked upÓ by exogenous C-type retroviruses, to create rapidly acting avian, murine or feline sarcoma viruses (6,7). In humans, by contrast, oncogenes are generally activated by mutations such as chromosomal translocations, rather than by retroviral infection, and no human sarcoma viruses have been discovered.

The presence of endogenous, ecotropic (i.e. infectious) C-type viruses in inbred chickens and mice is clearly an effect of inbreeding. In outbred feral mice (the progenitor of inbred lab mice), as found later in humans (i.e. HTLV), such leukemia viruses are transmitted solely by exogenous means, mainly by milk (13). Although a number of useful roles and possible pathogenic sequelae for endogenous virogenes have been postulated, it has not been possible, as yet, to demonstrate any biologic function for these genes in primates, including humans (17), despite the fact that over 1% of the human genome is represented by such endogenous retrovirus-like sequences (18).

Analysis of the newly available human genome reveals that coding sequences comprise less than 5% of the genome whereas repeat sequences account for at least 50% (19). Most human repeat sequences are derived from transposable elements, among which retrovirus-like elements are one major class. Among these transposable elements are LTR retrotransposons comprising LTRs that contain all of the necessary transcriptional regulatory elements ßanking gag and pol genes. Exogenous animal retroviruses probably have arisen from such retrotransposons by acquisition of a cellular envelope gene (*env*). In the human genome, in marked contrast to the mouse, LTR retrotransposon activity has been essentially extinct for the past 40 million years. Therefore, it is easily understandable why infectious endogenous retroviruses have not been generated in modern evolutionary times in human or the closely related non-human primates.

Baboon Endogenous Virus (BaEV)

The Þrst infectious, endogenous C-type virus isolate of primate origin was from baboon placenta (20). Subsequently, many independent but closely related isolates

were obtained from diverse normal adult and embryonic tissues of several different species of baboons. Like most other endogenous viruses, these could only be grown in permissive cells of certain species different from the species of origin (i.e. xenotropism). BaEV was not shown to have oncogenic activity and its biological role, if any, remains unknown. This prototype endogenous primate C-type virus (BaEV) became a useful tool for probing the evolutionary relationship of various primates. Non-infectious sequences related to those of BaEV virus were found in all other Old World monkeys with the degree of relatedness determined by the evolutionary distance between species (21).

More recently, PCR amplification and sequencing of two BAEV proviral DNAs in a baboon (*Papio cynocephalus*) genomic library revealed full length proviruses with little sequence variation and no evidence for the rearrangements or large deletions commonly observed in endogenous viral genomes from other mammalian species (22). A novel complete endogenous proviral genome was also isolated by PCR amplification from a yellow baboon genomic library (23). The *gag* and *pol* genes are closely related to BaEV, but the *env* gene was apparently contributed by an endogenous Type D provirus (SERV) also isolated by PCR from the baboon genomic library (24). PCR analysis of primate DNA suggests that SERV is ancestral to both BaEV and simian Type D retroviruses (SRV). In addition, the same library yielded proviral sequences with BaEV core and Type C *env* genes (PcEV) (25). Neither of these endogenous proviruses are expressed as complete virus particles. The distribution of these baboon endogenous proviruses among non-human primates appears to be the consequence of cross-species spread of exogenous viruses in antiquity among different primate species sharing the same habitat (26). Several similar examples were noted of suspected cross-species transmission of endogenous C-type viruses among animals in evolutionary times (27). For example, BAEV proved partially related to the endogenous, xenotropic feline C-type virus, RD114 (28). Because RD114 DNA was not present in the genomic DNA of most members of the Felidae family, it was concluded that BaEV spread from ancestral baboons to ancestors of the domestic cat about 5 million years ago (29). PCR sequence analyses now suggest that RD114 is actually a new recombinant between PcEV Type C core genes and the env gene of BaEV (30).

Sequence homology between HTLV-1 and STLV-1 isolates from specific geographic regions has also suggested the transmission of ancestral viruses of this retrovirus genus between primate species including humans (31) (see STLV section). In current times, it has been very difficult to document transpecies spread of any animal retrovirus, endogenous or exogenous, under natural conditions. However, four important exceptions, discussed later in this chapter, have become apparent in retrospect: (a) the inadvertent infection about 25 years ago of Asian macaques in USA primate centers by the exogenous lentivirus (SIV) from African sooty mangabeys, an event that caused simian AIDS (32); (b) the recent (□□30 years) spread of SIV from sooty mangabeys to humans in West Africa to give rise to HIV-2 (33); (c) the spread of SIV in the early or mid-1900s from chimpanzees to humans to give rise to HIV-1 (34); and (d) the accidental infection of several primate handlers with SIV or possibly SRV (35–37). It will be of interest to see how often humans given xenotransplants of baboon liver or bone marrow become infected with BaEV or other simian viruses, e.g. STLV, SIV, SERV or foamy virus. Indeed, retrospective PCR analysis of tissues from two human recipients of baboon liver transplants revealed the presence of BaEV and simian foamy virus in multiple tissues (37). The presence of baboon mitochondrial DNA in the same tissues suggested that infected baboon leukocytes in the liver transplants were the source of these viruses.

In recent years, several laboratories cloned the human receptor for BAEV, and identified it as a sodium-dependent neutral amino acid transporter (39–41). This same receptor functions for the feline endogenous retrovirus RD114, all strains of SRV and the avian reticuloendotheliosis group. This receptor was previously mapped in humans to chromosome 19q13.3 (42). Infection of cells with RD114 or SRV resulted in impaired amino acid transport suggesting a mechanism for virus toxicity and immunosuppression (41). Knowledge of this retrovirus receptor may facilitate gene therapy using vectors derived from this virus group.

In addition to BaEV, five other primate endogenous retroviruses, *in toto*, have been isolated and characterized, making a total of six distinct genetically transmitted retrovirus groups in primates (43) (Table 10.1). These isolates included C-type virions from tree shrew, stumptail and rhesus macaques and an owl monkey, and D-type viruses from the squirrel monkey and a langur monkey. Like BaEV, these other endogenous primate retroviruses have no known biologic role. Apart from the baboon and owl monkey, endogenous retroviral genes are powerfully repressed at the cellular level in their primate hosts, and, as in humans, are not expressed as complete infectious virus particles.

Gibbon Ape Leukemia Virus (GaLV) and Simian Sarcoma Virus (SSV)

The first exogenous primate C-type virus (GaLV) was isolated in the early 1970s from several captive gibbon apes that developed spontaneous leukemia (44). At about the same time, a closely related virus called simian sarcoma virus (SSV) was recovered from a pet wooly monkey with a spontaneous fibrosarcoma (45). GaLV was then shown to be fairly common in captive gibbon apes and to be spread horizontally as a purely exogenous virus (46). Molecular hybridization analysis suggested that it might have been acquired in the evolutionary past by

transpecies infection with an endogenous C-type virus of feral Asian mice (27). By contrast, SSV was found only in the one wooly monkey; it was neither endogenous nor exogenous in this species. Because the GaLV-infected gibbon ape and SSV-infected wooly monkey had frequent physical contact in the same household and because the GaLV and simian sarcoma associated or helper virus (SSAV) were so closely related, it was assumed that SSV was derived from GaLV by transpecies infection. The sarcoma-inducing property of SSV was later shown to be caused by the transduction of the *sis* oncogene (β-chain of platelet-derived growth factor) from the wooly monkey genomic DNA by infection with the GaLV (47). This was a rare one-time event, and still represents the only example of a sarcoma virus oncogene rescued from a solid tumor of a non-human primate. Experimental studies of SSAV/SSV cell transformation and induction of sarcomas or gliomas in marmosets (48) were very helpful in elucidating the molecular mechanisms underlying its rapid oncogenicity, e.g. defectivity of sarcoma viruses, rescued by helper type C viruses (such as GaLV), and transduction of the *sis* oncogene.

Because GaLV occurs naturally in gibbon apes in captivity, it was assumed to occur in gibbon apes in the wild, but this has not been studied. GaLV was isolated on several occasions and at different locations from additional cases of spontaneous malignant lymphoma or myelogenous leukemia occurring in captive gibbon apes (49). Subsequently, the virus was also isolated from healthy seropositive carriers. The virus causes a systemic bloodborne (cell-associated and cell-free) infection of multiple tissues, and can be readily isolated from many organs. It has a wide *in vitro* host range for a number of non-hematopoietic cell types of diverse species including humans and is non-transforming and non-cytopathic. All of the GaLV isolates are closely related one to another, but individual isolates can be distinguished by highly sensitive immunologic, *in vitro* infectivity, or nucleic acid hybridization assays (50). Experimental transmission studies showed that the GaLV isolates were very stable and could induce the same tumors (e.g. myelogenous leukemia with latent period of 6–14 months) as that seen in the animal from which the virus was initially isolated. Infectious molecular clones of GaLV were not then nor now available for *in vivo* pathogenicity study. Natural transmission of GaLV was documented several times in captive gibbon apes, but the route of spread was not shown (46). Congenital transmission also was observed. Neutralizing antibodies were protective against fulminating viremia and subsequent development of tumors. Despite extensive surveys, and numerous false alarms, GaLV infection of humans was never found (51). In the late 1970s, the NIH-supported gibbon ape colonies were disbanded and biologic studies of GaLV and SSV came to a halt. The cell surface receptor for GaLV and SSV in human cells has been characterized as a channel for sodium phosphate ions (52, 53). A closely related receptor functions for amphotropic murine leukemia viruses and feline leukemia virus subgroup B (54, 55). These receptors are expressed on human adult and fetal tissues including hematopoietic stem cells and umbilical cord blood cells. Thus, GaLV and amphotropic MuLV have provided a useful source of envelope for retroviral vectors frequently used in current gene therapy protocols (56–59).

A novel Type C retrovirus was recently detected by EM and PCR in the blood and tissues of many wild and captive koalas and in three animals with lymphoma (60). The virus (KORV) is apparently endogenous in koalas but not other marsupials and phylogenetic analysis showed that, paradoxically, it clusters with GaLV, thus suggesting yet another example of relatively recent cross-species transmission. Its pathogenicity remains to be determined.

Human Endogenous Retroviruses

In the last 20 years, since human endogenous retroviral elements (HERVs) were first isolated (61), a number of sequences related to non-human (i.e. chicken, mouse, cat, primate) endogenous and exogenous retroviruses have been cloned and sequenced or found by polymerase chain reaction (PCR) analysis in human genomic DNA (17). Most of these sequences are related to primate and murine Type C viruses (BAEV, GaLV/SSAV, MuLV), murine Type B viruses and intracisternal A particles (IAP) or HTLV (62–64). Phylogenetic analysis shows that the HERV-W family entered the human genome more than 25 million years ago (65). Sequences detected in the purified retrovirus-like particles from human placentae, have the highest homology to B- and D-type retroviruses (66). Historically, in 1973, it was the detection by electron microscopy (EM) of such budding C-type particles in baboon and human placentas (67) that prompted the isolation of BaEV from baboon placenta (20) and led to a massive search for replicating human endogenous retroviruses. However, no direct evidence for replication of complete, infectious endogenous retroviruses from human placenta or any other human tissue has ever been observed (17,18,51). The immunologic detection of HERV antigens or antibodies and the occasional sighting by EM of budding retrovirus particles that, when purified, contain reverse transcriptase (RT) activity, in human tissues, especially placenta and germ cell tumors, suggest a biologic function for some of these sequences (68–74). HERV envelope proteins are expressed in the syncytial trophoblast layer of the placenta and may be involved in the development of the structure (75). Several tumor cell lines, mainly teratocarcinoma, bladder carcinomas, testicular and lung tumors, also express significant levels of HERV transcripts including full length *gag*, *pol*, and *env* domains (76–79). Several HERVs with open envelope reading frames were further characterized by an *in vitro* transcription/translation approach (80). Premature stop

codons were present in the great majority of env genes of such HERVs. The results suggest the lack of conservation of a functional envelope gene of possible beneÞt for the host. Some of the HERV *env* genes including those expressed in the placenta contain sequences related to the so called immunosuppressive domain of other retroviruses (79, 81), and it has been suggested that this env domain protect the fetus from the maternal immune response and may allow tumor cells to escape immune rejection in immunocompetent hosts (75,82). Expression of a HERV has also been tentatively linked to patients with multiple sclerosis (83Ð85). Recently, the envelope glycoprotein of a placenta-related HERV-W was able to form pseudotypes *in vitro* with defective HIV-1 virions thus enforcing tropism for CD4 negative cells (86). Thereby, the HERV-W env gene was demonstrated for the Þrst time to encode functional properties of a retrovirus envelope glycoprotein. Nevertheless, no replication competent HERV has been described and the cumulative data suggest that the human genome does not contain an entire, intact proviral copy of any HERV. In conclusion, any health related signiÞcance of these endogenous retrovirus-like elements in humans, as in non-human primates, remains unknown, despite the many speculative roles assigned them (17).

SIMIAN T-LYMPHOTROPIC VIRUS, TYPE 1 (STLV-1)

STLV-1, the simian counterpart of HTLV, was discovered in Japanese macaques (*Macaca fuscata*) shortly after the discovery of HTLV-1 (87). STLV-1 and HTLV-1 share many molecular features and pathogenic properties (88). Naturally-occurring STLV infection is highly prevalent in feral populations of at least 30 species of African and Asian non-human primates (87Ð97). The genomic organization of both STLV-I and HTLV-I is similar consisting of LTR-gag-pol-env-tax/rex-LRT and genomic sequences exhibit an overall similarity of 90Ð95% (98). Because of their close phylogenetic relationship, these simian and human viruses have been classiÞed as primate T-cell lymphotropic virus Type 1 (PTLV-1). Indeed, simian strains cannot be distinguished from human strains by phylogenetic criteria (99).

Serosurveys and virus isolation indicate that STLV-1 is a common infection in feral Cercopithecoidea and apes in Africa and, in feral macaques and orang-utans in Asia and Indonesia (87Ð97). Infection is also frequent in captive monkeys and apes in primate centers and zoos (100Ð106). In Africa, infection rates of 10Ð60% are found among feral troops or captive colonies of African green monkeys, baboons, mandrills, Sykes, and patas monkeys, and in Indonesia, similar infection rates are found in Celebes and cynomolgus macaques and siamongs. In primate centers, approximately 20Ð50% of captive rhesus monkeys and sooty mangabeys and 10% of chimpanzees may be infected with STLV-1. An increasing seroprevalance with age suggests that the virus is horizontally spread; sexual transmission (107) and Þghting among males (97,108) may account for most of this spread. Vertical transmission may occur less frequently. Like HTLV (and BLV (109), STLV is transmitted in a T-lymphoid cell-associated and not cell-free manner. Almost all of the monkeys or apes carrying STLV remain healthy. However, the spontaneous occurrence of adult T-cell leukemia (ATL), similar to ATL in HTLV-1-infected humans has been documented in African green monkeys (110,111), baboons (90,102,112) and gorillas (113,114). Neurologic disease similar to that associated with HTLV-1 infection of humans (i.e. tropical spastic paraparesis) has not, as yet, been observed in STLV-1 infected monkeys or apes. In naturally infected African green monkeys, STLV-1 speciÞc killer T cells are activated *in vivo*, which may help hold the virus in check (115). In captive sooty mangabeys, coinfections with STLV-1 and SIV are also non-pathogenic (103). Experimental infection of macaques with STLV-1 resulted in a latent low level infection without seroconversion. Even in SIV experimentally infected macaques, STLV-1 infection apparently does not affect the course of SIV-disease (116). Nor does STLV apparently contribute to the development of lymphoma in naturally-infected macaques, despite a single report to the contrary (117). Recently, experimental infection of pigtailed macaques with STLVsm or HTLV-I resulted in an unexpected high mortality within days from a hypothermic syndrome unrelated to AIDS (118).

Because of the evolutionary age (> 25 M years), relative genetic stability (0.5% variation within an individual) and limited horizontal transmission of the PTLVs, molecular epidemiology and phylogenetic analyses of these viruses have revealed the movements and contacts of ancient human and non-human primate populations (94,99, 119Ð127). In general, and in marked contrast to the simian lentiviruses (SIV), PTLV sequences evolve slowly and segregate according to their geographical origin rather than according to host species, suggesting the repeated interspecies transmissions in the distant past of these viruses between primate species including human. The greater overall sequence variation between STLV-1 strains (1Ð18%) compared with HTLV-1 strains (0Ð9%) supports an ancient simian origin of the modern viruses in all species. Phylogenetic analysis reveals three major HTLV-1 clusters or clades: cosmopolitan (subtype A), Central Africa (Zaire) (subtype B) and Melanesian- (subtype C), and 7 STLV-1 clades in Asia and Africa with the most primitive (i.e. genetically diverse) clade involving several Asian primate species. Each of the three major HTLV-1 lineages appears to have arisen from separate ancient interspecies transmissions between STLV-1 infected monkeys and humans (128). The African STLV-1 isolates are more closely related to HTLV-1 than Asian STLV-1 isolates suggesting an African origin for most strains of HTLV-1 (129Ð131). Despite a higher evolutionary rate of PTLV-1 in Asia than Africa, phylogenetic analyses are compatible with an ancient origin of PTLV from either old

world continent (119,132). In humans, the cosmopolitan lineage (HTLV-1 subtype A) has the widest global distribution (Japan, Caribbean, South American, India, and West Africa) and probably arose from a West African STLV strain. The central African subtype (HTLV-1 subtype B) is clearly descendant from an STLV-1 chimpanzee-like ancestral simian strain in the former Zaire, and the Melanesian strain (HTLV-1 subtype C) probably descended from an Asian STLV-1 strain. The distribution of the cosmopolitan HTLV-1 clade suggests a close connection of Caribbean and South American natives with the Japanese, probably dating back to the migration of the lineage to the American continent via the Bering strains in the Paleolithic era. Consistent with this idea is the PCR detection, in 1,500 year old Andean mummies, of HTLV-1 proviral sequences that are similar to those in contemporary Andeans and Japanese (133). HTLV-1 subtype B remains focused in Central Africa. Clustering of HTLV-1 isolates from a B/C intermediate genotype (subtype D) in Central and West Africa with yet other Caribbean isolates suggest that some Caribbean isolates originated from Central and West Africa via the slave trade. Mandrills in Gabon, Central Africa, are apparently the natural reservoir of HTLV-1, subtype D (134). Close sequence relatedness between STLV-1 isolates in Celebes macaques in South East Asia and the HTLV-1 subtype C Melanesian strains suggest the interspecies transmission of STLV-1 to early Australoid settlers during their migration from South East Asia to the Australian continent (122). Interestingly, in South Africa, the known HTLV-1 strains do not share a common origin with non-human primates in that region (135,136). By contrast, as mentioned above, mandrills in Gabon carry the same PTLV-1 subtype D as found in humans in that area, and STLV-1 infected chimpanzees probably transmitted the virus (STLV-1 subtype B) to African green monkeys and baboons as well as humans in Central Africa (137). No speciÞc clustering of virus subtypes has been observed in relation to manifestations of HTLV-1-related diseases. Thus, the topology of the HTLV-1 phylogenetic tree reßects the interspecies transmission of STLV between non-human primates and humans and the movement of people carrying the viruses in the past.

At the Southwest Foundation for Biomedical Research (San Antonio, TX), about 40% of the 3,400 free-ranging baboons have been infected with STLV-1 (102). The risk of ATL developing in the infected animals is 3Ð4% during their lifetime; thus, in any single year a dozen or more lymphomas occur in this colony. Most of the tumor cell lines from these baboons have monoclonal integration of complete STLV-1 proviral DNA, whereas a few tumor cell lines show monoclonally integrated defective proviruses. A high incidence of STLV infection and associated lymphoma has also been observed in baboons at the former (now disbanded) Sukhumi Primate Center in southern Russia (Georgia) (90). Sequence analysis indicates that the baboons at this center acquired STLV-1 by recent cross-species transmission of the virus from rhesus macaques (112). In summary, STLV-1 has a natural history and biology in non-human primates that is very similar to HTLV-1 in humans (and BLV in cows) (109) and, as mentioned, the genome structure and degree of homology between STLV-1 and HTLV-1 (and BLV) are very similar. Clearly, the leukemogenic potential of STLV-1 is analogous to that of HTLV-1 and BLV.

Each of the PTLV has the same genetic constitution encoding typical *gag*, *pol* and *env* gene products as well as unique regulatory and accessory genes not present in the C type viruses, and located in four open reading frames (ORF) of the 3′ region (98,138). Two of these ORFs, analogous to *tat* and *rev* in HIV and SIV, encode the Tax and Rex proteins, respectively. Tax is a 40 kDa nuclear phosphoprotein, which activates viral transcription from the LTR and also activates many cellular genes involved in host cell proliferation and cell cycle control (139Ð143). Tax can also activate HIV-1 transcription *in vitro* (144). Rex is a 27 kDa nucleolus localizing phosphoprotein that increases the cytoplasmic accumulation of unspliced and singly spliced viral RNA. The other two ORFs are less well characterized, and encode various transcription factors (145). For both STLV-1 and HTLV-1, the natural target cells are mainly T-cells, mostly of CD4+ phenotype (146). Like HTLV-1, STLV-1 can immortalize cultured T-cells by a mechanism involving Tax activation of cellular transcription factors and other cellular genes (139,147). Several cytokines (IL-6, TGF-β, GM-CSF), involved in cellular proliferation and cell cycle control are released from STLV-1 transformed T-cell lines (140). The cellular receptor for STLV-1 or HTLV-1, thought to be located on human chromosome 17 (148), is still uncharacterized.

Obviously, STLV-1 could serve as an excellent experimental surrogate for HTLV-1 infection of man except that, like HTLV-1, the lymphoma/leukemia incidence is usually so low (1Ð5%) and the latent period so long (several decades), that it has not been used as a practical experimental model for studies focused on neoplasia. However, this opportunity could now be provided by the Southwest baboon colony described above (102). It is important to screen African and Asian monkeys in primate centers for infection with this virus, because it could yet prove to be a confounding cofactor in studies with SIV or other viruses.

An STLV-2 isolate, virtually identical to HTLV-2, has been recovered from New World Spider monkeys (*Ateles fusciceps*) (149). This is the Þrst link between HTLV-2 and a simian reservoir in the New World. PTLV-II viruses have been isolated, to date, from only IV drug users and their sexual partners (in which they are rapidly spreading (150), several Amerindian tribes (151) and Spider monkeys in the New World, and human Pygmys (152), and pygmy chimpanzees or bonobos (*Pan paniscus*) in Central Africa (153,154). So far, no disease has been found in association with PTLV-II infection of non-human primates or humans. Compared to PTLV-I viruses the PTLV-II viruses are evolving more slowly and exhibit less transcontinental

genetic differentiation (132). Several highly divergent STLV isolates have been recently isolated from feral Hamadryas baboon (155,156) and pygmy chimpanzee (153,154). These isolates are distinct in genetic sequences and genomic structure but are more closely related to HTLV-2 than HTLV-1 (157–159). These new STLV isolates constitute several distinct STLV-3 strains occurring in different African monkey species (160). It will be important to monitor whether such STLV variants can be transmitted to humans, especially following organ xenotransplantation. In contrast to SFV, SRV and SIV, no evidence has yet been found for transmission of STLV-1 to animal handlers in primate facilities.

The geographical prevalence of HTLV-1, and the low degree of variability compared to HIV-1, indicate that people in areas of high prevalence, such as southern Japan, and people of developing countries would beneÞt most from a vaccine against HTLV-1. Cynomolgus monkeys (*M. fascicularis*) have been used as an HTLV-1 vaccine model. In one experiment (161), four monkeys were immunized with a recombinant HTLV-1 *env* gene product produced in *E. coli* and challenged with live MT-2 cells, a high HTLV-1 producer cell line. After challenge, all four control non-immunized monkeys were infected and all four of the immunized monkeys were protected. Protection correlated best with vaccine induction of speciÞc antibody against envelope glycoproteins (gp68 and gp46) of HTLV-1, including high-titered syncytial inhibiting neutralizing antibody. A vaccinia-based HTLV-1 envelope vaccine also protected cynomolgus monkeys from infection by HTLV-1 (162). A highly attenuated HTLV-1 *env* poxvirus vaccine (ALVAC) induced protection against HTLV-1 infected cells in rabbits (163). In yet another study (164), three monkeys were immunized with an HTLV-1 gag and env subunit vaccine and challenged with an STLV-1 infected cell line. After challenge, the two controls were infected; the three vaccinates apparently were uninfected. However, an increase in antibody titers in the vaccinates after challenge suggests that they had been transiently infected. Protection correlated with induction of HTLV-1 (and to a lesser extent STLV-1) syncytial inhibition antibody and lymphocyte-mediated cytotoxicity (CTL) against STLV-1 infected cells. Thus, as previously shown in murine and feline C-type virus and BLV systems (165), vaccine protection via neutralizing antibody is also effective against the HTLV/ STLV group of primate retroviruses under laboratory conditions. Most recently, the squirrel monkey (*Saimiri sciureus*), a south American non-human primate, free of STLV, has been found susceptible to experimental infection with HTLV-1 immortalized syngeneic or allogeneic cells (166). The strong seroconversion and persistent latent infection, especially in lymphoid organs that ensues, indicates another promising animal model for evaluating candidate vaccines against HTLV-1 (166,167). An HTLV-1 vaccine protocol consisting of an env DNA primer followed by recombinant vaccinia (NYVAC) gag-env boosts protected all three squirrel monkeys against IV challenge with HTLV-1 infected allogeneic cells (168). It seems clear that, in contrast to HIV and SIV, an efÞcacious vaccine against HTLV (and STLV) should be quite feasible to develop, at least from the scientiÞc standpoint.

D-TYPE VIRUSES

Epidemiology, Natural History and Experimental Transmission

Exogenous Type D retroviruses, called simian retroviruses (SRV), of several serotypes (SRV 1–5), are indigenous in feral Asian macaques and cause a potentially fatal immunosuppressive disease resembling AIDS in captive macaques worldwide (2,169,170). In the early 1980s, before the discovery of SIV in 1985, the disease was called simian AIDS or SAIDS but this term is now generally restricted to the AIDS-like disease in macaques caused by simian immunodeÞciency lentivirus (SIV). Because SIV is more closely related to HIV in structure and function than is SRV, research on SIV is considered more relevant to AIDS and has largely replaced research on SRV. The original D-type virus, isolated in 1970 from a macaque with spontaneous breast cancer, was called Mason-PÞzer monkey virus (MPMV) (171). A serologic survey of U.S. primate centers, taken in the mid-1970s indicated that about 25% of all macaques had antibody reacting to MPMV; this observation revealed the widespread distribution of this infection in captive macaques prior to the 1980s (172).

Before the causative Type D retrovirus (i.e. SRV-1) was identiÞed in 1983 at the New England, Washington, and California Primate Centers and NIH, a cage exposure experiment was set up at the California Primate Research Center (CPRC) to prove the infectious nature of an immunosuppressive AIDS-like disease in an outdoor corral (NC-1) of rhesus macaques in which many deaths from SAIDS had occurred (173,174). Nineteen of 23 (83%) healthy tracer juvenile rhesus died of a fatal immunosuppressive disease within nine months of introduction into the resident affected population. In contrast, 21 healthy sentinel juvenile rhesus placed in the same outdoor enclosure but denied physical contact with the SAIDS affected group by a 10-foot wide buffer zone remained healthy and seronegative for 2 1/2 years. This result indicated that direct physical contact was required for spread of the disease. The most likely route of natural transmission was by percutaneous inoculation of virus-containing saliva and blood, via biting and scratching (175). Following the isolation of SRV-1 from affected rhesus in NC-1 and the development of appropriate serologic and virologic assays for its detection, it was found that all monkeys with SAIDS in NC-1 were persistently infected with this D-type virus. All of the healthy ÒsentinelÓ monkeys located within the same

enclosure, but denied physical contact with the affected animals, were free of infectious SRV-1 and antiviral antibody. In NC-1, the speciÞc mortality rate from simian AIDS was higher in juveniles than in adults and the overall prevalence of SRV-1 antibody in all ages ranged from 68Ð85%. Passive maternal immunity to SRV-1 may have protected some of the infants. Antibody prevalence increased with age to such a degree that, essentially, all animals over three years of age were seropositive. Seroconversion was found to be a poor indicator of current infection; about 50% of virus-positive juveniles had no antibody detectable by enzyme-linked immunosorbent assay (ELISA). In disease-free breeding colonies of rhesus monkeys, the prevalence of SRV-1 antibody was only 4% by ELISA.

Repeated viral isolations from all animals in NC-1 revealed the following patterns of infection: (a) SRV-1 viremia with clinical SAIDS; (b) transient viremia with clinical recovery; (c) intermittent viremia suggesting reactivation of latent infection; (d) viremia in a one-day-old infant, suggesting transplacental transmission; and (c) persistent viremia and virus shedding in several healthy animals. In a retrospective epidemiologic analysis, one healthy carrier in NC-1 was linked by direct physical contact to 34 cases of SAIDS over a three-year period (174). Simian AIDS was experimentally transmitted to two juvenile rhesus by inoculation of SRV-1, containing saliva from this adult female monkey (175). Although SRV-1 could be isolated from PBMC and most body secretions of infected animals, the most plentiful source of virus was saliva, which was the major natural source of virus transmission. Transmission of SRV in semen was not evaluated. Although SRV-1 is present in vaginal secretions, female-to-male sexual transmission of this virus also remains undetermined. Perinatal transmission of SRV-1 transplacentally or via milk appeared to occur infrequently.

Experimental transmission of SRV-1 from tissue culture media conÞrmed the virulence of this virus that had been indicated by the natural cage exposure observations and by experimental inoculations of infected blood, saliva and tissue homogenates (176). Intravenous inoculation of SRV-1 into 14 juvenile (9Ð11 months) rhesus led to the same spectrum of clinical disease as seen naturally in NC-1. All animals became infected; six died acutely 7Ð20 weeks after inoculation, six remained persistently infected up to one year after inoculation, and two developed neutralizing antibody, became non-viremic and remained healthy after one year. Monkeys dying acutely had a high level of persistent viremia and no serum antibody response by ELISA, whereas monkeys with a more indolent clinical course had a low grade viremia and only transient initial antibody response to the major core antigen (p27) (177). Monkeys that never became ill and were either non-viremic or transiently viremic and developed high levels of serum antibody, including neutralizing antibody to the virus envelope. Therefore, in the SRV simian AIDS model system, one can correlate disease resistance with humoral antibody levels and neutralizing activity. These observations further establish the etiologic role of SRV-1 in this fatal immunosuppressive disease. Conclusive proof of this etiology came later with induction of an identical, fatal disease spectrum, using molecularly-cloned infectious SRV-1 (178) and the prevention of this disease with SRV vaccines (see below).

Since 1983 D-type viruses (i.e. SRV) were identiÞed as the causative agents of a naturally-occurring infectious immunodeÞciency disease in eight species of macaques at Þve of the seven primate centers in the United States (170). The centers primarily affected with this disease were New England, California, Oregon, Washington, and Wisconsin. The Yerkes and Delta primate centers in the southeastern United States currently were largely spared of this problem. Infection appears to be highly prevalent in Asian macaques in captivity (179), the natural hosts of the SRV subfamily. SRV infection has been found in healthy feral macaques in India, but the prevalence of infection with the different serotypes in these feral animals remains to be determined. Nor has any disease linked to SRV infection in feral macaques yet been reported. SRVs are related to the endogenous D-Type retrovirus (PO-1-Lu) of the spectacled langur (*Presbytis obscuris*), another Asian monkey from which the exogenous SRVs may have had their evolutionary origin (180). Alternatively, the SRVs may have been derived evolutionarily from an endogenous Type D provirus (called SERV for simian endogenous retrovirus) recently detected by PCR ampliÞcation of genomic DNA in all Old World Monkeys of the subfamily Cercopithecinae, but not present in apes or humans (23). This intact endogenous Type D provirus is the putative ancestor of both non-pathogenic BaEV and the pathogenic SRVs. According to this hypothesis, the SRVs could be the products of recombination between the SERV gag-pol genes and a GP70 Env protein gene of unknown origin. Endogenous Type D retroviruses are also present in the New World squirrel monkey (181), mice (182,183) and the common brushtail possum (TuERV), an Australian marsupial (184). D-type viruses have not been isolated from any African monkey species trapped and caught in Africa. However, SRV antibodies have been detected in African talapoin monkeys (185) and in Indonesian orang-utans (186). D-type virus (i.e. SRV-2) has also been recovered from baboons at the Washington Primate Center, presumably by cross-species infection from SRV-2 infected macaques. All of the contemporary D-type virus isolates associated with simian AIDS are related to MPMV, but are distinct envelope variants falling into Þve major serotypes. SRV-1 is the serotype in macaques at the California and New England Primate Centers, and the SRV-2 serotype is present in macaques at the Oregon and Washington Primate Centers (187). The original MPMV is the third distinct serotype (SRV-3), now thought to be present in macaques at the Wisconsin Primate Center. Experimental transmission of MPMV in the early 1970s led to death in

many infant rhesus monkeys from a wasting syndrome with thymic atrophy and profound neutropenia, anemia, lymphoid depletion and opportunistic infections (188). Features of this immunosuppressive syndrome were the same as those observed occurring spontaneously in captive macaques in the early 1980s. In 1986 at the California Primate Center, re-isolation and experimental transmission of MPMV from its initial source, a frozen sample of the spontaneous rhesus mammary carcinoma, conÞrmed the earlier observation that this virus, like SRV-1 and SRV-2, was immunosuppressive and apparently non-oncogenic (189). Fatal SAIDS has been induced with an infectious molecular clone of SRV-1 (178). A molecular clone of SRV-2, recently obtained from rhesus monkeys at the Oregon Primate Center seems to be less pathogenic because it induces only mild immunosuppression *in vivo* and has a reduced ability to infect speciÞc T cell lines (190).

Clinical Features

The clinical features of SAIDS induced by SRV include generalized lymphadenopathy, splenomegaly, fever, weight loss, diarrhea, anemia, lymphopenia, granulocytopenia and thrombocytopenia (191). Necrotizing gingivitis (noma) is occasionally observed. Despite the striking depletion of peripheral blood cellular elements, the bone marrow is frequently hypercellular. Evidence of immune activation, so prominent in HIV/SIV infection is lacking in SRV-induced SAIDS. Electrophoresis of sera of ill animals reveals hypoproteinemia, hypoalbuminemia, and hypogammaglobulinemia, the latter consistent with the histologic absence of plasma cells in lymph nodes and the early impairment of B cell function. In contrast to SIV infection of macaques, lymphomas are not a feature of SRV induced SAIDS.

Concentrations of immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) are decreased, but the complement component C3 is not changed and C4 is increased (192). The absolute lymphocyte count decreases, but the OKT4/OKT8 ratio remains unchanged when compared to controls, reßecting an absolute decrease in both helper and suppressor T cells. Thus, in comparison to human AIDS and SIV-induced SAIDS, an inverted T-cell helper-to-suppressor ratio is not found in disease induced by the D-type virus. A decreased response to mitogens concanavalin A (Con A), phytohemaglutinin (PHA) and to allogeneic lymphoid cells, occurs early and becomes more severe near death. Response to pokeweed mitogen (PWM) is variable. Interleukin-2 causes a complete or partial restoration of the response to the mitogens Con A and PHA. A major difference between SRV, SIV, and HIV, is the broader immunosuppressive effect of the D-type virus on both T and B cells, with a consequent depression of both T and B-cell function (193). This difference is correlated with the broader tropism of the D-type virus for both T and B cells *in vitro* compared to the more restricted T4 tropism of HIV and SIV.

Numerous bacterial, protozoan and viral agents have been identiÞed in SRV immunosuppressed macaques, including cytomegalovirus (CMV) and leukocyte-associated herpes virus. Disseminated CMV has been the most frequent opportunistic infection (194). Retroperitoneal Þbrosis (RF), associated mainly with SRV-2 infection, apparently represents the simian counterpart of the herpes virus associated KaposiÕs sarcoma in immunosuppressed humans (see below). Elevated levels of neopterin in serum and CSF of monkeys with SAIDS probably reßected local inßammatory responses (195). Neuropathologic signs or symptoms do not occur in SRV infection. The major similarities and differences between SRV and SIV are listed in Table 10.2.

Pathology

At necropsy, affected animals show severe depletion of lymphocytes in both germinal centers and paracortical regions of lymph nodes, as well as an absence of plasma cells. The histopathology of the lymph nodes is virtually indistinguishable from that of lymph nodes in the terminal stage of human AIDS (196). The distribution of SRV-1 in tissues of infected macaques was studied by virus isolation, electron microscopy (EM), immunohistochemistry, and molecular hybridization (197). Virus could be isolated from PBMC, plasma, serum, urine, saliva, lymph nodes, tears, breast milk, cerebrospinal ßuid, and vaginal secretions of sick monkeys, as well as of some healthy carriers. Separation of peripheral blood T and B cells by panning and ßuorescent cell-sorting indicated that animals with SAIDS harbored infectious D-type virus in both T and B cells but more in T4 cells than in T8 cells (193). Virus was also detected in macrophages but not in mature neutrophils or platelets. Macrophage and neutrophil function was not impaired early in the course of disease (198). Serial titrations suggested that only 0.1% to 1% of lymphocytes were infected in peripheral blood. Testing of the susceptibility of normal macaque lymphocytes to infection with SRV *in vitro* was possible, but difÞcult, because of the inability to establish long-term cultures of macaque lymphocytes. SRV-1 will grow in established human T-cell lines such as human T-cell line 78 (HUT 78) as well as in certain Epstein-Barr virus (EBV) transformed human B cells such as Raji cells. The ability of simian D-type viruses to induce syncytia in Raji cells is the basis for an infectious virus assay (199), and serum inhibition of such syncytia is the basis for a neutralizing antibody assay (187). Apart from syncytia, the virus has no direct cytolytic effect and it is non-transforming. OKT4 antibodies do not block infection of Raji cells.

By immunohistochemistry with a monoclonal antibody to the SRV-1 transmembrane glycoprotein (gp20), viral

TABLE 10.2. *Comparison of simian retrovirus (SRV) and simian immunodeÞciency virus (SIV)*

Similarities
Exogenous lymphotropic retroviruses that induce fatal immunode ciency in macaques after a long incubation period
Pathogenic molecular clones have been derived
Persistent infection despite host immune response
Length of survival correlates directly with vigor of antiviral antibody response
Relatively high levels of virus expression *in vivo*; predominantly cell associated and systemic distribution
Neurotopic
Syncytia induction *in vitro*
Transmission by close physical contact with blood or body secretions
Differences
SIV does not cause disease in its natural host, i.e. African monkeys: SRV does cause disease in its natural host, i.e. Asian macaques
SIV is genetically more closely related to HIV than to SRV
SIV is more virulent for macaques than is SRV
SRV neutralizing antibody can allow for recovery from infection. SIV neutralizing antibody does not allow for recovery from infection. Vaccine induction of neutralizing antibodies will strongly protect against SRV; not true of SIV vaccines
SIV uses CD4 receptor and chemokine coreceptors; SRV has a different receptor, a neutral amino acid transporter, and wider cell tropism including epithelial cells.
SIV envelope mutates more rapidly than SRV, thus allowing for escape mutants.
SIV causes neuropathology; SRV does not.
SIV is more T-cell cytopathic than SRV.

protein was identiÞed in epithelial cells of the mouth, upper gastrointestinal (GI) tract, sweat glands, mammary glands, and choroid plexus, as well as in lymphoid cells in lymph node, spleen, and thymus, but not in the brain parenchyma (197). Viral antigen was commonly detected in germinal centers of lymph node and spleen, and viral particles were seen by EM at the same location, apparently in association with antigen-processing dendritic reticulum cells. The amount of viral antigen increased as the disease progressed, and it appeared predominantly in the peri-follicular capillary endothelial cells of the spleen. The only other *in vivo* site where abundant virus particles were detected by EM, was the salivary glands. Southern blot analysis revealed SRV-1 DNA in lymph nodes, salivary gland and brain and SRV-2 DNA in lymph nodes, spleen, PBMC and retroperitoneal Þbromatosis tissues, but not in the skeletal muscle or liver of affected macaques (200). By *in situ* hybridization, SRV-1 RNA was detected in salivary gland and brain parenchyma of rhesus monkeys with no overt neurological symptoms. A Þnding of SRV-1 nucleic acid in the absence of detectable core antigen or neuropathology in the brain of monkeys suggested viral latency in the central nervous system (CNS). A partial transcriptional block to SRV expression in the brain parenchyma was suggested by the Þnding that very few cells were positive by *in situ* hybridization for viral RNA, seemingly too few to account for the signal seen by Southern blot for viral DNA. The cell types infected have not been identiÞed, and macrophages or giant cells characteristic of HIV-infected human brains or SIV-infected macaque brains, have not been seen. No evidence of activation of D-type virus from this latent state in the CNS has been observed during 20 years of observation. Despite the evidence for latency in the CNS, cell-free SRV-1 could be isolated from cerebrospinal ßuid of over 50% of neurologically normal monkeys with SRV-1-induced SAIDS. A few scattered epithelial cells in the choroid plexus appear to be the source of this cell-free virus in the cerebrospinal ßuid (197).

Two further D-type serotypes, SRV-4 and SRV-5, consist respectively, of single isolates from a cynomolgus macaque at the University of California at Berkeley and a rhesus macaque imported to the Oregon Primate Center from Beijing, China. The SRV-4 isolate has not yet been further characterized for biologic activity, whereas the SRV-5 isolate has been partially sequenced and has produced generalized lymphoid hyperplasia when transmitted into a juvenile rhesus macaque (201). Serial epidemiologic and virologic surveys have shown that D-type viruses (SRV-1Ð8-serotypes) are the primary cause of almost all cases of spontaneous SAIDS in each of the Þve centers where endemic infection with these viruses exist (170). However, variations in severity of this disease and its manifestations with the different SRV serotypes and the different species of macaques at each center are observed under conditions of both natural and experimental exposure. Findings from the Washington Primate Center Medical Lake facility have conÞrmed the rapid natural transmission of SRV-2 among group-housed cynomolgous macaques and the same pattern of infection and immune response as observed with SRV-1 in rhesus macaques (202). In London, a small collection of mostly asymptomatic cynomolgous macaques (*Macaca fascicularis*), naturally infected with SRV-2 was followed virologically over an eight-month period (203). Virus loads in blood varied greatly but remained steady and no signiÞcant sequence variation was found within individuals over time. However, no clear disease pattern emerged in the period of observation.

The SRV-2 serotype is particularly associated, not only with immunosuppression, but also with a proliferative disorder called retroperitoneal Þbromatosis (RF), which has some features in common with KaposiÕs sarcoma (KS) (204,205). These features include an origin of RF cells from vascular smooth muscle (206) and the production by RF cells of basic Þbroblast growth factor (207), which serves as an autocrine growth factor. However, in contrast to HIV-1 associated KS, SRV-2 is detectable as a productive infection in RF lesions (200). Recent evidence indicates that a simian herpes-virus, related to Kaposi sarcoma-associated herpes-virus (KSHV), is associated with RF (208,209). Elevated levels of IL-6 are found in the herpes viremic animals. Clearly, a common pathogenesis appears to exist between the induction of RF by an activated herpes virus in macaques immunosuppressed by SRV infection and the induction of KS by an activated homologous herpes-virus in humans immunosuppressed by HIV infection (210).

A novel simian type D retrovirus was recently isolated from a feral Indian Hanuman langur (*Semnopithecus entellus*) (211). Based on sequence analysis from the 3′ orf and env regions of the viral genome, this SRV is phylogenetically related to, but distinct from, the Þve known SRV serotypes. This novel SRV is provisionally named SRV-6. The relatedness of SRV-6 to the endogenous Type D virus of the spectacled langur (PO-1 Lu) (180,212), obtained from a different langur species, could not be determined because the latter virus has not been sequenced. Because of the greater genetic distance (62% homology) of SRV-6 from the SERV sequences in the baboon (23) than from exogenous SRV-2 in macaques (72% homology), SRV-6 is considered an exogenous retrovirus in langurs. There is no evidence that macaques (or humans) are infected with SRV-6.

In the last two decades, extensive serologic surveys of humans, including primate center animal handlers, have shown no proof of D-type virus infection, despite numerous claims based on serologies and isolation results, which were not supported by further analysis. Many putative human D-type virus isolates were found to be contaminants of MPMV or SRV-1 growing in HeLa cells. The detection, by PCR ampliÞcation and Southern hybridization, of MPMV-like gag and env sequences in healthy Africans from Guinea (213) may or may not prove to be a contaminant. A survey of several hundred lymphoma patients, HIV-1 infected and uninfected, persons with unexplained low CD4 counts and persons with HTLV seroindeterminate test results failed to Þnd any serologic or PCR evidence of SRV infection (214,215). However, RNA sequences related to the protease and reverse transcriptase of Type B and Type D retroviruses were detected by reverse PCR in salivary gland or lymphoid cells of eight individuals with SjogrenÕs syndrome (216). Although these sequences were present in sucrose density gradient fraction corresponding to that of enveloped retrovirus particles, no complete virus could be isolated. Because these sequences were not detectable in human genomic DNA the viral sequences were considered exogenous. Assuming that these viral sequences might represent a natural human infection, the virus was provisionally designated HRV-5. Subsequently, however, this virus was determined not of human origin but, instead, an endogenous retrovirus of rabbits (217). A recent survey of 231 people occupationally exposed to non-human primates found two individuals (0.9%) strongly seropositive, showing reactivity by Western immunoblot against multiple SRV antigens representing *gag*, *pol*, and *env* gene products (37). Repeated attempts to isolate SRV in tissue cultures and to detect SRV genes by PCR were negative. Both individuals remain healthy and one has seroreverted to SRV negative. Inoculation of whole blood from the one persistently SRV-seropositive person into an SRV-negative macaque failed to transient the seropositivity. These results suggest that people occupationally exposed to SRV-infected macaques, may become immunized, perhaps by transient infection.

Cell Receptor

The receptor for the Type D viruses has recently been cloned and sequenced (218). This same receptor is utilized by the endogenous Type D viruses from the squirrel monkey and langur, by the endogenous Type C viruses of the cat (RD114) and baboon (BaEV) and by the exogenous avian C-type reticuloendotheliosis viruses (219,220). This receptor was localized to chromosome 19 in human cells (42). It was initially demonstrated that a cell receptor of indentical molecular weight was bound by the envelope proteins of all 5 SRV serotypes (221). Different amino acid regions for the envelope protein of SRV-1 as compared to SRV-2 interacted with the same cell receptor and infectivity of these viral serotypes could be speciÞcally blocked by antisera to these respective peptides. Recent cloning and sequencing of the receptor, based upon transfer and expression of cDNAs from susceptible to resistant cells, revealed that it was an allele of a previously cloned neutral amino acid transporter ATB (218). Interestingly, infection of cells with Type D retroviruses resulted in impaired neutral amino acid transport. This result could suppress the metabolism and proliferative capacity of the chronically infected B and T lymphocytes, such as occurs in the SRV induced immunodeÞciency.

Pathogenesis

The immune system suppression caused by SRV is not well understood at the molecular level. The general ablative effect on many lymphoid and other hematopoietic cell types resembles the natural history of infection with certain strains of feline leukemia virus (FeLV) (222). Insofar as neither SRV nor FELV are directly cytolytic, the

generalized cellular depletions that they cause must have indirect mechanisms, mediated by cytokines, e.g. tumor necrosis factors (TNF), released from infected or activated lymphocytes. Also, an effect of the so-called Òimmunosuppressive peptideÓ (81) present in the amino end of the transmembrous protein, or an autoimmune component such as antilymphocytic antibodies, could play a pathogenic role. Alternatively, the immunodepletion may be the result of lymphoid proliferative dysfunction resulting from blocking of the SRV cell receptor by the viral envelope (218). A pathogenic effect related to viral load is supported by the Þnding that SRV infection is fatal primarily in those monkeys that cannot mount an adequate immune response and in which the viral load is therefore high. A major difference between SRV and SIV and other lentiviruses is that SRV is much more genetically stable, and therefore escape mutants that can avoid immune surveillance seldom if ever arise. Consequently, in contrast to SIV infection, many SRV-infected monkeys can generate a sufÞciently strong immune response either to contain the virus in a latent state or to completely eliminate it.

SRV Genetic Structure and Function

SRV-1, SRV-2, and SRV-3 (MPMV) have been molecularly cloned and sequenced (223Ð225). All three viruses have a similar genetic organization with four separate translation frames encoding the group speciÞc antigen (*gag*), protease (*prt*), RNA-dependent DNA polymerase (*pol*), and envelope glycoprotein (*env*). Partial sequence analysis of PCR generated clones of SRV-4 and SRV-5 indicate that they are gentically distinct from SRV 1,2,3 and from each other. The putative SRV-6 from Indian langur is only distantly related to SRV-2. SRV-1 Gag-Pol ribosomal frameshifting take place efÞciently via a proposed secondary RNA pseudoknot structure, similar to that of the mouse mammary tumor virus (MMTV) and feline immunodeÞciency virus (FIV) (226Ð227). The three dimensional structure of the SRV-1 pseudoknot has been determined by NMR (228). The *prt* genes of HTLV-2, BLV, MMTV, and hamster intracisternal A particles (IAP) are also in separate translational frames from the *gag* and *pol* genes. A comparison of amino acid mismatches based on nucleotide sequences shows that SRV-1 is more closely related to MPMV than is SRV-2. Whereas the *gag*, *prt*, *pol* and c-terminal *env* domains of the three viruses differ only by 5% to 15%, the externally located N-terminal domains differ by 17% in comparison between SRV-1 and MPMV and by 42% between SRV-1 and SRV-2. The long terminal repeats (LTR) of SRV-1 and MPMV are 88% homologous; SRV-2 shows 70% LTR homology with either virus. In keeping with their distinct neutralization serotypes, SRV-1 and SRV-2 show more envelope amino acid variation (□40%) than seen so far between different HIV-1 isolates (□25%). Computer sequence analyses indicate that a segment of the *prt* gene has been transposed between SRV-1 and visna virus (229), and that SRV-1 and SRV-3 contain a gene segment in the polymerase gene that is lacking in the primate lentiviruses (230). This gene represents a protease-like element related to the aspartate proteinase and possessing dUTPase activity (231), a function that may enhance SRV replication in macrophages. Each D-type virus has a *gag* precursor polypeptide cleaved by the *prt* enzyme into six proteins identiÞed as p10, pp18, p12, p14, p27 and p4 (232,233). The Type D viral gag polyprotein interacts with the cytosolic chaperonin TriC which probably assists in gag folding and/or capsid assembly (234). Each D-type virus utilizes tRNA lysine as a primer for minus-strand DNA synthesis. Visna virus, MMTV, HIV-1 and SIV also utilize tRNA lysine as primer. Although similar in ultrastructural features, the D-Type particles are morphologically distinct from HIV and SIV particles (235). However, the simian D-type viruses have a markedly different genetic organization from HIV and SIV and lack extensive homology with these agents. Transactivation of LTR sequence-mediated gene expression is not a property of D-type virus replication (236). The D-type viruses contain a 219-nucleotide sequence called the constitutive transport element (CTE), located in an untranslated region near the 3′-end of the genome. The CTE is capable of substituting for the Rev and Rev-response element (RRE) of HIV and SIV in promoting transport from nucleus to cytoplasm of intron-containing viral RNA (237Ð240). The CTE thus can efÞciently substitute for Rev in expression of Gag/Pol and Env proteins from subgenomic constructs. It is suspected that CTE functions by interacting with evolutionary conserved cellular factors essential for cellular export (241), analogous to that of the Rev protein. Further molecular studies of the CTE reveal a possible interaction between the CTE and the polyadenylation signal at the 3′ untranslated region of the viral genome (242). Secondary structural determinants in this region and multiple protein interactions are involved in function of the CTR in nuclear export of unspliced SRV RNA. The properties of the SRV CTE have provided a valuable component for lentiviral vectors used in gene therapy (243,244) and for SIV DNA vaccine constructs (245).

Control of SRV

D-type virus infection can be eliminated in group-housed monkeys by serial testing for antiviral antibodies and virus isolation, and subsequent removal of positives (246). Recently, detection of SRV proviral DNA in macaque PBMCs has enhanced the efÞciency of SRV diagnostics (247). Inactivated whole SRV-1 vaccine and recombinant vaccines consisting of SRV-1, SRV-2 or SRV-3 envelope glycoproteins (gp70) expressed in live vaccinia virus, have been able to protect macaques against persistent infection and disease, following experimental challenge (248Ð250). Protection correlated with the induction of neutralizing antibodies and was long-lasting (251).

SRV-1 and SRV-3 separately immunized monkeys showed cross-protection against each of these virus strains, in keeping with their close genetic relationship. The SRV-1 and SRV-3 cross- reactive sera failed to neutralize SRV-2 *in vitro* because the latter is genetically more distinct. SRV-2 vaccinated monkeys have yet to be challenged with SRV-1 or SRV-3. A polyvalent vaccine consisting of SRV-1 (or SRV-3) and SRV-2 env antigens would probably be required to protect against these three most common SRV serotypes present in captive macaques. SRV vaccines have yet to be tested under cage exposure conditions in which the horizontal transmission of SRV continues to cause signiÞcant morbidity and mortality among these important research animals. Nucleoside analogues, especially 3TC, have a potent antiviral effect against SRV-1 and SRV-2 *in vitro*, but these drugs have not been tested *in vivo* (252).

In summary, simian D-type viruses and associated immunosuppressive disease are highly prevalent and account for over 99% of the related morbidity and mortality from naturally occurring simian AIDS in macaque colonies at various primate centers and breeding facilities. SRV infection differs from SIV and HIV infection in that SRV is genetically much more stable; resistance to infection and recovery from SRV is correlated with the presence of neutralizing antibodies, and SRV is not as restricted to or cytopathic for T4 cells. Despite these differences between D-type virus and SIV or HIV, the highly reproducible and rapid experimental transmission of SRV disease, along with the short turn around time (7–10 days for *in vitro* assays), makes this an attractive primate model for studying the mechanism of immune suppression from an acquired retrovirus. The availability of molecularly cloned and sequenced viruses representing each of the three major D-type virus serotypes, the ability to produce fatal simian AIDS with molecularly cloned SRV-1 and SRV-2, and cloning of the SRV receptor, are further attractive attributes of this model, because they make possible identiÞcation of the critical virus genes involved in pathogenesis. The herpesvirus associated retroperitoneal Þbromatosis (RF) in SRV-2 infected macaques represents an intriguing counterpart of KSHV associated Kaposi's sarcoma in HIV-1 infected humans. From a practical standpoint, SRV can be readily controlled by testing, and removal of infected animals or by vaccination. Adequate screening for SRV as well as STLV, SIV and SFV is critical to exclude infected monkeys and prevent potential confounding of research results (253).

LENTIVIRUSES

Simian ImmunodeÞciency Viruses (SIV)

Epidemiology and Natural History

The phylogenetic relationship of primate and other animal lentiviruses is shown in Fig. 10.2 (235). The SIV constitute a family of naturally occurring lentiviruses indigenous to certain simian species in Africa (2). In their natural African simian hosts, these viruses apparently cause no disease. In their natural hosts, SIV is probably horizontally transmitted by Þghting/biting and sexual contact, and less often vertically transmitted at birth via maternal blood and milk. By contrast, SIV infection of macaque species of Asian and Indonesian origin, does not occur naturally, but accidental or inadvertent infection in captivity with certain strains of SIV (mostly of sooty mangabey origin) causes persistent infection with eventual death (2 months–3 + years) from an AIDS-like disease showing many parallels to human AIDS (255). Both HIV and SIV achieve viral fusion and cell entry by an interaction of their external envelope glycoproteins with the CD4 cell surface protein and one of the chemokine receptors.

Altogether, at least 30 different species of African non-human primates harbor SIV asymptomatically; these include four species of African green monkeys (*Cercopithecus aethiops*) (256–258), sooty mangabey (*Cercocebus atys*) (259–263), redcapped or cherry-capped mangabey (*Cercocebus torquatus*) (264,265), Sykes' monkey (*Cercopithecus mitis*) (266), mandrill (*Papio sphinx*) (267,268), drill monkey (*Mandrillus leucophaeus*) (269), L'Hoest's monkeys (*Cercopiithecus lhoesti*) (270–272), talapoin monkey (*Myopithecus talapoin*) (273), colobus monkeys (*Colobus guereza*) (274), and chimpanzee (*Pan troglodytes*) (275–282). Complete sequence analysis of 13 of these isolates reveals a very large and heterogeneous family consisting of at least six approximately equidistant lineages of lentiviruses with considerable heterogeneity within species and extensive divergence between species. A recent serologic survey of 788 monkeys in the rainforests of Cameroon found SIV reactivity in 13 of 16 primate species – 16.6% of the animals tested, and molecular analysis identiÞed Þve new phylogenetic SIV lineages (283). Asymptomatic SIV infection of sooty mangabeys and, to a lesser extent, other African non-human primates are also quite prevalent in primate centers and zoos (100). Recombination and cross-species transmission in the wild have undoubtedly contributed to the genetic complexity of primate lentiviruses (268,284). For example, evidence indicates cross-species spread in nature of SIV from African green monkeys to baboons (285). The sooty mangabey SIV group, perhaps, is the most diverse in a single monkey species (286,287). The chimpanzee SIVs (SIV_{cpz}) are most closely related to HIV-1 (288), and almost certainly provided the origin of HIV-1 via the bushmeat trade in Central Africa (281). The prevalence of SIV in chimpanzee (approximately 1%) appears far lower than in other primates (10–90%) (282). In West Africa, none of 387 wild chimpanzees (Pan troglodytes) were infected with SIV_{cpz} (289). No pygmy chimps, i.e. bonobos (*Pan paniscus*) were found infected with SIV (290). None of 143 orangutans in Indonesia were infected with SIV (291). The envelope variation among SIV isolates within a

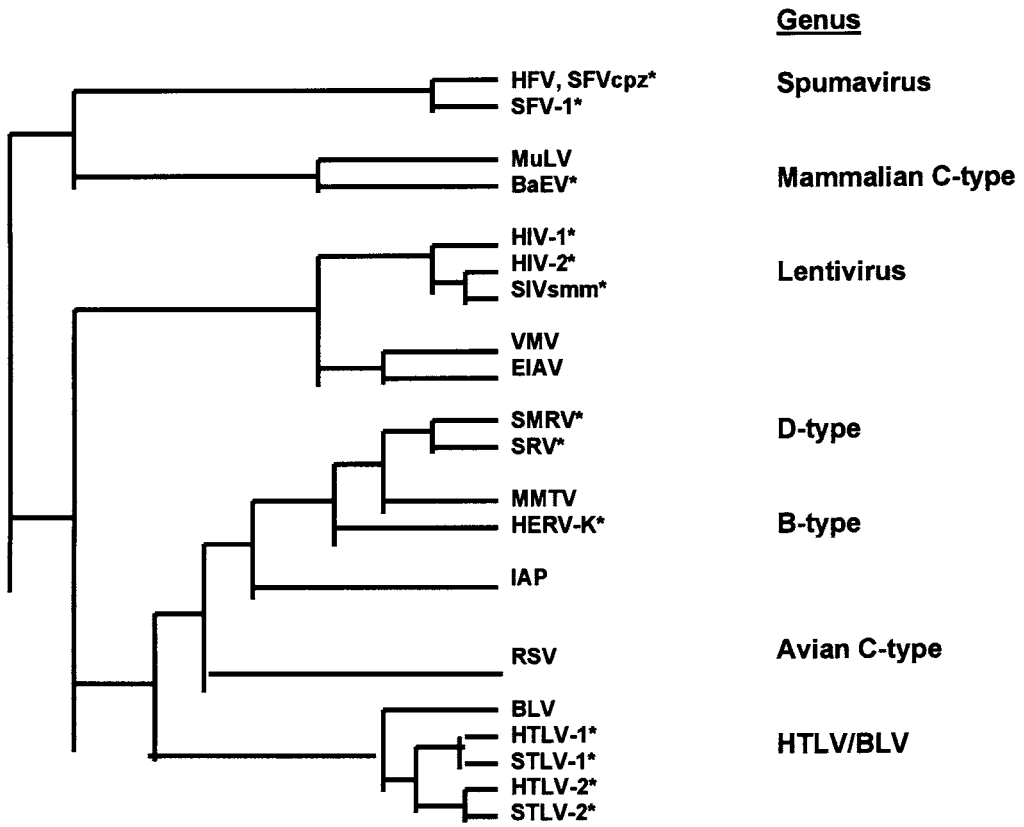

FIG. 10.2. Phylogenetic classi cation of representative retroviruses. The family Retroviridae contains seven genera (1). Asterisks identify primate retroviruses. Abbreviations for viruses are given in Table 10.1 and the Glossary. Figure adapted from Cof n 1992 (1).

single species appears at least as great as among HIV-1 isolates (25%), and the variation between species may possibly be as much as between HIV-1 and HIV-2 (50%). SIV isolates from African non-human primates other than the sooty mangabey generally have not been pathogenic for macaques, although they have not been extensively tested nor serial passaged in macaques. One SIV strain from an African green monkey has induced AIDS after serial passage in pigtailed macaques (292,293). The SIV isolates from African green monkeys (SIV_{agm}), Sykes monkeys (SIV_{syke}) and mandrills (SIV_{mdr}) are less closely related to SIV_{mac}, SIV_{sm} or HIV-2 and are about equidistant from HIV-1 and HIV-2 (256,257,261,268). This suggests that SIV may have existed in these African monkey species for a very long time.

Of all the SIV strains from African simian hosts, the sooty mangabey SIV isolates are the most closely related to SIV isolates from captive macaques in primate centers and to HIV-2 isolates from humans in West Africa (294D296). Geographic clustering in West Africa of SIV strains from feral sooty mangabeys with closely related HIV-2 strain, supports the likelihood that HIV-2 subtypes have arisen by recent (40D300 years) independent cross-species transmission events from monkey to human with divergent SIV_{sm} strains (286). That such cross-species infection can occur indeed has recently been documented by accidental infection of several laboratory workers with SIV_{sm} (35,36). Recent seroepidemiological evidence also suggests the possible cross species transmission of SIV from mandrill to a human in Cameroon (268). The close similarity between SIV_{sm} and SIV from macaques (SIV_{mac}), together with the knowledge that SIV is not indigenous in Asian macaques, suggested that SIV in macaques was acquired accidentally by cross-species spread from sooty mangabeys or closely related species in U.S. primate centers. Retrospective studies have conÞrmed this hypothesis (32,297). Two prototype strains of SIV isolated from rhesus macaques (SIV_{mac}) (255,298) and stumptailed macaques (SIV_{stp}) (299,300), originated in the early 1970s by cross species infection with SIV_{sm} from cohoused sooty mangabeys at the California Regional Primate Research Center. Because SIV infection and AIDS-like disease were then unrecognized, surviving carrier macaques were inadvertently sent to the New England and Yerkes Primate Centers, where they Ò seededÓ the viruses that were isolated a decade later after recognition of human AIDS (297,301). The closely related SIV_{mne} strain isolated from a single pigtailed macaque at the Washington Primate Center (302) in 1986 probably also came from contact with a SIV-infected sooty mangabey, although this transmission event is not documented. The isolation, also in 1986, of the closely related

SIV_{smB670} strain at the Tulane Primate Center occurred accidentally, following experimental inoculation of rhesus macaques with tissue extracts from natural SIV-infected sooty mangabeys with leprosy at the Yerkes Primate Center (260). Non-invasive detection of new SIV lineages in sooty mangabeys (303) and chimpanzees (304)Ñ by viral ampliÞcation from fecesÑ promises to further expand our knowledge of the epidemiology and phylogeny of the SIVs.

Based on viral sequence analysis to calibrate phylogenetic trees, the evolutionary origin of HIV-1 is almost certainly SIV from the chimpanzee (34,281), an event that probably occurred several times this century by contact with chimpanzee blood and body ßuids during the processing or eating of chimpanzee meat. The diversity of HIV-1 M subtypes and possible recombinant viruses and unclassiÞed strains circulating in the Democratic Republic of Congo suggests that the HIV-1 pandemic originated in Central Africa (305). The origin of the major HIV-1 group M viruses was calculated to be about 1930 (306). Inadvertent contamination of polio vaccines with SIV_{cpz} in the 1950s has been conclusively ruled out as a possible source of HIV-1, although the use of a common needle for mass vaccination at that time might have helped adapt the virus to humans (307Ð309).

Comparison of Natural and Experimental SIV Infection

A comparison of viral and host factors in the naturally SIV infected but asymptomatic African simian species with the macaque species that develop AIDS after experimental infection with the same SIV strain should provide insights into the pathogenesis of the disease. Most thoroughly studied of the naturally infected African non-human primates are sooty mangabeys (sm) (310Ð312), African green monkeys (313Ð319), and chimpanzees (275Ð282). Experimental pathogenicity for macaque species (which do not naturally harbor SIV) is most thoroughly demonstrated with SIV_{sm} (i.e. SIV_{mac}), and to a much lesser extent with SIV_{agm}. Based on viral sequence diversity in the different species and subspecies of naturally infected African non-human primates, it is apparent that these animals have been infected for eons, and thus are well-adapted to the virus. Contemporary African non-human primate populations presumably represent the survival of the Þttest selected in the distant past for resistance to AIDS, a hypothesis perhaps supported by the apparent reduction of HLA variation in chimpanzees (320). In short, the lack of disease in the natural host cannot be attributed to lack of replicating virus, even at high levels, to distinct target cells or viral reservoirs, to lack of viral variation or to induction of a strong protective antiviral immune response. Indeed, the humoral and cellular immune responses are much less active in the natural infection than in the experimentally infected macaques (317). HIV-1 or SIV_{cpz} infected chimpanzees do not have increased beta chemokine positive cells in contrast to HIV-1 infected human or SIV_{mac} infected macaques (321). Abnormal immune activation is thus strongly correlated with immune deÞciency in the pathogenesis of AIDS. The wide range of viral RNA levels in plasma and lymph node (2×103 to $> 6X\ 10^6$ copies/ml) of naturally infected AGMs (318,319) or SMs (322,323) often exceeds the minimum pathogenesis threshold value ($> 10^5$ RNA copies/ml) of SIV_{mac} infected macaques and HIV-1 infected humans during progression to AIDS (324). In the natural host, the gut is a major site for SIV replication *in vivo* and appreciable levels of virus are also found in brain parenchyma and CSF. However, T-cell turnover is normal in SIV infected sooty mangabeys compared with rapid turnover in SIV_{sm} infected macaques (325). High levels of virus replication during experimental primary infection of naive AGM with SIV_{agm} are non-pathogenic, even in newborns (326,327), and are rapidly controlled by host immune responses, although not neutralizing antibody (328). In AGMs, preinfected with a nef deÞcient SIV_{agm}, the plasma virus load was dramatically decreased after wild type SIV_{agm} challenge compared to naive controls, in the absence of neutralizing antibody (329). Uninfected AGMs vaccinated with inactivated whole SIV_{agm} vaccine or passively immunized with SIV_{Ig} were not protected against SIV_{agm} challenge infection, even in the presence of neutralizing antibody (330). Clearly, neutralizing antibodies do not control SIV infection in the natural host and cannot be responsible for the lack of disease. In contrast to HIV-1 infected humans or SIV-infected macaques, MHC-restricted CTL killing of SIV-infected cells is barely detectable and thus does not appear responsible for lack of disease in the natural host. Innate immunity, e.g. NK cells or viral-suppressor factors released by CD8+ lymphocytes may help hold the virus in check in the natural host (317,322). Apparently SIV is not cytopathic in its natural host and this resistance of CD4+ T-cells and dendritic reticuluin cells to virus induced lysis or apoptosis must be a major reason for the normal T-cell turnover and lack of pathogenicity in the natural host (310,331). The major difference between natural and pathogenic SIV infection may well lie in the indirect cell killing that results from abnormal T-cell activation.

Experimental Transmission of SIV and Pathogenesis of Simian AIDS

In so far as HIV-1 infection does not induce AIDS in chimpanzees or any other animal species, at least in a reasonably short time (10 years), the reliable and highly reproducible infection of macaques with SIV_{mac}, or closely related strains, and induction of SAIDS in a relatively short time (6 monthsÐ2 years), makes this the model of choice for studies of pathogenesis, antiviral drug testing, immunotherapy, and vaccination. Standardized stocks of

SIV, with dose titrations *in vitro* and *in vivo* and standardized virologic and immunologic assay systems and reagents, are in place at a number of primate centers. For rapid antiviral drug screening, one acutely lethal variant of SIV_{macPBJ} is also useful (332,333). The reproducible induction of simian AIDS with several SIV molecular clones or plasmid DNA (e.g. SIV_{mac239}) (334) conÞrms that this virus is both necessary and sufÞcient for inducing this fatal AIDS-like disease and provides a valuable tool for studying pathogenesis.

All species of macaques appear vulnerable to experimental infection and induction of SAIDS with SIV_{mac}, SIV_{stm}, SIV_{mne}, or macaque-adapted SIV_{sm} (2,4,5,335). However, species speciÞc variation in SIV disease, progression has been noted between Chinese and Indian subspecies of rhesus macaques (336). The age of the animal is not a critical factor, although newborns or fetuses are most susceptible to infection and disease (337,338). Infection readily occurs via intravenous inoculation with cell-free or PBMC-associated virus and across intact rectal (339Ð340), oral (337,341) or genital mucous membranes (342) with cell-free but not cell- associated virus. A higher dose of cell-free SIV is generally required for rectal or vaginal infection than for IV infection and the time required for systemic infection to occur is slightly prolonged after vaginal exposure. Oral exposure to cell-free SIV in adult macaques induces infection and SAIDS more readily than rectal exposure (341). CD4+ tonsillar T-cells become infected after oral SIV infection (343). The successful genital, oral or rectal mucosal transmission and induction of SAIDS with SIV present useful models for heterosexual or homosexual transmission of HIV. In the heterosexual model, Langerhans cells, macrophages and T-helper cells within or just beneath the vaginal mucosal membrane, have been shown to be primary cellular targets that carry the virus to regional lymph nodes (344,345). Thinning of the vaginal mucous membrane from progesterone therapy increases susceptibility to experimental SIV infection via this mucosal route (346) and thickening of the vaginal mucosa from estrogen therapy decreases susceptibility to experimental infection (347). Infection of newborn or fetal macaques by oral or systemic routes constitutes an excellent model for retardation of intrauterine growth and induction of pediatric SAIDS (337,338,348).

Most (90%) inoculated animals become persistently infected from about two weeks after inoculation until death from SAIDS, which occurs within two months to three or more years depending in part on the vigor of the antiviral and innate immune responses. Factors of innate immunity such as NK cells, gamma delta T cells and other T cells that spontaneously produce interferon, beta chemokines and soluble antiviral factors, are important defense mechanisms against diverse pathogens (349), but they have not been much investigated in the SIV macaque model. Psychosocial stress may also shorten survival in SIV-infected macaques (350). Once the threshold of infection is reached, the induction of disease and incubation period are essentially dose-independent. Transient infection with clearance of virus, or persistence of a non-productive latent infection, sometimes with evidence of residual SIV cell-mediated immunity, may occur following limiting dose inoculation (351,352), experimental infection via genital or rectal mucosa (339Ð342,351Ð356), infection with less virulent SIV strains (357) or non-pathogenic SIV molecular clones (358Ð360). Low doses of infectious SIV that fail to induce antibody or CTL responses fail to protect against subsequent IV inoculation of a high dose of SIV (361). The level of virus replication is also dependent on the properties of the infecting SIV and disease outcome is usually less variable among monkeys infected with the same virus strain than between groups of animals infected with different viruses. The route of inoculation is associated with the extent and breadth of the genetic complexity of the viral variant population in the acute stage of systemic infection (362). A more diverse population of SIV variants tends to occur after IV as compared to intravaginal infection. However, no speciÞc viral variants (e.g. macrophage vs. T cell tropic) are consistently transmitted by intravaginal inoculation. The length of survival of infected animals correlates directly with the viral load and the strength of the humoral, cell-mediated and innate immune responses during acute infection (363Ð369). MHC class I alleles inßuence viral load set point and survival time (370). Monkeys that express Mamu A-01 exhibit the best control of viral replication (371). SIV speciÞc T cells peak just after the level of viremia starts to fall suggesting that CTLs are responsible (372). Depletion of CTLs by infusion of anti-CD8+ antibody abrogates the early control of virus and increases the virus level during the chronic phase of infection (373). Virus levels are again suppressed with the appearance of SIV-speciÞc positive T cells. CTLs are thus critical in control of virus, a view supported by the frequent selection of CTL escape mutants *in vivo* (374), especially during acute infection (375,376). Experimental depletion of B cells with anti-CD20 antibody suggests that humoral immune response contribute to SIV control in the post acute phases of primary infection (376). About 20% of SIV-infected macaques make little or no detectable antiviral antibody responses; they show a persistently high virus load, progressive decline in total plasma Ig level and number of T4 cells and die from SAIDS after two to six months. Such animals are particularly proven to develop SIV encephalopathy (377). The longer survivors remain persistently infected but show higher antibody titers to both core and envelope proteins, including broadly reactive neutralizing antibodies, and have an increased total plasma Ig and only a late decrease in T4 cells (378). Complement Þxing antibodies and antibody dependent cell mediated cytotoxicity may also play a protective role (379,380).

There is strong selection for viruses that can evade humoral and cellular immune responses, and antibody and

CTL escape variants may have increased replicative Þtness (374Ð376,381Ð383). Neutralization escape variants of SIV_{mne} exhibit changes in the extracellular envelope glycoproteins (384) and cytopathicity is determined by mutations in *gag* and *env* intracytoplasmic tail (385). Monkeys infected with mutant forms of SIV lacking certain glycosylation sites in the external envelope glycoprotein exhibit a markedly increased neutralizing antibody response (386). A Òglycan shieldÓ may well protect SIV/HIV from neutralizing antibodies (387). No deÞnite role for complement-mediated antibody-dependent enhancement of SIV infection *in vivo* has been demonstrated, despite evidence of enhancing antibodies (388). The neutralizing epitopes of SIV, e.g. the V3 loop, differ somewhat from T-cell adapted strains of HIV-1 (389,390), but sequences in SIV_{mac}, that correspond to V3 in HIV-1 can affect entry, cell tropism and CD4 binding in a manner analogous to that of HIV-1 V3 (391Ð393). Antiviral CTLs in blood (364,365,396) and vaginal mucosa (395), and T-cell soluble cytokines (396,397) or chemokines (398) that suppress SIV (and HIV) infectivity, may contribute to survival but, eventually, virtually all SIV infected macaques die from SAIDS. High levels of IL-4, IL-10, MIP-1α, MIP-1β, MCP-1 and RANTES in lymph nodes correlate with increased virulence of the SIV strain (399). Elevated levels of TNF-α are present in serum of macaques during acute infection with SIV (400). SIV induced perturbations in chemokine expression and dendritic cell associated markers suggest a profound disruption of homeostasis (401). The cellular and humoral antigenic epitopes in SIV and the SIV neutralizing and binding activity of various monoclonal antibodies have been reviewed elsewhere (402Ð404). Many SIV CTL epitopes, their restricting MHC Class I molecules and inßuence on disease progression have been recently deÞned (405Ð408). However, differences exist between T cell epitopes recognized after immunization and after infection (409).

Throughout the course of infection, virus is widely disseminated and can be readily isolated from PBMCs, plasma, lymph nodes, GI tract, spleen, bone marrow, brain, cerebrospinal ßuid, salivary gland, placenta, genital organs and secretions, and other tissues. Infected cells include mainly CD4+ T cells, macrophages, microglial cells and, possibly, dendritic cells. As in HIV-1-infected humans (410,411), virus turnover in SIV-infected macaques is extensive (412), tissue-speciÞc viral variants accumulate (413Ð415), and a higher infectious virus burden is present in lymphoid organs and GI tract than in blood (416Ð421). IN SIV acutely infected monkey with high viral loads, the turnovers rate of T cells in blood and cell organs is increased about two-fold and that of memory T cells about three-fold; B cells and NK cells turnover is also increased about 3% per day (422,423). With the onset of SAIDS, proliferation rates return towards control levels. Progression to SAIDS tends to be associated with widespread distribution of proviral DNA in tissues (424) and occasionally, a change from macrophage-tropic non-cytopathic SIV variants to T-cell tropic syncytia-inducing variants (425). Variants with enhanced infectivity as well as CD4 down-regulation may emerge during the course of infection (426). On the other hand, CD4 independence and macrophage tropism are also associated with neutralization sensitivity and reduced pathogenicity (427). As in HIV-1 infection, the number of CD4+ lymphocytes in the peripheral blood decreases soon after infection, then plateaus within several months at $\square 1000/mm^3$ until progressively decreasing to less than $400/mm^3$ shortly before death. Similarly, T-cell memory and non-speciÞc mitogenic proliferative responses are diminished before the CD4+ cell count is markedly reduced and a marked CD8+ lymphocytosis occurs during acute SIV infection (417,418). In acute SIV infection of macaques, the decline in CD4 cells in the peripheral blood may, at least in part, be due to lymphokine (IFN-γ and TNF-α) mediated sequestration of these cells in lymphoid tissues (417). As mentioned, the appearance of CD8+ cells may help to curtail SIV replication by both cytotoxic activity and release of soluble antiviral factors. However, functional impairment of virus speciÞc CD8+ T cells during acute and chronic infection may impair their protective role (428,429).

Cell entry of SIV, like HIV, is mediated by interactions between the viral envelope glycoprotein and a cellular receptor complex that consists of CD4 and one or more members of the CC or CXC chemokine receptor family of proteins (430,431). All of the co-receptors so far identiÞed are G-protein coupled signaling receptors involved in controlling activation and immigration of various leukocytes. The speciÞcity of this interaction largely determines the cell tropism of the virus. Whereas the main co-receptors for HIV-1 are CCR-5 (ÒR5Ó), present on macrophages, and CXCR4 (ÒX4Ó), present on T cells, the main receptor for SIV is CCR5 (432Ð434). However, SIV gp120 can bind to rhesus CCR5 independent of CD4 (435) suggesting the presence of unindentiÞed receptors. Two new SIV coreceptors termed Bonzo and Bob, that appear to play only a minor role in infectivity, have been identiÞed (436,437); the natural ligands for these receptors remain unknown. SIV from a red-capped mangabey uses CCR2 as its primary coreceptor (438). Interestingly, the CXCR-4 gene is present and functional for X4 tropic SIV *in vitro* but is not used by primary SIV *in vivo* (436). Also, X4 tropism usually does not develop during progression to SAIDS in SIV or SIV infected macaques (384,439,440), in contrast to such a switch in tropism often seen in HIV-1-infected humans. Thus, the correlation between X4 tropism and AIDS may be more speciÞc to humans, (and domestic cats).

One to three weeks after inoculation and prior to the onset of clinical disease, SIV-infected macaques, like some HIV-1-infected humans, develop a transient skin rash (441). Immunohistochemical evidence suggests that this rash may be the consequence of injury to SIV-infected Langerhans cells by cytotoxic T cells. The main clinical

features of SAIDS are wasting with a loss of 15% to 60% of body weight (442) and persistent diarrhea. Lymph nodes may be initially enlarged with follicular hyperplasia associated with a polyclonal B-cell reaction and paracortical expansion of CD8+ lymphocytes, but in time the follicular framework becomes disrupted and hyalinized or "burned out." Immunohistochemical staining of lymph nodes in the early stages reveals a decrease in CD4+ cells and an increase in both CD8+ and B cells. Lung tissue is minimally infected during acute SIV infection (443). In chronic SIV infection of macaques, CD8+ cells and macrophages show signs of cell activation (444) and increased CD8+ cell activity may contribute to lymph node pathology (394,417). SIV antigen appears on dendritic reticulum cells within germinal centers as well as in scattered CD4+ cells, macrophages and giant cells. Just as in HIV-1 infected humans, macaque dendritic cells (DC) bind and internalize SIV and transmit the virus to CD4+ T cells (445), a process enhanced by DC-SIGN, a C-type lectin (446). Dendritic cells in the lymph nodes of SIV-infected macaques express elevated levels of monocyte chemoattractant protein 2 (447). The absence of intraepithelial DC-SIGN positive cells in mucosal tissues suggests that CD-SIGN does not play a significant role in transmucosal passage of HIV/SIV (448). Infection of lymphoid tissues and thymus is accompanied by depletion of follicular dendritic cells and cortical thymocytes, respectively (449–452). SIV-induced apoptosis of thymocytes (CD4+, CD8+) and lymphocytes, is associated with an increase of surface FAS expression and profound decrease of bcl-2 positive cells, indicating a disruption of the anti-apoptotic pathway (453–454). As mentioned, a high viral load in lymph node follicles and high plasma viral RNA or infectious virus load early in SIV infection, indicates a more rapid disease course. The dynamics of rapid and active virus replication and antigenic variation throughout the course of infection are alike in SIV-infected macaques and HIV-1 infected humans (410,411).

Clinical lesions associated with the respective organ sites of SIV infection include hematopoietic dysfunction (455), hemolytic anemia (456), enteropathy (457,458), arthritis (459), ocular disease (460), oral hairy leukoplakia (461), pneumonitis (462), cardiomyopathy (463,464) and encephalopathy (465–468). Insights into the molecular pathogenesis of these lesions are becoming evident. Encephalopathy and pneumonitis are associated with an *in vivo* selection of macrophage-tropic SIV variants (401–402), exhibiting nucleotide changes in the envelope (471–474), and increased blood and cerebrospinal fluid (CSF) levels of quinolinic acid from activated macrophages (475). Adaptation of SIV to replication in macrophages apparently results from its ability to infect these cells in a mostly CD4 independent fashion (476). The SIV-associated encephalopathy in macaques is indistinguishable from HIV encephalopathy and undoubtedly has a similar molecular pathogenesis (465–467). Increased glutamate and decrease γ-amino butyric acid concentrations in the CSF reflect excitotoxic damage with microglia as a major contributor (477). Viral proteins, e.g. gp120, Tat, may also exert both direct and indirect toxic effects on the CNS (478). Reproducible infection of the CNS with selection for specific *env* sequences has also been achieved by serial passage of neuroendothelial or microglial tropic strains of SIV (479,480). Microglial cells apparently select for replication of neurovirulent SIV genotype (481,482). Expression of endothelial cell adhesion molecules may affect viral and inflammatory-cell-trafficking to various tissues such as intestinal tract and CNS (483,484). Release of various cytokines, such as TNF alpha, IL-1, IL-6 and IFN alpha or gamma, from infected or activated macrophages, microglia, and T cells, may influence SIV production and undoubtedly contributes to the pathology of the CNS and other tissues (485–488). Elevated levels of benzodiazeprime receptor expression, a marker of microglial and macrophage activation,are found in the subcortical white matter of SIV infected macaques (489). Alcohol administration enhances SIV induced cognitive impairment (490). Elevated levels of indoleamine 2,3-dioxygenase in the CNS from activated macrophages can lead to neurotoxicity through the generation of quinolinic acid (491). The extensive viral replication and CD4+ T-cell loss that occurs in the GI tract during acute SIV infection depends on these cells coexpressing CCR-5 and having an activated memory phenotype (492–494). Such cells, including intraepithelial lymphocytes, are a prominent source of cytokines and antiviral cytotoxic activity (493,495). The envelope glycoprotein of SIV contains an enterotoxin domain that may contribute to diarrhea without histologic alterations (494). Vaginal CD4 T cells that express high levels of CCR5 are also rapidly depleted by SIV infection (495). The pathogenesis of SIV_{pbj} induced rapid death from diarrhea in pigtailed macaques (332) involves extensive T-lymphoid hyperplasia in the gut and an increase in plasma levels of IL-6 and tumor necrosis factor (498). The T-lymphoid proliferation induced by SIV_{pbj} appears to be a superantigen effect (499) and also involves an enhanced responsiveness of the SIV LTR to NFκβ (500).

Opportunistic infections may be caused by adenovirus, cytomegalovirus, Epstein-Barr-related herpes virus (EBV), papovavirus (SV40), candida, cryptosporidia, and mycobacteria (501). Experimental coinfection of macaques with SIV and BCG can induce a tuberculosis-like disease (502). Although seldom occurring spontaneously, pneumocystis carinii pneumonia can be experimentally induced in SIV infected macaques (503). Non-Hodgkin's lymphomas of B-cell origin occur sporadically as opportunistic tumors in SIV-infected macaques (504). The tumors are monoclonal, mostly extranodal, including the CNS, of high-grade malignancy and often associated with an EBV-like herpes virus. Cytokine gene transcription patterns appear similar in HIV-1 and SIV associated lymphomas (505). Unlike HIV-1-infected

humans, typical KS does not occur in SIV-induced SAIDS. Two divergent lineages of gammaherpes virus homologue (rhadinovirus) of the KS associated herpes virus (HHV-8) found in humans have been identiÞed in rhesus and pigtailed macaques (209). In macaques coinfected with SRV, the rhadinovirus is associated with KS-like retroperitoneal Þbromatosis (208), whereas in macaques coinfected with SIV the rhadinovirus is associated with B-cell lymphoproliferative diseases (506Ð508). Different strains of macaque rhadinovirus apparently contribute to these different retroviral diseases.

In summary, although the disease course is shorter, the clinical, histopathologic, virologic, immunologic manifestations and opportunistic infections in SAIDS are remarkably similar to the counterpart lesions in human AIDS, and the correlates of protective immunity, to the extent that they exist, appear similar in simian and human AIDS (509). Accordingly, the molecular pathogenesis of human and simian AIDS must be highly alike.

Genetic Organization and Regulation of SIV

The molecular biology of SIV and HIV has been recently reviewed (4,510) and, therefore, will only be summarized here. SIV has a complex genetic organization almost identical to HIV-2, and includes genes for virion precursor proteins (i.e. gag, pol, and env) as well as six accessory genes (*vif, tat, rev, vpr, vpx, and nef),* some of which (*tat* and *rev*) regulate viral gene expression in infected cells (Table 10.3; Fig. 10.3) (511). LTRs ßank the viral genes and contain critical sequence elements for viral RNA synthesis and integration. Genetic and sequence similarities of SIV/HIV-2 with HIV-1 indicate that the virion proteins of both virus groups have similar properties and functions. One difference from HIV-1, however, is that cyclophilin A does not bind to SIV gag polyproteins and is not required for SIV replication (512). Various SIV/HIV-2 isolates encode a gene designated *vpx*, which encodes a protein that is packaged into virus particles, whereas HIV-1 does not encode an open reading frame for the *vpx* gene. The HIV-1/SIV_{cpz} group of viruses encode *vpu*, a gene for a small non-virion protein, located between *tat* and *env,* whereas the SIV/HIV-2 group does not contain *vpu*. Knowledge of the molecular biology of SIV has been coupled with analysis of viral mutants and variants in experimentally infected non-human primates; such studies in animals offer critically important opportunities to determine the role of various viral genes in transmission, virus distribution within the host, persistence, and patterns of pathogenesis.

Transcriptional promoter elements in the LTR are a TATA box for initiation of viral RNA synthesis by cellular RNA polymerase II, and several upstream elements which include targets for the SP1 factor, and an enhancer sequence recognized by NFκβ (510). It has been hypothesized that cell activation signals, which function through the NFκβ site to augment viral gene expression, may be important for converting a low-level infection to a state of

TABLE 10.3. *Genes and proteins of primate lentivruses*

Gene[a]	Dispensable for Replication	Protein Localization	Function
gag	No	$pr55^{gag}$	polyprotein precursor for virion nucleocapsid proteins MA (p17), CA (p24), NC (p9), p7 Virion nucleocapsid
pol	No	$pr160^{gag\text{-}pol}$	polyprotein precursor for virion enzymes PR-p10, RT and RNAse-H-p51/p66, IN-p32 Virion (nucleocapsid?)
vif	yes[b]	p23	viral infectivity factor, function unresolved. Cell cytoplasm
vpx[c]	yes	p16	virion protein, related to vpr, function unresolved Virion
vpr	yes	p15	virion protein, cell cycle arrest, nuclear import Virion
tat	no	p14	transcriptional transactivator, binds TAR and cell factor(s) (initiation and elongation of viral transcripts). Primarily in cell nucleus
rev	no	p19	post-transcriptional transactivator, binds RRE and cell factor(s) (splicing and/or transport of viral mRNA). Primarily in cell nucleus
vpu	yes	p16	In uences virion release, augments turnover of CD4 antigen Integra cell membrane protein
env	no	gp160	precursor for envelope glycoprotein: SU (gp120)-CD4 receptor binding TM(gp41)-membrane fusion and entry Virion envelope, plasma membrane
nef	yes	p27	necessary effector for pathogenesis, down-regulates CD4 receptor, in uences T-cell activation, enhances virion infectivity Cell cytoplasm, plasma membrane

[a] gene nomenclature is based on that in 511
[b] dependent on cell type
[c] encoded only by HIV-2 and several SIV strains
[d] encoded only by HIV-1 and SIV_{cpz}

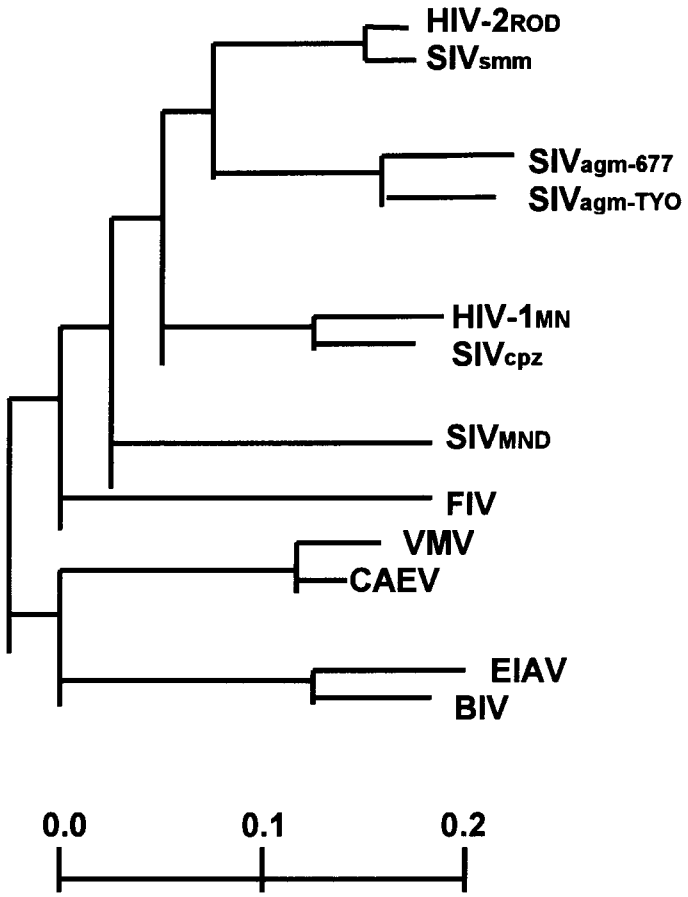

FIG. 10.3. Phylogenetic classi cation of the lentivirus genus of retroviruses. The scale indicates percentage difference in *pol* gene nucleotides. Abbreviations for viruses are given in Table 10.1 and the Glossary. Figure adapted from Myers and Pavlakis 1992 (235).

active viral replication. However, analysis of molecular clones of SIVmac239 containing deletions in the NFκβ site, reveals that this *cis*-acting element is not required for persistent infection and pathogenesis in macaques (513).

A regulatory gene found in all simian and human lentiviruses is the transactivator (*tat*) (514). The target (TAR) for *tat* is a sequence downstream from the cap site in the U5 portion of the LTR which folds into a stem-loop structure. The mechanism of *tat* function may involve both initiation of transcription and elongation of newly initiated transcripts; these effects are mediated by cellular proteins, which interact with *tat* (515).

The posttranscriptional transactivator designated the regulator of viral expression (*rev*), functions to shift viral replication from an early phase characterized by synthesis of spliced messages for viral regulatory proteins to a late phase in which unspliced and singly-spliced *(env)* messages for virion proteins accumulate (516). *Rev* may function by promoting transport of unspliced and singly spliced viral messages and by affecting the host cell's splicing mechanism. A target element responsive to *rev* (RRE) is a sequence about 200 bases long that adopts a secondary structure which is important for recognition by *rev*. A model for regulation of lentivirus gene expression with an early and late phase takes into account the properties of viral regulatory genes (516). Soon after infection, a low level of transcription produces double-spliced *tat* and *rev* messages. After accumulation of both tat and rev proteins, the splicing pattern changes so that synthesis of messages (unspliced and singly-spliced mRNA) predominates for virion structural proteins.

The *nef* is encoded by an open reading frame, which extends from the end of *env* into the 3′-LTR (517). *Nef* is dispensable for viral replication in a variety of tissue culture cells. Expression of this viral gene is associated with down-regulation of cell surface CD4 antigen, the binding receptor for virus. Some studies support the notion that *nef* down-regulates viral gene expression; however, other studies show that deletion of *nef* has no effect on or increases viral replication in tissue culture cells (517–519). Importantly, *in vivo* studies have demonstrated that SIV *nef* is essential for high level virus replication and pathogenesis in infected adult macaques (520). This pathogenesis function of *nef* may be attributed, at least in part, to its association with several cellular protein kinases leading to T-cell activation, which in turn augments viral replication in the infected host (521,522).

Primate lentiviruses also encode other accessory genes whose function in viral replication are under current investigation. The viral infectivity factor *(vif) is* encoded by the known primate lentiviruses and some of the animal lentiviruses (such as feline immunode ciency virus) but

not by the equine infectious anemia virus (523). *Vif* appears to play a role in production of infectious virus particles, perhaps by functioning at a step in virion assembly. The requirement for SIV *vif* in viral replication is cell-type dependent (524,525). Vif has recently been shown to overcome the effect of a cellular gene, CEM 15 that inhibits HIV-1 and presumably, SIV infection (526). A small gene, designated *vpr*, has been shown to augment expression directed by the LTR and to mediate cell cycle arrest (527). *Vpr* contains a nuclear localization signal, and appears to facilitate transport of viral preintegration complexes from the cytoplasm to the nucleus. The *vpr* gene is dispensable for SIV and HIV replication because viruses with deletion mutations in this gene productively infect some types of lymphoid cells; however, *vpr* is required for efficient replication in macrophage cultures. The *vpx* gene also encodes a small virion protein apparently required for nuclear import of viral DNA (528). Because *vpx* and *vpr* appear to be related genes that arose by duplication, *vpx* can substitute for *vpr* in some situations. Efficient viral replication *in vitro* in macrophages requires *vpx* (519); however, this gene is dispensable in lymphoid cell cultures. Molecular clones of SIV_{mac239} with a deletion in either *vpr* or *vpx* produce SAIDS in infected macaques (529). However, a viral clone with a deletion in both of these accessory genes is attenuated and does not produce high virus load or disease in macaques.

Several strains of SIV recovered from molecularly cloned viral genomes have been shown to cause fatal disease in experimentally-infected macaques (530–532). Other molecular clones of SIV are infectious for macaques but appear not to cause disease (533). Therefore, it is feasible to identify viral determinants of pathogenesis by analysis of recombinant genomes, constructed between SIV strains which show distinct biological properties either in tissue culture systems (e.g. macrophage-tropism) or *in vivo* (e.g. pathogenic potential). In addition, knowledge of the molecular biology of SIV and HIV-1 genes and proteins has made it possible to utilize genetic engineering methods to construct recombinant genomes between SIV and HIV-1 (SHIV) to investigate the roles of specific HIV-1 genes (e.g. *env*) in viral replication and pathogenesis in non-human primates, and to test anti-HIV vaccines and therapies in this animal model.

SIV Macaque Model: Molecular Pathogenesis

For a better understanding of the molecular pathways by which HIV evades immune clearance, continues to mutate, and eventually causes fatal CD4 cell depletion, the SIV macaque model appears indispensable, because only in such a relevant animal model can the critical experiments be done to define systematically viral and host determinants of viral persistence and pathogenesis. A unique advantage of this experimental model, not possible in humans, is the opportunity it provides to analyze in blood and other tissues the earliest stages of infection, following different routes of inoculation with well-defined biologic and molecular clones of SIV.

At the onset of infection, factors that affect the viral phenotype and level of replication appear critical to the clinical course (337,359,534–536). The fate of the infected animal appears to be determined within the first few weeks of infection, based on the virulence of the virus and how well the animal's immune response is able to control the initial level of replicating virus. A clear-cut dose relationship between infection and disease is not apparent; once the threshold of infection is reached the fate of individual animals appears to depend mostly on other viral and host factors. Host factors responsible for relative resistance or sensitivity to infection might involve MHC genes, β-chemokines and the balance of T-helper 1 (Th1) and T-helper 2 (Th2) lymphoid subsets and their cytokines (537). An important, as yet unanswered, question with direct relevance to HIV infection of humans that can perhaps best be answered in this model is whether or not it will be possible, by reducing but not necessarily eliminating virus replication at the onset of infection, by vaccination or therapy, to allow development of a long-lasting protective immune response that would otherwise be inhibited or overwhelmed by the virus itself. In other words, is it possible to permit a low level of virus replication, e.g. after vaccination or antiviral treatment, that remains too low to overwhelm host defense mechanisms but sufficient to induce a protective immune response to virulent virus? Although an affirmative answer to these questions is clearly the situation with most other viruses, including many animal oncoretrovirus infections, it remains to be seen whether this will apply to the immunosuppressive lentiviruses of animals and men in which even a little productive infection may inevitably, eventually, spell AIDS.

The opportunity to identify viral genes essential to pathogenesis is provided by the availability of several infectious, pathogenic, and closely related non-pathogenic, molecular clones of SIV that have been completely sequenced and characterized *in vitro* and *in vivo* (538). The prototype pathogenic molecular clone, SIV_{mac239} (530), and several closely related non-pathogenic molecular clones, e.g. $SIV_{mac1A11}$ (539) and BK28 (540), were independently derived from the same parental biologic isolate of SIV_{mac251}. A highly cytopathic variant of SIV_{BK28}, with several critical envelope mutations, was also derived (541). The SIV_{mac239} clone induces fatal AIDS in ~50% of macaques within one year, whereas monkeys infected with $SIV_{mac1A11}$ and SIV_{BK28} remain healthy for up to seven years. Pathogenic molecular clones are also available from the acutely pathogenic $SIV_{smPbj14}$ (531) and from the less virulent SIV_{mne} strains (542). From mutational studies of these molecular clones, several viral genes required for cell tropism and pathogenesis have been identified. It appears that *in vitro* growth characteristics of specific SIV

clones do not correlate well with differences in virulence observed *in vivo*; however, additional studies are needed to deÞne *in vitro* tissue culture conditions which might discern differences between pathogenic and non-pathogenic SIV clones. As is seen also with the oncoretroviruses, *env* gene sequences play a critical role in determining cell tropism, cytopathology and pathogenesis of SIV and other lentiviruses. The SIV biologic isolates consist of a mixture or Òswarm Óof related but genetically distinct virus variants (a quasispecies) which, when molecularly cloned, show unique degrees of lymphotropism or monocyte/macrophage-tropism and associated cytopathic effects. Selection for these viral variants may occur in different host cells and tissues and thus may account for different disease manifestations.

To attempt to identify viral functions essential to disease induction, macaques were infected with SIV_{mac239} clones in which various point and deletion mutations had been made in the accessory genes not required for virus replication (543). Results indicated that the *nef* gene is required, *in vivo*, for high viral replication and pathogenesis, because a stop codon or partial deletion of this gene (Δ *nef*) led to a reduction in virus load and absence of disease in adult macaques (520). In several animals, the stop codon in *nef* was repaired *in vivo* and this genetic event was associated with an increase in virus titer and disease. Thus, the virus selects for an intact *nef in vivo*. SIV (and HIV-1) Nef is a multifunctional protein known to downregulate CD4 and to associate with cellular kinases involved in cell activation (517,522,544Ð546). Mutations in the regions of SIV_{mac239} Nef that bind cellular serine kinases show reduced infectivity *in vivo* and a strong tendency to repair the mutation with reversion to high virus load and virulence (522). Thus, this association of Nef with a cellular serine kinase, tentatively identiÞed as a member of the P21-associated kinase (PAK) family, is one mechanism involved in the pathogenesis of SIV and HIV. Especially noteworthy was the observation that adult monkeys chronically infected with the SIV_{mac239} *nef* deletion mutant were protected against superinfection with a high dose (1000 animal infectious doses) of virulent SIV (547). This degree of protection, independently conÞrmed (548), was far more dramatic than that obtained with any of the previous conventional or novel vaccines tested in this model. The mechanism of protection afforded by *nef* deletion mutants remains unexplained although some aspect of cellular immunity is suspected. Of note, however, is that newborn macaques infected orally with the Δ *nef* mutant developed AIDS (549), without repair of the *nef* deletion, indicating that this SIV *nef* mutation alone does not guarantee safety in all age groups.

The involvement of other viral genes in addition to *nef* in pathogenesis and protection against superinfection was indicated by experiments in which rhesus macaques were infected with recombinants of attenuated ($SIV_{mac1A11}$) and pathogenic SIV_{mac239} clones (534,538). Genetically, $SIV_{mac1A11}$ is 98% related to SIV_{mac239} differing only by two 3 bp deletions within the envelope V1 region and stop codons in *Vpr* and in the cytoplasmic tail of the envelope transmembrane (TM) envelope (gp41) (539). In contrast to SIV_{mac239}, which is infectious for T cells but not macrophages. $SIV_{mac1A11}$ is both T-cell and macrophage-tropic *in vitro*. Macaques inoculated with the $SIV_{mac1A11}$ clone have only a transient infection lasting about six weeks, after which the virus can no longer be isolated from PBMC or tissues, and is only occasionally demonstrable at these sites by PCR. Low-level humoral and CTL immune responses persist, however, probably reßecting a low level of persistent virus expression. In general, the virus load of $SIV_{mac239/1A11}$ recombinant viruses in PBMC of infected monkeys was intermediate between the parental types, and the virus load and pathogenic potential *in vivo* most closely resembled the parental virus that made up most of the recombinant genome. For example, the recombinant carrying the SIV_{mac239} envelope on the $SIV_{mac1A11}$ background replicated only slightly better than $SIV_{mac1A11}$ and caused no disease. The reciprocal recombinant carrying the $SIV_{mac1A11}$ envelope on the SIV_{mac239} background replicated just a little less well (ten- to a hundredfold) than the SIV_{mac239} clone and was capable of causing disease within two years postinfection. The strength of the humoral and cellular immune response was directly proportionate to the level of virus replication in PBMC, thymus, and lymph nodes. In general, in animals chronically infected with these $SIV_{mac239/1A11}$ recombinants, the extent of resistance to superinfection with virulent uncloned SIV_{mac251} was also directly proportional to the level of virus replication. These Þndings add further credence to a concept that applies also to HIV-infected humans (550), that initial virus level is indeed an important determinant of pathogenicity, and also indicate that, in addition to *nef,* at least one or more viral genes are required for pathogenicity. These Þndings also suggest that some aspect of the immune response, yet to be determined, is responsible for protection against superinfection.

The role of other viral genes in pathogenesis has recently been tested via the same mutation strategy used to deÞne the critical role of *nef.* As mentioned in the preceding section, molecular clones of SIV, with deletions in *vpr* or *vpx*, were shown to infect macaques at only a slightly reduced level of viral replication, compared to SIV_{mac239} and both *vpr* and *vpx* deletion mutants were able to induce AIDS after a relatively short incubation period and without repair of the deletions (529,551). Thus, neither of these genes is required for *in vivo* replication or pathogenesis, although they may contribute a slight additive effect to these viral properties. Although the *vif* gene was shown to contribute to more rapid and efÞcient virus replication in certain cell types *in vitro* (524); its importance to *in vivo* pathogenesis has not yet been determined. Mutants of SIV_{mac239} bearing stop codons in the cytoplasmic tail of the TM protein (gp41) were infectious but non-pathogenic in macaques (552,553). However, in two of four monkeys, repair of these stop

codons was selected for *in vivo*, and this event correlated with an increase in virus titer and reversion to virulence. These findings suggest that this cytoplasmic portion of the viral envelope is critical to pathogenesis, and indicates yet another region of the viral genome, in addition to or instead of *nef,* that might be purposefully deleted to make an attenuated live virus vaccine. The cytoplasmic tail of SIV and HIV *env* TM contains two amphipathic alpha helices that alter cell membrane permeability (554) and bind calmodulin, an integral component of cellular signal transduction pathways (555). The lack of pathogenesis of $SIV_{mac1A11}$ clone may thus be attributed to the two stop codons in gp41 that preclude expression of these domains. Additionally, through studies of a viral mutant *in vivo* the intracytoplasmic domain of SIV_{mac239} Env glycoprotein was shown to be important for pathogenesis (556).

SIV Macaque Model: Vaccines

The remarkable similarities that exist between SIV infection and disease in macaques and HIV-1 infection and disease in humans have made this the animal model of choice for research towards a prophylactic AIDS vaccine (557). The species of macaques utilized have been mostly rhesus (*M. mulatta*), with fewer numbers of longtailed (*M. cynomolgus*) and pigtailed (*M. nemestrina*). The results, not surprisingly, have been mixed and no single candidate vaccine has yet emerged that appears consistently effective or highly promising for large scale, phase 3 human clinical trials. This sobering but realistic state of affairs reflects our continued uncertainty concerning the mechanisms of pathogenesis and immune protection against any of the animal or human lentiviruses. However, the vaccine successes, albeit limited, that have been achieved in the SIV macaque model, do engender some optimism (557). The best results in terms of strength and duration of protection against SIV infection and disease have been obtained with modified live SIV vaccines, but some degree of protection, such as lowering of the level of challenge virus infection and delay of disease onset, has also been achieved with other vaccine approaches, used alone or in combination vaccines, including inactivated whole virus, native envelope glycoprotein, recombinant envelope subunits, live vectors presenting SIV antigens, SIV peptides and, most recently, viral DNA. Four biologic strains of SIV, for which pathogenic molecular clones also exist, have primarily been used for vaccine research: SIV_{mac} and SIV_{sm} are closely related and usually cause a virulent infection, with about 95% mortality from AIDS within six months to two years (the average is 11–12 months). SIV_{mne} is less virulent and causes death from AIDS in about 50% of animals after two years of infection. SIV_{pbj} is highly virulent and causes death from diarrhea in 10–14 days. Most recently, recombinants of SIV and HIV-1 env (SHIV env) have been used as attenuated live vaccines or as a challenge for macaques that were immunized with various HIV-1 immunogens or modified live SIV. Encouraging results, summarized in the next section, have been obtained with SHIV DNA vaccine protocols against pathogenic SHIV challenge infection in rhesus macaques.

Inactivated Whole Virus

In early experiments (1989–1992) vaccine protection against SIV_{mac} challenge infection was unexpectedly achieved by use of inactivated whole SIV_{mac} or SIV_{sm} cell-free or cell-virus vaccines. A number of laboratories in the United States and Europe found that macaques immunized with inactivated whole SIV vaccines, in which vaccine and challenge virus were both propagated in human T-cell lines, could be completely protected against low dose (1–100 animal infectious doses) IV challenge infection with homologous or heterologous SIV strains (558,559). However, this protection did not extend to HIV-2, which differs by about 20% in nucleotide sequences. This complete protection was called "sterilizing immunity" because, using sensitive detection methods, the vaccinees had no virologic or serologic evidence of transient infection or anamnestic immune response after inoculation. This type of immunity appeared to last up to about one year, and was also effective against challenge infection by rectal application of SIV grown in human T cells (560), but, with one exception (561), not against vaginal mucosa inoculation with SIV grown in simian PBMCs (562,563). Interestingly, the best correlate of protection with the inactivated whole virus vaccines proved not to be antiviral neutralizing antibody, as might have been expected, but rather, antibodies against the human T-cellular antigens, viz, MHC class I and II proteins, present on the cells in which the vaccine and challenge viruses were grown (564, 565). It was shown that the same vaccinated animals that had been protected against live SIV_{mac}, grown in human T cells were not protected against the same dose of the same virus grown in rhesus PBMC. Nor was protection usually obtained against challenge with virus-infected rhesus PBMC. Some monkeys were completely protected against SIV_{mac} grown in human T cells by vaccination with uninfected human T cells (564), and even with semi-purified MHC I protein or MHC II DR proteins alone (566,567). Immunization with other cell surface antigens, such as β-2 microglobulin and CD4 protein, did not protect. Protection could be passively transferred by serum containing antibodies to the human T-cell antigens, with little or no SIV neutralizing antibody, possibly by the mechanism of antibody mediated complement lysis of virions (568). By contrast, protection could not be passively transferred by SIV neutralizing antibodies (569). Therefore, the immune protection achieved with the inactivated whole SIV vaccines could be attributed mostly to the xenogeneic MHC antigens picked up by the virions from the human T-cell lines in which the vaccine and challenge viruses had been propagated. Antibodies to the

CCR5 receptor may also have contributed to this protection (570). Some protection against SIV grown in rhesus PBMC was also obtained by immunization of macaques with uninfected allogeneic rhesus PBMC, indicating that alloantigens as well as xenoantigens could provide partial protective immunity against experimental SIV infection (571). Immunization of macaques with allogenic B cells expressing high levels of MHC Class I and Class II molecules elicited antibody responses to these antigens but did not protect against systemic infection with SIV_{mac} (572). However, the average virus-infected cell load was reduced in the immunized animals. This finding supports the idea that relative resistance to HIV-1 infection in certain high-risk individuals and the rate of progression of HIV-1 infection may be linked to MHC genes, and, possibly, alloantigen-induced autoimmunity (573). This notion is further supported by the observation that the rapidity of the clinical course in SIV-infected macaques is inversely correlated with the strength of MHC-II DR antigen expression on monocyte-macrophages and B cells (574). In HIV-1 infected humans, a single amino acid polymorphism in a MHC Class I molecule has a substantial effect on the rate of progression to AIDS (575). Perhaps, this resistance will be linked to increased β chemokine production or other aspects of innate immunity. The possibility of developing an efficacious HIV-1 whole virus vaccine using the SIV macaque model is still being investigated at the National Cancer Institutes (NIH) (576). However, because of safety concerns and patent issues, there is relatively little industrial interest in inactivated whole HIV vaccines.

Recombinant SIV Subunits and Live Vectors

Recombinant SIV subunit vaccines have, in general, not been able to provide complete protection ("sterilizing immunity") against systemic or mucosal SIV challenge infection. However, they have usually conferred partial protection, evidenced by a 1–2 log reduction in challenge virus level in comparison to naive controls. Only one example of apparently complete protection, using a recombinant SIV env (gpl60) vaccine, has been achieved (577); in this study pigtailed macaques were immunized with live vaccinia virus expressing gpl60 of SIV_{mne}, boosted one year later with subunit gpl60 and then challenged with a biologic clone (E11s) of the less virulent SIV_{mne} strain. None of these vaccinated animals had virus isolatable from PBMC following SIV challenge, or seroconversion to SIV proteins. Neutralizing antibody appeared to be the best correlate of protection of this study. CTL responses postchallenge revealed the induction of reactivity to SIV proteins not included in the subunit vaccine, suggesting that a low-level of SIV infection did occur following exposure of the vaccinated hosts to SIV (578). When these animals were boosted with the same vaccine and challenged with a genetically more diverse uncloned biologic SIV_{mne} strain, some of them became infected, indicating that the protection obtained in this system was primarily against a genetically uniform virus population. Using the SIV_{mne} pigtailed macaque model, a profound but not complete suppression of challenge virus was obtained by immunization with a mixture of four Env peptides expressed in E. coli as β-gal fusion proteins (579). Protection appeared to correlate with level of neutralizing antibody, which could passively protect a majority of recipients (580). But, again, animals immunized with this env peptide vaccine were not protected against challenge with a related but more diverse SIV strain. The same gp160 vaccine was highly effective against intrarectal infection with either cloned or uncloned SIV_{mne} (581). Protection appeared to correlate with neutralizing antibody activity and low level challenge infection. These results indicate that under ideal conditions envelope antigens alone can confer strong protective immunity to a genetically uniform, homologous SIV, but not to genetically diverse SIV, as present in natural SIV and HIV isolates (582). A summary of work in the SIV_{mne} pigtailed macaque model led to the conclusion that the best results in terms of preventing infection or reducing virus load after intravenous challenge are obtained with recombinant vaccines that include the entire env (gp 160) rather than only the external portion (gpl30), that combine core antigens with env and that use a live vaccinia vector priming, followed about a year later by a baculovirus expressed subunit gpl60 boost (583).

A subunit vaccine consisting of native gp130 oligomers but not of gp130 monomers protected rhesus macaques against productive infection with SIV_{mac} (584). Escape from neutralizing antibody was associated with the development of V-1 env mutation in the challenge virus (585). On the other hand, vaccines consisting of recombinant SIV_{mac} env ISCOMS alone or combined with gag ISCOMS and nef peptides were unable to protect macaques or lower the virus load following IV challenge with the homologous virus, despite the presence of vaccine induced neutralizing antibodies and CTLs (586).

A number of live vector systems, e.g. baculovirus (587,588), attenuated vaccinia virus (Ankara) (NYVAC) (589–593), Canary pox (ALVAC) (593–594), vesicular stomatitis virus (595), Semliki Forest virus (596,597), Mengovirus (598), Poliovirus (599,600), Adenovirus (601), Salmonella (602), and BCG (603) have been used for priming or boosting monkeys, different subunit expression systems; e.g. mammalian cells (CHO) (604,605), baculovirus (606), vaccinia virus, *E. coli*, yeast Ty, have also been used, together with a number of different adjuvants, cytokines (607) and vaccination protocols. Most of the recombinant vaccines expressed the SIV_{mac} *env* gene (gp160 or gp130), and many vaccines also expressed gag or pol genes (590,592,608–611). One recombinant env vaccine induced neutralizing antibodies selected for viral escape mutants and enhanced the infection, but such enhancement has not been commonly

observed (612). None of the recombinant live vaccinia or baculovirus vaccines, or recombinant CHO cell vaccines, conferred strong protection against IV challenge infection with pathogenic SIV_{mac} or SHIVs (see below), even when the recombinant antigens were expressed as virus-like particles and induced a high titered immune response. Although none of these recombinant live virus vaccines completely protected macaques against IV or mucosal challenge with a low to moderate infectious dose (10–100 ID/50) of cell-free homologous SIV_{mac}, the virus titer in PBMC and plasma of many recipients was reduced 1–2 logs during the acute infection phase after challenge, compared to controls. Most of the vaccinated animals developed antibodies and T-cell responses to the SIV antigen and some had neutralizing antibody, but the role of humoral and cellular immunity (as measured by T-cell proliferation and CTLs) in reducing the challenge virus titer during acute infection remains uncertain. Non-MHC restricted CD8+ T cell antiviral activity may have contributed to protective efficacy (613). An ALVAC SIV vaccine and the macaque MHC class I (A*01) genotype conferred partial protection against intrarectal challenge with SIV_{mac251} but no protection against pathogenic SHIVs (614). Immunization with the NYVAC SIV vaccine resulted in the appearance of CD8+T cells at mucosal sites even when the vaccine was delivered by non-mucosal routes (615). The same kind of vaccine partially protected cynomolgus monkeys from mucosal SIV_{sm} challenge (616). By contrast, using a non-virulent virus host model, vaccination of cynomolgous monkeys with a vaccinia virus expressing the SIV_{agm} *env* gene induced high titers of anti-SIV_{agm} antibodies and markedly reduced the replicability of the challenge polyclonal nonpathogenic SIV_{agm} (617). Inactivated SIV whole virus (grown in human T-cells) or a recombinant baculovirus envelope and core subunit vaccine injected into the iliac lymph nodes conferred complete or partial protection against rectal challenge with a molecular clone of SIV_{mac} (611). Protection was correlated with the induction of Th2 responses in circulating CD4+ T cells reflecting the expansion of SIV specific IgG producing cells followed by IgA secreting cells (618). Anti-SIV IgA secreting cells were also present in the iliac lymph nodes (619). The most significant correlate of protection with the targeted lymph node vaccines was the induction in the iliac lymph nodes of CD8 suppressor factors and β-chemokines (620), that prevent binding of SIV (or macrophage-tropic HIV) by the CCR5 coreceptor. Gamma-delta T cells may also have generated antiviral factors and β-chemokines (621).

Although not completely protected against challenge infection, vaccinated animals with reduced levels of acute viremia after challenge with pathogenic SIV have generally experienced a more prolonged asymptomatic infection than the non-vaccinated controls (622). Immunization of newborn rhesus monkeys with modified vaccinia virus expressing SIV gag, pol and env delayed the onset of AIDS after challenge at four weeks of age with virulent SIV_{mac251} (592). Similarly, low virus load in blood and lymph node during primary infection has repeatedly been shown to correlate with prolonged survival in HIV-1-infected individuals (623,624). Lessons learned from the SIV macaque models would indicate that partial vaccine protection, by lowering the level of initial infection, and thus, delaying or preventing disease onset for a long time, represents a more feasible and reasonable goal for an AIDS vaccine, rather than completely preventing infection (sterilizing immunity) (571, 625). The major reason for delaying Phase III human clinical trials was because the antibodies induced by the HIV-1 env vaccines did not neutralize field isolates (in contrast to T-cell line adapted HIV-1 strains) (626) and because of breakthrough infection of some high-risk individuals who had received these vaccines (627,628). Also, there is considerable concern that high-risk individuals, receiving candidate-unproven HIV vaccines, may feel that they are now protected against infection, and therefore will practice less safe sex. Nevertheless, Phase III trials are now underway with recombinant gp120 vaccines in the USA, Canada, Netherlands and Thailand.

SIV Peptide and DNA Vaccines

Immunization of macaques with a mixture of lipopeptides derived from nef and gag proteins or peptides representing CTL and neutralization epitopes elicited epitope specific antibodies and CTLs but did not protect against infection (629,630). The emergence of CTL escape mutants supported the necessity of eliciting broad CTL responses. With this goal in mind, a degenerative peptide cocktail representing the hypervariable epitopes of SIV (631,632) and HIV-1 (633) Env vaccine has induced broad and long-lasting humoral and cellular responses in rhesus macaques (631–634), but has not yet been tested for efficacy against SIV challenge. An SIV gp120 C4 domain peptide immunogen coupled with recombinant gp120 expressed in adenovirus did not protect against rectal SIV challenge (635). Immunization of macaques with SIV recombinant Nef protein revealed an inverse correlation between Nef specific CTL precursor frequency and virus load measured after challenge (636). Immunization with SIV Nef expressed by vaccinia virus or nef DNA elicited T cell responses and antibodies but did not protect against SIV infection (637). Oral immunization of macaques with SIV Gag protein and cholera toxin elicited both mucosal IgA and systemic IgG immune response but these animals were not challenged (638).

Genetic immunization with HIV-1 and SIV_{mac} *env* naked DNA has induced modest levels of anti-SIV humoral and cellular immunity in mice and macaques, and partial protection vs. SHIV or SIV challenge of macaques (639–642). The genetic immunization approach is considered especially attractive because it mimics the intracellular processing of a live virus by antigen presenting cells but is free of the safety concerns raised by

replicating virus (643). Incorporation of a lymphokine gene (e.g. IFN-g, IL-2, IL-4, IL-12, GMCSF) or MHC Class I or II genes in the recombinant vaccine may enhance the immune response or attenuate the potential virulence of the vector (644–648). Immunization of macaques with a CTL epitope gene-SIV DNA prime, and vaccinia virus (Ankara) boost regimen elicited high levels of epitope specific CTLs, that correlated with tetramer staining of PBMC and post challenge control of viremia (649,650). Vaccination with a SIV DNA gag-env prime and NYVAC gag, pol, env boost significantly suppressed viremia within two months after mucosal exposure to SIV_{mac251} (651) or SIV_{mac239} (652). However, most of these animals progressed to AIDS. Pigtailed macaques immunized with plasmid DNA expressing all SIV genes alone or boosted with recombinant gp160 plus gag-pol particles were partially protected against rectal challenge with SIV_{mne} (653). Protection seemed to correlate with a dominant Th1 response. Vaccination of macaques with SIV DNA producing inactivated virus-like-particles induced strong mucosal immune responses, including rectal IgA, and conferred partial protection against rectal infection with SIV_{mac239} (654). Another SIV DNA vaccine partially protected rhesus monkeys from intrerectal challenge with homologous virus (655,656). Vaccination of macaques with DNA of a chimeric SIV, in which SIV env was replaced with Friend MuLV env, together with DNA of the MuLV receptor, induced SIV gag specific cellular immune responses and partial resistance against SIV_{mac239} challenge (657). This novel strategy may be useful for development of safe and effective vaccines against various kinds of pathogenic viruses. Vaccination of macaques with SIV_{nef} DNA elicited multi-specific T cell responses recognizing variants present with the SIV_{mac251} isolate (658). These animals were not challenged with SIV. In summary, SIV DNA vaccination, often combined with subunit boosts, appears to be a promising strategy deserving of more research. The protection achieved is generally equal to or better than that obtained with recombinant SIV subunits, live vectors or peptides alone but not as strong as seen with attenuated live SIV vaccines.

Attenuated Live SIV Vaccines

Unquestionably, the best protection in the SIV macaque model has been obtained using genetically attenuated live virus vaccines. Such a result is perhaps not surprising in view of the historical precedence of successful modified live virus vaccines (659) and the reported success with modified live equine infectious anemia (a horse lentivirus) vaccines (660). Macaques immunized by infection with one of several different SIV_{mac} or HIV-2 molecular clones or recombinants that are relatively non-pathogenic, were uniformly protected as well or better against challenge with virulent SIV than observed with any of the other vaccine approaches (661–664). In general, the animals preinfected with the attenuated live SIV or HIV-2 clones are able to suppress the level of superinfection markedly, or even to prevent superinfection following challenge with high dose (100–1,000 ID/50) virulent homologous or heterologous virus strains, and such animals show a significant delay of disease (>4 years). Inoculation of macaques with subinfectious doses of SIV has also been considered as a way to induce primarily cell mediated virus responses (Th1) that might confer protection (665). The most thoroughly studied attenuated live vaccines are the nef-deleted (Δ*nef*) (661) and triple deletion mutants (Δ3) of SIV_{mac239} (666,667) and the closely related naturally attenuated 1A11 molecular clone of SIV_{mac239} (open nef) (668). The Δ*nef* clone 8 (C-8) has a 12-base pair deletion in the *nef/3′-LTR* and can revert to virulence after *in vivo* repair of this attenuating lesion (669).

The Δ*nef* and Δ3 clones replicate better *in vivo* than the 1A11 clone and protect better. Recombinants between the attenuated 1A11 clone and the intact virulent 239 clone of SIV_{mac}, replicate to various levels in rhesus macaques and the degree of protection against virulent virus challenge correlates with the relative level of replication (662). The same can be said for different nef deletion mutants of SIV_{mac}. Unfortunately, a window of safety has not been defined in which a level of replication can be tolerated that will not induce disease and yet will be protective in macaques of all ages. The higher the level of replication, the better the protection against superinfection with virulent virus, but the more likelihood that the recombinant virus eventually will cause disease. The lower the level of replication, the less will be the extent of protection against challenge with virulent virus, but also the less likelihood that the recombinant virus will cause disease. In newborn macaques, the SIVΔ3 clone induces a high level of virus replication and fatal AIDS (670). Given sufficient time (approximately six years), the SIVΔ3 vaccine is also pathogenic in most adult macaques (671). By contrast, infection of newborn macaques with the more attenuated $SIV_{mac1A11}$ clone induces only a transient viremia, yet completely protects some recipients against a low dose of SIV_{mac}, (10 ID/50) given orally, a dose that readily infects and eventually kills all controls (672). Perivaginal inoculation with the same attenuated live SIV clone, followed by a second perivaginal inoculation with whole inactivated SIV_{mac} substantially reduced viral load after intravaginal challenge with uncloned SIV_{mac251} (563). Adult macaques infected with the $SIV_{mac1A11}$ clone became superinfected when challenged with a high dose (1000 AID) of SIV_{mac} but they survived more than three times longer than controls (668). Similar results were obtained when macaques infected with nonpathogenic HIV-2 were superinfected with virulent SIV (663). SIV_{mac239} Δ*nef* infected adult monkeys withstood a very large dose (1000 ID) of virulent homologous SIV challenge infection given IV or intrarectally (547,548), and were also resistant to IV and IR infection with cell-free SIV_{mac} or SIV_{mac} infected rhesus

spleen cells (673,674). SIVΔ*nef* infected monkeys were also resistant to heterologous SIV challenge infection (675). None of the other SIV or modified live virus vaccines approach this magnitude of protection. Macaques infected with a macrophage tropic recombinant of SIV_{mac239} were protected against a heterologous SIV strain as well as against the homologous virus (676). Neutralizing antibodies appeared to correlate with this protection, which could also be passively transferred. By replicating in antigen-presenting cells, macrophage-tropic viruses may be more immunogenic, and such viruses are also implicated in the mucosal transmission of HIV-1 and SIV. SIV_{mac239} Δ*nef*-infected monkeys are also resistant to IV or rectal challenge with the more divergent SIV_{sm} or a SIV/HIV recombinants (SHIV) bearing the HIV-1 envelope (677,678). These latter results indicate an unexpectedly broad range of protection that cannot be attributed to envelope antibodies.

The mechanism of protection from these attenuated molecular clones of SIV is not clear. Protection does not appear to be by MHC alloantigens (678) or viral interference at the cell surface receptor level, as described with oncoretroviruses (679). Most likely the mechanism is immunogenic because a period of time (10–20 weeks) is required to obtain maximal protection (680). However, the mechanism may be distinct from classical humoral or cellular immunity and may involve factors of innate immunity e.g. NK cells, IFN-γ (681). The protection cannot be transferred with immune serum (682) and apparently does not involve neutralizing antibody (683,684). In those monkeys not protected from pathogenic virus challenge a minimum of viral diversity occurred with no selection for specific V-1–V-2 genotypes (685). Inhibition of SIV replication in SIV_{mac} Δ*nef* immunized monkeys apparently does involve T helper responses and CD8+ MHC restricted (i.e. CTL) and unrestricted mechanisms, the latter due to soluble factors other than known β-chemokines (686). Undoubtedly, by one means or another, CD8 T cells do play an important role in controlling replication of the live attenuated SIV *in vivo*. However, partial depletion of circulating CD8+ cells or total lymphocytes prior to challenge failed to abrogate the protection conferred by SIVΔ*nef* (687,688). Moreover, another study found no correlation between the anti-SIV CTL response and protection against infection in SIVΔ*nef* infected animals (689). Rechallenge of vaccine did not induce vigorous T-cell responses (690). Clearly, we do not as yet have a clear picture of the mechanisms of protection versus superinfection conferred by live attenuated SIV.

Whether attenuated live HIV vaccines will ever be acceptable for human use remains unlikely. Safety concerns, based on fears of recombination or reversion to virulence, or insertional activation of cellular oncogenes, are a major obstacle (691,692). The development of AIDS in newborn and adult macaques infected with the SIV_{mac239}Δ*nef* (670,671) certainly invites caution. However, the possibility remains that a safer, more attenuated SIV clone might yet be found that is immunogenic and protective in newborns as well as in adults. Also, the incorporation of lymphokine genes, e.g. IFN-τ (693), into the attenuated SIV might enhance its safety and efficacy. Other modified live virus vaccines might he constructed to have deletions in critical regions such as the integrase domain (694) of the *pol* gene, the cytoplasmic tail of the transmembrane envelope (695) or mutations in variable regions of the env gene (696). Good protection from mucosal challenge with SIV_{mac251} was achieved by live attenuated SIV with point mutations in the env transmembrane protein (697). Removing carbohydrate molecules from the viral envelope might also enhance the presentation of neutralizing antibody epitopes (698). Further study of macaques protected with genetically modified live SIV molecular clones is a most promising approach toward understanding the viral determinants of pathogenesis and the mechanism of apparent immunity. An annual update of worldwide SIV and SHIV challenge studies in vaccinated non-human primates, already about 250 in number (699), will provide information needed to guide the development of the most effective HIV-1 vaccines for phase III efficacy trials in men. Hopefully, effective AIDS vaccines may yet be deployed in high risk population around the world.

SIV Macaque Model. Antiviral Therapy

The urgency of the AIDS problem and the desperate desire of AIDS patients to try any potential treatment have prompted the application of most antiviral therapy directly to humans after only preliminary *in vitro* evaluation of toxicity and efficacy. Thus, preclinical testing of candidate drugs against HIV, and against most of the opportunistic pathogens encountered in AIDS, has not depended on the SIV macaque model. Fortunately, since 1994, these preclinical trials have led to new effective highly active antiretroviral therapy (HAART) employing potent combinations of three or more antiviral drugs directed at the RT and protease enzymes. HAART can accomplish lengthy and near complete suppression of virus replication in many HIV-1 infected persons, although multidrug resistance virus, latency and residual replication remain major problems (700). The nucleoside antireverse transcriptase (RT) inhibitors and peptidomimetic protease inhibitors (PI) are equally effective against the respective enzymes of HIV-1 and SIV (701). However, the non-nucleoside RT inhibitors do not inhibit SIV or HIV-2. Nonetheless, the SIV monkey model has served a useful purpose in confirming the efficacy and safety of the anti-HIV-1 RT nucleoside analogues, and in discovering and evaluating several other potent antiviral agents (i.e. PMPA), before clinical application (702–704). Currently the SIV macaque model is proving useful for evaluating the viral dynamics of primary viremia (705) and the efficacy of combined HAART and therapeutic vaccination or intermittent vs. continuous therapy against acute and chronic infection and

disease (706,707). The application of SIV/HIV recombinants (SHIVs) and HIV-2 infection of macaques for studies of antiviral therapy will also be highlighted in this section. Perhaps the major beneÞt of the monkey model is the ability to test the pathogenicity *in vivo* of drug resistant viral mutants although the speciÞc drug induced nucleotide mutations in RT or protease enzymes may not necessarily be identical in HIV-1 and SIV. For example, site speciÞc mutations made in RT residues, M184V and E89G, of SIV_{mac}, analogous to the drug resistant mutation found in clinical HIV-1 isolates, have been tested for ddc resistance (708) and SIV Þtness and virulence in macaques (709,710). Interestingly, a combination of PMPA with 3TC selects for the K65R mutation and against the M184V mutation in SIV RT (711). The major drawbacks of the monkey model are its expense, reluctance of drug companies to rely on non-human primate tests and the difÞculty in administering reliable oral doses of antiviral drugs.

In general, each of the RT nucleoside analogues inhibits HIV-1 and SIV RT with similar *in vitro* efÞcacy and, when started shortly before or shortly after SIV inoculation of macaques, these antivirals have been found to either prevent infection or suppress acute SIV infection (712Ð715). In early studies, AZT and ßuorodeoxythymidine (FLT) were shown to penetrate the brain and systemic tissues in extracellular concentrations that exceeded *in vitro* antiviral concentrations (716). In some of these studies (717Ð719), the onset of disease and time to death were delayed. Lowering the challenge dose of SIV in monkeys resulted in a more dramatic antiviral effect of FLT, including complete prevention of infection in some animals (720,721). However, FLT proved too toxic for human application. Long-term AZT treatment of SIV chronically infected macaques resulted in suppressed viremia and slightly prolonged survival (717), similar to results with AZT monotherapy of HIV-1-infected humans. Early treatment with AZT could even prevent infection of newborn macaques with a low dose of SIV given orally, but had no effect on the course of disease when treatment was started after infection had been established (718). These results in SIV-exposed macaques are consistent with the prophylactic effect of AZT on lowering incidence of infection in newborns of HIV-1 infected pregnant women (722). AZT treatment signiÞcantly decreased virus load within the CNS and delayed onset of CNS dysfunction and immunodeÞciency in rhesus monkeys perinatally infected with SIV_{sm} (719). AZT resistant mutants of SIV are derived by *in vitro* and *in vivo* selection but not necessarily with the same kinetics and same point mutations as occur with HIV-1 (Tom North, pers. comm.). The macaque model provides a unique opportunity to test the infectivity and pathogenicity of such AZT-resistant SIV mutants occurring *in vivo*; one such mutant, Q151M, has been shown to be pathogenic in newborn macaques (723). The toxic effects of AZT in SIV-infected monkeys are similar to those observed in AIDS patients treated with AZT. Prophylactic treatment of macaques with didanosine (ddI) completely protected against SIV_{mac} infection and ddI, but not hydroxyurea, protected macaques against a lethal dose of the highly pathogenic SIV_{pbj114} (724). During acute infection ddI markedly reduced the virus load in SIV_{mac} infected macaques (725). A lipophilic dideoxy nucleoside analogue (6-cl-ddg) had a beneÞcial antiviral effect in rhesus monkeys acutely and chronically infected with SIV_{mac} (726,727).

The nucleoside analogue RT inhibitors (PMEA, PMPA) had a prophylactic or long-lasting therapeutic effect on SIV infection *in vivo*, if given before or soon after infection, but less effect if given therapeutically after infection is established (728Ð732). In macaques acutely and chronically infected with SIV_{mne}, treatment with PMEA had a more dramatic anti-SIV effect compared to AZT (732). In the last eight years, the most dramatic protection against SIV infection in the macaque model has been achieved with PMPA (Tenofovir), an acyclic nucleoside phosphonate inhibitor of HIV-1 and SIV RT. One hundred percent (25 of 25) of pigtailed macaques (*M. nemestrina*) given PMPA either before or up to 24 hours after challenge were completely protected against infection (733). The effectiveness of postinoculation therapy with PMPA depended critically on the timing of initiation and duration of treatment (634). PMPA is already phosphorylated and does not require cell cycle dependent thymidine kinase to become phosphorylated, as does AZT. Therefore, PMPA is active in resting cells, a property that may help explain its antiviral potency. Also, drug-resistant mutants may arise more slowly against PMPA than against PMEA or AZT. Short term treatment of newborn infected macaques with PMPA reduced virus level and prolonged survival (735,736). Early after PMPA treatment of acutely infected macaques, activated memory CD4+ T-cells repopulated the intestinal mucosa but showed a decreased ability to produce IL-2 (494). Infant macaques receiving prolonged treatment with PMPA developed SIV mutants with Þve-fold reduced susceptibility to PMPA *in vitro* (737). These mutants had the characteristic K65R mutation in RT and were cytopathic *in vitro*. PMPA still offered a strong therapeutic effect on macaques infected with these mutant viruses (738). PMPA also had a beneÞcial prophylactic effect on preventing oral infection of newborn macaques by the mutant SIV with a reduced susceptibility to PMPA (737). A topical PMPA gel prevented vaginal transmission of SIV (739). However, topical administration of PMPA failed to protected infant macaques against multiple oral exposures of low dose SIV (740). Administration of PMPA to gravid and SIV-infected macaques showed a signiÞcant placental transport, a sustained reduction in viral load in SIV infected fetuses and infants and improved survival (741). Unfortunately, severe growth retardation and bone related toxicity were noted in about 25% of the infants (742) along with transient effect on material bone biomarkers (734).

Treatment of acutely and chronically SIV-infected monkeys with PMPA led to a several log reduction in viral load, not quite as dramatic as the antiviral effect of HAART therapy in HIV-1 infected individuals (700). However, viral loads rebounded to pretreatment level by 2 weeks after termination of therapy. Post-exposure macaques that were protected by PMPA treatment from persistent productive infection had preserved SIV-speciÞc immune function, especially CD8+ lymphocytes, and were partially or completely protected from subsequent homologous virus rechallenge (744,745). The degree of protection conferred by PMPA is unprecedented in the simian AIDS model and suggests a promising role for PMPA in HIV-1 prophylaxis and post-exposure therapy in humans, if toxicity is not too severe.

Because of considerable dissimilarity between the SIV and HIV-1 proteases in their active sites (746), it was generally assumed that SIV would be less susceptible than HIV-1 to protease inhibitors. Therefore, protease inhibitors have not extensively studied, so far, in the SIV macaque model. One peptidomimetic protease inhibitor (U-75875) was shown to have a modest prophylactic effect in SIV-infected monkeys, by slightly reducing virus replication and slightly delaying disease onset (747). Recently, the susceptibility of SIV was found similar to HIV-1 and to three protease inhibitors, indinovir, saquinavir and ritonavir (701). Therefore, the SIV macaque model will be useful for studies of HAART, such as reservoirs of residual replication. Whether protease-resistant mutants arise just as quickly in the SIV model as with HIV-1 infection (748) remains to be determined. HAART is now being tested in this model against both SIV (749) and SHIV (750) challenge infection either alone or in combination with therapeutic vaccination (749,751). Toxic effects of protease inhibitors seen in humans, e.g wasting, lipodystrophy, have not been reported in the macaque model.

Attempts at immunotherapy using non-speciÞc immune modulating drugs, such as phosphocarbohydrates (752), IFN-α (753), IFN-γ, IL-2 or IL-12 (754), have been reported in the SIV or SHIV model often in combination with vaccination. Recombinant rhesus IL-12 had a signiÞcant protective effect vs. SIV infection and disease (749). In pathogenesis and vaccine-related studies, recombinant live SIV and infectious SIV vectors, expressing IFN-γ (693), IL-2 (755) or IL-4 (647) in the place of nef, have been constructed and tested *in vivo*. SIV IFN-γ or IL-4 constructs were attenuated for virulence and gave partial protection against challenge infection with virulent SIV (693,647). In newborn macaques, an SIV IL-2 recombinant caused acute death with lymphoproliferation and high virus load (644). In adult macaques, a similar recombinant was non-pathogenic and prone to deletion of the IL-2 gene (756). IL-2 treatment helped to restore CD8+ T cell effects or function in SIV infected macaques (757). Short-term treatment of chronically SIV infected macaques with IL-12 was well tolerated but caused no measurable change in virus load (758). Recombinant rhesus IL-16 reduced the infectivity of SIV_{mac} for rhesus PBMC *in vitro* (759). In SIV infected cells interferon blocked an early step in viral replication, between virus binding and reverse transcription (760).

Non-speciÞc antigenic stimulation with conventional antigens during primary infection of macaques with SIV increased the virus load and, possibly, accelerated the disease (396). T-cell activation is well known to enhance replication of SIV and HIV in resting lymphocytes (761). HIV-1 chronically infected adults given IL-2 also had a sudden increase in virus load, but tended to also show an increase in CD4 T cells (762). Similarly, transient activation of HIV-1 replication has been observed after inßuenza vaccination or antigenic stimulation of HIV-1-infected individuals (763Ð765). Methadone, a treatment for opiod dependency, was found to induce CCR5 and activate SIV replication *in vitro* (766).

Other targets for therapeutic intervention, such as the CD4 receptor or chemokine coreceptors, and the viral Tat, Rev, Nef, Vpu, Vpr, Vif and nucleocapsid proteins, can also be targeted in the SIV macaque model (767). Both passive administration of recombinant soluble human CD4 to SIV-infected monkeys (768), or active immunization of infected or uninfected monkeys with this protein, which elicited CD4 binding antibodies, had only a transient beneÞcial effect on virus expression (769,770). A humanized anti-CD4 antibody transiently decreased plasma viremia in SIV infected macaques but its effect was limited by development of anti-antibody responses and CD4 independent SIV variants (771,772). Adaptive transfer of SIV naïve CD4+ cells to SIV chronically infected macaques rescued cell mediated immune responses and induced long-term non-progressor status, even in the absence of continued antiviral therapy (771). Gene therapy with antisense RNA against SIV, engineered into CD4+ or Cos-1 cells through a retrovirus vector or transfection, inhibited SIV replication *in vitro* (761,773). Macaques infused with autologous CD4 enriched lymphocytes, transduced with a retroviral vector expressing an antisense *tat/rev* gene, had a signiÞcant reduction in viral load and maintained stable CD4+ cells for more than one year after SIV challenge (774). Transduction of rhesus CD34+ hematopoietic stem cells with a hairpin anti-SIV ribozyme protected T cells and macrophages from SIV infection *in vitro* (775). Macaques infused with interferon engineered lymphocyte appeared to gain some protection against SIV disease (776). As a possible model for autotransfusion therapy, rhesus CD4+ T cells were activated and expanded with anti-CD3/CD28 coated beads; such cells maintained their immune function but exhibited an unstable anti-SIV effect *in vitro* requiring continuous exposure to the CD3/CD28 antibodies (777). A human chorionic gonadotropin associated factor in pregnant womenÕs urine had a beneÞcial effect on SIV infection *in vitro* and *in vivo* (778). For future antiviral and pathogenesis research, a full battery of macaque lymphokine genes has been cloned and sequenced (779,780).

Lentivirus-derived vectors are promising gene delivery systems because, unlike murine leukemia virus-based vectors, they are able to transduce nonproliferating differentiated cells. Highly efficient vectors derived from SIV_{mac251} were constructed and shown capable of transducing human primary dendritic cells, which might provide a novel approach to immunotherapy (781). A novel lentivirus vector consisting of an HIV-1 derived genome encapsidated by SIV_{mac} core particles and pseudotyped with VSV glycoprotein efficiently transduced human lymphocytes, macrophages and bone marrow stem cells (782). This vector may be safer than HIV-1-based vectors because of the decreased likelihood of recombination between transfer and packaging vectors.

The SIV macaque model has demonstrated the efficacy of vaginal microbicides as a strategy against HIV sexual transmission (783). Topical application of the spermicide non-oxynol-9 (784), PMPA (739), cellulose acetate phthalate cream (785) or estrogen (786), were effective in blocking transmission of SIV_{mac} across the macaque vaginal mucosa.

The need to evaluate other novel therapies in the SIV or HIV-2 macaque models will become even more imperative when we learn more about the molecular mechanisms of pathogenesis and are able to pinpoint more specific viral and host targets for drug intervention. The potential clinical benefit of early treatment, at or before seroconversion, can best be defined in the macaque. Such antiretroviral treatment may help control not only SIV disease but also opportunistic infections such as mycobacteria (787). Convincing evidence, obtained in the SIV macaque model, that enhancing cellular immune responses (788) and lowering the initial virus level by anti-SIV drug treatment or vaccination (or a combination of both) leads to a significant restriction of virus replication and significant delay in disease onset, provides further impetus for early drug and vaccine intervention in HIV-1 infected persons.

Antiviral Treatment Against SHIVs

Some non-nucleoside RT inhibitors now in clinical trials (delaviridine, nevirapine) are active only against HIV-1 RT and not against SIV or HIV-2 RT (789). To test RT inhibitors of this type, recombinants of SIV bearing a functional HIV-1 RT gene (RT-SHIV) have been constructed and shown to be infectious and pathogenic in macaques, and acute infection of macaques with this RT-SHIV has been suppressed by pretreatment with a novel HIV-1-specific RT inhibitor (790,791). Short term (4 weeks) post-exposure prophylaxis (PEP) commencing 8–24 hours post infection reduced viral load to undetectable levels for a prolonged period of time (90 weeks) and led to long term disease protection. In cell culture, the non-nucleoside RT inhibitors selected for similar mutations in SHIV-RT as in HIV-1 RT (792). As expected, RT-SHIV was also similar to SIV in susceptibility to protease inhibitors (701). The RT-SHIV macaque model thus offers the opportunity to study HAART with all classes of drugs currently approved for therapy of HIV-1.

Macaques treated with PMPA for eight weeks starting 7–14 days after infection with RT SHIV had suppressed viral replication (793). After chemotherapy ended, two of the six monkeys continued to maintain a low virus load and were able to resist infection with a highly pathogenic heterologous SIV. This treatment thus mimicked a vaccination effect similar to that induced by live attenuated SIV. In one of two RT-SHIV chronically infected macaques, PMPA treatment caused a decrease in viremia, which was apparently sustained after SIV subunit vaccination (794), thus giving further support for a combined chemotherapy vaccination approach. Cynomologus macaques infected with RT-SHIV and treated with the non-nucleoside RT inhibitor nevirapine developed RT mutants which corresponded to the HIV-1 mutations in nevirapine resistant HIV-1 patients (795).

PEP with PMPA was able to reduce perinatal infection with SHIV33 env given IV and SIV_{mac251} given orally (796). PMPA given 1 week after infection with SHIV-env (KU) and continuing daily for 83 days led to a reduction of virus burden below the level of detection and stabilization of CD4+ T cell levels (797,798). After cessation of therapy, animals subsequently controlled the virus, maintained normal T-cell counts and developed virus specific neutralizing antibody, T-helper cells and CTLs. These results suggest that antiviral treatment, if started early during infection, might help the host mount an effective immune response. However, PEP with HAART could not completely protect macaques from SHIV infection (799). By contrast, topical vaginal administration of a broadly neutralizing human monoclonal antibody could protect macaques from SHIV infection through the vagina (800). SHIV 89.6 P intravaginal infection has also been inhibited by novel chemical virucides that might prove useful in humans as "chemical condoms" (801).

HIV-2 Macaque Model: Antiviral Therapy

Early postinfection antiviral therapy with an RT nucleoside inhibitor (d4T), even of limited duration, has proven effective for long time (>1 year) control of pathogenic HIV-2287 challenge infection in pigtailed macaques (802). PMPA was able to prevent intravaginal infection of pigtailed macaques with pathogenic HIV-2287, if treatment was initiated 12–72 hours following HIV-2 exposure and was continued for 28 days (803). HAART therapy suppressed the maternal virus load and prevented the mother to fetus transmission of HIV-2287 in pigtailed macaques, if treatment was started 30 minutes after HIV-2 inoculation (804). None of these studies were carried out long enough to test for the emergence of drug resistant HIV-2 mutants.

SIV and SHIV Macaque Models. Therapeutic or Passive Immunization

In contrast to the well established rationale for passive immunotherapy with antibody or immunoglobulin, the concept of actively immunizing already infected individuals with viral immunogens from the same kind of virus causing the infection is novel and controversial (805). Proven successes in humans of a combination of passive and active vaccination against rabies and hepatitis viruses before infection becomes established, and against genital herpes virus infection in chronically infected guinea pigs (806), suggested that HIV-seropositive, asymptomatic humans, who were still immunocompetent, might be able to respond immunologically to inoculation with HIV immunogens and thereby hold the virus in longer abeyance. Historically, previous efforts at postinfection immunization of non-immunosuppressive lentivirus infections in sheep and goats were not successful and caused enhanced disease (807). Whether or not such therapeutic vaccination strategies will be effective or harmful in treatment of the immunosuppression lentiviral diseases of cats, monkeys, and man remains uncertain. Initial efforts to evaluate the feasibility of this therapeutic strategy in chronically infected macaques, using either inactivated whole SIV or live attenuated SIV were unsuccessful (808,809). No detectable effect on virus titer, immune response or clinical course was noted, even when the immunogen was administered soon and repeatedly after infection. Results of phase I and II trials of an inactivated whole HIV-1 preparation and of recombinant HIV-1 env gp120 immunogens in HIV-1 seropositive humans, have indicated that some of the volunteers do indeed register antibody and cell-mediated immune responses to additional HIV epitopes, not recognized during the natural infection (810,811). However, phase III trials have shown no clinical beneÞt in terms of virus load, CD4 count, opportunistic infections, or survival (812). Of current interest is the possibility that active vaccination after potent antiviral therapy has markedly reduced the viral load may prove of beneÞt to infected individuals. Support for the notion has been demonstrated in the SIV macaque model (751,813).

Passive immunization experiments in the SIV macaque model have given conßicting results, probably attributable to differences in the challenge virus, host species, age and quality or quantity of antibody transferred. In the prototypic SIV_{mac} rhesus macaque model, passive transfer of high-titered neutralizing antibodies from asymptomatic carriers failed to protect against challenge infection with the homologous virus (568,569) and, in one study (568) may have enhanced infection. Passive protection that was achieved using antibodies from macaques immunized with inactivated whole SIV_{mac} vaccines was attributed to antihuman cellular antibodies rather than to neutralizing antiviral antibody.

Infusion of serum immunoglobulins (SIVIG) puriÞed from SIV_{mac251} infected macaques into other SIV_{mac251}-infected macaques undergoing a rapid disease course had only a modest, transient effect on SIV RNA and cell-associated virus load, an effect that was not attributable to neutralizing antibody (814). Interestingly, passive immunoglobulin transfer failed to protect African green monkeys from SIV_{agm} infection despite the presence of neutralizing and ADCC antibodies (330). On the other hand, partial or complete passive protection of adult macaques against SIV has been achieved in other clinical settings using SIVIG (815) or inactivated serum-from SIV-infected macaques (816,817), and in newborns challenged orally with SIV after subcutaneous administration of SIV hyperimmune serum (818). Newborn macaques were also protected by the passive transfer of antibodies from SIV vaccinated mothers (819). An extensive passive immunotherapy study of symptomatic HIV-1 infected individuals, showed only a minimal viral suppressive effect, and no evidence of disease acceleration (820).

Passive protection in the SHIV-env macaque model has proven successful using anti-HIV-1 neutralizing monoclonal antibodies and HIV-1 immunoglobulin, alone or together (821Ð824). Antibody treated macaques were either completely protected against infection or against disease manifestations of SHIV infection following IV, oral or vaginal challenge. However, to achieve sterile protection vs vaginal SHIV exposure required serum neutralizing antibody titers of about 1:400 (825). Best results were obtained using three human monoclonal antibodies together, a contribution that was previously shown to the synergistic *in vitro* (826). By contrast, anti-SHIV neutralizing serum that protected macaques against IV inoculated pathogenic SHIV failed to protect against intrarectal challenge with the same virus (827). Anti-SHIV antibodies obtained from an HIV-1 infected chimpanzee were able to protect macaques against iv challenge with a pathogenic SHIV but only if the antibodies had anti-SHIV neutralizing activity as determined *in vitro* (828). The titer of neutralizing antibody in the plasma calculated to protect 99% of virus challenged monkeys was 1: 38 (829).

Taken together these passive immunization experiments against SIVs and SHIV envs indicate that under appropriate lab condition, passive protection can be successful against systemic or mucosal virus exposure. Neutralizing antibodies and possibly ADCC antibodies can be effective when they are directed at a sufÞcient narrow window of envelope epitopes. Just as with active immunization, achieving passive protection against the diversity of HIV-1 genotypes in circulation worldwide remains a formidable obstacle.

HIV-2 Infection of Macaques and Baboons

HIV-2 strains are closely related to the SIV_{mac}/SIV_{sm} subgroup of primate lentiviruses and probably originated

within the last 40–300 years (830) from multiple cross-species transmissions of SIV to humans from feral sooty mangabeys in West Africa (33,831). HIV-2 infection is still largely confined to West Africa (832), where it has reached epidemic proportions, but it has begun to spread worldwide (833). In humans, HIV-2 infection is less pathogenic than HIV-1 (834), and HIV-2 infection may confer partial protection against HIV-1 coinfection (835), although this is by no means certain (836). *In vitro* evidence suggests that B-chemokine mediated resistance may contribute to HIV-2 protection against HIV-1 infection (837). Cross-reactive CTLs could also play a role in cross protection (838). The genetic diversity and prevalence of HIV-2 isolates suggest that some strains may represent dead-end infections (831). *In vitro*, the HIV-2 Env protein gp105 has stronger immunosuppressive properties than HIV-1 Env gp120 (839). *In vivo*, this effect could be beneficial to the host by dampening immunocellular activities and bursts of viral replication that characterize HIV-1 infection. HIV-2 infection of humans is covered in the chapter by Kanki in this volume.

Initially, several different strains of HIV-2, including molecular clones, were inoculated into different species of macaques, sooty mangabey, and baboons, and although causing either transient or persistent infection and humoral and cellular immune responses, failed to induce AIDS (840–842). As also seen in SIV-infected macaques and HIV-1 infected humans, the CTL recognition in HIV-2-infected macaques was very broad, comprising the major structural virion proteins as well as regulatory proteins (844). Compared to pathogenic infection of macaques with SIV or humans with HIV-1, HIV-2-infected asymptomatic macaques showed little viral variation and lacked neutralization escape mutants (845,846). Such animals also showed helper T cell proliferative response to infection (847). In several HIV-2-infected monkeys antiviral CTL activity was detected only in lymphoid tissues and not in blood; therefore monitoring of PBMC only may not adequately represent the situation *in vivo* (848).

By contrast, highly reproducible induction of unequivocal simian AIDS has been accomplished at the University of Washington Primate Center by serial passage of the EHO strain of HIV-2 in pigtailed macaques (*Macaca nemestrina*) (849). The pathogenic strain, designated HIV-2287 and the parental HIV-2_{EH0} strain both predominantly use the CXCR4 co-receptor but the 287 strain shows greatly enhanced replicative capacity in macaque PBMCs (850). Within 10–14 days post infection with HIV-2_{287} the lymph nodes especially in the gastrointestinal tracts show a dramatic shift from disseminated infected cells to virus concentrated in follicular centers with cell destruction (851). This shift in virus localization indicates a narrow window for therapeutic intervention at this early stage of HIV infection. This is also a useful model for maternal-neonatal transmission (852). Another chronic disease causing HIV-2 strain, HIV-2_{GB122}, was shed in semen of acutely infected pigtailed macaques, thus providing a basis for evaluating antiviral therapies targeting male genital tract expression (853). Importantly, several other HIV-2 $strains_{UC\text{-}2,\ UC\text{-}14}$ have induced persistent infection, loss of CD4 lymphocytes and AIDS-like symptoms including retroperitoneal fibromatosis in baboons (854,855). Serial transmission of HIV-2 (UC-2) in baboons has led to increased kinetics of viral replication in baboon PBMC and cytopathicity (856). Control of HIV-2 infection in baboons may in part be attributed to soluble antiviral factors released from CD8+ cells (857), and other effects of immune activation (858). It thus appears that, given the right virus-host combination, persistent infection and progression to AIDS can result from HIV-2 induction of pigtailed macaques and, possibly, baboons. Asymptomatic baboons preinfected with one strain of HIV-2 were resistant to superinfection with another strain of HIV-2 (859). Pigtailed macaques preinfected with one non-pathogenic strain of HIV-2 could be superinfected with a second non-pathogenic strain of HIV-2 only within 8 hours of the first infection (860). Molecular clones of HIV-2, infectious but non-pathogenic for macaques and baboons, are available (861,862). Both the HIV-2 287 pigtailed macaque and baboon HIV-2 models appear suitable for AIDS pathogenesis and vaccine studies (863).

Vaccination of Macaques vs. HIV-2

Partial, or complete, long-lasting (>1 year) protection of cynomolgous or rhesus macaques against homologous HIV-2 strains grown on macaque PBMC has been achieved by vaccines made of inactivated whole virus (864,865), inactivated whole virus plus booster immunization with V3-derived synthetic peptides (866), native envelope (867–869), recombinant env, gag, and pol expressed in attenuated vaccinia virus or canary pox virus (870–872), and by passive immunization with plasma from whole virus immunized monkeys (873). Partial protection or delayed infection in rhesus or cynomolgous macaques immunized with the attenuated vaccinia virus or canary pox recombinant plus protein or peptide boosts has been associated with an anamnestic neutralizing antibody response on challenge although no clear cut immunologic correlation with protection was apparent (872,874,875). Protection against a heterologous strain of HIV-2 was not achieved with another recombinant vaccinia HIV-2 vaccine (876). An HIV-2 recombinant salmonella vaccine given orally failed to protect macaques against the homologous virus (870). In all of these studies, the correlates of immune protection remained uncertain. In particular, there was no correlation between the levels of neutralizing antibodies or the presence of virus specific CTL and protection against infection with HIV-2 or SIV_{mac} in the HIV-2 vaccinated monkeys (871). In contrast to the immune protection conferred by inactivated whole SIV vaccines, antihuman cellular antibodies were not involved

in protection since the challenge virus was grown in allogeneic monkey cells. Prior infection of some but not all macaques with live attenuated HIV-$2_{SBL-6669}$ or HIV-2_{rod} prevented superinfection with pathogenic SIVs given IV or intrarectally and markedly delayed disease in those animals that were superinfected (663,877,878). Disease was similarly retarded in macaques infected with HIV-2 and superinfected with SIV_{mac}, which also reactivated the latent HIV-2 infection (879). This partial protection appeared to depend on a speciÞc proliferative T-cell response early after infection. By contrast, preinfection of macaques with the weakly pathogenic HIV-2rod isolate protected only one of six macaques from intrarectal infection with SIV_{mac}, but no protection from superinfection or progression of disease was evidenced in the other Þve monkeys (880). Vaccination of cynomolgus monkeys with a canary pox (ALVAC) HIV-2 vaccine followed by exposure to live HIV-2 induced cross protection against mucosal infection with SIV_{sm} which seemed more effective than immunization with live HIV-2 alone (881). Pigtailed macaques infected with a non-pathogenic HIV-2 molecular clone (HIV-2KR) were largely protected from superinfection with the highly pathogenic HIV-2287 strain (882,883). Protection appeared to correlate with strong CTL responses and antigen speciÞc T-helper type 1 responses (884). Of note, crossprotection against HIV-2 challenge was obtained in rhesus macaques by immunization with an HIV-1 recombinant poxvirus vaccine (NYVAC) (885). No clear correlates of protection were discerned. However, rhesus macaques immunized with NYVAC HIV-2 were not protected against SHIV challenge (886). This demonstration of at least partial experimental cross protection between HIV-1 and HIV-2 adds further evidence that viral variability may not be an insurmountable obstacle in the design of a global AIDS vaccine.

SHIV Infection of Macaques

Chimpanzees are the only animals susceptible to HIV-1 infection, but they do not develop disease, at least within 10 years after infection, and they are too impractical and limited in number for AIDS research. After initial unsuccessful efforts to infect rhesus and other macaque species persistently with HIV-1, it was found that pigtailed macaques (*Macaca nemestrina*) were more susceptible to infection with certain T-cell-adapted HIV-1 strains (887). However, further research conÞrmed that only transient (8Ð24 weeks) productive infection and no disease occurred despite a high dose HIV-1 inoculum. Moreover, the majority of primary HIV-isolates were unable to infect these monkeys (888).

Therefore, for the purpose of developing a non-human primate model for replication of HIV-1 genes on a SIV genetic background, many recombinants (chimeras) between SIV and HIV-1 (SHIVs) have been constructed in the last 10 years and tested for infectivity and pathogenesis in macaques and baboons. Nomenclature of some of the SHIVs, confusing because of the lack of systemic designation, and their tropism and pathogenicity for macaques is shown in Table 10.4. The Þrst recombinants replaced the *env*, *tat*, *rev* and *vpu* genes of SIV_{mac239} with their counterparts from HIV-1 T-cell tropic IIIB strain and related subgroup B strains (SHIV*env*) and were non-pathogenic for macaques and baboons (889Ð891). Other non-pathogenic or weakly pathogenic SHIV envs were constructed from macrophage-tropic as well as T-cell-tropic strains of HIV-1 (892Ð895). The initial non-pathogenic HIV-1 SHIVenv constructs induced a high level of acute viremia, a transient decline in CD4 T cells and a low-level persistent infection. In baboons, the SHIV *env* recombinants induced only a transient infection and no disease. SHIV env-infected macaques made antibodies to the HIV-1 env and SIV core antigens and cytotoxic T lymphocytes speciÞc for HIV-1 env glycoprotein. In general, the viral load of the non-pathogenic SHIV-env recombinants in rhesus macaques was 10Ð100 times greater than that of HIV-1 IIIB infection of pigtailed macaques (887) and equal to the viral load seen during the acute stage of infection if rhesus macaques with SIV_{mac}, but after six to eight weeks, the level of SHIV-env infection dropped signiÞcantly. Virus persisted for more than one year, as demonstrated by PCR ampliÞcation of viral DNA in all animals and by virus isolation in some animals.

After serial passage (1Ð3X) of blood or bone marrow in macaques, at least Þve of the initially non-pathogenic SHIV envs reverted to virulence (896Ð902). These monkeys had a persistently high virus load, profound lymphoid activation (903,904), precipitous and irreversible fall of CD4 T cells in blood, lymph node and gut, and developed AIDS in two to four months. This dramatic loss of CD4+T cells was more severe than seen with HIV-1 infection of humans and its pathogenesis remains uncertain. Sequence analysis of SHIVs recovered from monkeys with accelerated AIDS revealed HIV-env mutations associated with escape from neutralizing antibodies and tissue speciÞc selection of HIV-1 env genotypes (905). These env mutations were also associated with increased chemokine receptor binding, increased CD4 cell apoptosis (906), and increased membrane-fusing capacity (907).

Pathogenic SHIVs have also been derived by SIV recombinants with HIV-1 RT (RT-SHIV) (902,908) (see above) and HIV-1 Nef (SHIV-nef) (909Ð911). Non-pathogenic SHIVs or weakly pathogenic SHIVs have been constructed from HIV-1 env subgroup A (912), HIV-1 env subgroup E (912,913), HIV-1 env subgroup C (914,915) and HIV-2 core and env (916). In baboons, one HIV-1 subgroup C SHIV env construct caused persistent infection but no disease (917).

Although useful for experimental transmission by various routes including mucosal (918Ð921), and for pathogenesis (905,922,923) and antiviral studies (see

TABLE 10.4. *SIV/HIV Recombinants (SHIVs)*

Designation	HIV-1 env	Co-receptor Usage	Pathogenic for Macaques	Reference
Subgroup B				
HXBc2	Cell line IIIB	X4	-	889
4	Cell line IIIB	X4	-	890
ACH320	Cell line Europe Isolates	X4	-	891, 892
Han-2	Cell line Europe Isolates	X4	-	891, 892
W6–1D	Cell line Europe Isolates	X4	-	891, 892
Sbg	Cell line IIIB	X4	-	896
KU1.2	serial passage of HXBc2	X4	+	897
89.6	primary isolate	X4, R5	-	893
89.6 P	serial passage	X4, R5	+	894
SF33	cell line	X4		895
SF33 a	serial passage	X4	+	898
SF13	cell line	X4	-	891
SF162	cell line	X4	-	895
SF162 P	serial passage	X4	+	901
DH12	primary isolate	X4, R5	-	899
DH12R	serial passage	X4, R5	+	900
Subgroup A				
SF170	Africa			912
Q23	Africa			912
Subgroup E				
CAR 402	primary isolate—Africa	X4	-	912
9466.33	primary isolate—Thailand	X4	-	1027
Subgroup C				
CHN 19	primary isolate—China	R5	-	914
MJ4	primary isolate—Africa	R5	-	915
	HIV-2 env, gag, pol	X4	-	916
SHIV RT	**SIV_{mac} env**	X4, R5	+	790, 792
SHIV nef	**SIV_{mac} env**	X4, R5	+	909–911

X4 = T-cell-tropic
R5 = macrophage-tropic
"-" = non-pathogenic
" + " = pathogenic

above), SHIVs Ð envs have been employed primarily for vaccine research directed at inducing immunity against HIV-env determinants. Particularly useful will be the SHIVs bearing HIV-1 env glycoproteins from non-B subgroups found commonly in Africa and Asia. In general, live attenuated SIV or SHIV envs or HIV-1 env ± gag or tat recombinant proteins have constituted the vaccines against challenge with non-pathogenic or pathogenic SHIV envs or pathogenic SIV. Not surprisingly, vaccine partial protection has been easier to achieve: (1) with live attenuated virus rather than recombinant vaccines; (2) against non-pathogenic rather than pathogenic SHIV variants; (3) and against homologous rather than heterologous SHIV challenge (Tables 10.5 and 10.6). Protection has been achieved against systemic or mucosal challenge and has been characterized by a persistent and signiÞcant (approximately 2Ð3 log) reduction of viral load and lack of disease (< 6 months) compared to sham vaccinated controls. To date, the vaccine and challenge HIV-1 env antigens have been homologous and from subgroup B isolates. In line with most SIV macaque vaccine studies no consistent correlates of protection have become apparent. Humoral, cellular and innate immunity all contribute to protection but broad protection against the diversity of viral variants faced in nature remains a formidable vaccine obstacle yet to be overcome.

SigniÞcant protection was achieved by SHIV env DNA vaccines against pathogenic SHIV env challenge (936,937). SHIV 89.6P challenge followed immunization with DNA expressing homologous SIV core and HIV-1 subgroup B env proteins. Boosts were given with either an IL-2/Ig fusion protein (936) or vaccinia vectors expressing the same immunogens (937). In the Þrst study (936), all IV challenged monkeys became infected but suppressed the virus and suffered no clinical disease for at least 140 days after challenge by which time half of the controls had developed SAIDS. In the second study (937), macaques were similarly partially protected against intrarectal challenge given seven months after the last boost. The virus was still under control 70 weeks post challenge (962). A modiÞed vaccinia (Ankara) SIV vaccine alone appeared to achieve protection equal to that of the monkeys Þrst

TABLE 10.5. *Vaccines conferring partial protection[1] against pathogenic SIV or SHIV challenge*

Vaccine	Challenge	Reference
Live Attenuated SHIV (IIIB)	SIV_{sm} IR	924
Live Attenuated SHIV (IIIB)	SIV_{mac239} Ivag	925, 926
Live Attenuated SIV_{mac} Δnef	SHIV 89.6P IV	927–931
HIV-1 Tat protein	SHIV 89.6P IV	932, 933
Live Attenuated SHIV (hu)	SHIV 89.6P IV	934
HIV-1 Tat DNA + CpG motifs	SHIV 89.6P IV	935
	SHIV 89.6 DNA + boosts	
	SHIV 89.6P IV	
	SHIV 89.6 IR	936,937
Live Attenuated ΔSIV or ΔSHIV	SHIV KU-1 IV	
	SHIV KU-1 Ivag	938,939
Live Attenuated ΔSIV or ΔSHIV	SHIV 89.6P Ivag	940
HIV-1 phage-displayed epitopes	SHIV 89.6P Ivag	941
HIV-1 env ΔV2 DNA	SHIV SF162P4	942
SHIV DNA + HPV-SHIV VLP	SHIV 12R IR	943
Live Attenuated SHIV 89.6	SIV_{mac239} Ivag	944
Ankara VV/SHIV	SHIV 89.6 IV	945, 946
SIV peptide	SHIV KU-2 IR	947
Adenovirus/SIV gag	SHIV 89.6P IV	594
Δ SHIV + rgag	SHIV 89.6P IV	948
HIV-1 r env, nef, tat + SIV nef	SHIV 89.6P IV	949
VV HIV-1 gag, env + HIV-1 and SIV IWV	SHIV IV	950
rHIV-1 Tat, rev ± Tat/Rev DNA	SHIV IR	951
HIV-1 env peptides	SHIV KU-2 IV	952

[1] Partial protection, in general implies a decrease of 1–2 logs in post-challenge acute phase viremia compared to controls
VV = vaccinia virus
Ivag = intravaginal
IR = intrarectal
IV = intravenous
Hu = human
HPV = human papilloma virus
VLPs = virus-like particles
Δ = mutation(s)
r = recombinant

TABLE 10.6. *Vaccines not conferring protection against pathogenic SIV or SHIV challenge*

Vaccine	Challenge	Reference
HIV-1 tat peptide	SHIV SF33A IV	826
Live Attenuated SHIV (IIIB)	SIV_{mac239} IV	827
HIV-1 V3 peptide	SHIV 89.6 P IV	828
HIV-1 rgag (Salmonella)	SHIV 89.6 P IV	829
HIV-1 rgp140/LN*	SHIV 89.6 P IR	830
HIV-1 renv	SHIV 89.6 P IV	831
HIV-1 renv	SHIV KU-2 IV	832

r = recombinant
IV = intravenous
* injected into iliac lymph nodes
IR = intrarectal

primed with SIV DNA (963). The same DNA + boost vaccines without env DNA did not protect as well (964). Evidence suggested that protection was mediated mainly by cellular immunity, viz, CTLs, but CD8 T cell depletion studies showed that neutralizing antibodies also contributed to virus control (966). Another SHIV DNA prime protein boost vaccine also showed some efÞcacy vs. pathogenic SHIV challenge (966). These and other SIV and SHIV vaccine results, raise hope that prophylactic HIV-1 vaccines may signiÞcantly prolong life of HIV-1 exposed humans even if they do not prevent infection. Unfortunately, these vaccines may fail because of the eventual development of viral CTL escape variants (967). However, it remains to be seen whether the same

beneÞcial effects on virus load and disease will be observed in vaccinated humans exposed to more heterogenous virus mixtures and where the pathogenesis may differ somewhat from the highly virulent, artiÞcial SHIV model.

SIMIAN FOAMY VIRUSES

Simian foamy viruses (SFV), members of the spumavirus genus of retroviruses, are prevalent in a variety of non-human primates and other animal species, including cats, hamsters, sea lions, horses and cows, in which they cause persistent latent and asymptomatic infections (968, 969). Non-human primates known to harbor multiple strains (serotypes) of these viruses include prosimians, macaques, African green monkeys, baboons, mandrills, apes and chimpanzees. In their natural host, these viruses are highly prevalent and the latent infection occurs in the presence of neutralizing antibody. Proviral DNA without detectable viral RNA or protein expression can be detected in all organs of the natural hosts and in peripheral blood lymphocytes, especially CD8+ T-lymphocytes, whereas minimal virus replication is restricted to the oral mucosa (970Ð972). Despite Þnding the same SFV serotype in different species, genetic comparisons imply that SFVs have generally coevolved with their hosts, rather than spreading between species (973Ð976). Although a putative human foamy virus (HFV) isolate was reported in 1971, it now seems, based on the lack of further independently derived human foamy virus isolates, and on close genomic and functional similarities between HVF and the chimpanzee foamy virus (SFVcpz), that HFV represents a human cell culture contaminant with a foamy virus from chimpanzee (977,978). Indeed, HFV has recently been renamed SFVcpz (hu) to identify its origin from a chimpanzee. Screening of over 10,000 human sera from all over the world using current serologic assays and PCR probes has failed to conÞrm any foamy virus prevalence in man (979). Moreover, the reported association of human foamy virus in the pathogenesis of Graves disease, autoimmune thyroiditis, chronic fatigue syndrome, amyotrophic lateral sclerosis, or as a cofactor in AIDS, could not be conÞrmed. However, this screening revealed a few cases of accidental human infection after monkey bites or laboratory contamination, thereby indicating that humans are susceptible to primate foamy viruses. Among 231 humans occupationally exposed to non-human primates in U.S. and Canadian primate facilities, four individuals (1.8%) were found infected with SFV from an African green monkey (one person) (980) and baboons (three people) (981). Among 133 zoo keepers who worked with primates, 4 individuals (3%) were seropositive for SFV, primarily with chimp-like viruses (982). Two of 46 primate facility workers were latently infected with macaque foamy virus (983). However, SFV infection was not detected in any of 17 hunters exposed to wild monkeys in West Africa (984). Importantly, no health problems were associated with these accidental infections, and no evidence was found for human-to-human transmission. Following accidental infection of humans, SFV persists as proviral DNA without any production of infectious virus (980,983). In the future, we cannot, of course, ignore the risks of SFV infection in transplant recipients of baboon organs (985).

In vitro, the foamy viruses (FV) are highly cytopathic, i.e. syncytia inducing, and have a broad host range, including cultured epithelial, Þbroblast and lymphoid cells. In common with oncoviruses, but unlike lentiviruses, SFV requires host cell proliferation for productive infection (986). The virus can be isolated from coculture of several organs and tissues including lymph nodes, brain and PBLs. Apoptosis of Þbroblast or lymphoid cell *in vitro* can be induced by SFV-1 (987). The fusogenic property of all foamy viruses is controlled by an evolutionarily conserved postively charged amino acid in the membrane spanning domain of the virus envelope protein (988). A critical cleavage site in HFV env controls virus maturation, infectivity and syncytia formation (989). Fine structural analysis of HFV env glycoprotein reveals a trimer with long tapering spikes 14 nm in length (990), which accounts for their characteristics appearance under the electron microscope. The cellular receptors for SFVs have not yet been identiÞed although they are ubiquitous and speciÞcally bind recombinant SFV envelope protein (991). Integration of SFV_{cpz} (hu) intact proviral DNA into HFV-infected human erythroleukemia-derived cells (H92) occurs at multiple independent sites on different chromosomes (992). Although these viruses have not been causatively linked to any histopathological changes or disease in any species (2), rabbits experimentally infected with SFV appeared to become transiently immunosuppressed (993), and transgenic mice expressing FV genes have developed neurological disease (994,995). The major signiÞcance of these viruses has been the annoying cytopathic effect that they cause *in vitro*. The derivation of herpes-virus saimirii-transformed T-cell lines from macaques, a necessary research tool for studies of cellular immunity in simian AIDS, has been markedly hampered by the reactivation of SFV with consequent cytopathic effect (996). Also, because they are retroviruses with reverse transcriptase activity, foamy viruses are apt to cause false alarms in the search for other potentially pathogenic retroviruses such as SRV, SIV or STLV. FV replication is resistant to most nucleoside analog RT inhibitors. Only zidovudine was equally efÞcient against HIV-1 and SFV (997,998).

The molecular biology of foamy viruses (FV) has revealed interesting and unique features of their replicative cycle and functional differences, including the method of packaging genomic RNA, the mechanism of pol expression and aspects of regulating viral gene expression (2). The genomes of simian foamy virus type 1 (SFV-1) and type 3 (SFV-3) isolates obtained from a rhesus macaque and African green monkey, respectively, have been molecularly cloned, completely sequenced, and compared

to the sequences of the cloned SIV_{cpz} (hu) (999–1002). More recently, another strain of SFV from the Taiwanese-*Macaca cyclopus* was isolated, cloned and sequenced (1003). The genomes of SFV-1 and SIV_{cpz} are closely related, with about 80% homology in the *pol* genes and about 70% homology in the *env* gene. The SFV-1 LTR is unusually long (1621 base pairs) and shows about 85% homology with SIV_{cpz} in the R and U5 regions but considerable divergence in the U3 region (25% homology). The FV genome encodes a large open reading frame (ORF) beyond the *env* gene that has been shown by transient expression assays *in vitro* to encode a transcriptional transactivator (*Tas*, also known as bel-l in SIV_{cpz} (hu). Tas is a DNA binding protein that acts on two separate and independent promoters, one in the U3 portion of the LTR and the other internal in the 3′ portion of the envelope gene (1004). Differential use of the LTR and internal promoters (IP) for initiating differently sized genomic transcripts appears to be a means of temporal regulation of foamy virus gene expression in different cell types (1005–1008). The IP direct expression of Tas and a second accessory protein, Bet, which functions as a negative regulator of basal IP activity (1009). The ony known inhibitor of FV replication, the promyelocytic leukemia protein, which binds Tas, does not mediate FV latency *in vitro* (1010). In lytic infections both the LTR and IP are efficiently transactivated by *Tas*, while in persistent latent infections, the IP is efficiently transactivated by *Tas*, but the LTR is not (1011). The DNA target elements in the IP which bind *Tas* and allow its transactivating function were further defined (1012). The SFV-1 tas DNA binding site has been narrowed down to a 25 bp consensus sequence in the IP (1013). A *Tas*-responsive enhancer element was recently identified at the 3′-end of *gag*, immediately upstream of the *gag-pol* overlap region (1014). Mutational analysis of the 5′ leaders region of SFV-1 has further defined separate *cis*-acting elements required for genome dimerization and packaging (1015). Certain cellular genes are also induced by Tas in HFV-infected human cells (1016).

An understanding of the regulation of FV gene expression will further our understanding of the molecular mechanisms that control retrovirus latency, persistence, and replication in the host animal. Also, because of their efficiency of transfection and large packaging capacity, non-infectious FV vectors made by replacing the *Tas* gene, a portion of the envelope gene and other modifications, have proven to be efficient gene transfer vehicles and, thus, may prove useful for a wide variety of gene transfer applications (1017,1018). At least two *cis*-acting elements, one in the leader region, one in the *pol* gene, are required for SFV-1 vector construction (1019,1020). The first stable packaging cell lines for SFV-1 vector are now available, a step toward the use of SFV-1 vector delivery systems for gene therapy (1021). A minimum SFV-1 genome vector system capable of accommodating a minimum 8930 base pair heterologous DNA fragment has been constructed (1022). SFV vectors have been able to stably transduce marker genes into the host cell genomic DNA *in vitro* (1023,1024), and an SFV-1 vector has successfully transduced human umbilical cord blood, CD34+ cells capable of repopulating NOD/SCID mice (1025,1026).

GLOSSARY

Primate Retroviruses

TRV = Tree shrew endogenous virus
BaEV = Baboon endogenous virus
Mac-1 = Stumptail macaque endogenous virus, strain 1
MMC-1 = Rhesus macaque endogenous virus, strain 1
CPC-1 = Colobus monkey endogenous virus, strain 1
OWC-1 = Owl monkey endogenous virus, strain 1
GaLV = Gibbon ape leukemia virus
SSAV/SSV = Simian sarcoma associated virus/simian sarcoma virus
STLV-1 = Simian T-lymphotropic virus, strain 1
HTLV-1 = Human T-lymphotropic virus, strain 1
STLV-2 = Simian T-lymphotropic virus, strain 2
HTLV-2 = Human T-lymphotropic virus, strain 2
SRV = Simian Type D retroviruses, strains 1–6
MPMV = Mason Pfizer monkey virus (SRV-3)
Po-1-Lu = Langur (Presbytis) endogenous virus
SMRV = Squirrel monkey endogenous retrovirus
HIV-1 = Human Immunodeficiency Virus, Strain 1
HIV-2 = Human Immunodeficiency Virus, Strain 2
SIV = Simian immunodeficiency virus
SFV = Simian foamy virus
HFV = Human foamy virus
SAIDS = Simian acquired immunodeficiency syndrome
HERV = Human endogenous retrovirus
SERV = Simian endogenous retrovirus
PcEV = Papiocynocephaleus retrovirus

Non-primate Retroviruses

MuLV = Murine leukemia virus
MMTV = Murine mammary tumor virus
ALV = Avian leukosis virus
BLV = Bovine leukemia virus
BIV = Bovine immunodeficiency virus
FeLV = Feline leukemia virus
FIV = Feline immunodeficiency virus
EIAV = Equine infectious anemia virus
SMRV = Squirrel monkey retrovirus
IAP = Intracisternal A particle
CAEV = Caprine arthritic encephalitis virus
KoRV = Koala retrovirus
TuERV = Bushtail possum endogenous retrovirus
RD114 = Feline endogenous retrovirus
VMV = Visna maede virus
RSV = Rous sarcoma virus

Antiviral Therapy

AZT = 3′-azido-3′-deoxythymidine
ddC = 2′,3′-dideoxycytidine
ddI = 2′3′dideoxyinosine
d4T = 2′3′-didehydro-2′Ð3′-dideoxythymidine
PMEA = 9-(2 phosphonylmethoxyethyl)adenine
PMPA = 9-(2 phosphonylmethoxypropyl)adenine
FLT = ßurodeoxythymidine

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III

IMMUNOLOGY OF HIV INFECTION

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Chapter 11

Immunode ciency in HIV -1 Infection

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Infection with the human immunodeÞciency virus type 1 (HIV-1) (1,2) initiates an intricate and fascinating series of host-virus interactions, the ultimate consequence of which is profound impairment of the host immune system. The extraordinary complexity of the host-virus interactions in HIV-1 infection results in part from the fact that HIV-1 infects two cell types that are involved in virtually all immune responses, CD4 + T lymphocytes and cells of the monocyte-macrophage lineage. How virus infection of these cell types leads to immunodeÞciency is the central question in AIDS immunology.

The acquired immunodeÞciency syndrome (AIDS) (3Ð7) resulting from HIV-1 infection is characterized by numerous immunologic abnormalities, the most prominent of which are severe quantitative and qualitative defects in the CD4 + T lymphocyte compartment. In their initial clinical studies describing an acquired immunodeÞciency condition in homosexual men, Gottlieb et al. (5) made the fundamental observation that affected individuals had a decreased concentration of CD4 + T cells in the peripheral blood. Subsequently, prospective studies have shown that much of the decline in CD4 + T cell counts occurs during the asymptomatic period between initial infection and the development of clinical immunodeÞciency. In adults, the average length of this asymptomatic period is 8Ð10 years. Opportunistic infections usually do not occur until the CD4 + T cell count has dropped from the normal level of 1,000 cells/μl. to below 200 cells/μl. Several studies have shown that the degree of loss of CD4 + T cells is an excellent predictor of progression to AIDS (8Ð11), and a CD4 + T cell count below 200 cells/μl is considered to be an AIDS-deÞning condition. As the CD4 count drops below 200 cells/μl, susceptibility to particular opportunistic infections appears in a surprisingly predictable way. Susceptibility to some infections such *Pneumocytsis carinii* pneumonia appears as the CD4 count falls below 200 cells/μl, while other infections are seen only in patients whose CD4 counts have fallen to below 100 cells/μl (disseminated *Mycobacterium avium* complex infection) or 50 cells/μl (cytomegalovirus retinitis). These Þndings suggest that the loss of CD4 + T cells is central to the development of clinical immunodeÞciency. Therefore, understanding the mechanism of CD4 depletion is the key problem in AIDS pathogenesis. In addition to the quantitative defects in the CD4 + T cell compartment, HIV-1-infected individuals also show defects in the functional capacity of the surviving CD4 + T cell population (12).

Although the numerical and functional defects in the CD4 + T cell compartment are particularly dramatic, functional defects in other cell types also occur in the disease. This chapter will consider recent advances in understanding the molecular mechanisms by which HIV-1 infection produces immunodeÞciency, focusing in particular on quantitative and qualitative defects in the CD4 + T cell compartment. Two questions will be considered in detail. First, what causes the loss of CD4 + T cells in HIV-1 infection? Second, what factors are responsible for the functional defects observed in those CD4 + T cells that are not depleted? As a prelude to a discussion of the mechanisms responsible for immunodeÞciency in HIV-1 infection, the molecular mechanism and cellular dynamics of HIV-1 infection of CD4 + cells are considered. The immune response to HIV-1 and the reasons that this response eventually fails to control the infection are also discussed. For recent reviews of the immunopathogenesis of HIV-1 infection, the reader is referred to the following articles (13,14).

MOLECULAR MECHANISM OF HIV-1 INFECTION OF CD4 + CELLS

HIV-1 selectively infects host cells expressing CD4, a 55 kd protein found on one subset of mature T lymphocytes. CD4 is also expressed on some subpopulations of

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thymocytes and at low levels on cells of the monocyte-macrophage lineage (15). The tropism of HIV-1 for CD4+ cells results from the fact that the HIV-1 envelope (env) glycoprotein has a high afÞnity for CD4 (16Ð19). The env protein consists of two subunits. The gp120 subunit, which contains the binding site for CD4, is present on the exterior of the virus particle where it is non-covalently associated with the second env subunit, the transmembrane protein gp41. The Kd for the CD4-gp120 interaction is in the nanomolar range. Oligomers of three gp120/gp41 complexes comprise the ÒspikesÓ seen to be protruding from the virion surface in electron micrographs. The high afÞnity of HIV-1 gp120 for CD4 permits viral attachment to CD4+ T cells and macrophages. This strong binding reaction is the critical initial step in the life cycle of the virus. As is discussed below, HIV-1 entry also requires in addition to CD4 a Òco-receptorÓ Although some cell types that do not express CD4 can be infected by HIV-1 *in vitro*, the infection appears to be limited to CD4+ cells *in vivo*.

Entry of the viral genetic information into the host cell cytoplasm requires fusion of the viral envelope with the host cell membrane. This membrane fusion event is triggered by a conformation change in the env protein that exposes a hydrophobic fusion domain located at the N terminus of gp41 (Fig. 11.1). This hydrophobic domain inserts into the membrane of the target cell, initiating the fusion of the viral envelope with the host cell membrane. For many other enveloped RNA viruses such as inßuenza, fusion occurs only after uptake of the virus particle into a low pH endocytic compartment. In the case of inßuenza virus, low pH triggers a conformational change in the inßuenza virus hemagglutinin that exposes a buried hydrophobic domain. This domain inserts into the membrane of the endosome. In contrast, entry of HIV-1 occurs at neutral pH and does not require endocytosis of the virus particle (20). Following binding of HIV-1 to CD4, the next step is a conformation change that allows binding to the Òco-receptorÓ Subsequently, there is a conformation

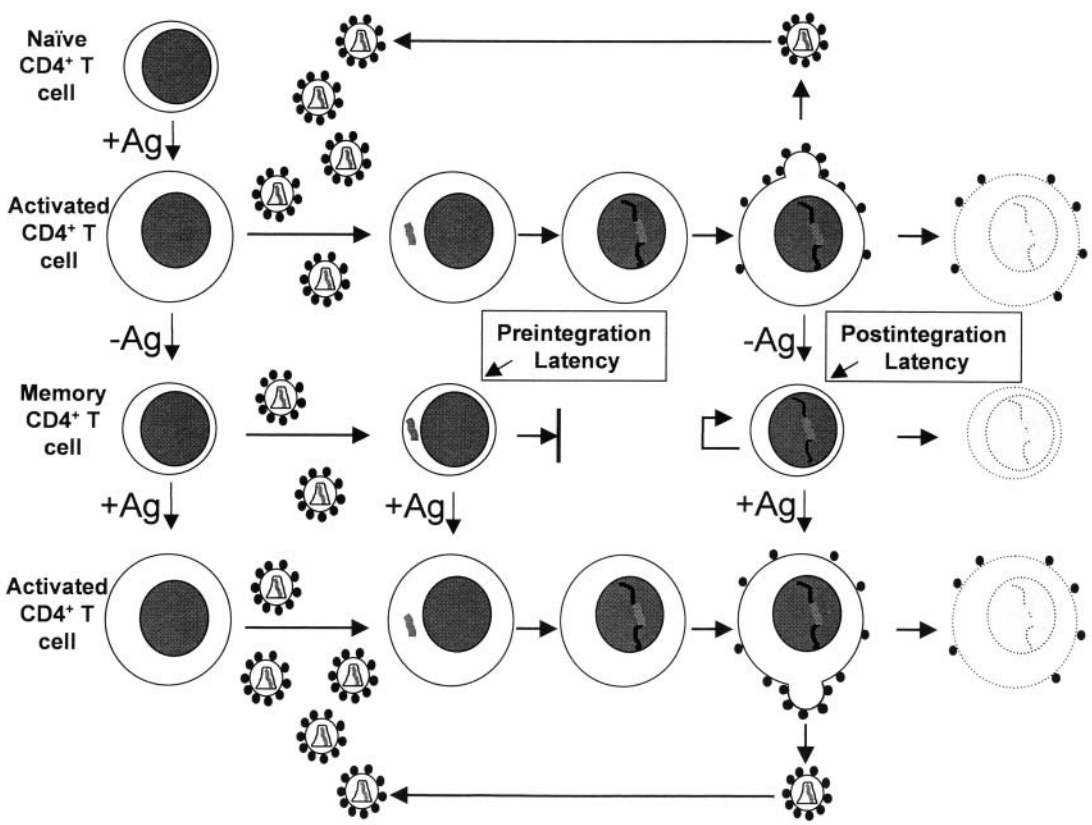

FIG. 11.1. Mechanism of infection. Step 1: HIV 1 attaches to CD4+ cells following high af nity interactions between the gp120 subunit of the env protein and CD4. The Kd of the binding reaction between gp120 and CD4 is $4 \times 10–9$ M. Note that on the surfaces of infected cells and HIV 1 virions, the env protein likely exists in the form of multimers of 3–4 gp120- gp41 complexes. It is probable that the co-receptors also play some role in the initial binding. Step 2: Binding of gp120 to CD4 and to the co-receptor induces a conformational change in the gp120-gp41 complex, resulting in exposure of the N-terminal hydrophobic domain of gp41. This hydrophobic domain inserts into the target cell membrane. Note that the chemokine receptor CCR5 can serve as a co-receptor for entry of macrophage-tropic isolates of HIV-1. Step 3: Fusion of the viral envelope with the plasma membrane and delivery of the viral capsid into the cytoplasm of the CD4+ cell. These events occur at neutral pH and do not require endocytosis of the virion prior to fusion.

change in the transmembrane subunit of the env protein (gp41) in which two alpha helical domains of the protein snap together, resulting in an intimate approximation of the viral envelope and the cell membrane and subsequent membrane fusion (Fig. 11.1).

One of the most exciting recent discoveries in the AIDS field has been the identification of "co-receptors" for HIV-1 entry that appear to serve as triggers for the fusion reaction. It has been clear for some time that the fusion reaction requires another host cell protein in addition to CD4. Mouse cells transfected with human CD4 can bind HIV-1 virions, but successful fusion and entry does not take place, presumably because the human version of this "co-receptor" is not present (19). In 1996, Edward Berger and colleagues showed that a seven transmembrane domain protein called CXCR4 or fusin, functions as a co-receptor for certain isolates of HIV-1 (21). CXCR4 is a member of a family of membrane proteins which serve as receptors for chemotactic cytokines (chemokines). Co-expression of human CD4 and CXCR4 allows infection of non-human cell types by certain isolates of HIV-1.

The discovery that chemokine receptors function as co-receptors for HIV-1 entry has also provided a coherent explanation for three seemingly unrelated sets of observations in the AIDS field. The first of these is that CD8+ T cells from HIV-1-infected donors can suppress virus replication *in vitro* through a non-cytolytic mechanism (22–24). It is now clear that the elaboration of chemokines by CD8+ T cells may account for the suppressive effect (25–27). Chemokines such as RANTES, MIP-1a, MIP-1b, and SDF-1 have been shown to suppress HIV-1 replication, presumably because interaction of these chemokines with their receptors on CD4+ T cells prevents an interaction of the HIV-1 env protein with chemokine receptors that is essential for fusion. Although dramatic suppression of HIV-1 replication *in vitro* can be achieved using chemokines, it is still unclear whether chemokines play a significant role in suppressing viral replication *in vivo*.

The second important phenomenon related to HIV-1 co-receptors involves differences in the capacity of different HIV-1 isolates to infect different types of CD4+ cells. Virus transmitted by the sexual route has the ability to infect activated T cells and macrophages and was referred to in the older literature as macrophage-tropic. In some infected individuals, sequence variation in the env protein gives rise to variants that infect CD4+ T cells but not macrophages (orignally termed T cell-tropic). Following the discovery of chemokine receptors as co-receptors for HIV-1, it rapidly became clear that macrophage-tropic viruses use different chemokine receptors than T cell-tropic viruses. The chemokine receptor CCR5 is a major co-receptor for macrophage-tropic viruses (28–30), while T cell-tropic viruses utilize CXCR4. Thus viral tropism reflects co-receptor usage (Table 11.1). In the new nomenclature, viruses that utilize CCR5 are referred to as R5 viruses while viruses that utilize CXCR4 are called X4 viruses. The "switch" from R5 to X4 viruses that is seen in some patients is associated with more rapid disease progression, perhaps reflecting the fact that X4 viruses are more pathogenic and/or infect a wider variety of cells (31,32). Infection by R5 viruses is inhibited by the chemokines that bind to CCR5 (RANTES, MIP-1a, MIP-1b), while infection by X4 viruses is inhibited by chemokines that bind to CXCR4 (SDF-1).

A third phenomenon that can now be at least partially explained in terms of co-receptors is the phenomenon of resistance to HIV-1 infection in certain repeatedly exposed individuals (33). A fraction of these individuals are homozygous for a 32 base pair deletion in the gene encoding CCR5 (34,35). This deletion is frequent in Caucasians, with 13% of the population being heterozygous and 1% being homozygous. There is no obvious immunologic impairment in individuals who are homozygous or heterozygous for the defective CCR5 gene. Homozygotes, but not heterozygotes, are protected from infection by the frequently transmitted R5 forms of HIV-1.

Because interactions between the HIV-1 env protein and its cellular receptor (CD4) and co-receptors (CXCR4, CCR5) are the critical initial events in the life cycle of the virus, they represent logical targets for therapeutic intervention. One conceptually attractive approach involves the use of recombinant soluble forms of CD4 which should bind to gp120 on free virus particles and thereby interfere with the initial step in infection. Clinical trials of various forms of soluble CD4 have thus far been disappointing. One problem is that clinical isolates of HIV-1 are less

TABLE 11.1. *Co-receptors for HIV-1 entry (see text for references).*

	Important HIV-1 Co-receptors	
Property	CCRS	CXCR4
Family	CC chemokine receptor family	CXC chemokine receptor family
Ligands	CC (β) chemokines (RANTES, MIP-1a, MIP-1b)	CXC (a) chemokines (SDF-1)
Expressed on CD4+ T cells	Yes (primarily on activated T cells)	Yes (resting and activated)
Expressed on macrophages	Yes	Not in a functional form
Used by	R5 ("macrophage-tropic") HIV-1 isolates	X4 ("T cell-tropic") HIV-1 isolates
Resistance conferring mutations	32 bp deletion in 1% of caucasians	?

susceptible to inhibition by soluble CD4 than are laboratory strains, for reasons that are not entirely clear (36). New multivalent forms of soluble CD4 are being developed. A very promising new drug called T20 interferes with the rearrangements of alpha helical domains of gp41 during fusion. Other new drugs in development block virus binding to CCR5 and CXCR4. These entry inhibitors are discussed in more detail elsewhere in this volume.

THE CELLULAR DYNAMICS OF HIV-1 INFECTION

As a result of the high afÞnity of gp120 for CD4, HIV-1 virions can attach to any CD4+ cell. Fusion occurs if appropriate co-receptors are expressed. However, the course of subsequent events in viral replication depends upon the cell type (CD4+ T cell vs. macrophage) and the state of activation of the cell.

The cellular dynamics of infection of CD4+ T lymphocytes by HIV-1 are illustrated in Fig. 11.2. In the periphery, the majority of mature CD4+ T lymphocytes are in a resting state. Activated T cells normally represent only a minority of cells in the peripheral T cell pool, although the proportion of activated cells is increased in infected individuals and increases with disease progression. HIV-1 virions can bind to and fuse with both resting and activated CD4+ T cells. The next step in the virus life cycle is reverse transcription of the viral genomic RNA by the reverse transcriptase (RT) enzyme carried by the virion. RT is a component of the high molecular weight preintegration complex that provides a structural framework for the reverse transcription and integration reactions. Reverse transcription can occur in both resting and activated T cells (37), but the next step, entry of the preintegration complex into the nucleus, does not occur in resting T cells, possibly due to the absence in resting cells of sufÞcient metabolic energy for transport of the complex through the nuclear pores (38). Thus, in resting T cells, partially or completely reverse transcribed HIV-1 genomes reside in the cytoplasm for a Þnite period of time (days) before being degraded. If the T cell is activated by antigen before the preintegration complex (PIC) becomes nonfunctional, then the subsequent steps of nuclear import, integration into host chromosomes, virus gene expression, and release of infectious virions can occur. In this sense, resting T cells carrying unintegrated HIV-1 DNA represent a labile reservoir for the virus (39,40). In asymptomatic HIV-1-infected individuals, most of the viral DNA present in circulating CD4+ T cells is in this unintegrated form (40Ð42). Because transcription of unintegrated viral DNA in the cytoplasm cannot occur, latently infected cells carrying this form of viral DNA presumably escape detection by immunologic mechanisms.

Following encounter with antigen, resting naive or memory CD4+ T cells undergo blast transformation and enter a state in which they are highly susceptible to productive infection by HIV-1. In activated T cells, there is no block to nuclear import and the infection progresses rapidly to integration, viral gene expression, and virus production. The HIV-1 LTR has sequence elements capable of binding some of the same host transcription factors that are upregulated in activated T cells (43,44). NFκB seems to be particularly important in this regard. As a result, viral gene expression from the HIV-1 LTR occurs

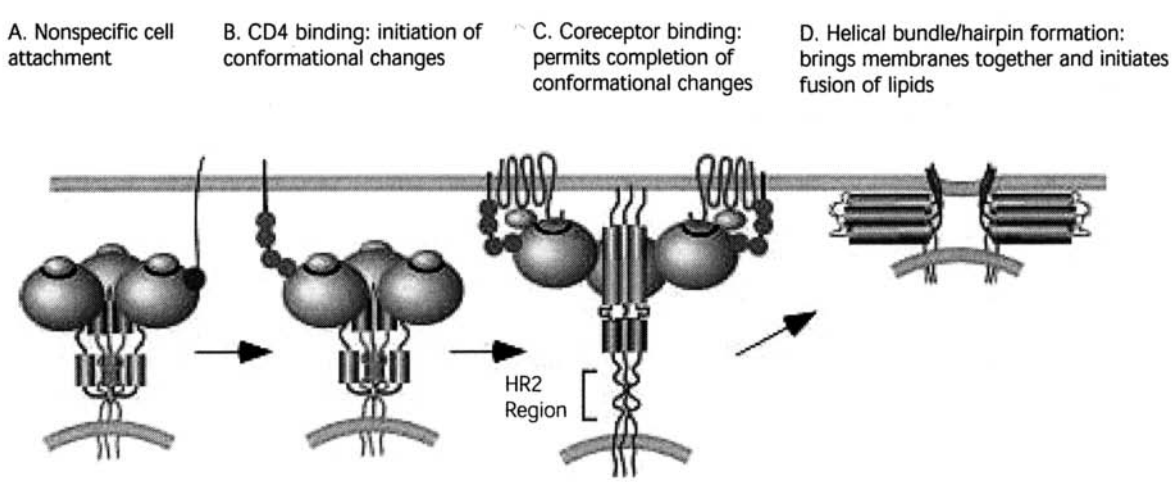

FIG. 11.2. Cellular dynamics of HIV 1 infection of CD4+ T cells. Successive steps in the life cycle of the virus are indicated by horizontal arrows. Transitions between resting (small) and activated (large) CD4+ T cells are illustrated by vertical arrows. HIV-1 can infect resting and activated CD4+ T cells, but integration of the reverse transcribed HIV-1 provirus, which is necessary for virus production, occurs only in antigen-activated T cells. Note that unintegrated HIV-1 DNA in the cytoplasm of resting cells is labile. Productive infection requires antigen-driven activation of recently infected resting CD4+ cells or infection of antigen-activated CD4+ T cells. Productively infected cells generally die within a few days from cytopathic effects of the infection, but some survive long enough to go back to a resting state, thereby establishing a stable latent reservoir.

readily in activated T cells. Thus infected activated CD4+ T cells begin to produce and release infectious virus.

From this point, there are several possible fates for cells in this state (Fig. 11.2). *In vitro* and *in vivo* studies show that in the case of activated CD4+ T cells, the infection can be highly cytopathic and can induce cell killing by mechanisms that are described below. Thus, many of these activated infected cells are likely to die from cytopathic effects of the virus. Some of these cells can also be destroyed by immunologic mechanisms including HIV-1-speciÞc cytolytic T lymphocytes (CTL). Studies (45,46) of the decay of plasma virus following the intiation of antiretroviral therapy have provided compelling evidence that the half-life of productively infected CD4+ T cells is relatively short, generally <2 days (see below). This short half-life reßects destruction by viral cytopathic effects and/or cytolytic host effector mechanisms.

Not all productively infected cells die. Some escape both the viral cytopathic effects and immunologic effector mechanisms and revert to a resting state carrying integrated provirus. In doing so, these cells are following a normal pathway in which some of the effector cells activated in response to a particular antigen revert back to a quiescent state and survive for long periods of time, allowing future responses to that same antigen. In a resting state, these memory CD4+ T cells are likely to have little or no virus transcription and are therefore also invisible to the immune system. Because the viral DNA is in an integrated state in these cells, it is highly stable. Thus these cells in the state of post-integration latency potentially represent a long term, stable latent reservoir for the virus. Upon subsequent exposure to antigen, these cells will become activated and release infectious virus (41,47). Thus, as is emphasized in Fig. 11.2, antigen plays a critical role in driving CD4+ T cells into states in which they are susceptible to productive infection by HIV-1 and subsequent destruction by viral cytopathic effects or immune mechanisms. For example, in the pre-HAART era, several studies showed that immunization of seropositive donors with antigens like tetanus toxoid results in a transient increase in plasma viremia (48). However, the generality and long term signiÞcance of these increases in viremia are currently unclear. At the present time, immunization of seropositive donors against other infectious diseases is still an important part of the medical management of HIV-1 infection (see Chapter ??).

For macrophages, the cellular dynamics of HIV-1 infection are different. It is of considerable interest that HIV-1 can replicate in macrophages (49–51). For most retroviruses, integration and replication can occur only in dividing cells. SpeciÞc amino acid sequences in several different HIV-1 proteins that are part of the preintegration complex target the complex for nuclear import, permitting integration and replication in some non-dividing cells such as macrophages (52,53). In macrophages, infection is not cytopathic. *In vitro* studies suggest that infected macrophages can continue to produce virus over long periods of time (49,50). This Þnding has led to the notion that infected macrophages may serve as a reservoir of virus *in vivo*. Virus production by macrophages is particularly apparent late in the course of disease when few CD4+ T cells remain and in the setting of opportunistic infections (54).

Important insights into the cellular dynamics of HIV-1 infection have come from studies (45,46) in which infected individuals have been treated with powerful antiretroviral agents that block infection of new cells but that do not inhibit release of virions by cells that are already infected (Fig. 11.3). In such individuals, plasma virus drops by approximately two logs within about two weeks, indicating that the half-life of plasma virus and the half-life of productively infected cells are both very short (<2 days). Thus, most of the plasma virus is produced by cells that are only recently infected. The infection is sustained by continuous new rounds of viral replication. Chronically infected macrophages or latently infected cells CD4+ T

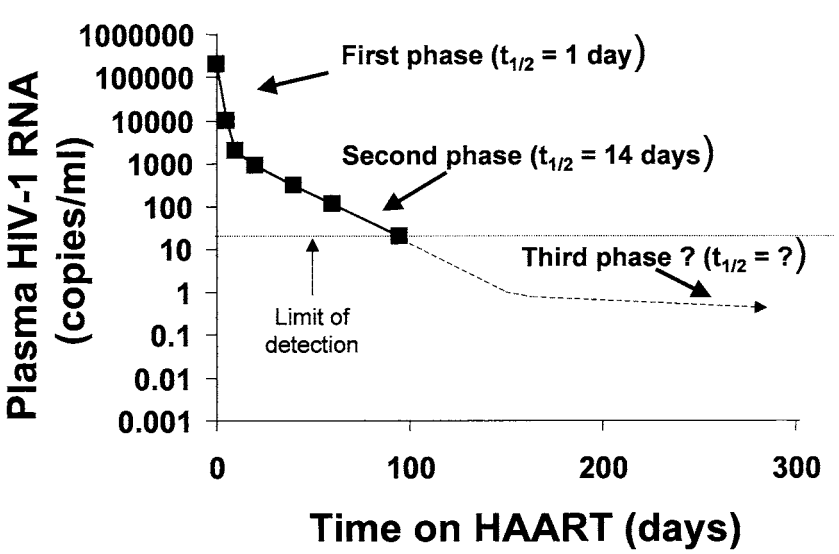

FIG. 11.3. Hypothetical plot of plasma virus in a patient who is started on an effective regimen of drugs that block infection of new cells. Plasma virus drops rapidly in the first two weeks of treatment, reflecting the short plasma half-life of the virus and the short half-life of productively infected cells. The decline in plasma virus shows a second, slower phase which is due to turnover of cells infected before initiation of therapy. These may be persistently infected macrophages or CD4+ T cells that have been reactivated from a state of post-integration latency. The second phase brings the viral load down to below the limit of detection, but the virus persists in reservoirs, including an extremely stable reservoir of latent virus in resting memory CD4+ T cells.

cells make a relatively minor contribution to plasma viremia in untreated individuals (1%). However, it is clear that eradication of the infection will require elimination of these reservoirs.

Having considered the dynamics of HIV-1 infection at the cellular level, we will now discuss the clinical characteristics of the infectious process and mechanisms by which HIV-1 infection leads to immunodeficiency.

HOST-VIRUS INTERACTIONS IN PRIMARY HIV-1 INFECTION

The natural history of HIV-1 infection may be divided into three phases (Fig. 11.4). During the initial phase, known as primary HIV-1 infection, virus present in the infecting inoculum replicates in the host, eventually producing a viremia that is controlled by the emergence of a vigorous anti-viral immune response. These events occur during the first several weeks following exposure to HIV-1. Primary HIV-1 infection has been studied most carefully in the 50–70% of individuals who develop constitutional symptoms (see reference #55 for a review). In those individuals, a transient illness resembling infectious mononucleosis appears one to twelve weeks after exposure. Symptomatic primary HIV-1 infection is characterized by fever, lymphadenopathy, pharyngitis, arthralgia, myalgia, rash, lethargy, and occasionally aseptic meningitis. Symptoms persist one to two weeks. The severity varies dramatically. Many infected individuals give no history of such an acute illness. At the other extreme are rare individuals who develop serious manifestations such as candida esophagitis. Severe symptomatic primary HIV-1 infection may be associated with a more rapid overall disease course (55). During the acute illness, assays for the HIV-1 gag protein p24 in the serum are typically positive, reflecting high levels of free virus and viral antigen in the circulation. Assays for antibodies to HIV-1 are initially negative. Seroconversion (the development of a detectable antibody response to HIV-1) usually occurs within a few weeks after onset of the acute illness. IgM antibodies precede IgG antibodies by about a week. During symptomatic primary HIV-1 infection, the levels of infectious virus and of infected cells in the circulation are both very high (56). This level is 30–300-fold higher than the levels typically observed during the asymptomatic phase of infection. It has been proposed that the initial systemic seeding of the peripheral lymphoid organs with HIV-1 occurs as a result of the high levels of viremia that develop during primary HIV-1 infection.

As the immune response to HIV-1 develops, there is a dramatic reduction in viremia and in the amount of viral antigen in the circulation in most patients. The CD4+ T cell count is typically reduced during symptomatic primary HIV-1 infection, but it is unclear whether this reflects virus-induced CD4+ T cell depletion or sequestration of circulating CD4+ T cells in the peripheral lymphoid organs. After the acute illness resolves, CD4+ T cell counts generally rise again but often not to preinfection levels. Another interesting observation is that of a CD8+ lymphocytosis during primary HIV-1 infection, perhaps reflecting a CTL response to the virus (57). Primary HIV-1 infection provides clues regarding the importance of various immune effector mechanisms in

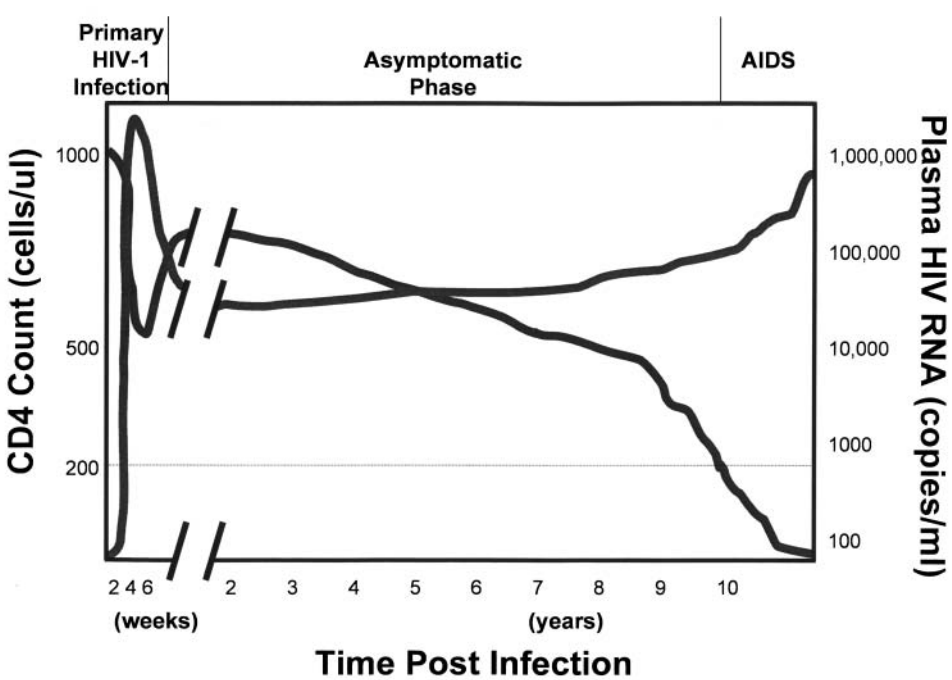

FIG. 11.4. The three stages of disease in a hypothetical case of HIV-1 infection.

controlling HIV-1 infection. Knowledge of factors responsible for the dramatic decline in the levels of infectious virus and infected cells in peripheral blood may be particularly valuable in the design of vaccines and immunotherapeutic strategies. Antibodies capable of neutralizing autologous virus isolates can be detected in serum samples taken 2–3 weeks after the onset of symptomatic primary HIV-1 infection (58,59). Several reports suggest that virus-specific CTL may appear even earlier and may represent a critical host factor in the control of acute HIV-1 infection. HIV-1-specific CTL can be detected in the first weeks after infection, prior to or coincident with the development of a detectable antibody response (60–63). Compelling evidence in this regard comes from recent studies of acute simian immunodeficiency virus (SIV) infection of rhesus macaques (64). SIV and HIV-1 show many striking similarities including virtually identical genomic organization, a tropism for CD4+ cells, and the capacity to induce a fatal immunodeficiency syndrome. SIV-specific CTL can be detected in the first weeks after inoculation of rhesus monkeys with the virus, prior to or coincident with the development of a detectable antibody response. In animals experimentally depleted of CD8+ T cells, control of viremia is not observed, and the animals progress quickly to AIDS (64). Thus is it very likely that through the lysis of infected cells and perhaps also through the release of chemokines like MIP-1α, MIP-1β and RANTES that inhibit HIV-1 entry, these cells help to reduce the level of circulating virus to the lower levels that are characteristic of the asymptomatic phase of infection.

The initial immune response to HIV-1 contributes to reducing the level of plasma virus to a lower plateau level (the "set point") that is different in different patients and that determines the rate of disease progression (65). Typical set point levels might be 10,000–100,000 copies of HIV-1 RNA/ml of plasma. This set point level of viremia reflects a balance between viral replication and host immune responses that plays out over the course of the second phase of HIV-1 infection.

HOST-VIRUS INTERACTIONS IN THE ASYMPTOMATIC PHASE: THE SIGNIFICANCE OF VIRAL LOAD

The second phase of HIV-1 infection is the long asymptomatic period between primary infection and the development of clinical disease. The most important and characteristic pathophysiologic feature of the asymptomatic phase of HIV-1 infection is the gradual loss of CD4+ T cells. Levels of CD4+ T cells may be initially stable but inevitably begin to drop. The fall is generally gradual and steady, with a more rapid drop in the 18 months preceding development of an AIDS-defining illness. The loss of CD4+ T cells is primarily responsible for the development of frank immunodeficiency observed in the final stage of the illness. It is important to stress that although the asymptomatic phase may represent a phase of clinical latency, the virus replicates continuously during this period as indicated by the "set point" level of viremia (66). While the CD4 count is the best predictor of clinically significant immunodeficiency, the level of viremia determines the rate at which CD4+ T cells are lost (65). The higher the set point, the more rapidly CD4+ T cells are lost. This suggests that viral infection is somehow involved in the destruction of CD4+ T cells, but the precise mechanism is unclear. Because HIV-1 is cytopathic for T cells *in vitro*, it was originally presumed that direct viral cytopathic effects were responsible for CD4 depletion (2). However, subsequent studies of virus load in HIV-1 infection have raised significant questions about whether the fraction of cells infected is high enough to account for the depletion of the entire CD4+ T cell compartment. Therefore, the issue of virus load is central to understanding the mechanisms by which HIV-1 produces immunodeficiency.

Clinically, viral load is measured by the determination of the level of HIV-1 RNA in the plasma (65,66). However, this is ultimately a reflection of the number of productively infected CD4+ T cells and macrophages in various sites throughout the body. Viral load has also been evaluated by measurement of infectious virus in plasma (67,68), limiting dilution analysis of the frequency of cells in the peripheral blood capable of producing infectious virus (41,67), PCR analysis of viral DNA in cells in the peripheral blood (40,41,47,69–71), lymph nodes (41,72,73), and central nervous system (74), and quantitation of viral mRNA species in productively infected cells (75–79). Although these approaches all confirm that in untreated individuals HIV-1 replicates actively and continuously, regardless of stage of disease, there remains some confusion over the fundamental question of what fraction of CD4+ T cells are infected, with widely divergent answers coming from different experimental approaches. Part of the confusion results from the fact that many of the experimental approaches used to study this issue do not provide information about the distribution of virus between latent and active states, between defective and replication-competent forms, between CD4+ T cell and macrophage compartments, and between circulating and tissue sites. All of these factors are important in understanding viral load.

CD4 depletion as a result of direct cytopathic effects can only occur for cells that are productively infected with HIV-1, and this enumeration of infected cells is important in understanding pathogenesis. An early *in situ* hybridization study suggested that even in patients with AIDS, only a small subset ($< 1/10{,}000$) of the total pool of lymphocytes was productively infected, as evidenced by high levels of viral mRNA (75). Subsequent PCR studies detected HIV-1 DNA in a larger fraction of CD4+ T cells (69–71) and were interpreted as indicating that most of the infected cells were in a state of latent infection. Careful analysis of the fraction of CD4+ T cells from which virus

can actually be cultured have shown that CD4+ T cells harboring replication-competent provirus are rare (<0.01%) (41). Since productive infection requires integration of reverse transcribed viral DNA into the host genome, the issue of virus load can also be addressed by analysis of the frequency of CD4+ T cells carrying integrated provirus. In the peripheral blood of seropositive individuals in the asymptomatic phase of infection, most of the detectable viral DNA is in an unintegrated form (40,41). Integrated virus is found in >0.01% of CD4+ T cells and macrophages (41). The notion that CD4 depletion is the direct result of infection and subsequent death of large numbers of CD4+ T cells is not consistent with the low fraction of infected cells.

Some evidence suggests that the extent of infection is greater in the lymph nodes rather than in the peripheral blood (72,73). Lymphocytes in the circulation represent only a small fraction (<2%) of the total lymphocyte pool. Complex factors, including expression of various cell adhesion molecules, determine the trafÞcking of lymphocytes between these compartments. During the asymptomatic phase of the infection, the proportion of cells carrying HIV-1 DNA is higher (3Ð10-fold) in the lymph node than in the peripheral blood (73). For example, HIV-1 DNA may be detected by a sensitive PCR assay in 1/10,000 peripheral blood mononuclear cells while in the same individual the proportion of infected cells in the lymph nodes is typically 10-fold higher (1/1000). However, analysis of virus burden by methods that distinguish integrated and unintegrated HIV-1 DNA has shown that only a small proportion of CD4+ T cells (<0.01%) harbor integrated provirus and that the proportion of CD4+ T cells harboring integrated virus is not dramatically different in the blood and lymph nodes (41).

During the asymptomatic period, virus particles are readily detected in the germinal centers of the lymph nodes where they are found associated with the network of follicular dendritic cells (FDC) (73,80). FDC are unique antigen presenting cells that form a complex cellular network within the germinal centers. These cells express Fc receptors and three types of complement receptors (CR1, CR2, and CR3) and as a result are capable of binding antigens that have bound antibody and/or activated the complement system. FDC may serve as Þlters that trap virus particles and thereby lower the level of infectious virus in the circulation. FDC normally play an important role in the activation of B lymphocytes in response to antigen. During the asymptomatic phase of HIV-1 infection, there is progressive disruption of the normal architecture of the lymph nodes, with loss of the FDC network. It is possible that the loss of FDC is in part responsible for the abnormal B cell function observed in HIV-1-infected individuals. In addition, it has been proposed that the concentration of extracellular virus on the membranes of FDC provides a reservoir of virus which can infect CD4+ T cells migrating through the lymph node. Blood-derived dendritic cells, which are distinct from FDC and which have an important role in the presentation of antigens to CD4+ T cells, are also capable of binding virus and of transmitting the virus to the CD4+ T cells with which they interact during the course of an immune response. Controversy exists over whether the extent to which these dendritic cells can be productively infected (81). Blood-derived dendritic cells (DC), which are distinct from FDC and which have an important role in the presentation of antigens to T cells, can bind virus and transmit the virus to the CD4+ T cells with which they interact during the course of an immune response (81Ð83). A recently discovered lectin-like receptor expressed by DC, called DS-SIGN, can interact with carbohydrate ligands on gp120, facilitating attachment of virions to DC (84). Following stimulation with imßammatory cytokines, immature tissue DC migrate to the lymph nodes where they present antigens taken up in the tissues to T cells in the lymph nodes. It has been proposed that DC can carry virions bound via DC-SIGN to the nodes where they mediate infection of CD4+ T cells (84).

In summary, although virus replication occurs throughout the asymptomatic phase of HIV-1 infection, the fraction of susceptible cells that are productively infected at any given time is low. It is thus unclear whether direct viral cytopathic effects can account for the depletion of CD4+ T cells. The next section of this chapter reviews potential mechanisms for depletion of infected CD4+ T cells. Mechanisms for the depletion of CD4+ T cells that are not infected are considered in a subsequent section.

MECHANISMS OF CD4 DEPLETION: PRODUCTION AND DESTRUCTION

In considering the mechanisms responsible for the quantitative and qualitative defects in the CD4+ T cell compartment in HIV-1 infection, it is important to note at the outset that this compartment is heterogeneous with respect to several critical parameters. As mentioned above, only a very small proportion of the CD4+ T cells in an HIV-1 seropositive individual are productively infected at any given time. Therefore, the CD4+ T cell compartment consists of a small number of infected cells and a much larger number of uninfected cells. In addition, CD4+ T cells differ in their states of activation (resting vs. activated), prior history of antigen exposure (naïve vs memory), and commitment to particular patterns of cytokine production (Th1 vs. Th2). This heterogeneity is important to keep in mind when considering mechanisms of CD4+ T cell depletion.

CD4+ T cell depletion can be viewed in terms of a virus-induced alteration in cell kinetics within this compartment (for a review, see reference 85). In infected individuals, the rate of CD4+ T cell loss exceeds the rate at which CD4+ T cells are produced through thymic differentiation and/or clonal expansion of peripheral CD4+ T cells. CD4+ T cells may be lost through a

number of potential mechanisms, some of which operate on infected cells. Interestingly, there are other mechanisms for CD4+ T cell depletion that operate on non-infected cells.

Before considering mechanisms for CD4+ T cell destruction, the possibility that the production of CD4+ T cells is decreased in HIV-1 infection will be discussed. The decline in naive CD4+ and CD8+ T cells in the peripheral blood of infected individuals has been interpreted as indicating a defect in thymopoeisis in HIV-1 infection (86,87), a conclusion that is supported by the Þnding that na•ve T cell levels increase upon control of viral replication with HAART (88). Unfortunately, it is difÞcult to directly measure the rate at which new T cells are produced in the thymus. Thymic size can be measured radiologically, but direct measurement of function requires other approaches (89). To monitor ongoing thymic production of new T cells, recent studies have quantitated T cell receptor excision circles (TRECs) produced as a by-product of the VDJ recombination reactions that occur in the thymus as new T cells are generated (90,91). These DNA circles are stable in cells after the gene rearrangements that produce functional T cell receptors occur. Lower than normal TREC levels in peripheral blood CD4+ and CD8+ T cells have been observed in some HIV-1-infected adults, with partial reversal upon treatment. Interpretation of TREC measurements is complicated by the fact that TRECs can be diluted out by proliferation of mature T cells (92).

Pathologic examination of thymuses from AIDS patients suggests that HIV-1 infection accelerates the thymic involution that normally occurs with age (for a review, see reference 93). The mechanism is unclear. Infection of progenitor cells from bone marrow has not been consistently demonstrated. HIV-1 provirus is not detected in circulating monocytes or B lymphocytes (69), suggesting that stable infection of bone marrow stem cells either does not occur *in vivo* or does not lead to vertical transmission of the virus to progeny cell populations. In immunodeÞcient mice reconstituted with fetal human thymus, infection and depletion of thymocytes can be demonstrated (94). However, in this system, the virus is often directly innoculated into the thymic graft, and it is not clear how closely this model mimicks HIV-1 infection in humans. Since HIV-1 provirus is not detected in circulating CD8+ T cells, infected double negative or double positive thymocytes apparently do not survive *in vivo* to produce mature T cells. Taken together, these results suggest that HIV-1 infection may interfere with T cell production. Because HIV-1 can induce the loss of mature T cells by other mechanisms, the decrease in thymic production of new T cells may be especially signiÞcant.

Most of the current evidence suggests that accelerated CD4+ T cell loss is a critical factor in CD4+ T cell depletion. Under some experimental conditions, HIV-1 infection of susceptible cell types *in vitro* results in death of the infected cell population. The initial Þnding that HIV-1 was cytopathic for CD4+ T cells (2) led to the notion that direct cytopathic effects of the virus on infected cells produced the CD4+ T cell depletion that is characteristic of HIV-1 infection in humans.

Two general types of HIV-1 induced cytopathic effects have been observed *in vitro*. In some experimental systems, syncytia or multinucleated giant cells form by the fusion of infected cells expressing env protein and non-infected cells expressing CD4 (95,96). Infected cells expressing gp120 were shown to fuse with non-infected cells expressing CD4. Inclusion of non-infected CD4+ T cells in short-lived syncytia provides a potential mechanism for CD4+ T cell depletion. The process of syncytium formation involves membrane fusion events initiated by the interaction of gp120 and CD4 on opposing cell surfaces. Co-receptors are essential for the fusion reaction. The extent to which syncytium formation contributes to CD4+ T cell depletion *in vivo* is unclear. In pathologic studies of lymph nodes and spleen from infected individuals, giant cells are observed only rarely, but this may reßect the fact that syncytia are labile structures. Recent studies suggest that syncytia may form in the mucosal lymphoid tissue (83).

In the older literature, HIV-1 isolates were classiÞed based on capacity to form syncytia in *in vitro* cultures of transfomed CD4+ T cell lines. Not all HIV-1 isolates induce syncytia in these *in vitro* culture systems. Isolates from different infected individuals show variation in biological properties including ability to induce syncytia in various cell types, rates of replication *in vitro*, and ability to induce cytopathic effects (97Ð100). In some infected individuals, the capacity of HIV-1 isolates to induce cytopathic effects *in vitro* increases with disease progression. Isolates with the capacity to induce syncytia in cell lines *in vitro* emerge with disease progression in certain individuals, although in about 50% of cases progression to AIDS is not accompanied by a transition from non-syncytia-inducing (NSI) to syncytia-inducing (SI) phenotype (100). High *in vitro* replication rates appear to correlate with the SI phenotype and with rapid CD4+ T cell loss *in vivo*. Several recent studies have conÞrmed that association of the SI phenotype with more rapid decline in CD4+ T cell counts and progression to AIDS. Nevertheless, CD4+ T cell depletion occurs during the asymptomatic phase of infection when the predominant viral species are usually of the NSI phenotype. Unfortunately, all of the older literature in this area requires reinterpretation in light of the discovery that different virus isolates utilize different co-receptors. Syncytium formation in cultures of T cell lines most likely reßects capacity of the viral isolate to infect those lines by utilizing the chemokine receptor CXCR4. However, use of transformed cells lines that express only CXCR4 will not provide information about the syncytium-inducing capacity of isolates that utilize CCR5. Thus, there is still considerable confusion about the role of syncytium formation in CD4

depletion. A careful study by Mullins and colleagues suggests that X4 viruses may emerge more commonly that previously thought but may not become dominant (31).

There are also cytopathic effects that operate at the level of individual infected cells; that is, under some conditions, HIV-1-infected T cells appear to die from the infection independent of any cell-cell fusion events (101–103). Most potential mechanisms for HIV-1-induced single cell killing involve the env glycoprotein, a protein that is poorly tolerated by many cell types. In some studies, susceptibility to cytopathic effects is related to levels of CD4 expression, perhaps reßecting toxic consequences of intracellular interactions between newly synthesized env protein and CD4 (104–105). Other studies suggest that the fusogenic properties of the env protein are an important determinant of the intrinsic toxicity of this protein for host cells (106). HIV-1 has evolved mechanisms to control the toxicity of this protein. For example, an intrinsic internalization signal sequence in the cytoplasmic tail of gp41 (107,108) mediates endocytosis of excess env protein that is not destined for incorporation into virions (109). This may reduce toxic effects of env protein expression on infected cells and allow the cells to survive longer and produce more virus. Other HIV-1 proteins including tat and vpr have also been implicated in the death of infected cells (110). Although the earlier literature suggested that infected cells die through apoptotic mechanisms, more recent studies suggest that infected cells undergo necrosis (106,111). Which viral proteins are most important in this process remains controversial, with a recent study implicating viral proteins other than the env protein (111).

Another potential mechanism for the loss of CD4+ T cells in HIV-1 infection involves the destruction of such cells by components of the immune system, particularly CD8+ CTL. As is discussed below, the natural immune response to HIV-1 infection includes a strong CD8+ CTL response, and it is quite likely that CD8+ CTL mediate destruction of infected cells *in vivo*. Most of the current evidence (reviewed in 112) suggests that CTL play a beneÞcial role by lysing infected host cells. An alternative view, proposed by Zinkernagel and Hengartner (113), is that CTL induce immunodeÞciency through the lysis of infected CD4+ T cells. This view is problematic for several reasons. First, it is based on the assumption that the virus is not cytopathic *in vivo*. If so, then the destruction of infected cells by CTL might do more harm than good. However, as discussed above, there is actually a great deal of evidence that the virus is cytopathic for T cells. Second, this view assumes that most of the CD4+ T cells that are lost are infected. There are several potential mechanisms for CD4+ depletion that operate on non-infected CD4+ T cells (see below). Third, the Þnding that many long-term nonprogressors have vigorous HIV-1-speciÞc CTL responses argues against a pathogenic role for CTL. A more popular view, which is consistent with much of the current evidence, assumes that infected cells are likely to die from the cytopathic effects of the virus. Therefore, the destruction of productively infected cells by CTL is beneÞcial to the host because it leads to a more rapid cessation of virus production from cells that are destined to die.

MECHANISMS FOR THE DEPLETION OF NON-INFECTED CD4+ T CELLS

While the the highly cytopathic nature of HIV-1 provides a potential explanation for the characteristic depletion of CD4+ T cells in patients with HIV-1 infection, the low frequency of infected cells makes it likely that direct cytopathic effects of the virus on infected CD4+ T cells are not the only factors. In the last several years, it has become clear that HIV-1 infection may contribute to a global dysregulation of T cell dynamics, affecting the turnover of both infected and uninfected CD4+ T cells. Initial studies by Ho and Shaw and their colleagues demonsrated a very rapid rebound in CD4+ T cells counts following the initation of antiretroviral therapy. Although it has subsequently been shown that CD4+ T cell rebound following initiation of therapy is due in part to the redistribution from lymphoid organs into the blood stream (114), these investigators used the rate of CD4+ T cell rebound as an indirect reßection of the rate of CD4+ T cell turnover *in vivo*. This analysis led to the suggestion that as many as 1–2 × 109 CD4+ T cells were produced and destroyed daily in HIV-1-infected individuals (115). Because this rapid destruction of CD4+ T cells had to be balanced in the short term by rapid T cell production, HIV-1 infection was likened to a sink with an open tap and an open drain. In other words, HIV-1 infection induces a state of high CD4+ turnover, eventually exhausting the bodyÕs proliferative T cell reserve and leading to an inevitable collapse of the immune system.

These results were partially supported by subsequent studies that directly measured the turnover rate of CD4+ T cells in both SIV-infected rhesus macaques and HIV-1-infected humans using radiolabeled nucleotide analogs or precursors that could potentially be incorporated into the DNA of dividing cells (116,117). Upon introduction of BrdU, a nucleoside analog, to the drinking water of SIV-infected rhesus macaques, the percentage of BrdU+ CD3+CD4+ lymphocytes increased from zero to a peak value, as expected. When BrdU was stopped, the percentage of BrdU+ CD3+CD4+ lymphocytes subsequently decreased. This rise-and-fall dynamic of BrdU+ lymphocytes was most pronounced for SIV-infected macaques with high viral loads. A graded response was observed for lower viral loads and the least pronounced dynamic was observed in normal macaques. When a system of differential equations was Þtted to the data, the best-Þt parameters indicated a statistically signiÞcant increase in

both the CD4+ T cell production rates and death rates in infected macaques as compared to uninfected macaques (116). Additionally, the replacement rate of CD4+ T cells (presumably by the thymus) was calculated as the difference between the best-Þt CD4+ death and production rates and shown to be two to three-fold higher in SIV-infected macaques versus controls. Thus, SIV infection was observed to be associated with a signiÞcantly increased CD4+ lymphocyte turnover rate as well as an accelerated proliferation rate of CD4+ T cells.

Additional studies with human subjects using deuterated glucose labeling of DNA in dividing cells showed similar results (118). In a series of experiments from the same group, labeling and delabeling kinetics of DNA in CD4+ T cells in the blood were studied in HIV-1-infected, treatment na•ve patients and normal volunteers. Detection of labeled DNA became possible with a half time of approximately 0.5 days. On infusion of 2H-glucuse, the group of normal volunteers demonstrated a slow increase of labeled CD4+ T-cells followed by a gradual decrease. Additionally, only a small peak fraction of CD4+ cells was labeled (~ 0.04). The kinetics of DNA labeling and delabeling of CD4+ T cells in HIV-1-infected patients were different. The most striking differences were the signiÞcantly higher rates of labeling and delabeling of the CD4+ T cells in HIV-1-infected patients and a peak fraction of labeled CD4+ T cells that was as much as Þve times greater than the normal control group.

Several subsequent experiments were carried out to conÞrm that the higher rate of DNA labeling and delabeling of CD4+ T cells in the HIV-1 infected represented increased CD4+ T cell turnover (118). A mathematical model of CD4+ T cell dynamics, which estimated CD4+ T cell proliferation and death rates, was formulated to Þt to the data from the described DNA labeling experiments and predicted signiÞcantly higher mean proliferation and death rates of CD4+ T cells in the HIV-1 infected group than in the normal control group. Analysis of the data using this model provided no evidence that HIV-1 infection decreases the introduction of CD4+ T cells from intrinsic sources, e.g. thymus or na•ve/memory CD4+ T cells that may be activated into clonal expansion. In order to test these predictions, some treatment-na•ve patients were placed on a potent four-drug regimen. Second and third periods of 2H-glucose labeling were carried out on days 35Ð77 and 245Ð875, respectively. For most patients, the second period episode yielded signiÞcantly slower rates of deuterium incorporation and CD4+ T cell loss. Additionally, the observed peak fraction of labeled cells was less than before treatment. Qualitatively similar effects were observed at the third labeling episode (although to a lesser degree), suggesting slower CD4+ T cell turnover after initiation of drug therapy.

Based on the results obtained from all of the aforementioned important studies, these investigators concluded that the loss of CD4+ T cells during HIV-1 infection was due to increased CD4+ T cell turnover, turnover at a rate which the body would inevitably be unable to maintain, leading to immune failure. There are, however, a number of additional complexities. First, the CD4+ T cell compartment is composed of several distinct subpopulations, including activated, memory and na•ve CD4+ T cells and cannot be realistically treated as a single homogeneous compartment. Another concern is whether CD4+ T cell dynamics observed in the blood can be extended to the whole body. It is now clear that the rapid rise in CD4+ T cell counts seen shortly after the initiation of therapy may simply reßect redistribution of T cells from the lymph nodes and spleen into the blood. At any given time, only 2% of the lymphocytes are in the blood, the remainder being distributed throughout the body in various lymphoid organs and in non-lymphoid tissues. A minor change in trafÞcking patterns can result in signiÞcant changes in blood levels. The redistribution seen after initiation of therapy may result from decreases in the overall activation state of the immune system (see below). Lastly, it is important to note that qualitative, functional defects in the CD4+ T cell compartment cannot be explained alone with a quantitative destruction of CD4+ T cells.

What is the cause of the increased CD4+ T cell turnover in HIV-1 infection? CD4+ T cell replication dynamics follow one of two pathways: either the cells undergo regenerative or ÒburstÐlikeÓ replication (119). Regenerative replication is exhibited by quiescent CD4+ cells as a means of maintaining a steady-state population size, while burst-like replication occurs after antigenic activation of a na•ve or memory T cell. During any sort of infection, HIV-1 included, CD4+ cells speciÞc for the relevant pathogen are stimulated, leading to activation and rapid replication of antigen-speciÞc cells. Some cells of irrelevant speciÞcity may also be stimulated by cytokines released in the process. Eventually, as the infection resolves, there is deletion of most of these activated cells by apoptosis. A small fraction of these activated CD4+ cells avoid clonal deletion and instead convert to resting memory cells. Some researchers have argued that CD4+ depletion during HIV-1 infection is due to CD4+ cell death from HIV-1 antigenic stimulation of CD4+ cells. Thus it is proposed that a substantial fraction of CD4+ cells are killed indirectly by HIV-1Ñ as bystandersÑ through apoptosis subsequent to the massive antigenic burden presented by HIV-1 infection. This would provide an explanation for the increased T cell turnover observed in the BrdU or (2H)-glucose labeling studies discussed above. The rapid rise of labeled cells is consistent with rapidly dividing activated cells while the rapid fall of labeled cells after ceasing infusion of the label is consistent with the rapid death of activated CD4+ cells. Ultimately, it is believed that the negative effect of chronic activation on the balance between CD4+ T cell production and death in the presence of HIV-1 mediated CD4+ cell death leads to CD4+ depletion, mostly through destruction of non-infected bystander CD4+ T cells.

FUNCTIONAL DEFECTS IN CD4+ T CELLS

In addition to the quantitative depletion of CD4+ T cells that occurs during the course of HIV-1 infection, there are also qualitative defects in the function of the surviving CD4+ T cells. In a pivotal early study, Lane and colleagues showed that unfractionated peripheral blood mononuclear cells (PBMC) from AIDS patients showed decreased responsiveness to mitogens and to soluble recall antigens (12). When puriÞed CD4+ T cells were tested, responses to mitogens were normal but responses to soluble antigens were depressed indicating that the lower response seen with unfractionated PBMC from patients was not simply due to a lower percentage of CD4+ T cells. Rather, CD4+ T cells from AIDS patients had a marked defect in responsiveness to soluble antigen presented by monocytes (12). This defect was shown to be intrinsic to the T cell and did not occur at the level of antigen-presenting cell.

One possible explanation for these functional defects is that activation with antigen renders the T cell highly susceptible to productive infection, particularly if the antigen presenting cell is infected with HIV-1. Macrophages and dendritic cells infected with or exposed to with HIV-1 *in vitro* can present antigen to T cells and the encounter results in transfer of infection to the responding T cell population (82).

DEFECTS IN B CELL FUNCTION

In addition to the effects on CD4+ T cells described above, infection with HIV-1 results in dramatic effects on B-lymphocyte function. Normal absolute numbers of circulating B cells are found in HIV-1-infected individuals. However, there are several major lines of evidence suggesting that HIV-1 infection results in abnormalities in B-cell function. First, there is strong evidence for an increased degree of B-cell activation in infected individuals. Increased B-cell activation occurs early in the disease process as evidenced by high levels of antibody-producing B cells and by hypergammaglobulinemia (120Ð122). There is a higher than normal percentage of immature B-cells and an increased number of activated B cells (123). Spontaneous *in vitro* B-cell proliferation is also characteristic of HIV-1 infection (120,124). Circulating immune complexes (125Ð127) can be detected in the sera of AIDS patients. Also part of the clinical picture of HIV-1 infection is immune complex-associated pathology including autoimmune thrombocytopenia (128) and anemia (129).

The polyclonal B-cell activation seen in HIV-1 infection is often termed Òspontaneous,Ó although there is some evidence that much of the hypergammaglobulinemia actually results from a strong humoral response to HIV itself (130). Other proposed mechanisms for nonspeciÞc B-cell activation in HIV-1 infection include direct effects of HIV-1 virions or HIV-1 proteins as well as activation mediated by EBV and activation by membrane forms of the cytokine TNF-α expressed on activated T cells (131). Importantly, B cell abnormalities in HIV-1 infection tend to normalize after effective therapy is started, suggesting that viral replication drives the B cell hyperactivity *in vivo* (132,133). Recent evidence suggests that abnormal B cell function in the setting of ongoing replication may be due to the presence of a subset of B cells with decreased expression of the complement receptor CD21, plasmacytoid features and poor proliferative responses (132). It is also important to note that patients with HIV-1 infection have an increased risk for B cell malignancies (134).

The polyclonal B-cell activation seen in HIV-1 infection is found in conjunction with decreased *in vivo* humoral responses to speciÞc antigens. In an important early study (120), Fauci and colleagues demonstrated that AIDS patients had reduced responses to a T-independent B-cell mitogen Staphylococcus aureus Cowan strain, indicating an intrinsic B-cell defect. Healthy seropositive individuals also show B-cell functional defects. These defects, in conjunction with defects in helper T cell function and with alternations in the germinal center environment where B cell responses originate, may explain the defects in speciÞc antibody responses in HIV-1 infection.

THE IMMUNE RESPONSE TO HIV-1: ANTIBODY RESPONSES

The immunologic abnormalities in HIV-1-infected individuals must be viewed in the context of the ongoing immune response to HIV-1. ImmunodeÞciency develops despite the presence of readily detectable B and Tlymphocyte responses to HIV-1 (for a review, see reference 112). In fact, in many ways, HIV-1 is a highly immunogenic virus. Virtually all infected individuals develop antibody responses to several of the protein products of the HIV-1 genome. Even more striking is the Þnding that most infected individuals also have very high levels of virus-speciÞc CTL activity (60Ð63,112,135Ð137). The frequency of HIV-1-speciÞc CTL in the peripheral blood of some seropositive individuals is sufÞciently high that cytolytic activity can be detected in freshly isolated PBMC preparations without any *in vitro* restimulation (135) and that CTL speciÞc for particular HIV-1 epitopes can be directly quantitated using recently developed tetramer technology (138). Given that HIV-1 infection generally induces vigorous B and T lymphocyte responses, it is important to consider whether these responses exert beneÞcial anti-viral effects.

The potential of speciÞc antibodies to control HIV-1 infection is measured in *in vitro* neutralization assays in which serum from an infected individual is incubated with known quantities of infectious virus and the reduction in infectivity of the virus sample is measured (139,140). In general, levels of neutralizing antibodies are low even

when high levels of antibodies to HIV-1 env glycoproteins are present, indicating that many anti-env antibodies are not neutralizing. There has been intense interest in determining the nature of neutralizing epitopes on the env protein. For some laboratory isolates of HIV-1, a significant proportion of the neutralizing antibody response is directed at an epitope located in the third hypervariable (V3) region of HIV-1 gp120 (141–143). This V3 loop has a conserved tip flanked on either side by variable residues. Current evidence suggests that this region of gp120 is involved in fusion events following the binding of virions to CD4+ cells. Mutations in the V3 loop decrease the capacity of the env protein to mediate membrane fusion events, but such mutations do not affect binding to CD4 (144). This region of gp120 is involved in interactions with the chemokine receptors that function as co-receptors for HIV-1. Regions of gp120 that are involved in binding to CD4 have been identified and can now be directly visualized due to the success of crystallographic studies (reviewed in reference 145). Neutralizing antibodies directed at the CD4 binding site have also been detected in HIV-1 seropositive individuals and are of particular interest because they neutralize diverse isolates of HIV-1 in contrast to V3 loop-directed antibodies, which are highly type-specific. Unfortunately, these antibodies appear slowly during natural infection and have proven difficult to induce with experimental AIDS vaccines, probably because the CD4 binding site on gp120 is located in a relatively "hidden" region protected by N-linked oligosachharides (146) and is surrounded by the variable loops which can accommodate mutations that allow escape from neutralizing antibodies (145).

There are several possible explanations for the failure of neutralizing antibodies to halt disease progression. One is that the neutralization of viral infectivity *in vivo* is more difficult than *in vitro* assays because of the much higher concentrations of infectable target cells in *in vivo* sites such as lymph nodes (147). If this is the case, high-titer neutralizing antibodies may be required to prevent infection and/or disease progression. A second explanation for the failure of neutralizing antibodies to prevent disease progression is that antibodies to the env protein may in some cases facilitate the uptake of HIV-1 by cells that express Fc receptors, such as monocytes and macrophages (148,149). This effect has been demonstrated *in vitro* but its *in vivo* significance remains to be established. Finally, as is discussed in the next section, failure of neutralizing antibodies to prevent disease progression may be due to the continuous emergence of neutralization-resistant variants, which is a reflection of the striking propensity of HIV-1 to undergo genetic change (reviewed in reference 150). Recent evidence suggests that the virus evolves so quickly that the neutralizing antibody response never "catches up" with the evolving virus. Late in the course of disease, there is also most likely a declining capacity to mount *de novo* antibody responses to new antigenic determinants. AIDS patients have greatly diminished antibody responses to novel and recall antigens both because of decreased T cell help and because of intrinsic B-cell abnormalities (120). One might, therefore, expect that late in the disease course infected individuals would have a decreased capacity to generate antibodies to newly emergent, neutralization-resistant variants.

CYTOLYTIC T-LYMPHOCYTE (CTL) RESPONSES TO HIV-1

The primary function of CD8+ cytolytic T lymphocytes is to lyse virally infected cells. HIV-1 specific CD8+ CTL are readily detected in healthy seropositive individuals and are sometimes detected in patients with AIDS (60–64,112,135–138). There is considerable current interest in whether these cells exert a beneficial antiviral effect by lysing HIV-1-infected T cells and macrophages (reviewed in 112). As discussed above, CTL appear early in response to acute HIV-1 infection and help to control the high level viremia characteristic of this stage of infection (60,64). CTL may also control viral replication through the release of chemokines (25). CTL isolated from infected individuals have been shown to lyse HIV-1-infected macrophages (136) and CD4+ T cells (151,152) *in vitro*. As discussed above, CTL may also control viral replication through the release of chemokines. Vigorous HIV-1-specific CTL responses are observed in many long term survivors of HIV-1 infection (153,154). Convincing evidence for the importance of CTL is the dramatic increases in viral load observed in SIV-infected macaques when CD8+ T cells are depleted (64). Interestingly, there is increasing evidence that MHC genotype can influence the rate of disease progression, likely through presentation of viral epitopes to CD8+ CTL (for a review, see 155). Particular class I MHC alleles have been associated with slower disease progression, probably as a result of the capacity of the relevant alleles to present conserved epitopes in HIV-1 proteins to CTL. The breadth of the CTL response is also important and perhaps as a consequence, heterogeneity at the class I loci is also associated with slower disease progression (156).

The frequency of HIV-1 specific CTL has been shown in some studies to decline as the clinical and immunological status of the patient deteriorates (136,157). In addition, there is evidence that the functional capacity of HIV-1-specific CTL is reduced in the setting of ongoing viral replication. Although further longitudinal studies will be needed to determine whether the drop in levels of virus-specific CTL is causally related to disease progression, it is quite likely that the CD8+ HIV-1-specific CTL response exerts a significant antiviral effect *in vivo* and is therefore beneficial to the host. Such a mechanism, while limiting the spread of infection, probably also contributes to CD4+ T cell depletion through the destruction of infected cells. As is discussed below, the emergence of viral variants with changes in epitopes recognized by CTL may permit viral escape from this component of the immune response.

HELPER T CELL RESPONSES TO HIV-1

The one component of the immune response to HIV-1 that is not readily demonstrable in most infected people is the helper T cell response to HIV-1 proteins (158). It appears that HIV-1-speciÞc CD4+ T cells are deleted or inactivated early in the course of the infection. They can be readily detected only in those rare individuals in whom the disease does not progress (long-term non-progressors). Interestingly, recent studies have shown that treatment of infected individual with HAART early in primary HIV-1 infection allows this HIV-1-speciÞc immune response to develop (158). In such individuals, multiple cycles of interruption of HAART has been shown to boost HIV-1-speciÞc immune responses, allowing eventual control of viremia at low levels in the absence of drugs (159).

In summary, vigorous B and CD8+ T cell responses to HIV-1 have been demonstrated in infected individuals. Although such responses ultimately fail to control the infection, they may delay the onset of symptomatic disease for a period of years. Subsequent sections discuss two principle mechanisms that allow HIV-1 to evade host immune responses. These are sequence variability and latency. The ability of the virus to evade these responses through these mechanisms, coupled with the progressive virus-induced destruction of CD4+ T cell compartment, eventually leads to immunodeÞciency.

SEQUENCE VARIABILITY IN HIV-1

HIV-1 exhibits a striking degree of genomic heterogeneity that is evident when the nucleotide sequences of viral isolates from different infected individuals are compared (160Ð165). This heterogeneity arises in part from the fact the HIV-1 reverse transcriptase has a high error rate (1/1,700Ð1/4,000 nucleotides) (166,167). Even distinct molecular clones of HIV-1 obtained from a single infected individual show sequence heterogeneity (168Ð170). Although viral clones from a given individual are more closely related to each other than to HIV-1 clones from other infected individuals, the level of genetic heterogeneity that occurs within a given individual is sufÞcient to produce variants with signiÞcantly different biological properties (97Ð99). Viral diversity can be assessed as divergence from the founder strain and as diversity of clones replicating at a particular time point. Both divergence and diversity increase with time although diversity eventually levels off as patients near the Þnal stages of the infection (31). Elegant studies by Mullins and colleagues have demonstrated a temporal relationship between changes in the degree of diversity and the rate of CD4 depletion (31). Of the protein products of the HIV-1 genome, the env glycoprotein gp120 shows by far the most sequence variability.

There is considerable current interest in the issue of whether or not this natural variation may contribute to the pathogenesis of AIDS by permitting the *in vivo* selection of variant viral clones that are not recognized by existing neutralizing antibodies or virus-speciÞc CTL (for a review, see reference 150). In other lentiviral systems, including visna virus infection in sheep and equine infectious anemia virus infection in horses, the emergence of viral variants resistant to neutralizing antibodies has been well documented (171). For HIV-1, neutralization-resistant variants have been obtained *in vitro* in infected cultures maintained in the presence of neutralizing antibody (172). In addition, neutralization-resistant variants have been isolated from gp120-immunized chimpanzees infected with HIV-1 (173). Neutralization resistance has also been studied extensively in a laboratory worker accidentally infected with a reference strain of HIV-1 (174). Numerous studies suggest that anti-gp120 antibodies arising in the setting of natural infection fail to neutralize contemporaneous virus; they have activity against previously circulating variants (150). With respect to CTL responses, viral escape has been documented in experimental systems (175) and in HIV-1-infected individuals (176) from the host CTL response. HIV-1 sequence variability may in principle affect T cell recognition of viral proteins in several ways. Mutations within an epitope may affect the binding of the processed viral antigen to the relevant MHC molecule or the recognition of the resulting peptide-MHC complex by the TCR. In the case of some epitopes, a considerable degree of naturally occurring sequence variability can be tolerated without loss of binding to the presenting MHC molecule, although for other epitopes even a single conservative substitution at a critical residue can prevent recognition in the context of the relevant MHC molecule. It has been proposed that mutations that lead to low afÞnity interactions with the TCR may actually antagonize the response (177). The role of CTL escape mutants in infections by HIV-1 and SIV has been controversial (150,175,178Ð181), but there is now substantial evidence that CTL exert selective pressure for mutations in critical epitopes (182) and that mutations can contribute to disease progression (183).

VIRAL LATENCY

Another factor that may contribute to the inability of the anti-viral response to eliminate HIV-1 infection is the latent reservoir of virus that is invisible to the immune system (reviewed in 184). The latently infected cells may contain viral nucleic acids in integrated or unintegrated forms as is discussed above. Cells in the post-integration state of latency provide a stable and inaccessible reservoir for the virus. Recent studies suggest that this latent pool persists even in individuals on effective antiretroviral therapy (184).

CONCLUSIONS

HIV-1 infection initiates an extraordinarily complex set of HIV-virus interactions that ultimately destroy the CD4+ T cell compartment of the immune system, resulting in profound immunodeÞciency. The key pathophysiological process is the loss of CD4+ T cells. CD4 depletion appears to be driven by viral replication. Most infected CD4+ T cells live only a short time due to viral cytopathic effects or destruction by host CTL. In addition to the death of infected cells, uninfected CD4+ T cells are depleted, probably as a result of the high levels of immune activation that are characteristic of HIV-1 infection. HIV-1 invokes a vigorous host immune response, but this response fails ultimately to control the virus due to the combined effects of viral variation, latency, and virus-induced immunodeÞciency.

Considerable progress has been made in *in vitro* analysis of the numerous potential immunologic and virologic mechanisms by which HIV-1 can induce immunodeÞciency. Critical areas for future research include analysis of which of the many potential mechanisms outlined above are actually responsible *in vivo* for the quantitative and qualitative defects in CD4+ T cells observed in HIV-1 infection. This will depend on the development of methods for monitoring these processes *in vivo* in natural infection in humans or in the closely related SIV model in macaques. Research in this direction is likely to be critical for the design of new therapeutic approaches and for the development of AIDS vaccines.

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Chapter 12

The Role of Host Genetic Variation in HIV Infection and its Manifestations

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PERSPECTIVE, APPROACHES AND LIMITATIONS

As with ÒresistanceÓ and ÒsusceptibilityÓ to many other infectious agents, the likelihood and the consequences of becoming infected with HIV-1 vary greatly among individuals. In general, nearly all humans have some resistance to HIV-1, but quantitative differences do exist. A very small proportion of uninfected Caucasians are nearly completely protected against infection by a double deletion in a viral coreceptor gene (see below). Among infected persons, although viral replication is extremely rapid, early fulminant disease is rare. A few individuals, when left untreated, do succumb to serious immune deÞciency in a matter of months after initial infection, while many more retain intact immunity for years.

The sequence, structure and expression of human genes that control the response to HIV-1 are also extraordinarily variable. This variation (i.e. polymorphism) is believed to have evolved under long and steady pressure from the microbial pathogens, culminating in a wide range of immune responses from highly advantageous to distinctly disadvantageous. The theme of this chapter is ÒintrinsicÓ resistance and susceptibility to HIV/AIDS as determined by those human genes. After a brief review of the general approaches and pitfalls in unraveling how this host genetic variability contributes to the differential response, we summarize current knowledge about speciÞc genetic determinants of (1) transmission of infection; (2) early equilibration of virus concentration and disease progression; (3) occurrence of AIDS-deÞning opportunistic diseases; and (4) response to treatment and vaccines.

While laboratory tools have simpliÞed sequencing of genes and screening for their variants, there are no standardized approaches to designing studies, identifying candidate variants to pursue, and analyzing the data generated. Family studies, ordinarily a basic tool of the geneticist, have been largely limited to mother-infant pairs because informative kinships with and without HIV infection have generally been unavailable in the settings where genetic techniques are most readily applicable. Nearly all of the work summarized and cited here has derived from studies of unrelated individuals (population studies), often based on specimens banked from cohorts including dozens to hundreds of volunteers monitored regularly. The principal patient groups include (1) homosexual and hemophilic men in American, European and Australian cities; (2) female sex workers or heterosexual discordant couples in East Africa and Asia; (3) heterosexual adolescents and adults in the U.S.; and (4) infants of infected mothers in the U.S., East Africa, and South America. The more reliable studies have been those large enough to offer homogeneous subgroups of uninfected persons with detailed HIV-1 exposure history or of infected persons with carefully deÞned disease categories, staging and rate of progression.

Even in larger population studies there are obstacles to the analysis and interpretation of genetic data. An almost universal concern is that unaffected individuals selected for comparison with the affected, even from within the same cohort, may have different genetic backgrounds that could account for differences observed for the target loci (1,2). Ethnic admixture in a population group as heterogeneous as North American Caucasians reinforces this

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concern. The distribution of genetic polymorphisms further complicates the analysis. Each of the two inherited copies of a gene may show different deletions, insertions, inversions, duplications or other simple allelic variation such as single-nucleotide polymorphisms (SNPs). These genetic variations are distributed throughout the genome, with sequences corresponding to untranscribed, promoter and transcribed regions (introns and exons). Individual SNPs seldom sufficiently reveal the extent of genetic diversity. Rather, alleles consisting of multiple SNPs within a single locus are generally more informative. Furthermore, closely adjacent variations, either SNPs or alleles, occur in varying degrees of linkage disequilibrium (LD) with each other; that is, they may be inherited more often together as a unit (haplotype) than would be predicted by their individual frequencies. Such units often have evolutionary significance, having been preserved by a usually unexplained historical advantage. In a sense, LD is one form of *cis* interaction. Alleles at homologous loci may be identical or differ; of course, they may show different kinds of *trans* interaction reflected in their dominant, co-dominant or recessive expression. Sorting out all of these properties and properly accounting for them in the design of genetic studies and analysis of genotyping data is obviously not a simple matter.

Beyond the difficulty of evaluating effects of genetic variation in any single study population, influences of host genetic variants on HIV/AIDS almost surely differ in different settings. Genetic effects may be modulated by viral characteristics (e.g. HIV major group, subtype and phenotype and steady-state plasma concentrations) and by non-genetic host factors (e.g. race, age, and perhaps other inadequately studied features like gender, nutrition, co-infection, and route of exposure). Moreover, the effects of any of these factors or their combinations may differ with timeÑ because the virus is evolving rapidly within individuals and within a population. Thus, while effects observed consistently across viral subtypes or racial boundaries can provide some reassurance about their likely biologic significance, inconsistently observed effects are difficult to reject without substantial iterative analysis.

Several broad categories of genetic systems have been examined in the context of HIV-1 transmission, pathogenesis and intervention (Table 12.1). Within the space

TABLE 12.1. *Quantity and quality of evidence for human genetic polymorphisms associated with a feature of HIV-1 infection*[1]

Locus	Disease Control/ Progression	Transmission/ Acquisition	Secondary/ opportunistic manifestations[2]	Treatment effects
Major histocompatibility complex				
HLA-A, -B, -C	++	++	KS ±	
HLA-DRB1, -DQB1	+	–	KS ± , DMAC ±	Abac ++[3]
HLA-DPB1	±			
TAP1	±	±		
TAP2	±	±		
Chemokine receptors/ligands				
CCR2	++	±		
CCR5	++	++	NHL ± , KS ± , Toxo ±[4]	
SCYA5 (RANTES)	+			
SCYA3 (MIP1a)	+			
SDF1	?	±		
CX3CR1	?			
Cytokines				
IL10	+	+		
TNF	+	?		Abac ++[3]
IL6			NHL ±	
Other				
MDR1 (P-glycoprotein)				±
CYP2D6 (cytochrome p450)				±

[1] ++, requires study of 2 separate patient populations, □2 independent investigators, and >100 main endpoints; +, ndings meet at least two of previous criteria; ±, suggestive but less stringent criteria; ?, con icting.
[2] KS = Kaposi's sarcoma, NHL = non-Hodgkin lymphoma; DMAC = disseminated *Mycobacterium avium* complex infection; Toxo = toxoplasmosis.
[3] Abac = relationship of extended haplotype in the HLA region with adverse reaction to abacavir.
[4] In single studies, Δ32 has been associated positively or negatively with each outcome.

constraints we discuss the most consistently reported or promising genetic variation in some detail, but only briefly mention other markers whose involvement seems less certain at this time.

MAJOR HISTOCOMPATIBILITY COMPLEX

Class I (*HLA-A*, *-B* and *-C*)

Background

Within the 4-megabase-long major histocompatibility complex (MHC) on human chromosome 6 are numerous genes responsible for both innate and adaptive immunity. Among them are the human leukocyte antigen (*HLA*) class I system, which consists of the major products of *HLA-A*, *-B*, and *-C* loci along with catalytic, transporter, chaperone and other components working in concert. Each A, B and C protein, coupled with a β_2-microglobulin molecule, has a pocketed groove that binds antigenic peptides arising intracellularly in the course of normal cell function or from tumors or viruses. Resting in the specific different binding grooves, peptides 8–11 amino acids long are cradled by *HLA* molecules as they are shuttled to the cell surface. There the protein-peptide complex interacts with receptors on CD8+ T-lymphocytes to initiate a variably effective, primarily cytolytic response.

Presumably through long, continuous and differential environmental exposure, a large number of variations (e.g. gene conversions, duplications, recombinations) have accumulated in the sequence of DNA and corresponding amino acids in *HLA* molecules, especially in the residues along the peptide-binding groove. There are more than 400 forms of the *HLA-B* molecule alone and dozens of variants at each of the other class I loci (3); this extreme *HLA* polymorphism has drawn great investigative attention. A sizeable body of experimental and population research has documented how allelic variants carried by individuals differentially influence their response to antigenic challenge, and increasingly refined techniques for molecular typing have improved the ability to discriminate subtle differences among the variants that may explain the diversity (4).

The evidence for involvement of the *HLA* class I system in the pathogenesis of viral infections is conclusive, and its importance in immunity to HIV infection is increasingly clear. Much effort is being devoted to elucidating the events by which class I molecules differentially restrict HIV peptide binding, control peptide transport and presentation, and evoke critical specific CD8+ cytotoxic T-lymphocyte (CTL) responses. Briefly, in human as well as non-human primates, efficient control of viral replication is lost as a result of deficiency in CD4+ cells coupled with a malfunction of CD8+ T-cells (5–11). These derangements may be delayed or reversed by efficacious vaccines (12–15) and effective antiretroviral therapy.

Disease Progression and Early Host/Virus Equilibration

Much early research on the effects of class I polymorphism in HIV infection was conducted in cohorts of Caucasians, who were infected with HIV-1B relatively early in the epidemic and followed long enough to observe divergence in late clinical outcomes (16–19). Only recently have results begun to emerge from investigations in populations of African ancestry infected with A, C, and D subtypes and Asian populations with subtype E and A/E recombinants. Careful comparisons of class I effects in groups infected more recently with those infected earlier with the same subtype have not yet been performed, and it is not clear whether "intra-clade" evolution has been paralleled by a drift in the effect of any specific class I marker associated with slower or faster progression (see below). Both similarities and differences across races and viral subtypes have been reported, but most will require considerably more work before meaningful inferences are possible. The few generalizable observations are summarized in Table 12.2.

Rather consistently, homozygosity at a single class I locus has been found to accelerate onset of disease, and further reduced diversity (at two or three class I loci) is even more disadvantageous (20–22). With similar but not complete consistency, certain individual class I alleles have been associated with a favorable (B*27 and 57) or an unfavorable (B*08, the B22 serogroup, B*35, and B*53) natural history of infection (21–33). Particularly intriguing is the persuasive demonstration in Caucasian and African ethnic groups with subtype A, B or C infections that B*57 is a uniformly favorable allele (21,22,27–31,33–35). It apparently mediates effective CTL responses to highly conserved Gag epitopes, presumably critical to viral replication (34–36). On the other hand, it has been suggested that a subset of B*35 alleles (B*3502 and 3503) along with B*53, which share a binding pocket motif that distinguish them from other B*35 alleles, fully account for the deleterious effects of B*35 seen rather consistently in Caucasians (32). The importance of that degree of allelic specificity has not been widely corroborated epidemiologically or confirmed experimentally. Finally, a number of other alleles have shown more variable effects, again documented largely in Caucasians infected by clade B virus and confirmed to varying degrees in African-Americans with clade B as well as Africans with clade A infection (37,38).

Efforts to summarize the multiple effects of markers more consistently associated with relatively extreme outcomes have taken fairly simple combinatorial approaches. One uses an algorithm that simply assigns individual values to each marker, sums the values to produce a profile for each individual, and categorizes individuals with similar profiles. It begins to capture the impressive collective impact of the most informative *HLA* class I (and *CCR2-CCR5*) markers (Figure 12.1), but this and other relatively simple approaches (39,40) will most

TABLE 12.2. *HLA class I markers showing consistent associations with control or progression of infection in HIV-1-positive individuals*

Established HLA class I marker[1]	Viral/immunologic control[2]			Disease progression[2]			References
	Cauc	Af-Am	Af	Cauc	Af-Am	Af	
Homozygosity	B, –	B, –	C, 0/–	B, –		A, –	(20–22)
*B*08*	B, 0/–	B, –		B, 0/–			(23,24,26,27)
*B22 (B*55, B*56)*	B, –			B, –			(29,228)
*B*27*	B, ++			B, ++			(22,26,27,29,31)
*B*35*	B, –	B, –	C, 0	B, –		A, –	(21,25,27,28)
*B*5301*		B, –	C, 0			A, –	(32,37)
*B*57*	B, ++	B, ++	C, ++	B, ++		A, ++	(21,22,27–31,33–35)
*B*5701*	B, ++			B, ++			(21,22,27–29,31,34)
*B*5703*		B, ++	C, ++			A, ++	(30,33,35,37)

[1] Based on two independently assembled and *HLA*-typed populations (seroconverters or seroprevalents with closely estimated duration of infection and median duration of follow-up > 6 years) with > 100 main endpoints (AIDS cases) in studies showing consistent associations.
[2] A, B and C = HIV-1 subtypes studied; 0, no effect; –, unfavorable effect; +, favorable effect; double symbols indicates greater strength and consistency of association.

likely be replaced by more sophisticated ones as understanding of these genetic effects increases.

Recent effort has begun to extend the Þndings to earlier stages of infection. Preliminary studies of subtype B infection suggest that homozygosity and many of those individual markers at class I loci clearly associated with a better or worse long-term prognosis exert a sizable portion of their effects on HIV-1 RNA concentration as early as six months after seroconversion. The strong linear relationships between early viral load and the algorithm-based proÞle seen in Caucasians (R. A. Kaslow et al., unpublished) appears to hold for African-Americans (37); however, as exempliÞed by Þndings with CCR, it is premature to predict the extent to which early and late effects can be expected to parallel each other within the same ethnic group or even in the same cohort (41,42). On the other hand, if activation of CD8+ CTLs and perhaps other related pathogenetic mechanisms mediated by class I gene products occurs soon after infection, and *HLA* class I polymorphism substantially inßuences the ultimate outcome of HIV infection by governing early viral replication dynamics, the implications for prognostic assessment and other applications are important.

Transmission and Acquisition

*a. Sexual*Ñ The evidence for a critical role of CTL response or differential effects of *HLA* class I markers in protecting against transmission of infection from person to person is accumulating, but the Þndings are not quite so persuasive as for control and progression of disease

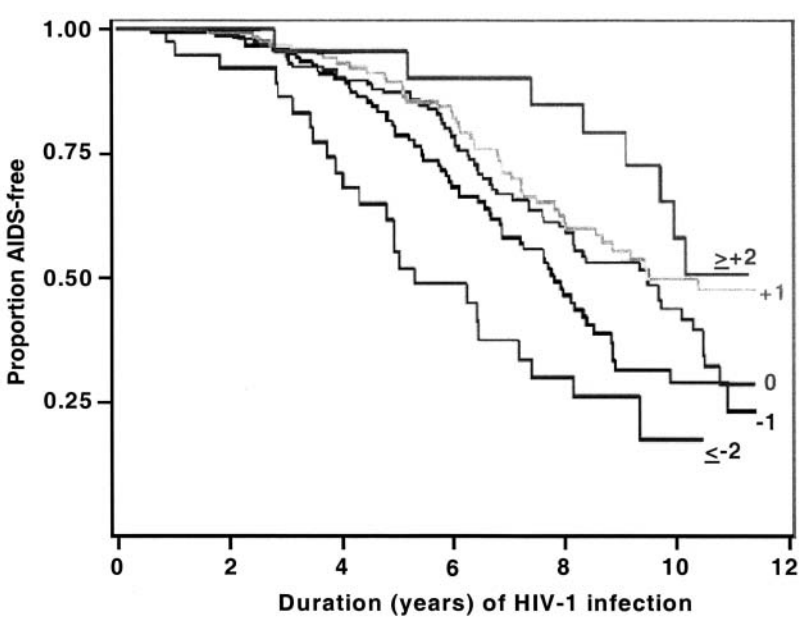

FIG. 12.1. Progression of HIV-1 infection according to pro le of polymorphisms in *HLA* class I and *CCR2-CCR5* genes in a cohort of 470 HIV-1B-infected Caucasian men. Each man was scored using an algorithm based on established marker effects (–1 or –2, respectively, for homozygosity at 1 or > 1 *HLA* class I locus; –1 for *HLA-B*54Þ56*, *B*35*, *B*53*, and *CCR5* promoter genotype E/E; +1 for *B*27*, *B*57*, *CCR5-D32*, *CCR2Þ64I*, and +2 for the Δ32/64I combination.

(43–48). Much of the information on acquisition in adults comes from highly exposed but persistently seronegative (HEPS) individuals—usually uninfected sex workers or gay men selected for intense sexual activity. HIV-specific CTL responses to viral products if not replication-competent virus have been detected in HEPS (47,49–52), but protection by those CTLs has been difficult to establish. Few studies have been designed to assess risk of transmission between members of HIV-discordant cohabiting couples (53,54). A serious obstacle to documenting the role of class I-mediated immunoregulation is the prominence of other elements of immunity (55,56) and particularly of non-genetic factors (e.g. gender, intensity of exposure to the virus, modes of transmission) that are often overlooked or omitted in studies of HEPS.

Enhanced transmission due to sharing of class I alleles has been examined in three studies comparing HIV-concordant and discordant heterosexual couples (45,53,54). A small, ethnically mixed group in the U.S. showed no difference in allele sharing by discordant and concordant couples. Caucasians in Edinburgh showed an association of transmission with overall concordance for any allele at the A, B or DR locus (53). Conclusions may have been limited by sample size, ethnic heterogeneity, serologic typing, or statistical analytic approach. In a larger and more comprehensive prospective investigation of cohabiting Zambian couples discordant for HIV-1C (57–59), sharing of one or more *HLA-B* alleles by partners was independently associated with heterosexual transmission of virus documented as identical in the donor and recipient (54), whereas class I homozygosity did not confer increased risk. The allele-sharing effect in partners with identical virus may be due to allogeneic "priming," in which susceptibles are exposed more and respond more intensely to infected partner cells bearing non-identical ("foreign") class I allelic forms than to cells that carry one or more shared forms. One way or another, donor virus may have "pre-adapted" to the likely CTL repertoire in the prospective recipient host, under close control of *HLA-B* and perhaps other class I alleles.

Individual class I alleles have also been associated with promotion or inhibition of transmission. However, again, small sample sizes, ethnic diversity, absence of details of behavioral or virologic exposure, and analytic differences have left uncertainty for many single alleles (45). An apparent protective relationship with A*6802 and the A2/6802 supertype to which it belongs was originally described in the primarily HIV-1A-infected Kenyan sex workers (48) and subsequently reported to inhibit transmission to their infants (60). A similar effect has been observed in HIV-1B-infected Caucasians in the large Multicenter AIDS Cohort Study (MACS) (61) but has not been reproduced in other settings. Correlative epitope mapping data now suggest that the critical viral antigenic peptides likely to stimulate a protective response are often preferentially targeted in HEPS (62,63). Another suggestive finding in the MACS was that B*3502 and *3503 subtypes of the broader risk marker, B*35, conferred increased risk of seroconversion (61) as similar to that observed for disease progression (64), ostensibly due to their distinctive peptide-binding profiles. Zambians differed, however, with little if any effect for B*53, despite its preference for epitopes identical to those bound by B*3502 and *3503 (33). The converse also occurs: although B*57 is consistently involved in suppressing viral replication and retarding development of immunodeficiency in infected individuals, no study of transmission recognized corresponding protection of uninfected individuals by this allele. Such distinctions could be crucial for vaccine development. As for other alleles, relationships in single and usually small populations have not yet been reliably replicated and thus should be viewed with particular caution.

b. Perinatal— In Kenyan sex workers, maternal-fetal allele sharing at class I loci in general has been associated with increased perinatal as well as with sexual transmission (65). The protective effect of the A2/A6802 supertype noted above with horizontal transmission was also observed for vertical transmission (48,60). No other studies have been reported.

Secondary Manifestations

Limited research into possible class I influences on secondary opportunistic features of HIV infection has been performed, most of it on Kaposi's sarcoma (KS) in infected men. To date the findings in several studies remain unconvincing because of their small size and typing techniques now considered imprecise. It must be emphasized that any investigation of determinants of a secondary event must exclude confounding by either the effect of the risk factor in modulating the course of HIV infection or by its independent association with one of the other frequent secondary outcomes.

Treatment and Vaccine Responses

a. Abacavir hypersensitivity— Concerted effort to evaluate host genetic influences on virologic and immunologic responses to treatment is impeded by the difficulty in excluding variable adherence to complicated antiretroviral regimens and in interpreting the significance of resistance mutations in HIV isolates. However, adverse responses can be examined more readily. Accordingly, two recent parallel studies in two separate groups of patients taking the nucleoside analogue abacavir have yielded impressively strong evidence for an association between drug hypersensitivity and *HLA-B**57, with or without other MHC genes in the haplotype including DRB1*0701 (66, 67). Hypersensitivity occurs in 10–15% of patients taking abacavir, but the two case-control studies implicated B*57 in the vast majority of cases, with relative odds >30 in

both. Withholding of the drug from individuals found for other reasons to carry B*57 would be prudent; however, the mechanism for this phenomenon is not yet known, and carriage of the allele is not considered a sufficiently reliable predictor of an adverse reaction to warrant routine HLA screening. Various other genes being screened in these studies did not show any appreciable effects on therapy.

b. HIV Vaccine Response—Vaccine-generated CTL response mediates protection against simian immunodeficiency virus (68–70), but CTL-dependent efficacy has not yet been demonstrated for any human vaccine (71). Differential restriction of responses by polymorphic *HLA* class I and/or class II gene products had been suggested with vaccines against other human viral pathogens (72–74). Early trials of an HIV-vaccinia combination (75) also hinted at such regulation. The most direct evidence comes from a trial of recombinant HIV-1-canarypox vaccine in uninfected recipients (71,76–78) in which class I alleles (B*27 and B*57) recognized as the most consistently advantageous in infected individuals generated earlier and/or more sustained HIV-1-specific CD8+ CTL responses than did the other alleles in aggregate (79).

Other Markers in the Class I Pathway—*TAP1/TAP2*

The transporters associated with antigen processing (TAP1 and TAP2) form a heterodimer that pumps enzymatically degraded cytoplasmic peptide fragments into the lumen of endoplasmic reticulum. There peptides are loaded to the class I heavy chain by tapasin and other chaperone molecules. *TAP1* and *TAP2* genes display only modest polymorphism, but variant forms are still being defined (80–82). Functional significance of TAP alterations and disease associations (83–89) fit with limited and not always consistent experimental evidence that certain polymorphisms alter peptide processing (90,91). Earlier positive findings with single variants of each gene (22,27,92) have not been examined in other cohorts, and these analyses did not fully account for all relevant alleles or extended haplotypes. It will be difficult to sort out concurrent relationships with HLA, TAP1, TAP2, tapasin and other accessory molecules in the class I pathway because close functional interactions among them may obscure the effect of any single marker.

Class II (*HLA-DRB1*, *-DQA1*, *-DQB1*, *-DPB1*)

Background

The class II response pathway and its principal effector cell, the CD4+ T-lymphocyte, is the critical coordinator of the immune response to HIV—regulating generation of CTLs, cytokine expression, antibody production, and other functions involved in control of the infection (93,94). Epitope-specific CD4+ cell enhancement in HIV infection has recently been emphasized (95). At the centromeric end of the *HLA* region, genes in the class II pathway encode products specialized for capturing lysosome-derived peptides and presenting them to regulatory or cytolytic CD4+ T-cells. The genetic structure and function of these genes governing interaction with CD4+ cells are well described (96–98). The highly polymorphic classical class II genes (*DRB1*, *DQA1*, *DQB1*, and *DPB1*) encode molecules have a longer binding groove, open (flexible) enough at each end to permit binding of peptides of more variable length and composition than for the class I products. Common alleles are readily sorted by standard molecular techniques that concentrate on the hypervariable region of exon 2 (4).

Disease Progression and Early Host/Virus Equilibration

Associations of specific *DR* or *DQ* alleles/haplotypes with variable control of early HIV infection or subsequent clinical course have been more difficult to demonstrate than for class I markers, and their differential impact remains unclear. In contrast to the importance of heterozygosity at class I loci, *DRB1* and *DQB1* heterozygosity appears to offer little or no advantage in delaying onset of disease (20). Inconsistencies have also been observed for relationships of individual alleles or haplotypes, particularly among earlier studies of smaller numbers of subjects, but larger cohort studies may help resolve them (22,28,29,99,100) (Kaslow, unpublished). The difficulty in establishing a role for class II polymorphism may be explained by the more accommodating class II binding grooves, which do not discriminate as precisely as those of class I among the large array of available peptides. High degree of redundancy in class II alpha and beta chains may further compensate for the specificity of certain allelic effects. However, these explanations are not entirely satisfying because for infection with the highly mutable hepatitis C virus (HCV), there is growing evidence of specific class II allele/haplotype-regulated control of viremia rather than indifference to such variation (101–103).

Transmission and Acquisition

Only minimal effort has been devoted to date to examining the role of class II markers in acquisition and transmission of infection (48,104) and no clear effects have yet been confirmed.

Secondary Manifestations

An initial study of disseminated Mycobacterium avium complex (DMAC) (105) suggested relationships with

*DRB1*1502-DQB1*0602* compatible with those observed in tuberculosis and leprosy and with *DRB1*0701*. They remain to be conÞrmed.

CHEMOKINE RECEPTOR AND LIGAND GENES

CCR2, *CCR5* and *SCYA3Ð5*

Background

The families of human C-C and C-X-C chemokine genes encode molecules that accumulate and act where inßammation occurs. These secretory products {e.g. regulated on activation, normal T expressed and secreted (RANTES), macrophage inßammatory proteins 1 alpha and beta (MIP-1α and MIP-1β), and others} bind to a range of receptors in the CCR family, while stromal cell-derived factor 1 (SDF-1) is the exclusive ligand for CXCR4 (106). These interactions govern cell adhesion, cell migration into sites of inßammation, internalization of receptors for recycling and/or degradation, and gene expression along the separate pathways of Th1 and Th2 lineages with their corresponding immunoregulatory functions. The majority of chemokine genes (in particular, *SCYA3-SCYA5*, encoding MIP-1β, MIP-1α and RANTES) reside on chromosomes 4 and 17 (107Ð113). *CCR* and *CXCR* genes encoding the corresponding receptor proteins are mostly arranged on chromosomes 2 and 3 (114Ð119).

The Þrst link between the chemokine receptor and ligand family members and the pathogenetic process of HIV infection was forged by the discovery of increased levels of RANTES, MIP-1α and MIP-1β in uninfected persons with suppressed replication of macrophage (M)-tropic HIV-1 (120Ð123). M-tropic HIV-1 competes with C-C chemokines for positions on any of several CCR receptors, but particularly CCR5; receptor preference has led to its alternative designation as R5 virus. T-cell-tropic HIV-1 competes with SDF-1 as the natural ligand for receptor CXCR4; this form of virus is designated X4 (124). Certain viruses are dual tropic (124Ð126). During transmission and initial stages of HIV infection, the non-syncytium inducing (NSI) and largely R5 phenotype in the virus population utilizes CCR5 as the preferred coreceptor (127Ð131). Later in the course of infection CXCR4-using X4 virus often becomes dominant. Several reviews provide additional detail (132,133). Another chemokine receptor, CX3CR1, has recently been implicated as a co-receptor for HIV; investigation of its role and that of its ligand fractalkine are at earlier stages.

Receptor ligand interaction have been linked to alteration of CD4+ cell counts and viral RNA concentration (viral load) during HIV-1 infection. However, the functions of chemokine receptors and their ligands in HIV-1 infection are obscure and complicated (134Ð136). Their full effects are not easily captured by simple correlations of circulating viral RNA or disease progression, or even by focusing on cell lineage-speciÞc chemokine production or receptor expression (137Ð140).

Polymorphisms in the genes encoding chemokine receptors and ligands have been extensively investigated for corresponding variation in their genetic, biochemical, and physiologic properties (141Ð143). This work has been difÞcult to follow because of the profusion of molecular variants and multiple simultaneous genetic designations; hopefully, an ongoing effort to catalogue the expanding number of genes and standardize their sequence nomenclatures (144) will soon reverse the confusion. The redundancy and ambiguity has affected population studies of receptor/ligand polymorphisms as well as the documentation of the basic research, but simplifying approaches have begun to resolve clinically and epidemiologically relevant contributions of *CCR2* and *CCR5* alleles and their haplotypic lineages (41,42,145).

Disease Progression and Early Host/Virus Equilibration

*a. CCR2 and CCR5*Ñ Polymorphisms in the coding or promoter and regulatory regions of certain chemokine receptors are unequivocally linked to critical events in the pathogenesis of HIV-1/AIDS. The most important observations and well established relationships are summarized in Fig. 12.2. The evidence for a causal relationship is strongest for the 32-base pair deletion (D32) at the distal end of the coding region of the *CCR5* gene. The deletion truncates the gene sequence and aborts the production of message. About 15Ð20% of Caucasians carry the deletion on one chromosome and 1Ð2% on both; in the latter homozygosity abolishes cell surface CCR5 expression altogether (129,146Ð149). Presence of a single copy of this variant has repeatedly been associated with more slowly evolving immunodeÞciency in HIV-1-infected individuals and with delayed disease onset (19,41,42,146Ð148,150). Reduced receptor expression due to this deletion appears responsible for lower equilibrium concentrations of HIV-1 RNA relatively early in the course of infection in essentially untreated (19,42,151,152) treated (153Ð155), or mixed (156) groups of patients. Elevated levels of CCR5 in Δ32 heterozygotes have likewise been associated with decreased HIV-1 RNA levels (157). Additonal mutations in the *CCR5* coding region include a point mutation encoding that confers *in vitro* resistance to HIV-1 when it occurs in conjunction with ΔD32 (158).

Although the receptor function of CCR2 in HIV infection remains uncertain, a 190G-to-A substitution produces a 64I variant in the gene for that receptor (Fig. 12.2) that has been associated with slower disease progression somewhat less consistently than D32 (19,41,42,156,159Ð166) and with establishment of a more favorable early viremic equilibrium in Caucasians (19,42). The effect may be due to its linkage with variants in the *CCR5* promoter region rather than to the 64I polymorphism itself (41,143,160,167,168).

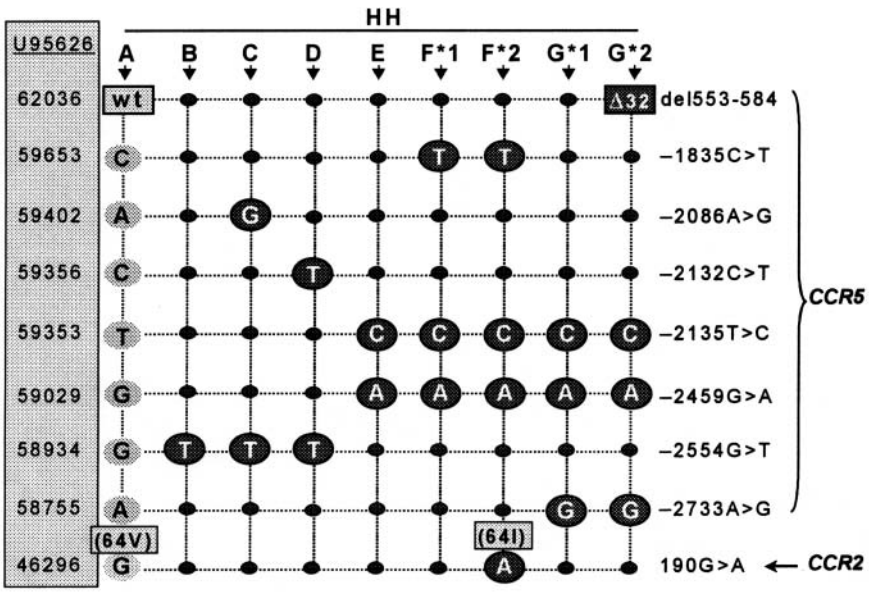

FIG. 12.2. *CCR2-CCR5* haplotypes on chromosome 3. Eight single-nucleotide polymorphisms (SNPs) and a 32-bp deletion (Δ32) de ne nine major human haplotypes (HH) known as A through G*2 (top). Sequences identical to these shown in haplotype A are indicated by dots. The 5-digit numbers (left) are based on Genbank sequence U95626, while 3- or 4-digit numbers (right) refer to positions relative to the ATG translation start site in the transcribed *CCR2* (lled box) and *CCR5* (open boxes) sequences. The open reading frame (ORF) of *CCR5* is part of exon 3. The *CCR2* 190G > A SNP has been commonly referred to 64V/I to re ect the predicted amino acid substitution.

The major CCR effects involve: (1) *CCR5-Δ32* (G*2 haplotype)—Homozygotes for this largely Caucasian variant show virtually completely protection against acquisition of HIV-1 infection, and in larger studies heterozygotes show partial protection. Heterozygotes have been consistently reported to experience slower disease progression (19,41,42, 146–148,150). (2) *CCR5* promoter genotype E/E—This is a more precise set of SNPs associated with susceptibility to infection and repeatedly with faster progression in both Caucasians and those of African descent (41,42,173,174). This is included in the less speci c genotype P1/P1 (combinations of 59029A, 59353C, and 59356C) reported rst with rapid progression (170). All of these are reciprocal to the 59029G SNP originally reported with slower disease progression (169). (3) *CCR2* SNP 190A encoding 64I (F*2 haplotype)—This variant has been repeatedly but not uniformly associated with slower progression (19,41,42,156,159–166). (4) *CCR5* promoter genotype D/D—This has been reported with vertical transmission in African-Americans (180). (5) Other promoter SNPs, haplotypes and genotypes—These have been reported to show relationships in single studies (158). Adapted from Ref (133).

TABLE 12.3. *Association of CCR2-CCR5 genotypes with acquisition of HIV-1 infection in MACS based on the comparison of heavily exposed and persistently seronegative (HEPS) caucasians with all or with heavily exposed caucasian seroconverters (Scs)*

			Seroconverters (SCs)[a]				Analysis with All SC[a]				Heavily exposed SCs[a]			
CCR2-CCr5 Genotype	HEPS (90)[a]		All (469)		Heavily exposed (270)		Model 1[b]		Model 2[b]		Model 1[b]		Model 2[b]	
	N	%	N	%	N	%	OR[c]		OC[c]		OR[c]		OR[c]	
G*2 (Δ32)/ANY	21	23.3	81	17.2	50	18.5	0.54				0.45			
A/G*2 (Δ32)	6	6.6	12	2.5	3	1.1		P_{LR} =	0.39	P_{LR} =		P_{LR} =	0.17	P_{LR} =
OTHERS	64	71.1	324	68.8	188	69.6	REF	0.010[c]	REF	0.033[c]	REF	0.004	REF	0.042[c]
E/E	5	5.5	56	11.9	32	11.9	1.95		2.21		1.87		2.15	

[a] As de ned in the text, HEPS had up to 500 + homosexual partners between 1984 and 1986 while heavily exposed SCs had 50–500 + (median = 113) partners during the same period.
[b] Model 1 includes G*2/any, E/E, and others (ref); A/G*2 replaces G*2 in model 2.
[c] Odds ratio (OR) and p values are based on logistic regression (LR) by maximum likelihood estimate.
Reproduced from Tang et al. (42), with permission.

Numerous efforts to demonstrate effects of one or more SNPs in the promoter region of *CCR5* have led to wide acceptance of a role for certain combinations of *CCR2* and *CCR5* markers in altering the regulation of HIV infection, but precise relationships remain uncertain. Several SNPs along the sequence of the *CCR5* promoter have been associated with either slower or faster disease progression (41,156,169–172). Adults treated variably and even aggressively with HAART also showed some effect of promoter polymorphism (153–156). However, the full array of common *CCR2* and *CCR5* single-nucleotide polymorphisms (SNPs) and their interrelationships had not been examined comprehensively until recently (41,42,173,174). Thorough documentation of the extended *CCR2* and *CCR5* haplotypes has suggested that speciÞc haplotypes, including the one carrying Δ32, either alone or paired as genotypes, inßuence the course of HIV-1 infection. Some more consistent relationships are summarized in Fig. 12.2.

Efforts to unravel these intricate receptor-virus relationships have still not deÞnitively shown how polymorphic variants might differentially alter *in vivo* HIV-1 pathogenesis at any speciÞc time or over the course of infection. Haplotype-speciÞc regulation of CCR receptor expression could shift production dynamics of viral phenotype toward ascendance of X4 virus using predominantly CXCR4 or dual-tropic virus (175). Thus, as a result of altered tropism, over time, earlier unequivocal effects of *CCR5* polymorphism may dwindle and previously less prominent ones may emerge. Finally, although most of the reported relationships were found in Caucasian populations, there is some but not total consistency in groups of African ancestry (41,133,174) as well as suggestions that the inßuences of these haplotype combinations may differ according their racial distribution (41,145,173). Such variations could have a differential impact on the course and the burden of disease in ethnically diverse populations (133,145).

*b. SCYA3–5 (MIP-1a, MIP-1b, RANTES)*Ñ Polymorphisms in genes encoding these ligands for *CCR5* have more recently been implicated directly in the control of expression. Two SNPs in the *SCYA5* (RANTES) promoter (–28C/G and –403G/A) reportedly alter transcription activity and differentially inßuence HIV disease progression in both Japanese and Caucasians (176,177). Although the two studies seem inconsistent at Þrst pass, the Þndings were typical of a combined (i.e. haplotype) effect, perhaps operating differently according to ethnic background. *SCYA5* haplotype effects have also been studied (145), as have *SCYA3* (MIP-1a) genotypes, deÞned by two SNPs (+113C/T and +459C/T). These promising preliminary observations will undoubtedly receive further attention.

Transmission and Acquisition

*a. CCR2 and CCR5*Ñ Innumerable studies have documented the effect of a single copy of Δ32 in inhibiting M-tropic HIV from penetrating an otherwise susceptible cell, and virtually complete protection from infection among the small fraction of Caucasians with both copies deleted (129,146–149). More recently, comparison of highly exposed but uninfected participants and seroconverters in the MACS (42), as well as analysis of horizontal and vertical transmission in other populations (178,179), have documented more subtle degrees of protection against initial HIV-1 infection among Δ32 heterozygotes. An independent role of the *CCR2*–64I mutation in acquisition has been less consistently documented (42,180,181) and remains rather uncertain. Homozygosity for a another haplotype (Fig. 12.2) found largely in ethnic Africans has been reported to be more frequent in perinatally infected than uninfected African-American (180).

*b. SCYA3–5*Ñ Less work on these variants has been done for transmission than for disease progression. In a preliminary study, the *SCYA3* CT haplotype, found mostly in those of African ancestry, was reported to be more frequent in HIV-1-negatives than -positives (145).

CXCR4 and *SDF1*

The Þnding that the common 801A variant in the 3′ untranslated region of *SDF1* may be involved in upregulation of its expression led to further observations on SDF-1 inhibition of binding of T-tropic HIV to CXCR4. As originally observed, infected homozygous A/A individuals experienced slower progression (182), but this relationship has not been readily reproducible (19,183–186). Some studies have actually described a more unfavorable disease course (183,187) higher rates of viral replication (188), or more complicated relationships (189).

Along with ambiguity surrounding the *SDF1* 3′A/A effect in HIV-infected individuals, it seems doubtful that heavily exposed uninfected homozygotes are protected. In a study of Kenyan infants, heterozygous mothers actually transmitted HIV to their infants, particularly those who were breast-fed, at signiÞcantly higher frequency than mothers carrying the wild type (190). Infant genotype appeared to play no role in acquisition. While the products of the wild type and *SDF1* variant may compete differentially with HIV for the CXCR4, the apparent involvement of the latter in perinatal transmission during breastfeeding but not in the immediate peripartum period would be difÞcult to explain biologically. Acquisition of HIV was also unaffected by infant carriage of the variant in an Italian study of vertical transmission (187).

CX3CR1

Reports from two Caucasian cohorts on this member of the chemokine receptor family provided contrasting results on the relationship of 249V/I and 280T/M genotypes or

the 249I-280M haplotype to infection and progression (191,192). The 280M homozygotes were concentrated in the seroconverters among European but not American patients, while 280M heterozygosity was associated with slower disease progression in the Americans and with either unaltered or faster progression in separate European subgroups. Those discrepant population effects of the *CX3CR1* polymorphisms remain unexplained (193). Reduced coreceptor activity was demonstrated in an initial study employing a cell-cell fusion assay with plasmid or vaccinia recombinants containing the 280M variant, but coreceptor surface expression was normal (192). No polymorphisms in the corresponding ligand fractalkine have been described in conjunction with HIV infection. In short, the importance of variations in the genes of this system is unclear.

Secondary Manifestations

*a. CCR2-CCR5*Ñ There is growing interest in the chemokine receptor/ligand systems as determinants of AIDS-related neoplasms (184,194,195). In a comparison of patients with non-Hodgkins lymphoma with other HIV-infected patients who were comparable in their course of infection, D32 was associated with a three-fold lower risk; no difference in risk was seen for Kaposiõ sarcoma (194). High levels of SDF-1 may occur in conjunction with the *SDF1* 3′A variant (homozygous and heterozygous) in non-Hodgkins lymphoma among HIV-infected but not among uninfected lymphoma patients (189).

CYTOKINES

TNF/LTA and *IL10*

Tumor necrosis factor alpha (TNFα) and the closely related lymphotoxin alpha (LTα) {formerly (TNFβ)} are potent pro-inßammatory cytokines. In a variety of circumstances the former is an antagonist of il-10. *TNF* and *LTA* are encoded together in the central MHC on chromosome 6 and share sequence motifs and function as trimers (196). Their complex mechanisms of action are not fully understood. TNFα appears to beneÞt the virus more than its host by increasing virus concentration, exacerbating treatment failure, and accelerating onset of AIDS (197Ð201). The *IL10* gene on chromosome 1 encodes a protein that down-regulates expression of MHC class I and II molecules and production of Th1 cytokines (202Ð206) and may upregulate *CCR5* expression and function (207). Both TNFα and *IL-10* also activate components of HIV-1 itself (i.e. LTR, Vpr, Rev, Gp120).

Well-described SNPs in the *TNF* promoter region (Ð238 and Ð308, also designated Ð237 and Ð307 (208)), microsatellites (*TNFa-e*), or other markers (e.g. *LTA*) in the extended MHC haplotype identify subsets of individuals with perturbed levels of circulating or locally produced TNFα (209). Disproportional distributions of certain *TNF* and *LTA* variants, repeatedly invoked to explain dozens of disease processes, have not been closely correlated with altered TNFα levels or occurrence of disease; moreover, the strong linkage disequilibrium with other alleles in extended MHC haplotypes has often been ignored (210Ð212). Reports of association of the Ð308A variant with enhanced transcriptional activity have conßicted (213,214). Overall, the few studies on differential effects of *TNF* polymorphism on HIV-1 occurrence and disease progression have been unpersuasive (215Ð217). Newly described variants in *TNF* require reassessment, and further claims for pathogenetic involvement in HIV must exclude the strong effects of disequilibrium with alleles at other MHC loci.

For *IL10*, three promoter SNPs (at Ð1082, Ð819, and Ð592 relative to the transcription start site (218)) form three combinations (ATA, ACC, GCC) that appear associated with differential IL-10 expression, and under certain circumstances the ATA combination codes for suppressed production (219). *IL10* SNPs individually or in the corresponding haplotype have been associated with both HCV and HIV-1 infection (220,221). In a large study of HIV-1 infection in multiple cohorts, the single Ð592 5′A SNP in the promoter was associated with increased risk of HIV infection and relatively rapid progression to AIDS (220). Because the Þndings were based on large numbers in multiple cohorts and internally consistent, it seems likely that reÞnement of the lineages involved and conÞrmation by other investigators will corroborate those relationships.

IL6

No work on the effects of *IL6* variants in HIV-1 infection has been presented to date. For KS, among several pro-inßammatory cytokines and growth factors thought to enhance its development, an *IL6* polymorphism has been associated with the disease both in experimental work and an unconÞrmed population study (222). The *IL6* Ð174 G/G promoter genotype found in conjunction with higher IL-6 production showed a signiÞcant association with that AIDS complication, in contrast to the C/C genotype ($p < 0.01$). Attempts to replicate this relationship are underway.

Other Cytokines

Single studies on such other cytokines as *IL4* (223) and *IL1RA* (224) have also begun to appear, but they cannot yet

be properly placed in the context of HIV/AIDS pathogenesis.

OTHER

MBL2 (Mannan Binding Lectin 2)

Relatively small individual studies of the gene encoding mannan binding lectin (MBL, also called mannan binding protein) have not produced readily interpretable Þndings. Among 131 HIV-1-infected homosexual seroconverters, men with a variant of the gene progressed relatively more slowly to AIDS (particularly Kaposiõs sarcoma) and death (225). In exposed HIV-uninfected as well as infected children compared with controls, a homozygous variant at position Ð550 of the *MBL2* gene altered vertically transmitted infection and its outcome (226). More work is obviously needed to reach any conclusion about the role of these variants.

ANTIRETROVIRAL DRUG METABOLISM

A recently published pharmacogenetic investigation of the multi-drug resistance transporter gene (*MDR1*) in European Caucasians undergoing protease inhibitor therapy (227) produced the Þrst evidence of host-mediated variation in response to antiretroviral agents. Patients homozygous for the T form of *MDR1* showed signiÞcantly lower expression of the encoded transcript for P-glycoprotein, lower plasma concentrations of nelÞnavir and efavirenz and greater increases in CD4+ cells after initiation of treatment than those with C/T or C/C genotypes. Although unconÞrmed, the results suggest that T/T homozygosity is advantageously associated with reduced expression of the transporter protein and redistribution of protease inhibitors in ßuid and cell compartments. In the same patient group measurements of sequence variants known to predict altered function of the cytochrome P450 gene *CYP2D6* also suggested a differential allelic effect on drug concentration.

SUMMARY

Human genetic polymorphisms (variants) contribute signiÞcantly to variability in the acquisition, course and complications of HIV infection. Two major gene systems, the classical *HLA* class I and the chemokine receptor/ligand systems, have well established roles in determining the variability in occurrence of infection and disease progression; for several others, supporting data are suggestive to different degrees but not yet conclusive. With *HLA* class I alleles, both HIV-negative sex partners of HIV-infected adults and infants of infected mothers who share the same allele are more prone to infection. Overall diversity (i.e. heterozygosity) of class I alleles is advantageous in delaying disease progression. Certain individual alleles clearly retard (*B*27* and *B*57*) or accelerate (some if not all *B*35* alleles) the disease process, probably by regulating the host-virus equilibrium relatively early in the infection. With the key chemokine receptor gene (*CCR5*), a deletion of a portion of its coding region reduces expression and function as the major HIV-1 coreceptor. Individuals who have inherited the 32-base pair deletion on both chromosomes (i.e. homozygotes) are almost completely protected from infection, and heterozygotes appear partially protected. Haplotypes containing combinations of mutations in the *CCR5* promoter region also differentially regulate receptor expression and host control of the virus. Similarly, variations in the genes for chemokine ligands appear to alter their expression and competition with HIV for their receptors. Investigations into these and other inßuences of variations in human genes on HIV infection have demonstrated the importance of cumulative small effects. Increased knowledge of genetic variation in response to should be increasingly useful for planning and interpreting research and ultimately for managing HIV/AIDS patients in the clinical setting.

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IV

CLINICAL MANIFESTATIONS

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AIDS and Other Manifestations of HIV Infection
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Chapter 13

Care of the Adult Patient with HIV Infection

Harold W. Horowitz and Gary P. Wormser

Skillful management of the HIV-infected patient involves blending common sense and compassion with knowledge of recent pertinent scientiÞc discoveries. Care of HIV-infected patients is constantly changing and improving, which is reßected by objective increases in patient survival (1Ð5). These rapid changes impose challenges on the clinician to remain updated on recent developments (6).

Until the latter part of the 1980s therapy was directed to treatment and prevention of opportunistic infections (OI) accompanying an inevitable immunologic deterioration. Patients also developed numerous other sequelae often more difÞcult to treat than OIs such as HIV-associated nephropathy, cardiomyopathy, neuropathy, and dementia among others. Today most of the challenges in the care of HIV-infected patients in the Western world revolve around decisions on how to use antiretroviral therapy (ART) effectively. Unfortunately, there are few evidence-based answers to many of the questions regarding treatment of HIV infection. Therefore, the clinician providing care to HIV-infected patients must practice with a great deal of uncertainty.

A partial list of drugs used in the care of HIV-infected patients, but that were not United States Food and Drug Administration (FDA) approved until after the epidemic began is found in Table 13.1 (7). Furthermore, older drugs such as dapsone and clindamycin have found new usages in the treatment or prevention of HIV-related infections. An understanding of the numerous interaction among drugs commonly employed in patients with HIV infection is essential and new information on this is rapidly emerging (see Chapter **??**) (8). Diagnostic procedures and methods are also in ßux, continually being tailored to the speciÞc needs of this patient population.

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Information on the natural history of HIV infection has demonstrated the importance and proper timing for beginning prophylaxis for prevention of *Pneumocystis carinii* pneumonia (PCP), central nervous system disease due to *Toxoplasma gondii* infection, and disseminated *Mycobacterium avium* complex (dMAC) infection (9). Use of measurements of CD4 + lymphocyte counts in conjunction with HIV in plasma (viral load), to determine when to initiate and change ART therapy is giving greater precision to clinical management. Introduction of highly active antiretroviral therapy (HAART) (loosely deÞned as the use of more active drug regimens consisting of multi-drug combinations) has had a major impact on the natural history of HIV infection and has introduced certain new challenges in the care of HIV-infected patients in terms of managing drug resistance and adverse effects. Because the complexities of patient management are much greater now than a decade ago, improved patient outcomes will likely depend even more on receiving care by experienced health care providers (6,10).

Furthermore, as the HIV-infected population ages, patients may develop illnesses such as coronary artery disease, diabetes mellitus, hypertension, hyperlipidemia and osteoporosis that may be related to HIV infection, to ART, or to the aging process itself. Health care providers specializing in HIV disease who may not have been practicing primary care medicine are Þnding themselves in need of refresher courses on managing these illnesses.

INITIAL ASSESSMENT AND MONITORING OF PATIENTS

Table 13.2 outlines one approach to the initial assessment of the HIV-infected adult patient (11) Although the diagnostic term AIDS is rigorously deÞned (albeit with several revisions (12Ð15) and has been immensely helpful for surveillance in tracking the epidemic (especially prior to the discovery of HIV), it is of limited value to clinicians in the care of individual patients. It is preferable to think in

TABLE 13.1. *Partial list of drugs used in the care of HIV-infected patients and given FDA approval in the United States after 1981*

Antiretrovirals (year)[a]	Antiinfectives (year)[a]	Others (year)[a]
Zidovudine (AZT) (1987)[b]	Intravenous and oral ganciclovir (1989, 1996)	Interferon-alfa 2 (1986)
Didanosine (ddI) (1991)[b]	Fluconazole (1990)	Epoetin alfa (1989)
Zalcitabine (ddC) (1992)	Foscarnet sodium (1991)	G-CSF (Filigastram) (1991)
Stavudine (D4T) (1994)	Azithromycin (1991)	Peg interferon alfa-2B (2001)
Lamivudine (3TC) (1995)	Clarithromycin (1991)	
Saquinavir (1995)[b]	Atovaquone (1992)[b]	
Ritonavir (1996)	Itraconazole (1992)[b]	
Indinavir (1996)	Rifabutin (1992)	
Nevirapine (1996)	Trimetrexate (1993)	
Nel navir (1997)	Ganciclovir ocular implants (1996)	
Delavirdine (1997)[b]	Cidofovir (1996)	
Combivir (1997)	Fomivirsen (1998)	
Efavirenz (1998)[b]	Valganciclovir (2001)	
Amprenavir (1999)		
Abacavir (1999)		
Lopinavir/ritonavir (2000)		
Trizivir (2000)		
Tenofovir (2001)		

[a] Year approval granted by the U.S. Food and Drug Administration; [b] Medications with newer preparations since initial FDA approval.

TABLE 13.2. *Assessment of HIV-infected patients (frequency of follow-up)*[a]

Strongly recommended for all patients
History and complete physical examination (at each visit)
Complete blood count with differential and platelet count (every three months)
Chemistry pro le, including LDH, CPK, liver function tests (every three months)
Fasting cholesterol and triglyceride levels (every three to six months, especially if on protease inhibitor)
CD4 + cell count and percentage (every three months)
HIV quantitative plasma assay (every three months)
HIV resistance testing (if within six months of acute infection or switching therapy due to failure)
Treponemal antibody test (yearly if at risk)
IgG for *Toxoplasma gondii* (if negative repeat every one to two years)
Hepatitis B surface antigen and antibody, core antibody (if core and surface antibody negative, repeat every one to two years if at risk)
Hepatitis C virus antibody (if negative, repeat yearly depending upon risk)
Puri ed protein derivative of tuberculin (if negative, repeat yearly)
Papanicolaou smear for women (twice during rst year , then yearly)
Chest roentgenogram if from high-risk tuberculosis population (repeat as determined by clinical necessity)
Tests to be considered
Hepatitis A IgG antibody (if considering vaccination)
Glucose-6 phosphate dehydrogenase enzyme level (if initiating dapsone or primaquine)
Rectal papanicolaou smear (if receptive anal intercourse)
Stool for ova and parasites (for homosexual men and for those with risk due to geographic exposure)

CPK = creatine phosphokinase, LDH = lactate dehydrogenase; [a] Testing may be needed at different frequencies depending upon the clinical situation.

terms of HIV infection per se and the clinical, virologic and immunologic consequences thereof. The deÞnition of AIDS has been principally based on events that occur secondary to a state of advanced immunodeÞciency. For example, using prior deÞnitions, the day after the diagnosis of PCP was made, the patient had AIDS; the day before the patient did not, despite the same degree of immunodeÞciency. An updated case deÞnition now includes cases based solely on the presence of immunodeÞciency, as deÞned by certain laboratory criteria, in the absence of an opportunistic infection or neoplasm (15). Since 1993 all HIV-infected patients with a helper T-cell (CD4 +) count of < 200 cells/mm^3 or a CD4 percentage of $< 14\%$ are considered to have AIDS (see Chapter **??**). Once a patient has been diagnosed with AIDS, he/she will always carry this diagnosis even if immune reconstitution occurs with a rise in the CD4 + lymphocyte count to levels > 200 cells/mm^3.

Other diagnostic entities, such as ÒAIDS-related complexÓ(ARC) or Òpersistent generalized lymphadenopathyÓ

(PGL), previously used to characterize subsets of HIV-infected individuals, are no longer helpful in patient management (16).

CD4+ Lymphocyte Count

An important component of the management approach for the HIV-infected patient is to focus attention on and monitor the course of HIV-induced immune deficiency. The most helpful, readily available laboratory test to do this is the number of helper T-cells (CD4+) in peripheral blood. The CD4+ lymphocyte count is critical in deciding both when to begin and when to discontinue various prophylactic strategies to prevent opportunistic infections, and when to initiate ART (9,17,18). Although some studies suggest that viral load measurements early in course of HIV infection are lower in women than men, there are no documented gender differences in the relation of CD4 cell count to risk of opportunistic infections (18–20).

CD4+ cells are expressed either as a percentage of the total lymphocyte count, or as an absolute number, which is calculated value derived from the percentage of CD4+ cells multiplied times the total lymphocyte count. For healthy non-HIV-infected adults, the average CD4+ cell count is approximately 1,000 cells/mm^3. However, the values may range widely, from as low as approximately 500 to as high as 1,500 cells/mm^3 (21). A CD4+ cell count of 200/mm^3 marks an especially important point in the course of HIV-infected patients, since serious opportunistic infections infrequently occur before this level of immune deficiency is reached (2,22–24).

As vital as the CD4+ cell count has become in the general management of the HIV-infected patient, there are several noteworthy limitations. First, any single determination may be aberrant. The reasons why a particular count may be inconsistent with other values for a specific patient are not always apparent. Since the CD4+ count is dependent on the total lymphocyte count, fluctuations and variability in the total white blood cell or differential count determinations will greatly impact test results (25–31). Inappropriate storage conditions and delayed transportation of blood samples to the laboratory may adversely affect CD4+ lymphocyte determinations (32). In addition, factors such as test methodology, quality control, and type of laboratory equipment employed may influence the accuracy of the test (33–36).

Stress, exercise, season, use of exogenous glucocorticoids, serum cortisol level, and the presence of acute or chronic illness, have all been reported to affect CD4+ cell counts (31,37). In addition, there is a normal diurnal variation of CD4+ cells/mm^3 in HIV-infected patients, with the highest values present in the evening (30,38–42), implying that specimen collection times should be standarized. The effect of diurnal variation is greatest for persons who have relatively high CD4+ cell counts. Splenectomy is associated with a large and prolonged increase in peripheral blood CD4+ lymphocyte count, often several hundred cells/mm^3 in magnitude (43,44). In these patients, the CD4+ lymphocyte percentage reflects the level of immunodeficiency more accurately (44).

When OKT4 monoclonal antibodies are used to identify T-helper cells, falsely low CD4+ cell counts are observed in up to 11% of Blacks (and in lesser numbers of Asians and Caucasians) who lack or have a partial deficiency of the OKT4 epitope (34–36). These individuals may appear to have no CD4+ cells when in fact they have normal numbers of T-helper cells if identified by other monoclonal antibody markers (e.g. Leu 3a).

The initial CD4+ lymphocyte count for a patient, or counts significantly out of line with prior determinations, should be confirmed by repeat testing. During the first year of HIV infection, in the absence of antiretroviral drug therapy, the CD4+ cell count falls approximately 400 cells/mm^3 (45,46). Thereafter, the average decline in CD4+ cells in untreated patients is approximately 60–100 cells/mm^3 per year (47–49), except for those patients who have entered into an accelerated phase (in which a decline of approximately 160 CD4+ cells/mm^3 per year may be anticipated) (50). Patients with an accelerated CD4+ lymphocyte decline are more likely to have detectable p24 antigen in serum, higher viral loads, syncytium forming strains of virus, and develop a serious opportunistic infection (50–53) (see Chapter **??**). A small proportion of patients (up to 7%) maintain near normal numbers of CD4+ cells over many years (54). These "long-term nonprogressors" usually have fewer than 103 HIV RNA copies/ml of plasma (55–57). Factors including defective HIV (54,58), a strong cytotoxic T cell response (59,60), deletions of HIV coreceptors on CD4+ lymphocytes (making these cells more resistant to infection by HIV) (61–63), and certain HLA haplotypes (63–68), seem to be important in keeping long-term nonprogressors well.

In view of the expected slow rate of decline of the CD4+ cell count, it is not surprising that the estimated mean duration of survival from time of onset of HIV infection to death in patients receiving standard preventive therapies (as described below), is more than 10 years, even in the absence of ART (69).

Caution should be exercised in over interpreting small changes in CD4+ lymphocyte test results. Patients often attach undue significance to minor rises or falls in these counts. They should be counseled that the overall trend of the CD4+ lymphocyte count is more important than any single value. In general, there is less test-to-test fluctuation in CD4+ cell percentage compared with absolute number (25–27). The frequency of CD4+ cell count testing is dependent in part on the rapidity of the drop in counts and upon the need for testing after a change in antiretroviral therapy. For patients with CD4+ cell counts around 300 cells/mm^3 testing should be done at least four times annually. At counts of under 50 CD4+ cells/mm^3, however, there is no need to do additional testing routinely, unless further management changes are made that might

lead to higher values (see section on antiretroviral therapy below).

Viral Load Measurement

Testing for the amount of HIV in plasma by measuring viral RNA is a standard component in the management of HIV-infected patients. The amount of virus in plasma has been demonstrated to have important prognostic implications (53,57,70–81). Patients with higher viral loads tend to progress more rapidly both immunologically in terms of the rate of CD4+ cell count decline and clinically in developing opportunistic infections. The plasma viral load correlates with the risk of disease progression after the acute retroviral syndrome (82), in early symptomatic disease (76,83–85), and in chronically infected patients (71,73,75). Furthermore, plasma levels of HIV directly correlate with the risk of perinatal transmission in pregnant women (86–88), and with the risk for heterosexual transmission to uninfected partners (89). Patients with high CD4+ lymphocyte counts, even above 600 cells/mm^3, can be placed into prognostic groups based upon the plasma viral load (90). Patients with viral loads $< 5{,}000$ HIV RNA copies/ml copies/ml of plasma have the lowest risk of progression. Plasma viral load measurements are used in evaluating the effectiveness of antiretroviral drug regimens (17,18,91,92). Due to measurement variation, only a change of 0.5 logs (three-fold) is considered significant evidence of a change in viral load (17,93). For example a patient with a true viral RNA of 10,000 copies/ml could have, on a single measurement, an HIV-1 RNA value that ranged from 3,100 to 32,000 RNA copies/ml (93).

Several different methods to quantitate plasma viral RNA measurements are commercially available at present. All use amplification techniques to determine the amount of HIV in plasma. It should be kept in mind that these tests measure total viral RNA copies, not infectious virions. The lower limit for detection of HIV RNA by reverse transcriptase polymerase chain reaction (RT-PCR) is approximately 50 copies/ml. Although the various assays generally correlate well, they may not give identical numerical results (94–98). Therefore, it is prudent to use the same assay when following the course of individual patients.

Subtype B is the predominant subtype of HIV-1 found in the United States (see Chapter **??**) (99). Since only a single primer set is used, it is important to recognize that RT-PCR may be less accurate than other methods for detection of certain HIV-1 subtypes uncommonly found in the United States, including A, E, and F (99,100). Inclusion of additional primers for HIV subtypes other than subtype B (101), will enhance sensitivity for non-type B strains.

HIV plasma RNA measurements should be done on initial assessment. Some authorities prefer to measure the baseline viral load twice, two to four weeks apart (17,18). After initiating ART or changing therapy, a measurement should be performed approximately four weeks later. Thereafter, measurements should be done every three to four months, but may be done sooner if there is reason to believe that the patient's clinical or immunologic status has deteriorated.

It is important for the clinician to recognize that recent immunizations and acute illnesses may have consequences for viral load testing (102–108). Immunizations against tetanus (103) and pneumococcus (102) may cause a transient increase in HIV plasma levels (up to 300 times) that usually return to baseline levels by four weeks post immunization. Several opportunistic infections (106), as well as active tuberculosis (107) and reactivated genital herpes infection (108), have also been associated with transient elevations in viral load. It is likely that other intercurrent infections will do the same. Therefore, it is probably best to postpone viral load testing for approximately one month after either immunization or acute infections.

Appropriate specimen handling is critical to ensure accurate HIV plasma load measurements (109–111). Samples must be processed and frozen within six hours so that RNA degradation does not occur, thereby leading to falsely low viral load measurements. The appropriate anticoagulant for the particular assay must be used, and plasma rather than serum should be assayed (109).

History and Physical Examinations

A thorough history and physical examination is necessary to uncover symptoms and physical findings that may at the outset require further evaluation. Particular attention should be given to systemic complaints, visual disturbances, neurologic abnormalities, and gastroenterologic or respiratory problems. Inquiry should be made into finding out where a person has lived and traveled, whether there is exposure to animals, drug use history, and the nature of sexual activities, in order to determine whether the patient may be at risk for exposure to certain infectious agents. For instance, exposure to *Coccidioides immitis* or *Histoplasma capsulatum* is limited to certain geographic regions. Intravenous drug use places the patient at risk for bacteremia as well as endocarditis due to several bacterial pathogens (112). These histories must be updated as they change over time. Symptoms related to potential drug toxicities should be explored at each visit. For patients on ART, it is critical to assess patient adherence regularly, as poor adherence may promote the development of HIV resistance to antiretroviral drugs leading to failure of viral suppression (113).

Physical examination should carefully document the patient's weight, cutaneous or oral mucosal abnormalities, and presence of enlarged lymph nodes. It is also important to look for evidence of lipohypertrophy and lipoatrophy,

TABLE 13.3. *Selected test results in HIV-infected patients*

Test	Expected result
Hemoglobin	Progressive reduction as immunode ciency worsens: 10–20% of asymptomatic HIV-infected persons are anemic and up to 70% of AIDS patients
Leukocyte count	Progressive reduction as immunode ciency worsens: leukopenia present in 10% of asymptomatic HIV-infected persons and up to 65% of AIDS patients
Platelet count	Reduced in 10% of asymptomatic HIV-infected persons and in up to 45% of AIDS patients
Neutrophil count	Reduced in 20–50% of AIDS patients
Lymphocyte count	Reduced in 70% of AIDS patients with opportunistic infections
Serum sodium	Hyponatremia present in □30% of AIDS patients, often attributed to renal salt wasting and less commonly to hypoadrenalism
Serum potassium	Hyperkalemia may complicate therapy with trimethoprim-containing regimens or pentamidine because these drugs are sodium channel inhibitors and therefore function as potassium-sparing diuretics
Serum glucose	Pentamidine (especially parenteral) and a PI-based antiretroviral regimen may cause hyperglycemia and diabetes
Creatinine	Increased in a small proportion of cases, especially among black men who are intravenous drug users
Liver function tests	Abnormal in 90% but usually mildly, i.e. □two-fold above upper limits of normal; also may be abnormal due to antiretroviral therapy especially PIs and nevirapine
Creatine phosphokinase	Usually normal; when elevated may indicate the presence of myopathy due to either HIV or AZT
Lactate dehydrogenase	Frequently mildly elevated; elevated in 90% of patients with *Pneumocystis carinii* pneumonia
Serum lactate	Elevated in approximately 10% and associated with lactic acidosis less often; associated with NRTI use
Serum globulins	Hypergammaglobulinemia (polyclonal) is usual
Amylase	Increased in 8–30%
Triglycerides	Elevated in 50% of HIV-infected patients pre-HAART and may be a complication of HAART
Cholesterol	Elevated on PI-based antiretroviral therapy
HbsAg	Present in approximately 10%
HbcAb	Present in 90%
PPD	Positivity rates high in Haitians, intravenous drug users, prisoners, and the homeless
Treponemal antibody	Positive up to in 45% of HIV-infected homosexual men
Toxoplasma IgG	Positive in 15–80%
Cytomegalovirus antibody	Positive in 70–100%

PI = protease inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, HAART = highly active antiretroviral therapy, HbsAg = hepatitis B surface antigen, HbcAb = hepatitis B core antibody, PPD = puri ed protein derivative (of tuberculin).

which are potential adverse effects of ART (see Chapter **??**).

Laboratory Testing: Complete Blood Count and Blood Chemistry Testing

A complete blood count with platelet count is recommended as an initial screening test and at approximately three to six month intervals in the routine care of HIV-infected patients (Table 13.3). Mild leukopenia and anemia may be seen and are especially common in the setting of advanced immunodeficiency (CD4+ cell count $<200/mm^3$) (114–118). These hematologic abnormalities complicate the use of drugs that may further suppress the bone marrow, such as zidovudine (AZT) or ganciclovir.

Depressed platelet counts may occur secondary to a variety of opportunistic processes including infections and lymphoma, or as an adverse effect of many of the drugs used to manage the HIV-infected patient (see Chapter **??**) (119–121). Equally or more common as a cause of thrombocytopenia, however, is the entity termed HIV-associated idiopathic thrombocytopenia purpura (ITP), which arises as a result of immune destruction of platelets that are coated with either immune complexes or antiplatelet antibody (122,123). Impaired marrow production of platelets may also be a contributing factor (124). Thrombocytopenia may occur at any level of CD4+ cell count but is more common in advanced disease (120,121,125).

In the absence of bleeding, no treatment is needed for patients with platelet counts above 20,000/mm^3 (126). When therapy is needed, a variety of options are available, which are discussed in Chapter **??**.

Blood chemistry testing is done in the initial assessment of the HIV-infected patient and should be followed at approximately three month intervals. This testing is useful to pinpoint abnormalities of liver function, which may be due to a variety of causes including chronic viral hepatitis, many of the drugs used to treat HIV infection or its complications, and opportunistic infections (Table 13.3) (127–132). Patients with chronic hepatitis are more likely to have liver function abnormalities when given ART, particularly protease inhibitor (PI) drugs (133,134). Liver dysfunction may affect the pharmacokinetics of medications, which should be taken into account when any drug is prescribed.

A baseline lactate dehydrogenase level (LDH) is useful to serve as a basis for comparison during suspected bouts of PCP since the LDH level is characteristically elevated in this form of pneumonia (135). A baseline creatine phosphokinase (CPK) may be helpful in detecting HIV-related myopathy or to serve as a basis for comparison should muscle pain or weakness develop during AZT therapy (AZT-induced myopathy) (see Chapter **??**) (136–138).

Serum glucose level is an important blood chemistry to evaluate and follow with serial determinations. For patients with an elevated level, fasting glucose levels should be assessed. The incidence of hyperglycemia appears to be increased in patients taking PIs (139–143).

Syphilis Serology

It has been observed that the prevalence of a positive serologic test for syphilis is up to five times higher among HIV-infected homosexual men compared to those without HIV infection (144). For this reason testing is recommended at baseline and possibly on an annual basis for those patients who remain at risk for this infection. HIV-infected individuals with latent syphilis, based on the isolated finding of a positive serology, who have never been adequately treated or evaluated, should undergo a lumbar puncture (145). Cerebrospinal fluid abnormalities, even if nonspecific, warrant strong consideration for the parenteral administration of penicillin in doses adequate for the treatment of neurosyphilis (145,146). In the absence of cerebrospinal fluid abnormalities, benzathine penicillin G at a dose of 2.4 million units should be given intramuscularly weekly for three consecutive weeks to those patients judged to have late latent syphilis (145). However, benzathine penicillin cannot be relied on in patients with neurosyphilis due to very low cerebrospinal fluid drug levels (146a,147). HIV-infected patients with neurosyphilis should be treated with aqueous crystalline penicillin G at a dose of approximately three million units IV every four hours for 10 to 14 days (145). The serum Venereal Disease Research Laboratory (VDRL) or Rapid Plasma Reagin (RPR) values should be closely followed after treatment in all patients, with particularly frequent testing done for patients with early syphilis (primary, secondary, or early latent, i.e. a year or less) (145,148–150).

Tests for Hepatitis B

Hepatitis B (HB) surface antigen is present in approximately 10% of HIV-infected patients (151,152). This infection may be associated with chronic liver disease and may pose an additional occupational hazard for health care workers with parenteral blood exposures to these patients. Patients without prior hepatitis B infection (i.e. with a negative test for HBcAb) should be considered for hepatitis B vaccination (see below).

Hepatitis C Antibody

Coinfection with hepatitis C is very common among HIV-infected intravenous drug users and hemophiliacs (>50%) and disease progression is exacerbated by HIV infection (153,154). This form of hepatitis may explain abnormalities of liver function studies in these patients and, like hepatitis B, may pose an additional occupational risk for health care workers with needle-stick accidents (155,156). Therapy for hepatitis C which is routine for non-HIV-infected individuals (157), should be considered for HIV-infected patients without advanced immunodeficiency (see Chapter **??**) (158,159).

Purified Protein Derivative (PPD) Testing

PPD is recommended at baseline for HIV-infected patients with no history of a prior positive skin test or active tuberculosis (9). Thereafter, especially, in persons at high-risk for tuberculosis such as prisoners, intravenous drug users and the homeless, testing should be done yearly (9,160,161). Patients who have low CD4+ lymphocytes may be anergic and test negative on PPD testing. When the CD4+ lymphocyte count rises it is worthwhile to repeat PPD testing on these individuals.

The PPD skin test-positive HIV-infected patient has a 5–10% risk per year of developing active tuberculosis (162) and consequently should receive chemoprophylaxis (160,161,163). Induration at the PPD skin test site of 5 mm (versus the usual 10 mm) is regarded as indicative of tuberculous infection for HIV-infected patients (160). Patients who have had a positive tuberculin skin test in the past and did not receive prior antituberculous treatment or chemoprophylaxis should also be given chemoprophylaxis (see Chapter **??**) (160). Presently, it is impossible to judge

the need for prophylactic therapy in an anergic person whose skin test history is unknown (164).

Stool Examination for Ova and Parasites

Homosexual men, or persons who have resided in areas endemic for *Strongyloides stercoralis* (most tropical and subtropical areas), should have stool examinations for ova and parasites. Patients who are infected with *S. stercoralis* may be at risk for developing dissemination of this helminth as the immunodeficiency progresses (165). Consequently, all carriers should be treated, with ivermectin being the treatment of choice (166). Homosexual men infected with *Giardia lamblia* or *Entamoeba histolytica* should also receive appropriate chemotherapy (166).

Serum Triglyceride Level

The fasting serum triglyceride level is often elevated in HIV-infected patients even in the absence of ART (167) and may be one factor contributing to the increased incidence of pancreatitis in this patient population (168–170). Elevated serum triglyceride and/or cholesterol levels also are an adverse reaction of ART (140,141,143,171–175), particularly with PI therapy. Fasting of at least eight, and preferably twelve hours, is important for accurate measurement of lipid levels. Testing for fasting triglycerides, total cholesterol, and HDL cholesterol should be done at baseline with follow-up every three to six months.

Lactic acid

Lactic acid levels may increase in patients with HIV who are treated with ART. This has been associated most directly with the use of nucleoside RTIs, in particular D4T (176–179). Patients may complain of fatigue, muscle aches, weight loss, vomiting, and abdominal pain (180,181). Hepatic and muscle enzymes including transaminases, CPK, and LDH may be elevated. When venous lactic levels rise to >10 mmole/L, symptoms are universal and the disease is often fatal (182). Despite the severity of this illness, routine evaluation of the asymptomatic patient is not recommended because mild lactic acid elevations are frequent in HIV-infected patients on ART and not predictive of more severe disease (183), and because substantial technical problems are associated with lactic acid testing (17). Measurement of lactic acid requires a standardized mode of sample handling, including prechilled fluoride-oxalate tubes, which should be transported immediately on ice to the laboratory and processed within four hours after collection; blood should be collected without using a tourniquet, without fist clinching, and if possible, without stasis (17,184).

Papanicolaou Smear

Because of the high incidence of papillomavirus infection (31–67%) (185–187), cervical dysplasia (32%) (188,189), and cervical cancer (190) among women with HIV infection, Papanicolaou smears should be performed at initial evaluation, repeated at six months and annually thereafter if normal (9,145). See Chapter **??** on the management of HIV-infected women.

Anal PAP smears should be considered in gay men and in women who have had anal receptive intercourse or anogenital warts, at baseline and then yearly. As with cervical carcinoma in women, squamous intraepithelial lesions (SIL) and anal carcinoma are linked to infection with HPV (191). In gay men the prevalence of HPV is in the range of 60% to 75% (192). The frequency of anal carcinoma is markedly higher than in the non-HIV infected population (193–195).

Chest Roentgenogram

In asymptomatic HIV-infected patients the chest roentgenogram is typically normal. A baseline film may be useful for comparison with later studies done for the evaluation of respiratory tract symptoms.

Toxoplasma Gondii Serology

Toxoplasma gondii serology (IgG) should be performed at the time of initial evaluation and if negative, when the CD4+ lymphocyte count falls to <100 to 200 cells/mm^3 when patients become at risk for toxoplasmosis. The seroprevalence of toxoplasma antibody in adults varies widely according to geographic location (from 15% to 80%) (196). Since most cases of active toxoplasmosis among HIV-infected patients represent reactivation of latent infection, only those with a positive IgG antibody test are, in general, at risk (197–199). This serologic result is helpful in the evaluation of neurologic abnormalities and may be a factor in guiding the choice of chemoprophylactic regimen for prevention of PCP (see below). Seronegative persons should be counseled on how toxoplasmosis is acquired (i.e. through ingestion of undercooked meat containing tissue cysts of *Toxoplasma gondii* or any food contaminated with oocysts that originate in cat feces) and on ways to reduce this occurrence (9,196).

Serum Amylase

For unclear reasons, serum amylase levels are modestly elevated in approximately 8–30% of HIV-infected patients who have no clinical signs of pancreatic disease (200,201). Results of a baseline amylase level may be helpful for use

in comparison to subsequent testing in patients with abdominal pain, nausea, or vomiting, and in patients prior to treatment with didanosine, which is associated with the development of pancreatitis in up to 10% of patients receiving the drug (202).

Vitamin B12 and Folate Levels

Vitamin B12 levels are modestly depressed in up to 15% of HIV-infected patients due to malabsorption, which may occur even in the absence of diarrhea or other clinical or laboratory abnormalities indicative of intestinal disease (203). Because low B12 or folate levels may be associated with increased hematologic toxicity from trimethoprim-sulfamethoxazole, pyrimethamine, trimetrexate, or AZT, replacement therapy should be given (see Chapter **??**). Vitamin B12 and folate levels should also be determined for patients with peripheral neuropathy.

Glucose-6-Phosphate Dehydrogenase Levels

Regimens including the drugs dapsone or primaquine are sometimes utilized in prevention and treatment of PCP for patients intolerant of trimethoprim-sulfamethoxazole (Chapter) (204–208). Because of the risk of hemolysis in glucose-6-phosphate dehydrogenase (G-6-PD)-deficient patients, this enzyme should be assayed prior to administration of these agents (doses of dapsone of 50 mg or less per day, however, are not associated with G-6-PD-related hemolysis (209)).

INTERVENTIONS FOR HIV-INFECTED ADULTS (Table 13.4)

Counseling

Various counseling activities are an important part of the care of HIV-infected persons (Table 13.5) (210). Counseling often first begins at the time that an individual requests or is advised to have HIV antibody testing. Because of the extraordinary psychosocial impact of a diagnosis of HIV infection, it is essential for the person being tested to have a clear understanding of the implications of the test results. The purpose and meaning of the test, the testing procedure itself, and issues of potential discrimination should be explained to the patient (see Chapter **??**). Informed consent is obtained prior to testing.

HIV test results should generally be communicated in person, at which time posttest counseling is done. This should include a discussion of coping strategies after learning of a positive result, issues regarding potential discrimination, behavioral changes to reduce the risk of secondary transmission, available medical treatments, and the importance of notifying needle-sharing and/or sexual partners. Food safety issues to reduce transmission of *Toxoplasma gondii* and enteric pathogens such as *Salmonella sp*. and Cryptosporidia should also be discussed (9). These discussions should not be limited to the first or second patient visit but should continue thoughout the long-term care of the individual. It is also important that patients understand that measures for risk-reduction for transmission of HIV must be continued even if undetectable plasma viral loads have been achieved while on ART because HIV may be still present in certain cells and genital secretions. The importance of adherence to medication regimens and various appointments that may need to be scheduled should also be stressed.

Vaccinations

HIV-infected adults respond less well than their HIV-uninfected counterparts to many vaccines. Response to hepatitis B vaccine, influenza A and B vaccine, pneumococcal vaccine and hepatitis A vaccine depends upon the degree of immunodeficiency present at the time of vaccination (211–214). Asymptomatic HIV-infected patients with CD4+ cell counts well in excess of 200/mm^3 respond the best.

TABLE 13.4. *Standard interventions for HIV-infected patients*

Counseling
Pneumococcal vaccine, with single revaccination after five years
Influenza A and B vaccine yearly
Isoniazid (or other therapy as indicated) for nine months if PPD positive (now or untreated in past)
Evaluation and treatment of syphilis (including asymptomatic seropositive patients)
Initiation of highly active antiretroviral therapy
Initiation of PCP prophylaxis for patients with CD4+ lymphocyte counts < 200 cells/mm^3
Initiation of prophylaxis for cerebral toxoplasmosis for toxoplasma IgG positive patients with CD4+ lymphocyte count < 100 cells/mm^3
Initiation of prophylaxis for disseminated *Mycobacterium avium* complex infections for patients with < 50 CD4+ cells/mm^3
Discontinuation of chemoprophylaxis for opportunistic infections when the CD4+ lymphocyte count rises to appropriate levels
Caloric dietary supplementation, as needed

PPD = purified protein derivative (of tuberculin), PCP = *Pneumocystis carinii* pneumonia.

TABLE 13.5. *Topics to be covered during pre- and post-HIV test counseling*

Pre-test counseling
Methods of HIV transmission and how to reduce the risk of infection
Purpose of testing and the meaning of test results
Partner noti cation (sexual partners and needle-sharing partners)
Psychosocial issues related to a diagnosis of HIV infection
Post-test counseling—test negative
Meaning of test results
Methods of reducing risk of acquiring HIV infection
Post-test counseling—test positive
Meaning of test results
Partner noti cation
Methods to reduce transmission
Psychosocial issues related to a diagnosis of HIV infection
Food safety issues to prevent toxoplasmosis, cryptosporidiosis, and salmonellosis
Natural disease progression and impact of antiretroviral therapy
Timing of various interventions and use of laboratory data to make therapeutic decisions
Importance of adherence with medication usage

Since inactivated vaccine preparations appear safe and potentially beneÞcial, they should be routinely administered to HIV-infected individuals (see Chapter **??**). The pneumococcal vaccine is given once with a subsequent single revaccination after Þve years (215,216). The latest preparation of the inßuenza A and B vaccine is given annually during the fall (217). Patients who are susceptible to hepatitis B (HBcAb-negative) and who remain at risk of exposure to this virus should be vaccinated. An exception would be the patient with a recent exposure to hepatitis B who may be incubating the virus. For unclear reasons, hepatitis B vaccination of such individuals has been associated with an increased rate of chronic HBsAg carriage (218) HIV-infected patients who are at risk for hepatitis A infection, particularly those who are infected with hepatitis C, should be vaccinated against hepatitis A (219). Patients should be informed that the extent and duration of protective efÞcacy of these vaccines are still uncertain. Whether there may be a role for booster doses of hepatitis B vaccines in this patient population is unknown.

Antiretroviral Therapy (ART)

Antiretroviral therapy began in March 1987 when the FDA gave approval for the nucleoside RTI, zidovudine (AZT). Since then additional nucleoside RTIs (NRTIs) have been approved, such as didanosine (ddI), zalcitabine (ddC), stavudine (D4T), abacavir and lamivudine (3TC) (7,17,18,220). Tenofovir is a nucleotide analogue RTA (NtRTA). The drugs, nevirapine, delavirdine and efavirenz, also inhibit reverse transcriptase but structurally are not nucleoside analogues (NNRTI) (221). A second major target for anti-HIV chemotherapeutic agents is the viral protease enzyme. Drugs which inhibit this enzyme (PIs) are also structurally different from nucleoside analogues and include such agents as saquinavir, ritonavir, indinavir, nelÞnavir, amprenavir and lopinavir (see Chapter **??**) (222,223). The status of ART is rapidly changing and much improved over even just a few years ago (Tables 13.6Ð13.8). It is clear that with current agents, more than two drugs must be used and that when on therapy, adherence to taking all of the medications should be at least 95% (113,224). Basic questions, however, remain unanswered such as: When should therapy be started? What combination of drugs should be used? When should therapy be changed and to what? And can or should therapy be stopped for various periods?

The primary goal of ART is to achieve prolonged suppression of HIV replication (17,18,225). Current multidrug regimens decrease the HIV level not only in the peripheral blood but also in lymphoid tissue (226,227), the central nervous system (227), and the genital tract (228). However, these regimens do not completely eliminate HIV, even when the viral load in plasma remains below 50 copies/ml for several years (229Ð231). HIV can still be reactivated in lymphocytes even after suppression in the peripheral blood for several years (229,231) and can be found in some patients in genital secretions when no longer detected in plasma (232). The current paradigm is that treatment for HIV will need to be for life until new strategies for eliminating HIV from the body or appropriately stimulating the immune system to suppress HIV are developed.

Clinicians involved in the care of HIV-infected patients may Þnd the use of antiretroviral drugs particularly challenging for several reasons. First, because new drugs are continuously being added to the antiretroviral armamentarium, physicians may lack familiarity with some of these drugs and may not have a clear idea how to integrate them into their practice. These new drugs may inßuence the toxicity, dosing, and viral susceptibility of older drugs.

TABLE 13.6. *Selected features of antiretroviral medications*

Generic Name (common name)	Brand Name	Preparations	Recommended Doses	Food
Nucleoside Reverse Transcriptase Inhibitors (NRTI)				
Zidovudine (AZT, ZDV)	Retrovir	capsules: 100 mg, 300 mg syrup: 50 mg/5ml IV: 10 mg/ml	300 mg bid	with or without food
	Combivir	combination: 300 mg with 150 mg lamivudine	1 tablet bid	with or without food
	Trizivir	combination: 300 mg AZT with 150 mg lamivudine plus 300 mg abacavir	1 tablet bid	with or without food
Didanosine (ddI)	Videx and Videx EC (enteric coated)	Buffered tablets: 25, 50, 100, 150, 200 mg Powder packets for oral solution: 167, 250 mg Enteric coated capsules (Videx EC): 125 mg, 200 mg, 250 mg, 400 mg	≥60 kg weight— tablets 200 mg bid powder 250 mg bid EC 400 mg qd <60 kg weight— tablets 125 mg bid powder 167 mg bid EC 250 mg qd	30 minutes before or two hours after a meal
Zalcitabine (ddC)	HIVID	Tablets: 0.375 mg, 0.75 mg	0.75 mg po q8h	with or without food
Stavudine (D4T)	Zerit	Capsules: 15, 20, 30, 40 mg Oral solution: 1 mg/mL solution	≥60 kg, 40 mg bid <60 kg, 30 mg bid	with or without food
Lamivudine (3TC)	Epivir	Tablet: 150 mg Oral solution: 10 mg/mL	150 mg bid or 300 mg qd	with or without food
Abacavir	Ziagen	Tablet: 300 mg Oral solution: 20 mg/mL	300 mg bid	with or without food alcohol increases levels 40%
Nucleotide Reverse Transcriptase Inhibitors (NtRTI)				
Tenofovir	Viread	Tablet: 300 mg	300 mg qd	with meal
Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI)				
Nevirapine	Viramune	Tablet: 200 mg Oral suspension: 50 mg/5 mg	200 mg qd x 14d then 200 mg bid	with or without food
Delavirdine	Rescriptor	Tablet: 100 mg, 200 mg	400 mg tid	with or without food; separate from antacids or buffered ddI by one hour
Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI)				
Efavirenz	Sustiva	Capsules: 50 mg, 100 mg, 200 mg 600 mg	600 mg qd (before sleep to minimize central nervous system side effects)	without food

TABLE 13.6. *continued*

Generic Name (common name)	Brand Name	Preparations	Recommended Doses	Food
Protease Inhibitors (PI)				
Saquinavir	Fortovase	Soft-gelatin capsules: 200 mg	1,200 mg tid[a]	with a meal or up to two hours after a meal to increase absorption
Saquinavir	Invirase	Hard-gel capsules: 200 mg	400 mg bid with ritonavir[b]	with ritonavir no meal effects
Ritonavir	Norvir	Capsules: 100 mg Solution: 600 mg/7.5 mL	600 mg bid[c]	take with food to decrease adverse reactions; separate by two hours from buffered ddI
Indinavir	Crixivan	Capsules: 100 mg, 200 mg, 333 mg, 400 mg	800 mg q 8 h	take one hour before or two hours after meals or with lowfat snack; separate by one hour from buffered ddI
Nel navir	Viracept	Tablets: 250 mg Oral powder: 50 mg/g	1,250 mg bid or 750 mg tid	take with a meal to increase absorption
Amprenavir	Agenerase	Capsules: 50 mg, 150 mg Oral solution: 150 mg/mL	1,200 mg bid capsules[d] 1,400 mg bid oral solutiond	avoid high fat meal but can be taken with or without food
Lopinavir/ Ritonavir	Kaletra	Capsules: 133 mg lopinavir plus 33 mg ritonavir Oral solution: 80 mg lopinavir plus 20 mg ritonavir/mL	400/100 mg bid	take with food to increase absorption

mg = milligram, mL = milliliter, bid = twice daily, qd = daily, kg = kilogram, q8h = every eight hours.
[a] = standard doses; when used with ritonavir doses decreased—see Table 13.14.
[b] = generally not prescribed without ritonavir.
[c] = rarely used in full doses, most often lower doses to enhance other drugs—see Table 13.14.
[d] = if body weight > 50 kg.

TABLE 13.7. *Dose adjustments of antiretroviral medications for renal and hepatic disease*

Drug	Dosage in Renal Disease	Dosage in Hepatic Disease
Nucleoside Reverse Transcriptase Inhibitors (NRTI)		
Zidovudine	CrCl < 10 mL/min 100 mg q8h hemodialysis = 100 mg q8 peritoneal dialysis = 100 mg q8h	Insuf cient data but prudent to reduce dose with severe liver disease
Combivir	Do not give if CrCl < 50, instead give drugs individually	Do not give as combination in patients with severe liver disease
Trizivir	Combination not recommended if CrCl < 50 ml/min—give individual drugs	Not recommended with severe liver disease
Didanosine	*Dosage* *CrCl (mL/min)* — *60 kg* — *< 60 kg* 30–59 — tablets 200 mg qd or 100 mg bid; powder 100 mg bid — tablets 150 mg qd or 75 mg bid; powder 100 mg bid 10–29 — tablets 150 mg qd; powder 167 mg qd — tablets 100 mg qd; powder 100 mg qd < 10 — tablets 100 mg qd; powder 100 mg qd — tablets 75 mg qd; powder 100 mg qd	Monitor closely for toxicity, dosage adjustment not certain
Zalcitabine	*CrCl (mL/min)* — *Dose* 10–50 — 0.75 mg q 12 h < 10 — 0.75 mg q 24 h Insuf cient data regarding hemo- or peritoneal dialysis	No dosage adjustment but use cautiously
Stavudine	*Dosage* *CrCl (mL/min)* — *> 60 kg* — *< 60 kg* > 50 ml/min — No change — No change 26–50 ml/min — 20 mg q12h — 15 mg q12h 10–25 ml/min — 20 mg q24h — 15 mg q24h < 10 ml/min — Insuf cient data Hemodialysis or peritoneal dialysis — Insuf cient data	No dosage adjustment but use cautiously
Lamivudine	*CrCl (mL/min)* — *Dose* 30–49 — 150 mg qd 15–29 — 150 mg × 1 then 100 mg qd 5–14 — 150 mg × 1 then 50 mg qd	No dosage adjustment anticipated
Nucleoside Reverse Transcriptase Inhibitors (NtRTI)		
Abacavir	Minimal renal excretion so dosage adjustment not anticipated; data not suf cient for speci c recommendation	Data insuf cient

TABLE 13.7. *continued*

Drug	Dosage in Renal Disease	Dosage in Hepatic Disease
Nucleotide Reverse Transcriptase Inhibitors (NtRTI)		
Tenofovir	Pharmacokinetics not evaluated in renal insuf ciency but dosage decrease expected due to primary renal excretion	Not evaluated but little hepatic metabolism so dosage adjustment not anticipated
Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI)		
Nevirapine	Pharmacokinetics not evaluated in renal failure; give standard doses cautiously because metabolites largely eliminated by kidney	Pharmacokinetics not evaluated in liver failure, nevirapine mostly liver metabolized. Use standard doses cautiously
Delavirdine	Pharmacokinetics not evaluated in renal failure; give standard doses cautiously because metabolites largely eliminated by kidney	Consider dose reduction with severe liver disease; pharmacokinetics not evaluated
Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI)		
Efavirenz	Standard doses likely due to hepatic metabolism but data lacking Hemodialysis 600 mg qd Peritoneal dialysis—unknown	Consider empiric dose reduction
Protease Inhibitors (PI)		
Saquinavir	Standard doses likely but data lacking Hemodialysis—standard doses likely Peritoneal dialysis—standard doses likely	Primarily metabolized in liver. Consider dose reduction with severe liver disease
Ritonavir	Standard doses likely but data lacking Hemodialysis—standard doses likely Peritoneal dialysis—standard doses likely	Primarily metabolized in liver. Consider dose reduction with severe liver disease
Indinavir	Standard doses likely but data lacking Hemodialysis—standard doses likely Peritoneal dialysis—standard doses likely	In mild to moderate hepatic failure 600 mg q 8 h
Nel navir	Standard doses likely but data lacking Hemodialysis—standard doses likely Peritoneal dialysis—standard doses likely	Consider dose reduction
Amprenavir	Standard doses likely but data lacking Hemodialysis—standard doses likely Peritoneal dialysis—standard doses likely	Moderate hepatic failure 450 mg BID Severe hepatic failure 300 mg BID
Lopinavir/Ritonavir	Standard doses likely but data lacking Hemodialysis—standard doses likely Peritoneal dialysis—standard doses likely	Data insuf cient

Cr/Cl = creatinine clearance, mL = milliliters, min = minute, mg = milligram, q8h = every 8 hours, d = day, bid = twice a day.

TABLE 13.8. *Selected adverse reactions and drug interactions of antiretroviral drugs*[a]

Drug	Selected Adverse Effects	Selected Drug Interactions
Nucleoside Reverse Transcriptase Inhibitors (NRTI)		
Zidovudine (AZT)	Anemia, granulocytopenia, myalgia, myopathy, nausea, headache, darkening of nger nails, lactic acidosis	Avoid if possible drugs that suppress the bone marrow; probenecid increases blood levels 100%; avoid with D4T[b]
Combivir	Similar to AZT and 3TC	Similar to AZT and 3TC
Trizivir	Similar to AZT, 3TC, and abacavir	Similar to AZT, 3TC, and abacavir
Didanosine (ddI)	Pancreatitis, lactic acidosis, peripheral neuropathy,[c] diarrhea	Try to avoid with other drugs that cause pancreatitis or neuropathy; allopurinal increases levels four-fold; itraconazole and ketoconazole should be administered two hours after ddI; administer two hours after or six hours before cipro oxacin; administer indinavir or delavirdine before ddI; tenofovir increases levels ~40%—uncertain signi cance consider decreasing ddI dose by 50% when used with tenofovir and separate time of administration
Zalcitabine (ddC)	Peripheral neuropathyc, pancreatitis, lactic acidosis	Avoid drugs that cause pancreatitis or peripheral neuropathy; probenecid and cimetidine reduce ddC elimination; bioavailability reduced with magnesium/aluminum containing antacids; avoid use with 3TC due to theoretical risk of antagonism
Stavudine (D4T)	Peripheral neuropathy[c], lactic acidosis, lipoatrophy, (reduce dose to 20 mg bid if peripheral neuropathy)	Avoid use with AZT;[b] use with caution with drugs that cause pancreatitis or peripheral neuropathy.
Lamivudine (3TC)	Lactic acidosis, headache, nausea	Avoid use with ddC due to theoretical risk of antagonism.
Abacavir	Hypersensitivity reaction[d], lactic acidosis, nausea	Alcohol increases levels 40%
Nucleotide Reverse Transcriptase Inhibitors (NtRTI)		
Tenofovir	Abdominal pain, diarrhea, nausea elevated liver function tests; uncertain if causes lactic acidosis	Increases ddI levels 40%; decrease ddI dose 50%
Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI)		
Nevirapine	Fatal hepatotoxicity,[d] fever, rash,[e] Stevens-Johnson syndrome, nausea, headache	Induces CYP3A hepatic enzyme and should be used with caution with drugs metabolized by this enzyme; contraindicated with St. John's Wort, ketoconazole, rifampin; increase dose of indinavir to 1,000 mg q8h and lopinavir/ritonavir to 533/133 mg bid; lowers ethinyl estradiol levels so use other contraceptive methods; lowers methadone levels and if withdrawal symptoms, increase methadone dose

TABLE 13.8. *continued.*

Drug	Selected Adverse Effects	Selected Drug Interactions
Delavirdine	Rash,[f] Stevens-Johnson syndrome, fever, headaches, increased liver function studies	Inhibits cytochrome P450 liver enzymes; avoid use with rifampin, rifabutin, ergot derivatives, astemizole, terfenadine, cisapride, midazolam, triazolam, simvastatin, lovastatin, H_2 blockers, proton pump inhibitors; may increase level sildena l—use with caution and start with low doses sildena l; drugs that decrease the level of delavirdine are carbamazepine, phenytoin, phenobarbitol, rifampin, rifabutin; administer one hour apart from antacids or buffered ddI due to decreased absorption; H_2 blockers decrease absorption; delavirdine 400 mg tid with lower dose; indinavir 600 mg tid or fortovase 800 mg tid
Efavirenz	Rash;[f] Stevens-Johnson syndrome rarely; central nervous system effects[g] including confusion, impaired thinking and concentration, abnormal dreams, depersonalization; hyperlipidemia; elevated liver function studies; teratogenic in animals	Induces and inhibits CY3A hepatic enzyme so that effects on drugs metabolized by this pathway variable; concurrent use with astemizole, terfenadine, midazolam, triazolam, cisapride, clarithromycin, and ergot alkaloids contraindicated; for most interactions, efavirenz dose does not need to be changed; increase dose rifabutin to 450–600 mg/day or 600 mg two to three times per week; may reduce levels phenobarbital, phenytoin, carbamazepine, warfarin—monitor levels; when used with indinavir, increase indinavir dose to 1,000 mg q8H; increase lopinavir/ritonavir to 533/133 mg bid; not recommended with saquinavir because decreases levels of saquinavir by 60%
Protease Inhibitors (PI)		
Saquinavir (soft or hard gel preparations)	Nausea, abdominal pain, diarrhea, headache, hepatic toxicity; class adverse reactions include: increased triglycerides and/or cholesterol, hyperglycemia, osteoporosis, possible increased bleeding among hemophiliacs, fat maldistribution	Although the ability of different PIs to inhibit CYP3A4 varies, in general the warnings and contraindications for all of these drugs should be similar. Contraindications: terfenidine, astemizole, cisapride, triazolam, midazolam, rifampin, ergot alkaloids, simvastatin, lovastatin, pimozide, St. John's Wart; the anticonvulsants phenytoin, phenobarbitol, carbamazepine may lower PI levels; in most instances birth control other than ethinyl estradiol should be used due to decreased ethinyl estradiol levels, however, indinavir increases levels; rifabutin doses should be 150 mg daily or 300 mg twice weekly and indinavir and nel navir should be increased to 1,000 mg tid with rifabutin; closely monitor patients on methadone for withdrawal due to decreased levels; close attention to toxicity while patient taking itraconazole or ketoconazole; antiarrhythmics ecainide, propafenone, amiodarone, lidocaine, encainide should be used with caution due to increased levels and possible increased arrhythmias; calcium channel blocker levels may increase so use with caution; sildena l levels increased so use with caution starting at 25 mg and slowly increase doses; immunosuppressants such as cyclosporine, tacrolimus, rapamicin levels increase so monitor levels closely reducing dosage as needed; monitor levels of warfarin closely

TABLE 13.8. *continued.*

Drug	Selected Adverse Effects	Selected Drug Interactions
Ritonavir	Nausea, vomiting, anorexia, abdominal pain, taste perversion, circumoral or peripheral paresthesias, hepatotoxicity (see Saquinavir for class adverse reactions)	See Saquinavir
Indinavir	Nephrolithiasis, urolithiasis, interstitial nephritis, asymptomatic hyperbilirubinemia; hepatitis; gastroenterologic intolerance; paronychia; dry skin, eyes, mouth; ingrown toenails (see Saquinavir for class adverse reactions)	See Saquinavir
Nel navir	Diarrhea (see Saquinavir for class adverse reactions)	See Saquinavir
Amprenavir	Gastroenterologic intolerance with diarrhea, nausea, vomiting; rash; headache; perioral paresthesias (see Saquinavir for class adverse reactions). Note that drug is a sulfonamide	See Saquinavir Oral solution contains 55% propylene glycol and is contraindicated in renal or hepatic failure, pregnant women, or patients receiving disul ram or metronidazole; avoid vitamin E
Lopinavir/Ritonavir	Diarrhea (see Saquinavir for class adverse reactions)	See Saquinavir

mg = milligram, bid = twice daily, tid = three times daily; [a] for more speci c information, package inserts should be reviewed; [b] D4T and AZT compete for same phosphorylase and therefore may be antagonistic; [c] peripheral neuropathy may be more likely when D4T and ddI used concurrently, or if ddI and ddC are used together; [d] abacavir must be stopped and not reinitiated if a hypersensitivity reaction occurs; [e] when initiating nevirapine, liver enzyme levels should be followed closely; [f] rashes due to NNRTIs may not necessitate drug class discontinuation if mild because administration of other NNRTIs may not lead to same reaction; [g] central nervous system adverse reactions may be decreased if given before sleep, and these symptoms may improve over several weeks.

TABLE 13.9. *Status of antiretroviral drug therapy*

1.	Combination antiretroviral therapy can suppress HIV replication for several years but no regimen has been demonstrated to eliminate the virus.
2.	Maximal duration of HIV suppression is not known.
3.	Adherence to antiretroviral therapy should be > 95% to maximize the chances for viral suppression.
4.	Whether long-term survival will be possible with combination therapy is not known.
5.	When during the course of HIV infection to initiate therapy has not been determined but the CD4 + cell count should not be allowed to drop to < 200 cells/mm^3.
6.	Which combination of drugs (and how many drugs) is best to start therapy with is not known.
7.	Which speci c changes to make to "optimize" a failing antiretroviral regimen is not known.
8.	The bene ts of HIV resistance testing for designing antiretroviral therapy appear to be maximized when used after a rst or at most second failing treatment regimen.
9.	All antiretroviral drugs have adverse effects and the long-term consequences of these drugs alone or in combination is not known. The impact on quality of life should be considered when recommending therapy and in devising regimens.
10.	Although treatment interruption is being studied as a method of reducing long-term side effects of antiretroviral therapy, there is insuf cient information to recommend this approach.
11.	It is likely that management approaches for the treatment of HIV-infected patients will continue to change and improve as knowledge of how to use antiretroviral therapy improves.

Second, clinicians may not be accustomed to the tendency for decisions about the clinical management of patients to be made on the basis of what seems biologically plausible rather than on the results of rigorous long-term clinical trials. Third, a sizable number of patients request placement on speciÞc (sometimes investigational) regimens on the basis of optimistic but preliminary observations that have been published in the lay literature. Fourth, physicians may Þnd that interpretation of the signiÞcance of certain clinical studies is confusing because of conßicting results, the small number of patients upon which studies are based and the use of surrogate markers rather than clinical endpoints to measure efÞcacy. Furthermore, because of different basic inclusion criteria for patients under studyÑ such as prior antiretroviral drug exposure, CD4+ lymphocyte counts, and viral loadsÑ comparison between studies frequently is not possible. Fifth, the extremely high-pressure marketing that the pharmaceutical industry employs to inßuence health care providers to adopt particular drugs also creates confusion. And sixth, health care providers realize that with over 1,000 possible combinations of available antiretroviral agents, the number of studies being reported is burgeoning and impossible to keep up with even for those integrally involved with the Þeld.

The status of ART is summarized in Table 13.9. Use of a single antiretroviral drug has caused resistance to emerge during therapy to all tested drugs and may occur within days to weeks for drugs for which only a single mutation leads to resistance such as the NNRTIs and lamivudine (51). Therefore, only combination drug therapy is recommended (17,18). Although studies have demonstrated viral suppression for as long as four years, whether or not this efÞcacy will last for more prolonged periods of time is not known. The single exception to the use of multi-drug regimens may be the pregnant woman for whom the choice of multiple drugs versus either AZT or nevirapine alone can be offered (17). However, even in pregnancy, three drug regimens are preferred (17).

Because of the extraordinary results that have been seen using combination therapy, the paradigm for HIV infection has shifted from a slowly but relentlessly progressive immunodeÞciency associated with OIs, frequent in-patient hospitalizations, and inevitable mortality, to that of a chronic, manageable illness (233). In this setting, ART may need to be taken for prolonged periods of time. The consequences of such prolonged therapy are not known. This has led investigators to test the concept of treatment interruptions (see below) in order to minimize the amount of antiretroviral agents taken, thereby reducing toxicity.

In a Þeld that is changing so rapidly, speciÞc recommendations regarding a particular combination ART regimen to use are difÞcult to make. However, the options are wide and increasing. Frequently updated recommendations from a National Institute of Health panel can be obtained at the HIV/AIDS Treatment Information Service Web site, http://www.hivatis.org. Combinations that require fewer pills and fewer daily doses, factors that may contribute to better adherence with medication usage, are now available. Furthermore, regimens can be developed that spare the use of PIs, thereby reducing the possibility of adverse reactions associated with this class of drugs. However, it is important that clinicians keep in mind that all drugs have associated toxicities and that long-term toxicities may not be known at this time. Table 13.10 outlines one approach to using antiretroviral therapy.

With the increasing appreciation of the potential for long-term drug toxicities, initiation of antiretroviral therapy is being delayed to later time points. Recent guidelines suggest withholding therapy until the CD4+ cell count falls to < 350 cells/mm^3 and/or the plasma viral load by RT-PCR is > 55,000 copies/ml (or bDNA > 55,000 copies/ml) (17). However, many authorities do not use the viral load when determining when to begin therapy because although higher viral loads may lead to a more rapid

TABLE 13.10. *Antiretroviral drug regimens*

Regimen	Uses
Monotherapy	
AZT Nevirapine	Generally not recommended; can be considered for pregnant women who prefer not to use combination therapy and for women who present in labor
Two-drug therapy	Not recommended to treat HIV infection but sometimes recommended for post-exposure prophylaxis of a low-risk exposure[a] from a low-risk source
Three-drug therapy	
3 NRTI[b]	Preferred initial regimen for most patients Initial therapy for patients with low viral load; alternative to PI-based regimen for patients with adverse reactions to PIs or NNRTIs, for patients with adherence problems due to many pills
2 NRTI plus 1 NNRTI	Initial therapy to spare PIs; for patients with adverse reactions to PIs; may allow single daily dosing
2 NRTI plus 1 PI	Initial therapy (particularly if low CD4 + cell count and/or high viral load); for salvage of patients with resistance using 3 NRTI or 2 NRTI plus NNRTI regimen
1 NRTI, 1 NNRTI, 1 PI[c]	Possible salvage therapy; use with caution in early treatment because this regimen has toxicity of all three drug classes and if regimen fails may have resistance to all antiretroviral classes
Four-drug therapy	
Ritonavir (low dose) plus 2nd PI plus either 2 NRTI or 1 NRTI plus 1 NNRTI;	Possible initial therapy for patients with high viral load and low CD4 cells; allows twice daily dosing PIs; salvage therapy for patients failing 3 NRTI or 2NRTI plus NNRTI regimen
1 PI plus 3 NRTI or 1 PI plus 2 NRTI plus 1 NNRTI	Salvage therapy
3 NRTI plus 1 NNRTI	Intensi ed antiretroviral therapy regimens
> Four drugs	
2 PI[d] plus 2 NRTI plus 1 NNRTI or 2 PI plus 3 NRTI	Salvage therapy

[a] Low-risk exposure from a non-severe, super cial or solid needle injury.
[b] Certain NRTI combinations, such as AZT plus D4T, or ddC plus 3TC, are not recommended to be used together because these drugs appear to share the same phosphorylation pathway, possibly leading to a decrease in the intracellular concentrations of active drug. ddI plus ddC is not recommended because of the theoretical risk that this combination will increase the risk of peripheral neuropathy, a well-recognized adverse effect of each drug individually. d4T plus ddI should be used with caution due to the potential for increased neuropathy and lactic acidosis.
[c] Saquinavir plus efavirenz is not recommended due to lower serum levels of saquinavir.
[d] Indinavir plus saquinavir is not recommended because of *in vitro* antagonism.
NRTI = Nucleoside or nucleotide reverse transcriptase inhibitor.
NNRTI = Non-nucleoside (or nucleotide) reverse transcriptase inhibitor.
PI = Protease inhibitor.

CD4 + cell count declines, they are not associated with a higher risk of OIs independent of the CD4 + lymphocyte count. Studies are currently being performed to compare prospectively the strategy of beginning therapy at 350 CD4 + cells/mm^3 versus delaying treatment to 250 CD4 + cells/mm^3.

The decision to initiate ART must be made on the basis of a patientÕs desire for treatment, an assessment of the likelihood that the patient will take the prescribed medications, and a consideration of the patientÕs underlying diseases that may affect drug tolerability (eg, ddI should be avoided if possible in patients with a history of pancreatitis). Advances, such as a better understanding of the pharmacokinetics of some of the RTIs, utilization of drug-drug interactions for pharmacokinetic advantage, and new drug discovery by the pharmaceutical industry, have led to the development of therapeutic regimens that can be taken twice daily and even once daily (234,235). These changes should translate into improved adherence.

Although no single combination regimen is guaranteed to work for any one individual, for patients beginning their Þrst antiretroviral regimen (Ònai•veÓ), the chances are quite good (approaching 80Ð90%) (235Ð240), that HIV can be suppressed at least in the short-term using any of multiple

TABLE 13.11. *Advantages and disadvantages of different HIV resistance tests*

Assay	Advantage	Disadvantage
Genotype	Low cost	Interpretation dif cult
	Rapid	Requires viral load > 1,000 copies/ml
	Mutations may be detected before resistance found on phenotypic assay	HIV strains with < 20% representation not detected Interplay of multiple mutations not accounted for
Phenotype	Easy interpretation—similar to bacterial testing	High cost Longer turnaround time
	Effects of multiple mutations can be assessed	Arbitrary testing cut-offs
		Requires viral load > 500–1,000 copies/ml
		HIV strains with < 20% representation not detected
Virtual Phenotype	Less expensive than phenotype	If few matches in database, dif cult to determine if resistance present
	Rapid Rapid turnaround time	Requires viral load > 1,000 copies/ml
	Effects of multiple mutations can be assessed	HIVstrains with < 20% representation not detected
	Easy to interpret—similar to bacterial testing	

regimens, provided that the patient is adherent. Figures 13.1a and 13.1b demonstrate such responses with the use of different ART combinations. For instance, antiretroviral regimens using three NRTIs (such as AZT, 3TC, and abacavir), two NRTIs plus one NNRTI (such as AZT, 3TC, and efavirenz; or ddI, D4T, and nevirapine), two NRTIs plus a PI (such as AZT, 3TC, and indinavir; or ddI, AZT, and nelfnavir), or a drug from each class (such as AZT, delavirdine, and indinavir), have all led to HIV viral suppression in approximately 80% to 90% of patients during the short term (235–240). There are currently no data on the preferred sequencing of NRTIs (18).

Susceptibility Testing

The susceptibility of a patient's HIV strain can be measured *in vitro* by a variety of methods: genotype, phenotype, or virtual phenotype (Table 13.11) (see Chapter **??**) (241). Within the past few years, resistance testing has gained widespread use in the management of HIV-infected patients despite the lack of long-term data demonstrating improved clinical outcome. However, data from several studies indicate that during the short-term (six to twelve months) basing treatment decisions on the results of resistance testing leads to a higher proportion of patients with viral load suppression than if such testing is not performed (242–244). However, not all studies demonstrate that resistance testing improves outcome as assessed by plasma viral load suppression (245). The ability of resistance testing to aid in successful drug selection is increasingly compromised as the number of prior failed regimens increase (246,247). By the time of the third failed ART regimen, resistance testing provides few options for devising new regimens (247).

Resistance testing has several noteworthy limitations: (1) Testing cannot be performed unless there are greater than approximately 500–1,000 viral copies/ml (248). Therefore, testing cannot be performed for patients with very early failure of an antiretroviral regimen. (2) Only the dominant viral strain at the time of testing is assayed. Strains that comprise < 10–20% of the viral population are not tested. (3) Strains that may be present in sequestered areas such as semen, vaginal secretions, the central nervous system or in non-replicating states in peripheral blood lymphocytes will not be assayed. (4) For resistance to antiretroviral drugs other that 3TC and NNRTIs, multiple mutations are required. Using the genotype assay, results may be difficult to interpret because the interplay among various mutations may lead to altered phenotypic expression of resistance or susceptibility in a manner not readily predicted. (5) Resistance to one agent may enhance sensitivity to another agent. This has been particularly well studied with the codon 184 mutation in the reverse transcriptase gene that leads to 3TC resistance but increases susceptibility to AZT. The genotype assay may not report this interaction. (6) Phenotypic assays use a threshold for defining susceptibility that may not always reflect antiretroviral drug concentrations, at least in part because of protein-drug interactions that occur *in vivo* but not *in vitro*. Threshold values have changed several times

in the past few years and are likely to change in the future as more is learned about the assay.

Despite the availability of resistance testing, it is still important for health care providers to take into consideration prior antiretroviral drug regimens. Drugs used in prior failing regimens must be used with caution. Resistance testing helps indicate which drugs should be avoided, but is less useful in determining which drugs should be included in a treatment regimen.

An unanswered question is whether to use resistance testing to determine what agents to use for initial drug therapy. Current guidelines do not strongly recommend such testing for chronically infected patients because data are limited regarding its utility (17). However, for newly infected patients (within six months of infection), resistance testing may be helpful because resistance to at least one antiretroviral drug can be found in up to 20% of cases (241,249–252). In contrast, in the chronically infected patient, it is more likely that the predominant HIV strain in the blood has reverted to wildtype (lacking resistance mutations) (253). A test demonstrating no resistance may lead to a false sense of security regarding the usefulness of various drugs.

Therapeutic Drug Monitoring

Therapeutic drug monitoring (TDM) is the terminology applied to the concept of measuring the plasma level of antiretroviral agents. It can be used to determine whether the level of a particular drug is in the therapeutic range as well as to determine potentially toxic levels of a drug. It is hoped that the use of TDM will explain some of the therapeutic failures among patients who are adherent to their medication regimens (254). TDM has not been tested in large clinical trials. Theoretically, its major role will be to measure the levels of drugs that have wide plasma ranges, particularly PIs (254). For instance, the trough level of indinavir is frequently below the therapeutic level needed to prevent HIV replication (255). Increasing the dose of indinavir or nelfinavir based upon a low trough level may help to improve viral suppression. In addition, TDM may be useful to both improve efficacy and decrease toxicity associated with efavirenz therapy, since treatment failure has been associated with low blood levels and toxicity with high blood levels of this drug (256). Because activity of NRTIs depends upon intracellular activation and because intracellular concentrations are more difficult to measure than plasma levels (18), TDM may have limited use with this class of antiretroviral drugs. Data are still emerging on how to account for the protein binding of various agents. Although TDM is commercially available, there is interlaboratory variability and the interpretation of results is still being formulated (254,256).

Practical Antiretroviral Drug Therapeutics

Despite susceptibility of HIV, some authorities are apprehensive about using a triple nucleoside regimen as first line therapy for patients with a very low CD4+ lymphocyte count (< 50 to 100 cells/mm^3) and high viral loads (> 100,000 copies/ml). Limited data have suggested a trend toward inferiority of the NRTI combination of AZT plus 3TC plus abacavir compared to AZT plus 3TC plus a PI (indinavir) in patients with a plasma HIV viral load of > 100,000 copies/ml (257). Because of concerns that PI containing regimens may predispose to adverse reactions such as lipodystrophy, hypertriglyceridemia, hypercholesterolemia, and glucose intolerance, some authorities will switch to a PI-sparing regimen when the viral load is stably suppressed on the PI-containing regimen. This appears to be effective, i.e. breakthrough viremia does not seem to occur in a higher proportion of patients whose therapy has been switched compared to those continued on the PI-containing regimen (258–260). Fig. 13.1a demonstrates the success of such a switch in a patient with lipoatrophy.

Preliminary data regarding the use of antiretroviral drug regimens with four and five agents have shown a more rapid decrease in viral load than with three-drug regimens (261), but are not currently recommended for initial therapy. It is of note that many regimens in use today with PI therapy use a low dose of ritonavir to "boost" the drug levels of the second PI. This allows for PIs to be given no more often than twice daily, with or without food, and with fewer pills. However, the ritonavir should not be considered a third or fourth drug when designing an antiretroviral regimen because the dose given for this purpose is usually not therapeutic for HIV.

Optimally, the goal of therapy is to reduce the plasma HIV viral load to undetectable levels (i.e. < 50 copies/ml). However, even a 0.5 log reduction (70%) in viral load leads to a decrease in the incidence of adverse HIV-related clinical endpoints (51,262), and this should be considered the minimally acceptable level of drug activity. By eight weeks of ART, a viral load reduction of at least 1 log is expected and by four to six months there should be no detectable virus.

In general, unless a patient develops drug toxicity, an antiretroviral drug regimen that has resulted in undetectable virus should be continued. Several randomized studies have tested whether "maintenance" therapy with fewer drugs could be used to simplify the therapeutic regimen after initial "induction" therapy with three (263–265) or four (267) drugs. Data from these studies in which one or more drugs were discontinued demonstrated that such patients had more frequent recurrence of HIV viral detection in plasma compared to those who continued the original regimen (263–266). Thus, this strategy is currently not recommended.

Despite rather remarkable decreases in viral load, increases in the CD4+ lymphocyte count can be more

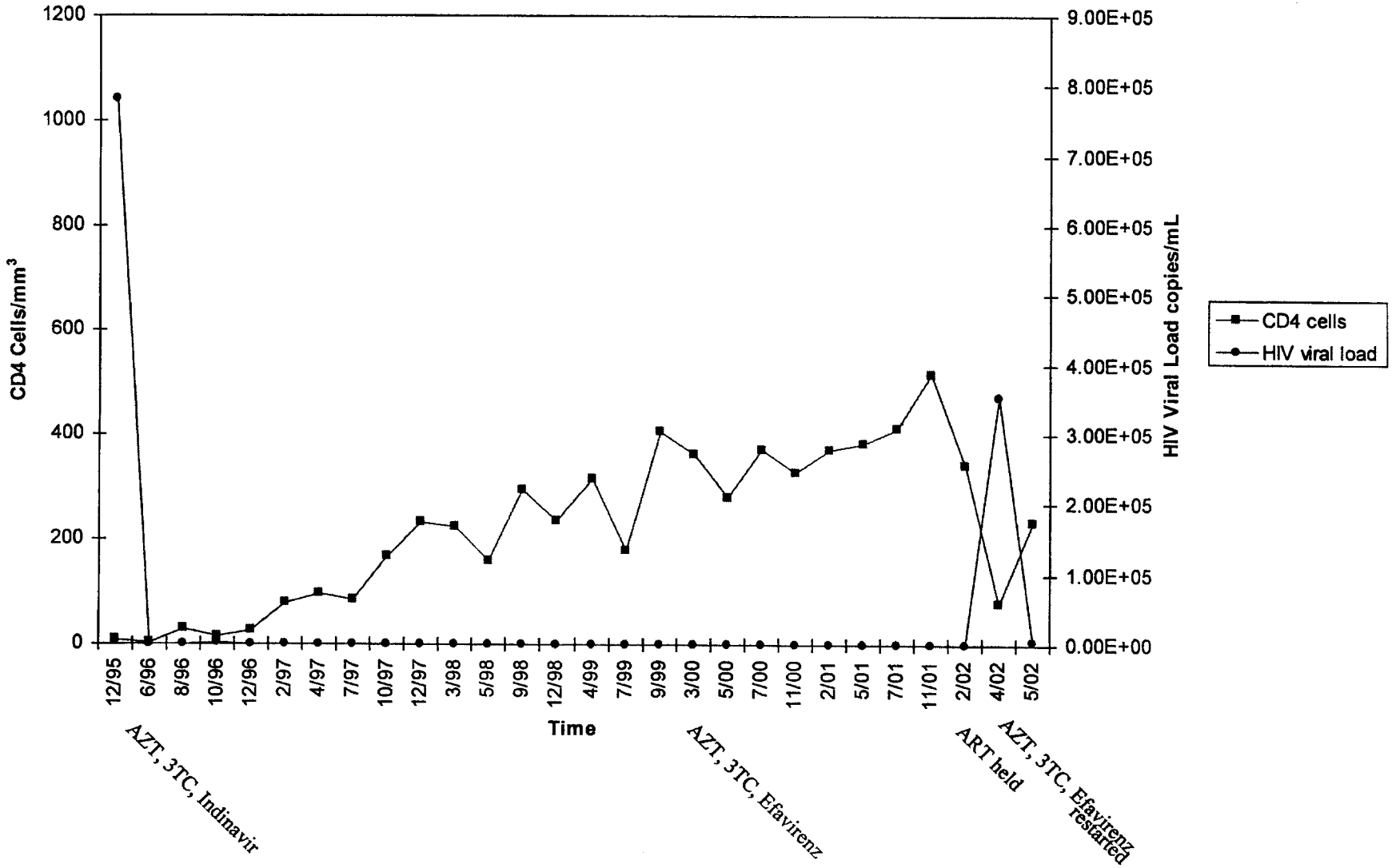

FIG. 13.1. Figures 1a and 1c demonstrate the rapid fall of CD4 + lymphocytes and rise in viral load that can occur when patients with previously very low CD4 + lymphocyte counts have therapy interrupted. Figure 1a also shows how reintroducing therapy can successfully re-suppress HIV and lead to a rise in CD4 + cells. Figure 1b demonstrates that the CD4 + lymphocyte count can be maintained after ART has been stopped despite viral load rebound in a patient who never had a low CD4 + cell count.
ART = antiretroviral therapy; AZT = zidovudine; D4T = stavudine; 3TC = lamivudine; ddI = didanosine.

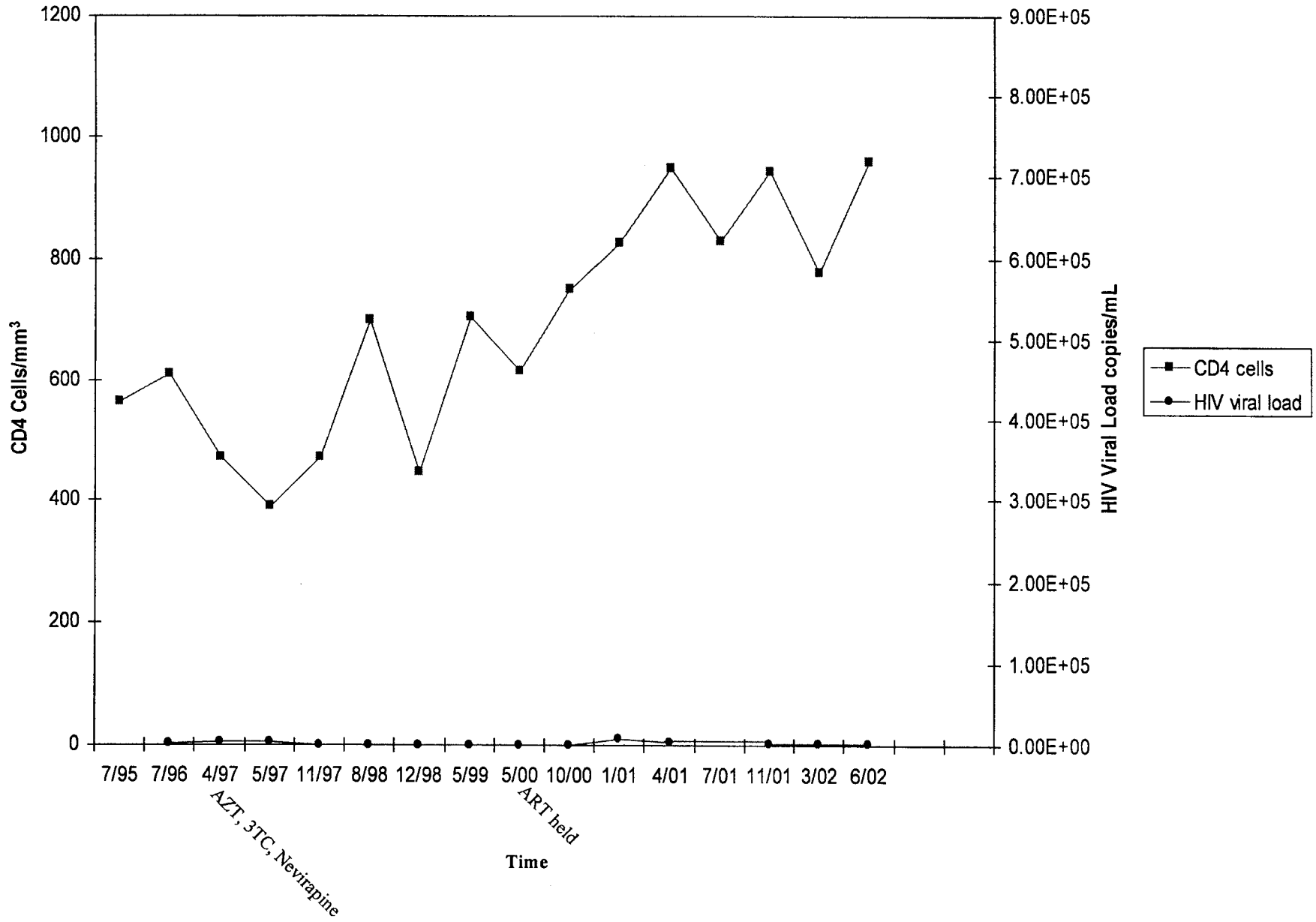

FIG. 13.1. continued.

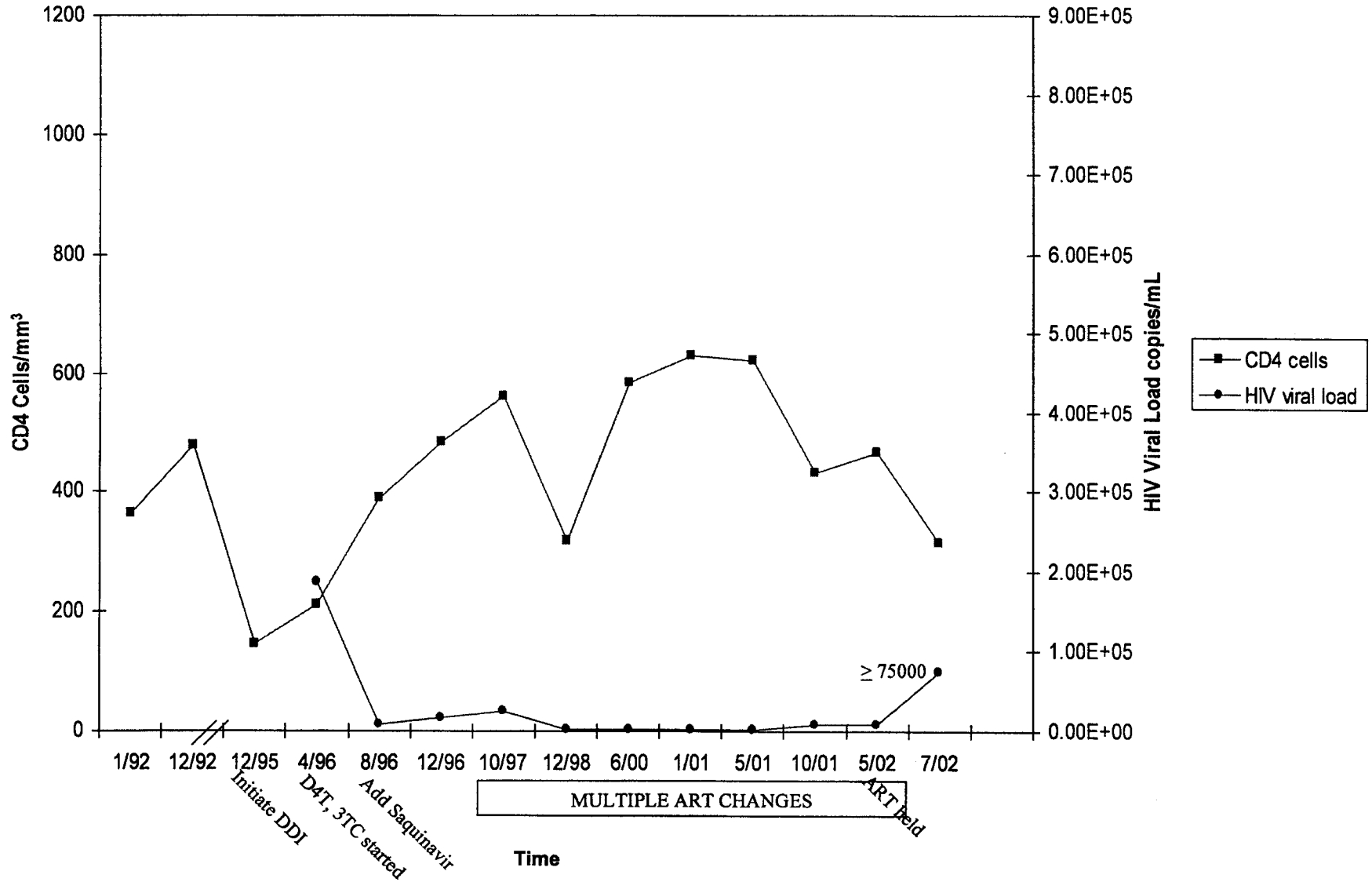

FIG. 13.1. continued.

TABLE 13.12. *Reasons to change antiretroviral therapy*

Failure to decrease viral load by □0.5 $\log_{10}$ within four weeks or 1 $\log_{10}$ by eight weeks after therapy initiation
Failure to decrease viral load to undetectable levels by four to six months of therapy[a]
Repeated detection of HIV after initial suppression to undetectable levels, possibly indicating resistance
Three-fold or greater increase in HIV load in plasma from nadir levels not attributable to other factors
Drug toxicity
Sustained and signi cant drop in CD4 + lymphocyte counts
Non-adherence (eg. high pill burden)
Clinical disease progression

[a] Patients with very high viral loads to begin with and those receiving salvage therapy may not suppress to undetectable levels. For these patients it is more realistic for health care providers to allow low (and sometimes even high levels depending upon the CD4 + lymphocyte count) of HIV in plasma without changing therapy.

TABLE 13.13. *Principles of changing antiretroviral drug therapy*

1.	Substitute □2 new drugs to a failing regimen
2.	For patients on antiretroviral therapy, resistance testing in combination with knowledge of prior therapy should be used to help in the design of a new regimen
3.	Single drug substitution is reasonable when HIV is suppressed and the change is made due to toxicity
4.	Changes for resistance between currently available NNRTIs is of doubtful bene t due to within class cross-resistance
5.	Switches are particularly problematic among patients who have been exposed to drugs from all three classes of antiretrovirals
6.	After a therapeutic change, drug-drug interactions and adverse reactions must be reevaluated
7.	Approximately four to six weeks after a change in therapy, ef cacy should be evaluated by a plasma viral load study
8.	If therapy is to be stopped for any reason, all drugs should be stopped
9.	For some patients who have received numerous drugs and have resistance to many antiretroviral agents and an increased CD4 + lymphocyte count despite active viral replication, continuing the current regimen may provide clinical bene t.
10.	When switches are made, particularly if a regimen is failing, reexamine adherence problems and solutions to these problems

NNRTI = Non-nucleoside reverse transcriptase inhibitor.

modest. The CD4 + cell count typically increases by more than 50 cells/mm^3 at four to eight weeks after starting an effective antiretroviral drug regimen, followed by an additional 50Ð100 cells/mm^3 per year thereafter (18,267Ð270). This point is demonstrated in Figs. 13.1a and 13.1b. Some patients have minimal initial CD4 + lymphocyte responses only to have marked increases years into treatment. In any one individual the maximum CD4 + cell response cannot be predicted. Individuals with a nadir CD4 + cell count that is extremely low (< 50 cells/mm^3), those with lack of optimal viral load suppression, or elderly patients may have more limited increases in CD4 + lymphocyte counts than others (271Ð274). Despite the fact that CD4 + cell increases are more likely to be sustained with optimal viral load suppression, relatively high CD4 + lymphocyte counts and clinical improvement may persist despite increases in viral load (termed discordant responder) (271,275Ð280). The patient results depicted in Fig. 1c (8/96Ð10/97) demonstrates this point. CD4 + lymphocyte counts may remain elevated for three years before they begin to decline. The marked decrease in OIs, and the ability to safely discontinue primary and secondary suppressive therapy for OIs among patients with CD4 + cell rises to > 200 CD4 + cells/mm^3 (9,281,282), suggests that these new CD4 + cells are functional (see below).

Changes in ART

Changes in ART are predicated on a variety of interrelated considerations. These include: drug tolerability, adverse reactions, clinical progression (evidenced by the development of new OIs), a declining CD4 + cell count, failure to suppress viral load adequately or viral load breakthrough after adequate suppression, and available options for treatment based upon prior therapy and resistance testing. At times it may be advisable to change therapy in order to improve adherence, i.e. decrease the number of pills or the frequency of dosing. Table 13.12 lists reasons to change therapy and Table 13.13 the principles that govern such changes.

Depending upon the reason for switching therapy, either a single drug substitution or a multiple drug change will be required. Patients with adequate viral suppression may need to have drugs changed because of a speci Þc toxicity. For instance, a patient placed on AZT, 3TC, and abacavir may develop an abacavir hypersensitivity reaction. In these instances, a single drug substitution is allowable. Rashes associated with NNRTI use also may require substitution of the NNRTI with an NRTI or PI. Other patients may develop adverse reactions to the PIs. In these instances, a simple switch of the PI to an NNRTI or a third NRTI may markedly improve glycemic control or help to

TABLE 13.14. *Adjustments in protease inhibitor doses when ritonavir enhancement is used*[a]

Drug dose[b]	Modi ed PI dose	Ritonavir dose
Saquinavir		
Invirase 600 mg TID	400 mg BID	400mg BID
Fortovase 1,200 mg TID	800–1,000 mg BID	100 mg BID
1,200 mg TID	1,600 mg QD 100 mg QD	100 mg QD
Indinavir 800 mg Q 8 hours[c]	800 mg BID—with food	100–200 mg BID
	400 mg BID—with food	400 mg BID
Amprenavir 1,200 mg BID	600 mg BID	100–200mg BID

BID = twice daily, TID = three times daily.
[a] Data on which these adjustments are based are limited; lopinavir is formulated with 100 mg ritonavir and prescribed daily (Kaletra).
[b] FDA approved non-enhanced dose.
[c] When taken without ritonavir should be taken on empty stomach or with light meal. However, when taken with ritonavir should be taken with food.

decrease elevated triglyceride or cholesterol levels (283). Unfortunately, to date, such a switch for lipoatrophy or lipohypertrophy does not appear to improve the situation (283–286).

Addition of a single drug to a three-drug regimen can be considered in several settings. In certain patients, ART may reduce viral loads to levels below 10,000 copies/ml, but not to undetectable levels. In this situation, ART "intensification", addition of a single drug in order to attempt to suppress the viral load completely, has been used by some investigators. Data for this strategy are limited (287–289), and more information on safety and efficacy are needed before this approach can be recommended.

Ritonavir, in lower than therapeutic doses, may also be added as a single drug to a multi-drug combination regimen. By inhibiting the CYP450 enzymes in the gastrointestinal tract and liver which are responsible for the metabolism of the PIs, ritonavir in doses as low as 100 mg twice daily can markedly increase drug levels of other PIs. This allows for a reduction in the frequency of administration and in the number of pills for PIs such as saquinavir, amprenavir, lopinavir, and indinavir (Table 13.14).

Addition of low-dose ritonavir to an indinavir-containing regimen may also be considered for patients who have an early HIV viral breakthrough after suppression, or for whom there was lack of complete suppression in the first place. Use of ritonavir to "intensify" therapy in this manner, before a multiple drug switch, has been shown to lead to viral suppression (288). The pharmacokinetics of indinavir are such that there is great variability in the trough level when 800 mg is given three times daily without ritonavir (290). When the indinavir concentration drops below the level necessary to inhibit the HIV strain, intermittent viral replication might occur until adequate levels are restored with the subsequent dose. Low trough indinavir levels have been associated with ART virologic failure (291). Low-dose ritonavir leads to higher and more constant blood levels of indinavir and avoids low trough levels (292), although the incidence of renal adverse effects of indinavir (eg stones, flank pain or hematuria) may be higher (293).

At least 50% of the time the reason for changing an antiretroviral regimen is due to failure to either reach or durably sustain viral load suppression (294). When changes are to be entertained for this reason, in most instances at least two new drugs to which the virus is susceptible should be included in the new regimen (17,18). The options for making such changes are numerous and no single regimen is "best" for all patients. In this particular area of therapy available study data are quite limited. Prior to making a switch, health care providers should try to ascertain which drugs the patient may have received earlier as part of a failing drug regimen, since the patient may harbor HIV strains resistant to these agents, that are not identified by drug susceptibility testing methods (see above) (241). Table 13.15 provides guidance regarding some of the changes to consider in this circumstance.

Other considerations to be kept in mind when making drug changes are prior intolerance of specific drug classes (such as NNRTI-induced rashes), co-existing illnesses (such as diabetes or pancreatitis), adherence problems, and prior reports of patient dissatisfaction with certain drugs. In some instances prescribing newer preparations of the same drug may make them much more tolerable. For instance, many patients in the past had an aversion to mixing ddI powder and taking it twice daily on an empty stomach. Now, a single pill can be taken daily. If ART failure is due to poor adherence, prior to switching medications, attempts should be made to ameliorate the problem.

Before making a change, it is important to assess the significance of the increased viral load. Among patients who have had adequate viral suppression, transient viremia may occur. Transient viremia is frequently low grade, i.e. <500 copies/ml, but can be higher. This phenomenon occurs in 35% to 40% of patients who have been suppressed to a level of <50 copies/ml (295). Although vaccination and intercurrent illness may be the etiology of transient viremia, frequently the cause is not known. The significance of such "blips" is not clear, but

TABLE 13.15. *Possible empiric changes in therapy for a failing regimen*[a]

If a 3 NRTI regimen has been unsuccessful consider:

switching 2 NRTIs (eg, AZT plus ddI to d4T plus 3TC; or d4T plus ddI to AZT plus 3TC) and adding an NNRTI or a PI (with or without ritonavir enhancement)

If a 2 NRTI plus NNRTI regimen has been unsuccessful consider:

switching 1 or both NRTIs (eg, AZT plus 3TC to ddI plus D4T; or d4T plus 3TC to AZT plus tenofovir) and switching the NNRTI to a PI (with or without ritonavir enhancement)

If a 2 NRTI plus PI regimen has been unsuccessful consider:

switching one or both NRTIs and
changing the PI to a NNRTI (if the patient has not been exposed to NNRTIs)

or

switching one or both NRTI's
and switching the PI (eg, saquinavir or nel navir to indinavir or lopinavir/ritonavir or amprenavir)

or

changing to a triple class regimen (if patient not exposed to NNRTIs)—switching one or both NRTI's and switching the PI and adding a NNRTI

[a] There are few data to recommend one speci c regimen over another when making such switches.
NRTI = Nucleoside reverse transcriptase inhibitor.
NNRTI = Non-nucleoside reverse transcriptase inhibitor.
PI = Protease inhibitor.
AZT = Zidovudine; ddI = didanosine; d4T = stavudine; 3TC = lamivudine.

patients who have frequent ÒblipsÓ to >400 copies/ml have an increased risk of eventually failing therapy (296Ð298).

Patients who are discordant responders (as described above) may also bene Þt from continuing their current regimen and not switching drugs, particularly if they have had prior problems with various drugs or failed other regimens, since the available antiretroviral drug options may be limited. These patients appear to have a reduced clinical progression of disease (277,278). It is hypothesized that the mutated virus that is replicating in the face of antiretroviral drugs is less ÒfitÓ (replicates more slowly) than wild-type virus (299). The decision regarding when to change therapy for these patients is complex and should be based upon available drug options and the patientÕs desire to make changes.

After changing ART it is advisable to obtain a complete blood count and blood chemistry studies (including liver function testing) approximately two to four weeks later to look for adverse effects that may not be clinically apparent. The viral load should be assessed between four and eight weeks after changing therapy.

Salvage Therapy

Some patients who have received numerous antiretroviral drugs will have persistently high or newly rising viral loads and either a poor CD4+ lymphocyte response or a falling CD4+ lymphocyte count. For these patients, the concept of salvage therapy (sometimes referred to as Òmega-HAARTÓ when four to Þve or more drugs are used) has entered the treatment lexicon. These regimens tend to be empiric due to broad antiretroviral drug resistance and sometimes consist of six or more drugs many of them recycled from prior use. There are few data to recommend any one of these regimens, many patients will not tolerate them for any prolonged length of time, and if viral load suppression is achieved, it is often short-lived (300Ð303). Furthermore, when changes are made at high viral loads i.e. >50,000 copies/ml, salvage therapy is more likely to fail (302). When devising a salvage regimen, it is advisable not to use new Òexpanded accessÓ drugs (drugs made available to health care providers under research protocols after completion of phase 3 studies while FDA approval is pending) unless other agents to which the HIV strain is susceptible can also be added at the same time, to avoid ÒwastingÓ potentially important and effective agents if they are used as monotherapy.

Even among patients with advanced disease who are not responding to therapy there seems to be an advantage to continuing therapy unless toxicity precludes doing so. However, there are some settings in which stopping therapy may be considered. Some patients with later stage disease choose to stop therapy to avoid taking medications when they are very ill. Some authorities also stop therapy for patients who were started on ART when their CD4+ lymphocyte count was >500 cells/mm^3 if the counts have been maintained at a high level. It is hoped that long-term toxicity will be avoided. When the CD4+ lymphocyte count falls to the threshold used to initiate ART, therapy is then restarted (17). Antiretroviral medications should be stopped and initiated together except in circumstances

when sequential initiation is used to determine which drug may be causing a particular toxicity.

Strategic Treatment Interruption

In recent years there has been a growing interest in intermittently stopping therapy, particularly for those patients with suppressed viral loads. The concept of strategic treatment interruption (STI) is that prolonged therapy may lead to unacceptable long-term adverse reactions. Furthermore, it is also hoped that stopping therapy will lead to a stronger immune response to the patientÕs HIV strain when the virus rebounds. Cost savings also would be considerable both due to lower expenditures for drugs and lower costs related to treating medication side effects.

Among chronically HIV-infected patients with prolonged viral suppression, the cell-mediated CD8+ anti-HIV immune response wanes (304,305). This can be boosted when the virus is allowed to replicate (304,306–308). There have been several small studies using differing strategies for stopping therapy (304). One is based upon a deÞned time period of drug discontinuation (for example, cycling one month on therapy then two months off). A second strategy is based upon the height of the viral load rebound (for example, therapy is restarted when the virus rebounds to a predeÞned level). It has been demonstrated that time to viral rebound increases in some patients with each drug treatment interruption (304,309). This supports the concept of Òself-immunizationÓ when therapy is stopped. However, the vast majority of patients still require intermittent treatment because the viral load eventually rises and the CD4+ lymphocyte count eventually falls when therapy is discontinued (309). Importantly, with re-initiation of therapy the virus is readily suppressed (304,306,308–310). Whether the hoped for decrease in toxicity can be realized using intermittent therapy, is an active area of investigation (311,312).

Among patients who have viral load suppression on ART, a syndrome that mimics the signs and symptoms of the acute antiretroviral syndrome may occur in a small percentage of patients when ART is stopped (see below) (313–315).

Treatment of Early HIV Infection

Patients with acute HIV infection and those infected within six months of diagnosis are two subgroups of patients whom many authorities would treat despite high CD4+ cell counts (17,316). Approximately 50% of patients with acute HIV infection manifest symptoms of the acute retroviral syndrome. This is manifested by an acute illness that has been characterized as mononucleosis-like (316,317). In fact, heterophile antibody studies may be positive (318). Common Þndings include: fever, lymphadenopathy, an erythematous maculopapular rash on the face and trunk, headaches, pharyngitis, myalgias and/or arthralgias (316,317,319). Although it is not common to make this diagnosis, physicians should consider it when encountering a patient from a high-risk population who presents with the above described illness.

At the time of the acute syndrome, antibodies to HIV are frequently negative (316,317). Diagnosis can be made by the p24 antigen assay or by Þnding HIV RNA in high levels in plasma (316). Although HIV RNA assays in general should not be used to make a diagnosis of HIV infection, in the setting of acute infection they may be helpful. Low levels of HIV in an HIV seronegative individual should be interpreted cautiously because of the potential for false positivity of HIV RNA assays (320). Among patients with a recent diagnosis of HIV infection who have high CD4+ cell counts, it is important to question when they may have most recently tested negative for HIV infection. In retrospect, these patients may recall a distinct exposure or illness compatible with acute HIV infection.

Data on the use of ART in early HIV infection are limited. However, preliminary data suggest that both disease progression and outcome are improved when therapy is begun during the acute antiretroviral infection or within the Þrst six months of infection (321–323). ART recommendations for these patients are similar to those outlined above for chronically infected patients, but should be based upon viral resistance testing (17). Patients should be informed that treatment is being offered based upon theoretical considerations with little scientiÞc data. It is hoped that early intervention will decrease viral dissemination, reduce viral load, alleviate the symptoms of the acute retroviral syndrome, and preserve immune function. The major risks for treating at this early time point are related to drug toxicities and their effect on quality of life among individuals who otherwise feel well. If the viral load cannot be adequately suppressed, resistance may limit antiretroviral options in the future when ART may be needed more.

If and when to stop therapy among these patients has not been Þrmly established. Some health care providers choose to treat indeÞnitely until new data are forthcoming. Others prefer to treat for 12 months before stopping therapy.

Treatment of Pregnant Women

The decision of when and with what drugs to treat HIV-infected pregnant women should be made in conjunction with an obstetrician, a health care provider well versed in the care of HIV-infected patients, and the patient. With some notable exceptions (see below), basic principles regarding treatment are similar for pregnant and non-pregnant HIV-infected individuals (324).

A nearly 70% reduction of HIV transmission from mother to infant can be achieved using monotherapy with

AZT, when the drug is given orally at 14 weeks of pregnancy, IV during labor, and then orally to the infant during the Þrst six weeks of life (325). However, more recent United States Public Health Service (U.S.PHS) task force guidelines (available at http://www.hivatis.org) recommend that health care providers discuss the use of combination antiretroviral drug therapies with their HIV-infected pregnant patients (326). In order to minimize the risk of vertical HIV transmission to the newborn, all HIV-infected pregnant women should receive ART regardless of CD4+ lymphocyte count and HIV viral load levels. Pregnant women must be counseled at length, however, about the lack of knowledge regarding toxicity to mother and child of multi-drug therapy. Pre-term delivery has been reported to be associated with combination antiretroviral therapy in one study (327), but not in others (328,329), and is probably not a major concern.

Due to limited enrollment of pregnant women in drug treatment trials, there are insufÞcient data concerning the effects of the physiologic and hormonal state of pregnancy on antiretroviral drug metabolism and distribution. However, based upon available data, combination ART appears safe in pregnant women (329) and can decrease the rate of vertical transmission of HIV to 1% to 5% (328,330,331). With the exceptions of ritonavir, saquinavir, nelÞnavir, and didanosine, which are category B (animal data fail to demonstrate a risk to the fetus, but human studies in pregnancy have not been done), all other approved antiretroviral drugs are category C (animal studies are either positive for fetal risk or have not been conducted, and safety in human pregnancy has not been determined) (17,324). Efavirenz, zalcitabine (ddC), and hydroxyurea should be avoided in pregnancy due to the development of fetal abnormalities in animal studies (17,326). The combination of D4T and ddI also should not be given to pregnant women, since this combination has caused severe lactic acidosis and death in three pregnant women (332).

Current recommendations for use of ART in pregnancy contemplate several potential scenarios (17). In all scenarios and regardless of the results of resistance testing to AZT, the three-part AZT regimen should be included if tolerated. HIV-infected pregnant women who have $<1{,}000$ copies/ml of plasma HIV and who have not previously received ART should be recommended to receive at least the three part AZT regimen described above beginning in the second trimester of pregnancy. Pregnant women without previous ART with $>1{,}000$ copies/ml of plasma HIV should receive three-drug combination therapy including, if tolerated, AZT. If an HIV-infected pregnant woman is already on ART and has $<1{,}000$ copies/ml, then the drug regimen may be continued with appropriate counseling about potential risks, unless it includes an agent contraindicated during pregnancy. HIV-infected pregnant women already on ART with $>1{,}000$ copies/ml HIV in plasma should have resistance testing done and changes made accordingly. The three part AZT regimen should be included when possible. If the patient is found to be HIV-infected while pregnant or is known to be HIV-infected but na•ve to ART, then therapy can be initiated immediately or deferred until the second trimester of pregnancy.

HIV-infected women who present in labor and are not taking ART present a special situation. Four options are recommended including: nevirapine (1 dose), AZT orally plus 3TC, intravenous AZT, or nevirapine (1 dose) plus intravenous AZT (326).

Cesarean section for delivery of infants of HIV-infected mothers combined with the use of AZT during pregnancy decreased the transmission of HIV from mother to infant to approximately 1% in one large French study (333). However, it is not clear that these rates are better than those achieved with combination therapy in the pregnant HIV-infected woman (328,330,331). Combination therapy without cesarean section avoids the potential morbidity and mortality associated with surgery (334,335). Among HIV-infected women undergoing cesarean section the risk of complications appears to be similar to that of non-HIV-infected women but is several fold higher than that for vaginal delivery (334,335).

Management of Adverse Effects of ART

Management of ART-related toxicity (Table 13.8) usually, but not uniformly, requires discontinuation of the medication. In many instances, complex decisions will need to be made regarding the beneÞts of continuing medication in order to maximize viral suppression and immune function versus stopping therapy. Some adverse reactions may be manifested by abnormal laboratory values such as anemia or elevated liver function studies that may not cause symptoms until the problem is severe. This is why most physicians monitor complete blood counts and blood chemistries including liver function studies at three-month intervals. Selected adverse-reactions and possible management approaches are listed in Table 13.16 (see Chapter **??**).

Adverse events can be categorized as follows. Symptomatic complaints that can occur with almost any drug are common with ART. These symptoms include nausea, vomiting, gastrointestinal discomfort, headaches, and fatigue, among others. They frequently occur early in treatment, are not life threatening, and may resolve without speciÞc treatment. Unless the problem is severe, it is worthwhile continuing the medication for several weeks to determine whether the symptoms improve. If not, then one or more medications will need to be changed. Unfortunately, if these problems occur after introduction of a new multi-drug regimen, it can be very difÞcult determining which drug caused the problem. At times, after stopping therapy, health care providers will need to reintroduce the medications sequentially in order to determine the offending drug.

Other adverse reactions tend to be more drug or drug-class speciÞc. Several NRTIs including ddI, d4T, and ddC

TABLE 13.16. *Selected adverse reactions and their treatments related to selected drugs used to treat HIV and HIV-related illnesses*

Adverse Reaction	Drugs	Evaluation and Treatment Options
Bone marrow suppression	AZT, cidofovir, cytotoxic chemotherapy, dapsone, ganciclovir, hydroxyurea, interferon, primaquine, pyrimethamine, ribavirin, sulfadiazine, TMP-SMX	Consider discontinuation of drug; Supportive therapy such as erythropoietin, G-CSF; Rule out other causes besides medication such as infection due to parvovirus or dMAC
Peripheral neuropathy	ddI, D4T, ddC, isoniazid	Consider discontinuation of drug; Rule out other causes such as vitamin B12 or folate de ciency; treat with analgesics, NSAIDS, tricyclics (e.g. nortriptyline, amitriptyline), gabapentin, phenytoin or carbamazepine; if severe, morphine sulfate or fentanyl patch; consider pain consult
Pancreatitis	ddI, pentamidine	Discontinue medication; Evaluate contributing factors such as hyperlipidemia, alcohol use or infections
Renal toxicity	indinavir, foscarnet, cidofovir, pentamidine, aminoglycosides, amphotericin B	Discontinue medication; Rule out HIVAN, other contributing medical conditions e.g. diabetes, hypertension, sepsis
Liver toxicity	NNRTIs[a], PIs, NRTIs, azole antifungals, rifampin, rifabutin, isoniazid, androgens	Consider drug substitution—NRTI for NNRTI or PI; or NRTI or PI for NNRTI; Switch from ritonavir-enhanced to non-enhanced ART; If HCV co-infection consider possible immune reconstitution if on new ART and continue medications; If severe, discontinue medications and evaluate for other medical causes and acute viral hepatitis
Rash	abacavir, PIs, NNRTIs, dapsone, TMP-SMX, sulfadiazine, clindamycin other drugs	If abacavir-related, stop drug and do not rechallenge[b]; If mild NNRTI-related rash, can observe closely and continue drug; Often must stop drug
Diarrhea	nel navir, ritonavir, lopinavir/ritonavir, amprenavir, clindamycin, among numerous other drugs	If severe and associated with a new PI containing regimen, consider switch of PI or change to another class of drugs; If mild and need or desire to continue drug then treat symptoms eg, loperamide If severe, evaluate for *Clostridium difÞcile* and other infections including OIs depending upon CD4 + cell count—likely will need to hold ART while evaluating; If evaluation negative, may need colonoscopy and symptomatic treatment
Hyperlipidemia	PIs[c], NNRTIs (less commonly)	Evaluate risk vs. bene t of continuing PI-based regimen; if HIV viral load suppressed consider switch to NRTI or NNRTI from PId If continue PI or after switch lipids remain elevated, treat according to guidelines with statins or brate,[e] dietary modi cation and exercise,[f]

TABLE 13.16. *continued*

Adverse Reaction	Drugs	Evaluation and Treatment Options
Fat maldistribution	PIs, NRTIs (particularly D4T)	Consider switch from PI to another class, particularly if associated with hyperlipidemia and HIV has been suppressed; Few data on successful management
Hyperglycemia	PIs[g]	Evaluate risk vs. bene t of continuing PI-based regimen; if viral load suppressed consider switch to NRTI or NNRTI from PI If elevated random blood glucose level, measure fasting blood glucose; Treat per diabetes guidelines
Lactic acidosis	NRTIs	Discontinue medications if symptoms
Nausea/vomiting	Any agent	If felt related to new ART regimen try to continue medications if mild and treat with antiemetics—problem may resolve within rst few weeks without need to stop medication; If severe, stop medications

g-CSF = granulocyte colony stimulating factor, TMP-SMX = trimethoprim-sulfamethoxazole, dMAC = disseminated *Mycobacterium avium* complex, NSAIDS = nonsteroidal anti-in ammatory drugs, HIVAN = HIV-associated nephropathy, NRTI = nucleoside reverse transcriptase inhibitor, NNRTI = nonnucleoside reverse transcriptase inhibitor, PI = protease inhibitor, ART = antiretroviral therapy, HCV = hepatitis C virus.

[a] although all NNRTIs can cause hepatotoxicity, nevirapine has been associated with severe liver disease.

[b] hypersensitivity reactions with the use of abacavir have been reported in up to 5% of patients, rechallenge has caused death in several patients; patients developing a rash on nevirapine frequently tolerate efavirenz.

[c] Hyperlipidemia most directly associated with PIs with the greatest incidence in patients taking ritonavir, even at low "boosting" doses but has been reported in patients taking NNRTIs regimens.

[d] Among patients with suppressed HIV viral loads, switching from a PI-based regimen to a NNRTI-based regimen appears to be safe with continued viral suppression.

[e] Statins and brates should be used in combination cautiously in HIV-infected patients, particularly if taking NRTIs due to concern about myopathy.

[f] Few data support these "lifestyle" changing measures but it seems advisable to use these in conjunction with other measures.

[g] Hyperglycemia occurs with all currently available PIs.

cause peripheral neuropathy (336). The distribution may be Ògglove and stockingÓ or may involve the lower extremities alone (337). NRTI-associated neuropathy may be mild or severe enough to interfere with activities of daily living. Other causes of peripheral neuropathy should be considered before attributing neuropathy to NRTI medications. If the inciting drug is stopped it can take months for the neuropathy to resolve. Some patients with neuropathy do not need medication for this problem. For those who do, initial management should include mild analgesic therapy and non-steroidal anti-inßammatory medications (336). Nortriptyline, amitriptyline, and gabapentin have been used with some success in a manner similar to the treatment of diabetic neuropathy (336). Some patients are in enough pain to warrant evaluation by a pain control service. An ascending Guillain-Barre-like polyneuropathy has been reported with D4T in rare instances (332) and ART should be stopped in patients who present with this syndrome.

Didanosine has been associated with pancreatitis. This drug should be stopped immediately if a patient has abdominal pain or symptoms consistent with this diagnosis, pending evaluation since this complication may be life-threatening.

Up to 3.7% of patients started on abacavir will develop a hypersensitivity reaction that includes such signs and symptoms as rash, fever, malaise, and abdominal complaints including diarrhea, nausea, vomiting, and pain (338). Recent data suggest that abacavir toxicity occurs predominantly among patients with HLA-B57 (339). However, routine screening for this HLA group is not recommended currently. In some instances respiratory symptoms such as cough, tachypnea, and pharyngitis may predominate (338). More than 90% of these reactions occur within the Þrst six weeks of therapy (338). Abacavir should be stopped if a patient is suspected of experiencing this syndrome (338). Due to the possibility of a severe reaction including death, patients who have had this reaction should never be rechallenged with abacavir (338).

Severe lactic acidosis is an infrequent adverse reaction associated with NRTIs alone or in combination, but may

be more frequent with D4T and ddI (340). This adverse reaction has been attributed to mitochondrial toxicity (341). The incidence of elevated lactic acid levels (8.7Ð14.5/1,000 patient-years) (181,183,342,343) exceeds that of symptomatic lactic acidosis (3.9/1,000 person-years) (178). Symptomatic lactic acidosis carries a high mortality rate (344); however, chronic mild, asymptomatic hyperlactatemia with levels in the range of 1.5 mmol/L to 3.5 mmol/L appears to be well tolerated (183). For lactate levels in the range of 2.1 mmol/L to 5.0 mmol/L, the lactate should be repeated, an arterial blood gas should be performed, and symptoms evaluated. If the patient becomes symptomatic, and the lactate level is persistently elevated, or the arterial pH is abnormal, ART should be stopped. Initial symptoms may be vague with complaints of fatigue most prominent. However, left untreated, patients may develop tachypnea, increasing fatigue, weight loss, nausea and vomiting, and abdominal pain (180,181). Laboratory studies reveal elevated lactate levels with a low serum bicarbonate with or without metabolic acidosis. CPK, ALT, and/or LDH all may be elevated (176). Hepatic steatosis is often demonstrated on liver biopsy. Discontinuation of therapy is critical, after which there may be slow resolution of the disease process over three to six months. Despite aggressive supportive therapy, patients frequently do not survive. Treatment for ART-associated lactic acidosis is supportive. Antioxidants such as riboßavin, carnitine, and ubiquinone have been suggested but data are limited regarding their efÞcacy (345). Once patients have improved, introduction of other NRTIs has been successful in some patients (180), but the safety of this approach has not been adequately studied.

All NNRTIs may cause rashes (346). This is particularly common in patients receiving nevirapine, occurring in nearly 20% of patients. If the rash is mild, therapy can be continued, and frequently the rash will resolve. However, patients must be observed carefully if therapy is continued; fatal Stevens-Johnson syndrome has been reported (347). Cross-reactivity between agents within this class occurs, but not in the majority of patients (347,348). Therefore, administration of a different NNRTI can be attempted but should be done cautiously. Use of corticosteroids, antihistamines, and/or desensitization techniques to decrease the incidence of skin rashes with nevirapine have met with variable results and cannot currently be recommended (349). Insomnia, dizziness, central nervous system irritability, and very vivid, often disturbing dreams have been associated with efavirenz (336,340,346). To reduce the risk for dizziness, the drug is usually taken at bedtime.

Diarrhea is a common problem with the PIs, especially ritonavir, amprenavir and nelÞnavir (340,346). Although this problem is generally mild, it can necessitate discontinuation of the medication. Drugs which slow gastrointestinal motility such as loperamide can be tried at the lowest dose that demonstrates efÞcacy after other more serious causes of diarrhea (such as infectious diarrhea) have been excluded.

Indinavir has two side effects peculiar to it: asymptomatic indirect hyperbilirubinemia that is of no consequence (340) and kidney disease including crystalluria, renal stones, and interstitial nephritis due to indinavir crystals (350,351). Renal stones can cause severe pain and hydronephrosis (350), while interstitial nephritis is frequently asymptomatic and leads to increased creatinine levels as a result of a reduction in kidney function (see Chapter **??**) (351). In order to prevent renal complications, at least two liters of ßuids should be drunk each day. Although indinavir does not necessarily need to be discontinued in the face of kidney stones, many authorities stop ART until the acute episode resolves. Afterwards, the health care provider and patient must decide whether to reintroduce indinavir and improve hydration. Indinavir should be stopped for patients with interstitial nephritis (351).

In some studies male sexual dysfunction has been associated with PI use (352) and has improved in most patients with discontinuation of the PI. Indinavir has been more strongly associated with this problem than other PIs (352).

Several metabolic adverse reactions that have been linked most closely with PI therapy, although they occur with non-PI containing regimens (175). These include lipohypertrophy, hyperlipidemia, and hyperglycemia (171) (see Chapter **??**). These metabolic abnormalities can occur together or separately and it is difÞcult if not impossible to predict which patients will develop any or all of these problems. The long-term consequences of these metabolic abnormalities such as coronary artery disease, peripheral vascular disease, and pancreatitis have been suspected, but data proving causation have not been consistent (353,354). This may in part be due to the fact that many HIV-infected patients who develop medical conditions such as coronary artery disease have other risk factors such as smoking. Another explanation for the lack of a clear association between PI therapy and consequences of metabolic problems is that the relatively brief duration of follow-up may be too short for development of clinically apparent disease.

Abnormal glucose metabolism among patients taking PIs has led to new onset diabetes, rarely with ketoacidosis, or exacerbation of pre-existing diabetes mellitus (139Ð141). It generally presents like type 2 diabetes. The mechanism for glucose dysregulation is not clear but may be multi-factorial. Some HIV-infected patients may be predisposed to diabetes mellitus because they are living longer. However, peripheral insulin resistance, pancreatic beta cell dysfunction, and accelerated conversion of proinsulin to insulin due to PI therapy are some of the proposed mechanisms for this problem (139Ð141). Fasting blood glucose levels should be monitored at three to four month intervals among patients taking PI-based ART. Glucose metabolism will improve in many patients if either ART is stopped or patients are switched to a non-PI-based regimen (283). If it is not possible to change therapy,

then patients should be managed according to the recommendations of the American Diabetes Association (284,355). Diet and exercise are important adjuncts to drug therapy. The indications for diabetic therapy and the agents that are chosen are similar to those for the non-HIV-infected patient with certain caveats. Health care providers must follow liver functions closely when biguanides and thiazolidinediones are used due to their potential to cause hepatotoxicity, especially among patients on ART or those with chronic underlying liver disease. These drug classes also may have beneÞcial effects upon lipid metabolism. Metformin, a biguanide, can also cause lactic acidosis. Careful symptomatic follow-up is required among patients taking NRTIs and metformin because lactic acidosis in this setting is often fatal.

Hyperlipidemia can be manifested by elevated cholesterol and/or elevated triglyceride levels in HIV-infected patients and has been associated with all PIs, in particular ritonavir. Fat maldistribution may or may not accompany hyperlipidemia. Co-morbid risks that may contribute to these problems such as smoking and alcohol use should be modiÞed. Factors such as liver disease, hypothyroidism, diabetes, and medications such as anabolic steroids and corticosteroids that may cause or exacerbate hyperlipidemia should also be addressed. When needed, medication for hyperlipidemia is similar to that of non-HIV-infected patients (356) except that several drugs must be avoided due to their metabolism by the CYP3A hepatic enzyme and the potential for elevated blood levels. Fluvastatin, lovastatin, and simvastatin should be avoided in patients receiving PIs, while atorvastatin and pravastatin can be used (17,357). Because levels of atorvastatin are increased six-fold, it is best to start at a low dose and then increase the dose as tolerated. Data regarding the efÞcacy of these agents are limited, and they may not be as effective as similar therapy in the non-HIV-infected patient. Statins are generally used for isolated high LDL cholesterol levels, a statin or Þbrate for high cholesterol and triglyceride levels, and Þbrates for isolated high triglyceride levels. Among non-HIV-infected patients when Þrst-line therapy fails or is not optimally successful, a member of the other drug class is added. However, the combination of a Þbrate and statin can lead to myopathy and should be used with extreme caution among patients on NRTIs. Diet and life-style changes incorporating more exercise are important adjuncts to medications for the treatment of hyperlipidemia.

Fat maldistribution sometimes termed ÒlipodystrophyÓ has been reported to occur in 17% to 75% of HIV-infected patients on ART (see Chapter **??**) depending upon how the syndrome is deÞned or ascertained and is often accompanied by hyperlipidemia and abnormal glucose metabolism (171,172,358,359). Although fat maldistribution is not in and of itself dangerous, it can be quite disabling due to the psychological impact associated with the patientÕs altered appearance. Patients may have lipohypertrophy and lipoatrophy simultaneously and all fat regions usually are not involved simultaneously.

To date, management of this problem has been difÞcult at best and no speciÞc therapies lead to consistent improvements. Discontinuation of all antiretroviral drugs can lead to minor improvement (17). Switching from a PI-based regimen to a NNRTI- or NRTI-based therapy rarely leads to signiÞcant improvement of body fat changes (360,361). Exercise, diet, and treatment of the lipid and glucose problems may help to avoid some of the body fat accumulation (284,346). Anabolic steroids, testosterone, and human growth hormone have been studied with modest effects (284,346). These therapies are expensive and have their own associated toxicity including glucose metabolism abnormalities, liver disease, and lipid disorders (346). Some patients have employed liposuction as a means of reducing fat accumulations while others have tried injections of collagen or collagen-like materials to augment facial fat loss. These are costly therapies that may not be covered by insurance policies. The long-term outcomes of such therapy are not known. In the short term, some improvement can be seen in the areas of the body that have had liposuction or injections (see Chapter **??**) (284,346).

Hepatocellular dysfunction may develop with the use of NNRTIs and PIs. In particular ritonavir and nevirapine have been associated with this problem (17,18). Fatal hepatic necrosis has been reported due to nevirapine in health care workers taking post-exposure prophylaxis and this drug is no longer indicated for that use (362). For mild liver function abnormalities, ART can generally be continued. If liver function studies rise to more than four to Þve times the upper limit of normal, health care providers should discontinue ART and consider a new regimen when the liver functions have normalized. In patients with HCV and HIV co-infection who are receiving a new ART regimen, the incidence of liver function abnormalities may be increased either because of drug toxicity (363) or because of an enhanced anti-HCV effect due to a stronger immune response, somewhat analogous to the immune reconstitution syndrome (see below). There is no deÞnitive way to distinguish between these two separate phenomena. Health care workers should therefore closely observe the patient with follow-up liver function studies. ART does not necessarily need to be withdrawn immediately.

Accelerated bone loss leading to osteopenia and osteoporosis has been reported to occur at an increased frequency in HIV-infected patients compared with the general population (364). The cause for this is not known. Although a relationship to ART has been hypothesized, this has not been established (364Ð368). When osteonecrosis (avascular necrosis) occurs, the femoral head is the most common site. However, avascular necrosis of the humeral head and multi-site disease also occurs. The association of ART and osteonecrosis is confounded by the fact that many patients have other risk factors such as

hyperlipidemia, alcoholism, pancreatitis, corticosteroids, smoking, and hypercoaguability (365,366). Patients who present with moderate to severe bone or joint pain should be evaluated for the presence of avascular necrosis. Magnetic resonance imaging is the most sensitive diagnostic test in the early stages of disease. Treatment of pain and physical therapy can help to reduce loss of mobility, and possibly the need for surgery. Whether management of osteoporosis should be similar to that of HIV-negative patients and include biphosphonates, raloxifene, calcium, calcitonin, and/or estrogens is not known at this time.

Two other adverse reactions associated with ART but not necessarily directly caused by these medications are galactorrhea and hyperprolactinemia (369), and gynecomastia among men (370). The former resolves with discontinuation of PIs.

The Immune Reconstitution Syndrome

Within weeks of starting ART, patients may become ill as new inßammation develops localizing to areas of preexisting infection (371,372). This unanticipated and unwanted consequence of ART results in troubling symptoms that occur because of the improved immune function. The frequency with which these problems arise is not known, but is probably less than 2%. Patients with inactive CMV retinitis can develop immune recovery vitritis (373,374). IntensiÞed immune responses causing symptoms in HIV-infected patients placed on ART have also been reported in patients with dMAC (375,376), herpes zoster (377), cryptococcal meningitis (378), toxoplasma encephalitis (379), tuberculosis (380,381), and progressive multifocal leukoencephalopathy (382,383). Although optimal treatment has not been deÞned, anecdotal treatment with anti-inßammatory agents such as steroids (local for vitritis or systemic for MAC) or nonsteroidal anti-inßammatory agents (MTb) plus speciÞc treatment of the inciting infection has been employed with some success. ART should not be stopped because of this problem.

The role of drugs with immunomodulating activity for use in conjunction with antiretroviral therapies is also under active investigation (384–388). Interleukin-2 administered by intravenous infusion or subcutaneously is the most well studied (389,390). No immunomodulating drug, however, can be recommended at present (see chapter). Continued changes in the approach to ART therapy can be expected over the next few years, representing perhaps the most dynamic area in clinical management.

CHEMOPROPHYLAXIS OF OPPORTUNISTIC INFECTIONS

As HIV induced immunodeÞciency advances and the CD4+ lymphocyte count falls to <200 cells/mm^3, patients become at risk for an increasing array of OIs including PCP, central nervous system toxoplasmosis, cryptococcosis, dMAC infection, oral and esophageal candidiasis, CMV retinitis, and many others (9). The CD4+ cell count is the single best indicator of when to offer and stop various preventive therapies (22–24). Unless the CD4+ lymphocyte count rises to levels at which it is safe to stop therapy, chemoprophylaxis should be continued indeÞnitely. Table 13.17 indicates the CD4+ lymphocyte levels at which primary prophylaxis should be started and stopped and the preferred antimicrobial choices for prophylaxis. Chemoprophylaxis can be considered primary (given to patients who never have had the particular infection) or secondary (given to patients who have had an episode of the infection).

If chemoprophylaxis is discontinued because of a rise in CD4+ cell count, it should be remembered that it may need to be resumed in the future if CD4+ cell counts decline. Discontinuation of chemoprophylaxis reduces: pill burden, potential drug-drug interactions, toxicity, potential for drug resistance to develop, and drug costs. HIV replication does not have to be completely suppressed in order to discontinue prophylaxis.

Primary prophylaxis is routinely recommended for prevention of infection due to: *P. carinii*, *T. gondii*, *Mycobacterium-avium* complex, and *M. tuberculosis*. For other infections, primary prophylaxis is not currently recommended.

Pneumocystis carinii Pneumonia (PCP) Chemoprophylaxis

Initiating prophylaxis

Without speciÞc chemoprophylaxis, it has been estimated that up to 75% of HIV-infected individuals will develop PCP when the CD4+ lymphocyte count falls to below 200 cells/mm^3 (23,391). Although PCP is rare among patients with a CD4+ lymphocyte count >200 cells/mm^3, approximately 5% have higher counts (22). Patients with CD4+ cell counts in the range of 200 to 250 cells/mm^3 with thrush or a CD4+ lymphocyte percentage of $<14\%$ are at higher risk for developing PCP and should receive PCP prophylaxis. The discovery that this form of pneumonia can be largely prevented by chemoprophylaxis was a major achievement in the care of HIV-infected patients (9,392–397).

The preferred agent for prophylaxis is trimethoprim-sulfamethoxazole (160 mg trimethoprim, 800 mg sulfamethoxazole) (TMP-SMX) given orally once per day, either daily, on alternate days or three times weekly (Table 13.17) (see Chapter **??**) (9,395–397). Daily therapy may be more effective than three times weekly (398) but is tolerated less-well than intermittent regimens (399). Advantages of TMP-SMX over other prophylactic therapies include greater efÞcacy, lower cost, convenience of

TABLE 13.17. *Prophylaxis of opportunistic infections*

Infection	Indication to start therapy	Duration of therapy[a]	Preferred regimen	Alternative regimen
Tuberculosis	Positive tuberculin skin test or recent contact with active case Mtb	Completion of course	INH 300 mg + pyridoxine 50 mg q.d. × 9 mo	INH 900mg + pyridoxine 100 mg BIW × 9 mo; Rifampin[b] 600 mg + pyrazinamide 15–20 mg/kg q.d. × 2 mo; Rifampin 600 mg q.d. × 4 mo
Suspected INH resistant *Mycobacterium tuberculosis*	As above	Completion of course	Rifampin[b] 600 mg + pyrazinamide 15–20 mg/kg q.d. × 2 mo	Rifampin 600 mg q.d. × 4 mo
Pneumocystis carinii	< 200 CD4 + cells/mm^3	Until CD4 + increases to 200–300 cells/mm^3	TMP-SMX DS q.d. or TIW TMP-SMX SS q.d.	Dapsone 100 mg q.d.; Atovaquone 1500 mg q.d. with meals; Dapsone 200 mg/wk + pyrimethamine 75 mg/wk + folinic acid 25 mg/wk; Dapsone 50 mg q.d. + pyrimethamine 50 mg/wk + folinic acid
Pneumocystis carinii				25 mg/wk; Pentamidine aerosol 300 mg q mo by Respirgard II$^{(TM)}$ nebulizer
Toxoplasma gondii	< 100 CD4 + cells/mm^3 and *T. gondii* IgG positive in serum	Until CD4 + increases to 200 cells/mm^3	TMP-SMX DS q.d. or TIW	TMP-SMZ SS q.d.; Dapsone 200 mg/wk + pyrimethamine 75 mg/wk + folinic acid 25 mg/wk; Dapsone 50 mg q.d. + pyrimethamine 50 mg/wk + folinic 25 mg/wk; Atovaquone 1500 mg q.d. with meals ± pyrimethamine 50 mg/wk + folinic acid 25 mg/wk
Mycobacterium avium complex	< 50 CD4 + cells/mm^3	Until CD4 + increases to 100 cells/mm^3	Azithromycin 1,200 mg/wk	Clarithromycin 500 mg b.i.d.; Rifabutin[b] 300 mg q.d.

INH = isoniazid; q.d. = daily; mo = months; TMP-SMX = trimethoprim-sulfamethoxazole; DS = double strength; SS = single strength; TIW = three times a week; wk = week, q = every.

[a] Time duration that CD4 + cell count should exceed the numbers given in this chart are not known but should be in the range of three to six months; If the CD4 + cell count drops to levels lower than the number for stopping medication, prophylaxis should be restarted.

[b] Rifampin should not be used concurrently with amprenavir, indinavir, nel navir, saquinavir, lopinavir/ritonavir, or delavirdine due to decreased levels of these drugs. If efavirenz is used with rifampin, the dose of efavirenz should be increased to 800 mg daily. Rifabutin should be considered in place of rifampin for patients on above drugs. When used with nel navir, indinavir, or amprenavir, the rifabutin dose is 150 mg daily or 300 mg two to three times per week; indinavir and nel navir dosages should be increased to 1,000 mg q 8 hours. With ritonavir-containing regimens, rifabutin should be decreased to 150 mg two to three times per week. Increase rifabutin to 450–600 mg daily when prescribed with efavirenz. Rifabutin should not be given with delavirdine and use with caution with saquinavir due to decreased saquinavir levels.

oral administration, and potential effectiveness in preventing extrapulmonary pneumocystosis and other infections such as salmonellosis, shigellosis, pneumococcal infection, *Haemophilus inßuenzae* infection, isospora infection, cyclospora infection, nocardiosis, and listeriosis (400). A particularly useful beneÞt of TMP-SMX is its effectiveness in preventing cerebral toxoplasmosis (401Ð403). The principal disadvantage of TMP-SMX is that it is not tolerated well by approximately 50% of patients. The most frequent causes of intolerance are hypersensitivity reactions, particularly rashes and fever (396,404).

There are a number of alternatives to TMP-SMX (9) (Table 13.17). Pentamidine by aerosol 300 mg per month by *Respirgard II* nebulizer (Maarquest, Englewood, Colorado) was widely used at the beginning of the HIV epidemic but has largely been replaced by oral regimens. Because dapsone provides systemic therapy and may provide some protection from reactivation of *T. gondii* infection (particularly when combined with pyrimethamine), it has become, for many physicians, the Þrst alternative to TMP-SMX (9,395,397,405). Although dapsone is a sulfone antibiotic with some structural similarity to sulfonamides, approximately 60% of patients who react to TMP-SMX will tolerate dapsone (406).

Several caveats should be noted when considering the use of dapsone. Any patient with a prior life-threatening reaction to a sulfonamide, such as anaphylaxis or Stevens-Johnson syndrome, should not be challenged with dapsone. Furthermore, before using dapsone, glucose-6-phosphate dehydrogenase (G-6-PD) levels should be measured to avoid the problem of hemolytic anemia that can occur in G-6-PD-deÞcient patients. Dapsone should be used with caution in patients who are concomitantly receiving rifampin or rifabutin due to hepatic microsomal enzyme induction by these drugs leading to a decrease in dapsone levels (407).

Atovaquone is an effective preventive therapy for PCP (408,409) and may be better tolerated than dapsone (409). However, it is markedly more expensive than dapsone and therefore some authorities reserve it for patients intolerant of dapsone (410). Use of dapsone in preference to atovaquone has been evaluated and felt to be cost-effective (411).

Aerosol pentamidine should probably be reserved for patients who are intolerant of TMP-SMX, dapsone, and atovaquone. It is more costly than TMP-SMX and dapsone but less than atovaquone. Major drawbacks to pentamidine therapy are that it requires special equipment, is less efÞcacious than other regimens (especially at CD4+ counts <100 cells/mm^3), is not systemic and therefore extrapulmonary *P. carinii* infection may occur, and it does not protect against *T. gondii* infection (397,412,413). Because of aerosolization, pentamidine treatment poses potential risks of transmission of respiratory tract pathogens such as *M. tuberculosis*. Bronchospasm and coughing, which occur in about one-third of patients during inhalation, are the most common toxicities of aerosolized pentamidine (392). These problems can largely be prevented by pretreatment with an inhaled beta-adrenergic agonist. Hypoglycemia (414) and pancreatitis (415,416) are much less common with aerosol pentamidine than with IV administration, but they may still rarely occur.

Pentamidine given IV at a dose of 300 mg once monthly also has been used successfully to prevent PCP (417). This regimen may be useful for hospitalized patients with TMP-SMX intolerance who cannot ingest oral medications.

Some authorities prefer to desensitize TMP-SMX-intolerant patients with the use of an escalating dosage regimen administered over a time period ranging from as short as Þve hours to as long as several weeks (418Ð420). Success rates of 50% to 80% have been reported based on small numbers of patients. Two regimens for desensitization are listed in Table 13.18. Patients who have experienced only liver function abnormalities or cytopenia with high dose intravenous TMP-SMX can usually tolerate the lower doses used for prophylaxis and desensitization is not required (396). After desensitization has been completed, it is important that the patient not interrupt the TMP-SMX regimen.

It is important to emphasize that when patients who are compliant with TMP-SMX prophylaxis develop respiratory tract symptoms and an abnormal chest roentgenogram, PCP is unlikely to be the cause. In contrast, PCP may develop in patients receiving aerosol pentamidine,

TABLE 13.18. *Examples of trimethoprim-sulfamethoxazole desensitization regimens*

Regimen		Trimethoprim-sulfamethoxazole
10-day		
Day	1	1 ml of 1:20 dilution pediatric suspension[a]
	2	2 ml of 1:20 dilution pediatric suspension
	3	4 ml of 1:20 dilution pediatric suspension
	4	8 ml of 1:20 dilution pediatric suspension
	5	1 ml of pediatric suspension
	6	2 ml of pediatric suspension
	7	4 ml of pediatric suspension
	8	8 ml of pediatric suspension
	9	1 tablet of single-strength[a]
	10	1 tablet of double-strength (DS)[b]
5-hour		
Hour	0	5 ml of 1:10,000 dilution pediatric suspension[a]
	1	5 ml of 1:1,000 dilution pediatric suspension
	2	5 ml of 1:100 dilution pediatric suspension
	3	5 ml of 1:10 dilution pediatric suspension
	4	5 ml of undiluted pediatric suspension
	5	1 tablet, double-strengt[b]

Adapted from Gluckstein and Ruskin, ref. 419, and Absar et al., ref. 420.

[a] Pediatric oral suspension has 40 mg trimethoprim-200 mg sulfamethoxazole per 5 ml; single strength tablet (80 mg trimethoprim/400 mg sulfamethoxazole).

[b] Thereafter, 1DS tablet of trimethoprim-sulfamethoxazole (160 mg trimethoprim/800 mg sulfamethoxazole) every other day or daily for *Pneumocystis carinii* pneumonia prophylaxis.

although the roentgenographic Þndings may be atypical for this form of pneumonia (eg, upper lobe inÞltrates) (421Ð423).

Discontinuation of prophylaxis

Several studies have now demonstrated the safety of discontinuing both primary (424Ð428) and secondary (427Ð428) PCP prophylaxis when the CD4+ lymphocyte count has risen to >200 cells/mm^3. Recent U.S.PHS/IDSA guidelines reßect this, recommending stopping therapy when the CD4+ lymphocyte count is stable for 3 months at a level >200 cells/mm^3 (9) (Table 13.17). Although the guidelines recommend stopping prophylaxis at 200 cells/mm^3, in the largest studies addressing this question, the mean CD4+ cell count was 350 cells/mm^3 at the time of stopping therapy (424Ð428). A simulation model of HIV infection evaluating various times to stop PCP prophylaxis found that waiting to discontinue prophylaxis at a CD4+ lymphocyte count of >300 cells/mm^3 instead of 200 cells/mm^3 was cost effective (411). If the CD4+ lymphocyte count falls to below 200 cells/mm^3 among patients who have stopped prophylaxis, then prophylaxis should be resumed.

Toxoplasma gondii Chemoprophylaxis

Initiating prophylaxis

The risk of developing cerebral toxoplasmosis among HIV-infected individuals with positive *T. gondii* serology (positive IgG) is estimated to be 25% to 50% (430,431), with the highest risk occurring in patients with a CD4+ lymphocyte count <100 cells/mm^3 (432). Patients with CD4+ lymphocytes <100 cells/mm^3 who demonstrate no prior exposure to *T. gondii* demonstrated by lack of seropositivity, do not need chemoprophylaxis. However, they should be intermittently tested for seroconversion and counseled on how to avoid acute infection.

Preventive chemotherapy is recommended for toxoplasma antibody seropositive patients with a CD4+ cell count <100 cells/mm^3 (9). TMP-SMX in doses of one double strength tablet daily or on alternating days has been demonstrated to be extremely effective for prevention of cerebral toxoplasmosis (Table 13.17) (401,402).

For the TMP-SMX intolerant patient, dapsone 50 mg daily plus pyrimethamine 50 mg weekly plus leucovorin 25 mg weekly or a once weekly regimen of dapsone 200 mg plus pyrimethamine 75 mg plus leucovorin 25 mg are also effective for prevention of toxoplasmosis (9,433,434). Atovaquone with or without the addition of pyrimethamine and leucovorin may also be considered for prevention of *T. gondii* reactivation (9). Although clindamycin plus pyrimethamine is less well studied as primary chemoprophylaxis for toxoplasmosis, it may well be effective because this is an alternative regimen for the treatment of cerebral toxoplasmosis (435,436). This regimen should be considered second-line due to the large number of pills that must be used, frequency of administration, and adverse reactions associated with clindamycin (437). In addition, clindamycin plus pyrimethamine is not protective against PCP infection.

Discontinuation of prophylaxis

Patients who have responded to ART with an increase in the CD4+ cell count to >200 cells/mm^3 for at least three months can have primary prophylaxis discontinued (9). These recommendations are supported by both observational (427,438Ð441) and randomized trials (442,443). Although the critical CD4+ lymphocyte count at which to begin therapy is 100 cells/mm^3, in the studies of prophylaxis discontinuation, most patients have had CD4+ lymphocyte counts >300 cells/mm^3.

It has been recommended that secondary prevention of *T. gondii* cerebral infection can be discontinued after the CD4+ lymphocyte count has risen to >200 cells/mm^3 and the patient has had more than six months of therapy (9). These recommendations, however, are based upon data from only a limited number of patients (427,429,444). Although uncommon, relapses can occur despite CD4+ lymphocyte counts above 200 cells/mm^3 (445). Prior to discontinuing therapy many authorities repeat a cranial imaging study to ensure that prior lesions have either completely resolved or are markedly smaller.

Primary and secondary prophylaxis should be reintroduced when the CD4+ lymphocyte counts drops to <200 cells/mm^3 (9). Although there are no published data regarding which drugs to restart for patients who have had secondary prophylaxis stopped, it is probably safe to treat with TMP-SMX alone for patients who tolerate this medication.

Disseminated *Mycobacterium-avium* complex (dMAC) chemoprophylaxis

Initiation of chemoprophylaxis

Disseminated *M. avium* complex (dMAC) infections are almost uniquely associated with AIDS patients as compared with other immunocompromised populations. In the United States, dMAC infections occur in up to 40% of HIV-infected patients who have CD4+ lymphocyte counts <100 cells/mm^3 (446). Because treatment of active infection requires a multi-drug regimen that provides only modest success, prevention is greatly preferred. *M. avium* complex organisms are ubiquitous in nature and most of the transmission probably occurs through exposure to contaminated water. Because there are no proven ways to predict who will develop dMAC, all patients who reach the

critical CD4+ lymphocyte count at which to begin prophylaxis should receive prophylaxis.

Current USPHS/IDSA guidelines recommend that chemoprophylaxis begin at a CD4+ lymphocyte count of <50 CD4+ cells/mm^3 (9). Three drugsÑ azithromycin, clarithromycin, and rifabutinÑ are FDA approved for prophylaxis of MAC infection on the basis of randomized multicenter studies (447,448) (Table 13.17). Azithromycin (1,200 mg weekly) is the drug of choice because it lacks several of the drug-drug interactions found with rifabutin or clarithromycin, can be taken once a week, is equally as effective as rifabutin or clarithromycin, and is less expensive. Clarithromycin is prescribed in doses of 500 mg twice daily and rifabutin at a dose of 300 mg daily (Table 13.17).

Combination therapy with azithromycin plus rifabutin may be more effective than azithromycin alone for prevention of dMAC (448). However, adverse reactions occur more commonly with this combination, there is greater potential for drug-drug interactions, and the costs are higher. Furthermore, there was no difference in survival between patients who received combination prophylaxis and those who received azithromycin monotherapy (448). Therefore, combination therapy is not recommended.

When initiating chemoprophylaxis for dMAC, many authorities Þrst exclude active dMAC infection by evaluating the patient clinically and obtaining mycobacterial blood cultures. For patients who are intolerant of macrolide antibiotics and are going to be prescribed rifabutin, infection with *M. tuberculosis* should be ruled out clinically, by PPD testing (if not previously already done) and by chest roentgenogram. This is to avoid giving rifabutin inadvertently as a single agent for active tuberculosis. This would pose a substantial risk for the emergence of drug resistance to both rifampin and rifabutin (449).

If clarithromycin is prescribed for chemoprophylaxis, several drug-drug interactions should be considered despite the fact that their clinical signiÞcance has not been proven. Clarithromycin levels are increased by PIs, while efavirenz decreases the levels of clarithromycin while increasing those of its active metabolite 14-OH (450).

Rifabutin has been relegated to the third option for dMAC prevention mainly because of the potential problem with *M. tuberculosis* resistance and because of numerous drug-drug interactions. Like rifampin, rifabutin may cause an orange-brown discoloration of the urine (which should be mentioned to the patient prior to starting the drug) and may induce hepatic microsomal enzymes, thereby affecting the metabolism of a wide array of drugs. When administered with PIs with the exception of saquinavir, the dose of rifabutin should be 150 mg daily. However, if rifabutin is prescribed with efavirenz, the dose should be increased to 450 mg daily.

Discontinuation of prophylaxis

When the CD4+ lymphocyte count has risen to >100 cells/mm^3, primary MAC prophylaxis can safely be stopped (9,438,451Ð453). Secondary prophylaxis (which is a multi-drug treatment regimen given for dMAC infection) can be discontinued when the CD4+ lymphocyte count has risen to >100 cells/mm^3 on ART for approximately six months and the patient has received >12 months of therapy for MAC (9). These recommendations are based upon relatively few patients who have stopped therapy (429, 444). Discontinuation of secondary prophylaxis has major advantages to the patient in terms of decreased pill burden, toxicity, and reduced drug-drug interactions.

If the CD4+ lymphocyte count drops below 100 cells/mm^3, then dMAC prophylaxis should be restarted (9). Data on what therapy to begin among patients who have been treated previously for dMAC is lacking. However, if the patient has already received >12 months of multi-drug therapy, single drug therapy is probably safe.

Cytomegalovirus Infection Chemoprophylaxis

Initiation of prophylaxis

Cytomegalovirus infection, in particular retinitis, was a devastating OI for HIV-infected patients in the pre-HAART era (454). Infection developed in severely immunocompromised patients with CD4+ lymphocyte counts <50 cells/mm^3 who had positive serology for prior exposure to CMV (454Ð456). At necropsy, $>80\%$ of patients with such low CD4+ cells could be demonstrated to have evidence of CMV infection, although many of these infections were not clinically apparent (456). Retinitis occurred in 25% to 40% of patients and gastrointestinal disease, encephalitis, polyradiculitis, and rarely pneumonia were also well described (456). Use of primary prophylaxis to prevent CMV was hampered by the lack of a well-tolerated oral agent that was effective and moderately priced.

Acyclovir was not effective for prevention of CMV retinitis among patients with AIDS (457,458) and valacyclovir led to increased deaths among persons with AIDS receiving this medication despite the fact that it was modestly effective in preventing CMV disease (459). Of the systemic agents approved by the FDA for treatment of CMV disease (ganciclovir, foscarnet sodium, and cidofovir), all require IV administration except for ganciclovir, which can be taken orally. All have signiÞcant toxicities. For prophylaxis, oral ganciclovir is extremely costly, requires pills to be taken three times daily, and is only modestly effective (460,461). Furthermore, patients who either develop CMV disease or have a recrudescence while taking oral ganciclovir may then have ganciclovir-resistant strains of virus (462).

Due to the toxicity and costs associated with CMV primary prevention, numerous studies were undertaken to identify a target population of AIDS patients at highest risk for developing CMV disease. Blood or urine cultures for CMV have limited utility because of their poor positive predictive value (463). On the other hand, several studies have suggested that detection of increasing amounts of CMV antigen or DNA in peripheral blood is predictive of those patients who will develop CMV disease (464–467). These tests are not well standardized and their role in selecting patients for prophylaxis has not been determined.

In 2001, the FDA approved valganciclovir, a prodrug of ganciclovir that is taken orally for the treatment of CMV disease. In a randomized trial it was as effective as IV ganciclovir for treatment of CMV retinitis (468). This drug can be taken twice daily and is well absorbed. There are no studies to document the efficacy of valganciclovir to prevent CMV disease in severely immunocompromised patients with AIDS. Furthermore, with the advent of ART, it is not likely that a large trial can be easily undertaken to demonstrate its utility.

USPHS/IDSA guidelines do not recommend primary chemoprophylaxis for CMV disease (9). It is recommended to educate patients with CD4+ lymphocytes < 50 cells/mm^3 about the early signs of CMV retinitis such as floaters and to have regular (at least yearly) funduscopic examinations. By employing this strategy, early disease can be detected and treated.

Secondary prophylaxis for CMV retinitis relies upon continuation of the initial medication used for treatment and must be continued indefinitely unless the CD4+ lymphocyte count improves on ART (Table 13.22). Unfortunately, except for the use of oral ganciclovir or valganciclovir, therapy with other agents requires either prolonged IV infusions (foscarnet or cidofovir), or repeated intravitreous injections with fomivirsen or intraocular implants with ganciclovir (9). Foscarnet and cidofovir are problematic to use due to renal toxicity. Unless the CD4+ cell count increases, even with secondary prevention, recrudescent disease is common (469–471). Limited data exist supporting the use of secondary prophylaxis for CMV infection of the gastrointestinal tract after an initial three weeks of treatment (472). Due to the toxicity of traditional therapies, however, secondary prophylaxis has not been recommended. With the advent of valganciclovir, longer therapy or secondary prophylaxis may be warranted for CMV gastroenterologic disease until ART leads to CD4+ cell increases.

Discontinuation of prophylaxis

Patients who have a rise in CD4+ lymphocyte counts to > 100–150 cells/mm^3 for > 6 months on ART can safely stop CMV treatment (429,444,473–476). When stopping therapy it is important to consider the extent of ocular damage, activity of disease, and vision in the contralateral eye. Patients who have had CMV retinitis should continue to have regular follow-up examinations by an ophthalmologist (9). CD4+ lymphocyte counts must be followed closely among patients who have had secondary prophylaxis discontinued. Relapse may occur when the CD4+ lymphocyte count drops to < 50 cells/mm^3 (444). Therefore, therapy should be restarted when the CD4+ lymphocyte count drops to < 100–150 cells/mm^3 (9). Patients who have recurrent symptoms after therapy has been stopped should be evaluated immediately because CMV retinitis has recurred in rare instances even when the CD4+ lymphocyte count was > 100 cells/mm^3 (477,478).

Mycobacterium tuberculosis Chemoprophylaxis

Initiation of prophylaxis

Part of the initial assessment of patients with HIV infection includes evaluation of a patient's current potential risk for exposure to *M. tuberculosis* (their occupational risks and risks related to habitation) as well as their prior exposure to *M. tuberculosis* (by history and prior PPD studies).

HIV-infected patients who warrant primary anti-tuberculous chemoprophylaxis include those who have: close contact with an individual with active tuberculosis; a positive skin test; a prior positive skin test; or chest roentgenogram findings consistent with old disease, if not previously treated (9) (Table 13.17). Although individuals from regions of the world with a high incidence of tuberculosis may have a higher risk of exposure to *M. tuberculosis*, if they are skin test negative, they do not need prophylaxis since preventive therapy has not been demonstrated to improve outcome (478–480). Patients who have had prior tuberculosis and were adequately treated do not need preventive therapy. Recommendations for prophylaxis are independent of CD4+ lymphocyte count and patient age (9,160).

Isoniazid 300 mg daily for nine months is the most commonly used prophylactic regimen, although intermittent regimens can be prescribed (9). Isoniazid has excellent efficacy in preventing tuberculosis in HIV-infected patients (481). Rifampin or rifabutin alone for four months or one of these agents plus pyrazinamide for two months should be considered for patients at high risk for isoniazid resistant *M. tuberculosis*. However, rifampin-based regimens are contraindicated in patients on certain antiretroviral agents, and doses of rifabutin as well as some PIs must be modified if taken concurrently (163). Due to the potential for hepatotoxicity, the two-month rifampin plus pyrazinamide regimen should not be considered a preferred regimen (482), although it may be as effective as a nine-month isoniazid regimen for preventing tuberculosis (483).

Health authorities should be consulted to develop an appropriate chemoprophylactic regimen among patients exposed to strains of multiply drug resistant *M. tuberculosis*.

Discontinuation of prophylaxis

The duration of chemoprophylaxis for tuberculosis depends upon the selected regimen as described in the preceding section (Table 13.17), not upon the CD4+ cell count.

Fungal Chemoprophylaxis

Initiation of prophylaxis

Infections caused by fungi are a major problem for HIV-infected patients. *Candida* species (particularly *Candida albicans*) cause recurrent mucocutaneous infections including thrush and esophagitis at some point in the majority of HIV-infected patients with advanced immunodeficiency (484). *Candida* vaginitis is also a considerable problem for women with AIDS (485,486).

Cryptococcus neoformans is responsible for meningitis in approximately 5% to 10% of HIV-infected patients with advanced immunodeficiency in the United States (487). The incidence of disseminated infection caused by *Histoplasma capsulatum* among AIDS patients has been reported to be as high as 27% in endemic areas such as the Mississippi and Ohio River valleys in the United States, Puerto Rico, and Central America (488). In endemic areas it is not certain whether the majority of patients with disseminated histoplasmosis have primary infection, reinfection, or reactivation of old disease. In nonendemic areas, histoplasmosis in AIDS patients appears to be reactivated from previous foci of infection (488). Disseminated infection due to *Coccidiodes immitis* is another fungal infection that generally occurs during later stage HIV infection (489). Patients living in or traveling to endemic regions, such as southwestern United States, are at risk for developing coccidioidomycosis.

There are no reliable serologic or skin test studies for fungi to guide selective therapy for higher-risk patients. It is important to counsel patients regarding the avoidance of exposure to these organisms when possible. For instance, patients should be warned about the risk of histoplasmosis in areas of building excavation or in caves or areas with heavy bird droppings and the risk for coccidiodomycosis in areas of building excavation (9).

Several studies have demonstrated the efficacy of long-term prophylaxis against recurrent oral or vaginal candidiasis using a variety of azole antifungal agents including ketoconazole, fluconazole, and itraconazole (490–493). A randomized trial comparing fluconazole (200 mg per day) to clotrimazole troches (10 mg five times per day) showed that invasive fungal infection was significantly reduced during a 35-month follow-up period from 10.9% in the clotrimazole arm to 4.1% in the fluconazole arm (490). The protective effect of fluconazole was most marked for prevention of cryptococcosis, but it was also found for esophageal and oropharyngeal candidiasis. The protective effect was greatest among patients with <50 CD4+ lymphocytes/mm^3. However, survival differences were not found between the two groups. Other studies have demonstrated that administration of fluconazole in doses varying from as little as 100 mg weekly to 100 mg to 200 mg per day significantly reduced the incidence of cryptococcal meningitis when compared with historical controls (494–496). This effect may be dose-related (497).

In another study, itraconazole at 200 mg per day significantly lowered the rates of histoplasmosis and cryptococcosis when compared with placebo (498). This effect was confined to the subset of patients with <100 CD4+ cells/mm^3. However, the frequency of recurrent or refractory oral or esophageal candidiasis was not altered, toxicities were higher in the itraconazole group, and there was no survival benefit to prophylaxis (498). Data regarding the use of antifungal drugs for prevention of coccidioidomycosis are lacking. However, the incidence of this infection appears to be considerably lower than that of cryptococcosis or histoplasmosis.

Although it is tempting to consider antifungal prophylaxis for patients with extremely low CD4+ cell counts, the emergence of resistance in *C. albicans* to fluconazole and superinfection with non-*albicans* candida fungal infections that are inherently resistant to fluconazole is cause for concern (484,499,500). Intravenous amphotericin B may be necessary for patients with azole-resistant *Candida* infections, thereby exposing patients to the inconvenience and hazards of long-term intravenous catheter use and to more toxic antifungal drugs. Primary prophylaxis for these fungal infections is currently not recommended (9) and generally should not be offered.

Secondary prophylaxis for candidal oropharyngeal or vaginal infection is not recommended for episodic disease. However, in some patients with very low CD4+ lymphocyte counts, recurrence can occur frequently enough that chronic suppressive therapy is warranted. Fluconazole 100 to 200 mg daily should be considered, recognizing the risk for developing azole-resistant disease.

Secondary prophylaxis, or maintenance therapy, is indicated for life for patients with cryptococcosis, histoplasmosis, or coccidioidomycosis unless the CD4+ cell count rises. For cryptococcosis, fluconazole is the preferred drug because of its excellent activity (501,502) and low risk for drug-drug interactions (compared to itraconazole). In addition, fluconazole is associated with a lower relapse rate than itraconazole (503). However, itraconazole is an acceptable alternative (9,503). Coccidioidomycosis can be treated chronically with either fluconazole 400 mg daily or itraconazole 200 mg twice daily (504). However,

ßuconazole should be considered the preferred agent due to the greater potential for drug-drug interactions with itraconazole. For histoplasmosis, itraconazole 200 mg per day is the recommended drug of choice (471).

Discontinuation of prophylaxis

Data regarding discontinuation of secondary prophylaxis are extremely limited for these infections. The most data are available for cryptococcosis. It appears that therapy can safely be stopped after six to twelve months of ART if the CD4+ cell count has risen to >100 to 200 CD4+ lymphocytes/mm^3 (444,505,506). Some authorities prefer to perform a spinal tap to ensure that CSF cultures are negative before stopping anti-cryptococcal therapy (9). Before considering stopping therapy, the health care provider must explain the lack of data and the need for prompt evaluation if symptoms recur. Discontinuation of prophylaxis for recurrent candidal esophagitis was safe for a small number of patients with a good CD4+ count response to ART (429). Data regarding the safety of stopping secondary prophylaxis for histoplasmosis and coccidioidomycosis are even more limited. Discontinuation can be considered if the patient has been treated for the fungal infection for six to twelve months, and while taking ART the CD4+ lymphocyte count has risen to >100 to 200 cells/mm^3. However, current U.S.PHS/IDSA guidelines do not recommend stopping therapy (9).

If the CD4+ lymphocyte count drops to <100 cells/mm^3, it is probably wise to reinitiate antifungal therapy despite the absence of data on this subject.

Chemoprophylaxis of Varicella-Zoster Virus (VZV) Infection

Data are limited on the efÞcacy and safety of the varicella-zoster vaccine in adults, and it is currently not recommended (9). HIV-infected patients who are susceptible to varicella, ie have not had varicella or shingles or who have no detectable antibody to VZV, should be given varicella-zoster immune globulin within 96 hours of exposure to an active case of varicella (varicella or shingles) (9). Use of acyclovir cannot be recommended after exposure due to lack of data demonstrating efÞcacy.

Primary chemoprophylaxis is not recommended regardless of CD4+ cell count (9). After an episode or recurrent episodes of shingles in an HIV-infected patient, secondary prophylaxis is not warranted when initial treatment is completed. Among HIV-infected patients, no drug has been proven to be effective in preventing recurrent disease.

Chemoprophylaxis of Herpes Simplex Virus (HSV) Infection

Primary prophylaxis for HSV infection is not recommended regardless of CD4+ lymphocyte count, even for HIV-infected patients potentially exposed to HSV (9). Secondary prevention is generally only offered to patients with severe, frequently recurrent disease. Daily suppressive therapy with acyclovir, famciclovir or valacyclovir should be considered in these situations (Table 13.19). These recommendations are similar to those for non-HIV-infected patients and are independent of CD4+ cell count.

TABLE 13.19. *General principles in the approach to the HIV-infected patient with fever*

Except for primary HIV infection, sustained fevers are due to causes other than HIV infection itself
Serious opportunistic infections usually Þrst occur in patients with CD4+ lymphocyte counts <200 cells/mm^3
Multiple opportunistic infections and neoplasia may occur simultaneously, even in the same organ or tissue
Serodiagnostic studies and tuberculin skin testing may be ambiguous
Growth of some opportunistic organisms, e.g. cytomegalovirus or *Mycobacterium avium* complex, from urine or sputum samples may not indicate active infection.

DRUG-DRUG INTERACTIONS

No chapter on the management of HIV-infected patients would be complete without some discussion of drug-drug interactions and their potential impact upon devising treatment regimens (see Chapter **??**). There is perhaps no area more complicated than this because of the lack of data upon which to base decisions. This is due at least in part to the numerous combinations of medications that may be prescribed for: (1) treatment of HIV, (2) complications related to both HIV and its treatment, (3) prevention and treatment of OIs, and (4) co-morbid illnesses such as psychiatric problems, drug use treatment, and HCV among others. HIV-infected patients also may use alternative therapies such as St. JohnÕs wart or garlic tablets that can directly impact upon drug levels because of drug-drug interactions. Because two different drugs may both interact with the same liver enzyme, it is often impossible to say how the interaction will affect the blood levels of each without direct measurements. Data in this Þeld are emerging rapidly (7,507). Some drug-drug interactions may actually be quite useful as for example in the boosting of PI levels with ritonavir. The major interactions to consider are those related to the effects of various drugs on the cytochrome p450 hepatic enzyme system by PIs, NNRTIs, rifamycins, and itraconzole. This enzyme system is involved in the metabolism of numerous drugs. Other drug interactions to consider are related to potential additive toxicities of two or more drugs.

Sources of information include drug package inserts, HIV-related periodicals, fax reports, and internet sites.

MANAGEMENT OF SELECTED SPECIFIC PROBLEMS

Most HIV-infected patients will develop fever at some point in the course of their illness. Aside from primary HIV infection, sustained fevers are rarely, if ever attributable to HIV infection itself and are usually due to serious but treatable superimposed infections (Table 13.19) (508–510). Management of the febrile HIV-infected patient is integrally related to establishing a specific etiology for the fever. Determining the source of fever, however, at times can be extremely challenging. Fever may be related to nonopportunistic infections presenting typically or atypically, opportunistic infections (sometimes multiple (511)), hypersensitivity reactions to medications and malignancies, particularly lymphomas. Furthermore, nosocomial infections from intravascular catheters, pressure sores, Clostridium difficile diarrhea, and urinary tract infections should not be overlooked in the hospitalized, febrile HIV-infected patient (508).

It is important at the outset to know whether the patient is highly immunocompromised (i.e. has a CD4+ lymphocyte count <200 cells/mm^3) and is thus predisposed to serious opportunistic infections. If not, evaluation may be directed toward the discovery of less unusual microbial pathogens and conditions, for example, sinusitis (512), herpes zoster infection (513), or tuberculosis (514). In patients who are highly immunocompromised, it is essential to determine which chemoprophylactic therapies were prescribed and the level of compliance. Infections related to granulocytopenia, such as bacteremia, also may occur in HIV-infected patients with low white blood cell counts and should be taken into consideration when prescribing empiric antibiotic therapy (515–517).

It is important to note epidemiologic clues that may help in identifying specific infectious risks (518). The etiology of fever in the various reported series was greatly dependent on where the study was performed (519–523). For example, Haitians, African natives, Southeast Asians, and other patients from the developing world, as well as intravenous drug users from major Western metropolitan areas, have an increased risk of tuberculosis (524,525). Patients from the southwestern United States (and occasionally those who have previously resided there) are at risk for disseminated *Coccidioides immitis* infection (526). Histoplasmosis may be a consideration in persons who have resided in areas endemic for this fungus in the United States (527) or elsewhere, including certain areas of the Caribbean (such as Puerto Rico), and Central America (528,529). Visceral leishmaniasis is endemic in parts of the Mediterranean basin and may be responsible for fever in patients who have lived in these areas (520).

An approach to the evaluation of fever in the HIV-infected patient with advanced immunodeficiency is found in Table 13.20. If the source of fever has not been identified on routine diagnostic studies, such as blood and urine cultures or chest roentgenogram, then further testing is necessary. Blood cultures for fungi and mycobacteria should be obtained (530) (two are sufficient (531)). Mycobacterial blood cultures should include lysis of the cellular fraction of the specimen, followed by plating on a standard solid mycobacterial culture medium or by liquid culture with radiometric detection (532). Multiple cultures of stool, urine, and sputum for mycobacteria also should be considered (533). Stool acid-fast smears and cultures are helpful in the diagnosis of dMAC infection because the intestinal tract is often involved in the disease process (532,534). Positive cultures from stool and sputum for nontuberculous mycobacteria my represent contamination or colonization, however, and not indicate true infection. Stool acid-fast cultures also may be useful in the diagnosis of *M. tuberculosis* infection, although usually the sputum is culture positive in these instances (524).

Although CMV infection may be a cause of fever in these patients, positive cultures for this virus from urine, pharynx, bronchial lavage fluid or even blood are so frequent that they cannot be relied on to pinpoint the cause

TABLE 13.20. *A diagnostic approach for evaluation of fever in HIV-infected patients*

Evaluate whether the patient falls into a group at high risk for serious opportunistic infections (i.e. CD4+ cell count <200 cells/mm^3)
Direct history to determine possible geographic, ethnic, or lifestyle risk factors for specific infections; pay particular attention to neurologic, respiratory, dermatologic, visual, and gastrointestinal complaints
Note medication list
Physical examination
Laboratory tests: complete blood count; chemistry profile, including liver function tests; urine analysis; cultures of blood, urine, and sputum for bacteria; chest roentgenogram; stool culture if diarrhea is present
If initial cultures are negative, culture blood, urine, sputum, and stool for mycobacteria and blood, urine, and sputum for fungi; serum cryptococcal antigen, toxoplasma titer, and VDRL; PPD with controls unless known to be anergic; sputum for IFA test for *Pneumocystis carinii*; urine for histoplasma antigen
If no source yet identified, consider: Gallium scan of lung Computed tomography of abdomen Examination by ophthalmologist for CMV retinitis Lymph node biopsy if enlarged node is accessible Bone marrow biopsy and aspiration (especially if anemic) Skin lesion biopsy (if lesion present) Lumbar puncture if clinical signs warrant Bronchoscopy with bronchial alveolar lavage and/or biopsy (if respiratory tract signs or abnormalities are present) Liver biopsy (if liver function studies are abnormal)

CMV, cytomegalovirus; IFA, immunofluorescent antibody; PPD, purified protein derivative (of tuberculin); VDRL, Venereal Disease Research Laboratory.

of fever (535–537). For the same reasons serology for CMV is also nondiagnostic (535,537a,537b). It is of utmost importance to attempt to establish the pathogenicity of an organism that has been cultured, as specific treatment of microorganisms such as CMV or MAC may be at best partially effective, potentially toxic, inconvenient (i.e. require multiple drugs or intravenous administration), or prolonged. Further, administration of ganciclovir for CMV infection may preclude the use of AZT because of potential additive bone marrow suppression (538).

Additional diagnostic studies should include a test for serum cryptococcal antigen, which is positive in 75% to 95% of HIV-infected patients with central nervous system cryptococcosis (539,540) (this test is less likely to be positive when cryptococcal infection is confined to the lungs (541)). Sputum should be obtained (induced if necessary) for detection of *P. carinii* using an immunofluorescent monoclonal antibody assay (542,543), especially in patients with respiratory complaints, an abnormal chest roentgenogram, or gallium-67 uptake by the lung on scintigraphy (544). If an induced sputum sample is nondiagnostic or unavailable, bronchoscopy for bronchial alveolar lavage or biopsy should be considered for patients with objective pulmonary signs or test abnormalities (545).

Serodiagnosis of *Histoplasma capsulatum* and *Coccidioides immitis* is helpful for selected patients (526,527). Also, Giemsa-stained preparations of the buffy coat of peripheral blood may be useful in the diagnosis of disseminated histoplasmosis (546). A test for histoplasma polysaccharide antigen in urine is commercially available and appears to have excellent sensitivity and specificity in AIDS patients with histoplasmosis (547).

A careful funduscopic examination by an ophthalmologist may help establish the diagnosis of disseminated CMV infection. Although the patient with CMV retinitis usually reports visual disturbances, this is not uniformly true, particularly in patients with an altered mental status (see Chapter **??**) (548).

Biopsies for culture, special stains, and histologic examination of sites such as bone marrow, lymph nodes, gastrointestinal tract, or skin lesions may be helpful in the diagnosis of disseminated fungal, mycobacterial, or CMV infection, bacillary epithelioid angiomatosis, lymphoma and Kaposi's sarcoma, when other modalities of diagnosis are either nondiagnostic or negative. Biopsies may provide a rapid presumptive diagnosis of a fungal or mycobacterial infection based on the staining characteristics of these microorganisms. It is particularly helpful to perform a lymph node biopsy in patients who have a single group of lymph nodes that are disproportionately enlarged or that have rapidly increased in size (549).

Computed tomography may be used to locate intra-abdominal masses or enlarged lymph nodes for biopsy. In patients with an elevated alkaline phosphatase level and fever, a liver biopsy for histologic examination and culture may be useful (530,550–552). This procedure is not often necessary, however, as other sources of culture material are usually diagnostic.

Before embarking on any biopsy procedure, it is important to determine whether the patient has a potential bleeding diathesis, such as thrombocytopenia. Platelet transfusions, intravenous infusions of immunoglobulin G (IgG) preparations or other measures to elevate the platelet count at least transiently may be required for patients with platelet counts below 50,000 to 100,000/mm^3, depending on the bleeding time and the type and urgency of the invasive procedure to be done. Partial thromboplastin times and (less often) prothrombin times may be prolonged in HIV-infected patients, usually due to the presence of a lupus-like anticoagulant, found in up to 70% of HIV-infected patients particularly those with opportunistic infections (553–555). The "anticoagulants" are immunoglobulins that interfere with several *in vitro* coagulation assays. The presence of these anticoagulants can be confirmed by several diagnostic tests, such as the $^1/_2 + ^1/_2$ correction of the Russell's viper venom time (556). Most patients who have these factors are not predisposed to bleeding unless the level of coagulation factors is abnormal or qualitative/quantitative platelet abnormalities coexist (553).

Empiric Treatment of Infection

The empiric use of broad-spectrum antibiotics is not routinely indicated for the febrile HIV-infected patient because these drugs may be toxic, lead to increasingly resistant organisms, or confound the diagnostic evaluation. If the patient is clinically unstable or profoundly granulocytopenic (< 500 granulocytes/mm^3), however, broad-spectrum antibiotics should be administered promptly. Certain other indications for empiric therapy are listed in Table 13.21.

When an opportunistic infection (or infections) is diagnosed, specific therapy should be initiated (Table 13.22) (160,163,166,208,539,540,557–702). It is important to note that treatment of opportunistic infections in HIV-infected patients is rarely curative (703,704). More often, chronic maintenance therapy (secondary prophylaxis) for an indefinite period is necessary after completion of the acute treatment, unless the CD4 cell count increases secondary to ART.

Rheumatologic Manifestations

Arthralgia, seronegative spondyloarthropathy, arthritis, and connective tissue-like disorders including various forms of vasculitis, a sicca syndrome, and numerous other autoimmune phenomena are the principal rheumatologic complications of HIV infection (Table 13.23) (705–712). Rheumatologic complications are more common in HIV-infected patients with advanced immunodeficiency (709,710,713). Consequently, for those patients with

TABLE 13.21. *Clinical situations in which empiric antimicrobial therapy may be useful in HIV-infected adults prior to conÞrmation of the diagnosis*

Condition	Microorganism to which therapy is directed	Recommended therapy
Clinically unstable (septic appearing) or profoundly neutropenic (< 500 PMNs/mm3) with fever	*Staphylococcus aureus* *Streptococcus pneumoniae* *Haemophilus inßuenzae* *Salmonella sp.* Aerobic gram-negative rods	Third or fourth generation cephalosporin with or without an aminoglycosidea[a]
Lobar pneumonia	*Streptococcus pneumoniae* *Haemophilus inßuenzae* *Moraxella catarrhalis* *Staphylococcus aureus* *Legionella sp.* *Klebsiella sp.* *Escherichia coli*	Third-generation cephalosporin plus erythromycin or a quinolone
Ring-enhancing mass lesion(s) on cranial MRI or CT, especially if serum toxoplasma titer is positive	*Toxoplasma gondii*	Pyrimethamine plus sulfadiazine plus folinic acid
Diffuse pulmonary in ltrates and hypoxia, especially if not on trimethoprim-sulfamethoxazole chemoprophylaxis	*Pneumocystis carinii*	Trimethoprim-sulfamethoxazole
Dysphagia or odynophagia	*Candida albicans*	Fluconazole
PPD + and ill-appearing, especially with abnormal chest roentgenogram (or if PPD-negative and anergic, but at high epidemiologic risk for tuberculosis)	*Mycobacterium tuberculosis*	Isoniazid, rifampin,[b] pyrazinamide, ethambutol

CT, computed tomography; MRI, magnetic resonance imaging; PPD, puri ed protein derivative (of tuberculin); PMN, polymorphonuclear leukocytes.

[a] A third or fourth generation cephalosporin, or extended spectrum penicillin, with *Pseudomonas aeruginosa* coverage (e.g. cefepime) is essential unless an aminoglycoside is included in the drug regimen.
Vancomycin should be added in patients with a central line or in other clinical situations in which methicillin resistant staphylococci are suspected.

[b] Note that there are many drug-drug interactions between rifampin and antiretroviral agents, particularly protease inhibitors and non-nucleoside reverse transcriptase inhibitors (9,17,163)—see Table 13.17.

access to effective ART, these manifestations are becoming less common, although other complications such as osteopenia, osteoporosis and avascular necrosis that appear to be associated with ART (see above), are being increasingly recognized (364Ð368,714).

Intermittent arthralgias without synovitis may occur during primary HIV infection and in up to 45% of patients at a later time (706,715,716). Although sometimes intensely painful (715), the cause is unknown and treatment is symptomatic.

Joint infections are surprisingly uncommon among HIV-infected adults. Anecdotally, we have seen a case due to *Staphylococcus aureus*, and other reported pathogens include *Sporothrix schenckii Cryptococcus neoformans*, *Histoplasma capsulatum*, *Pseudomonas sp*., *Neisseria gonorrhoeae*, *Nocardia asteroides*, *Mycobacterium haemophilum*, *Salmonella sp*., and *Campylobacter fetus* (706,708,710,713,717Ð722). *Helicobacter cinaedi* may cause multifocal cellulitis or arthritis (697,708). Microbiologic studies are an essential component in the evaluation of joint ßuid in the HIV-infected patient with arthritis.

Reactive arthritides and Reiterõs syndrome are the most frequently recognized forms of arthritis in HIV infection, occurring in 2Ð10% of adults depending on geographic area (705,706,715,723Ð725). The majority of Caucasian patients with Reiterõs syndrome have the human leukocyte antigen (HLA)-B27 allele (705). Organisms known to trigger reactive arthritis are, however, rarely discovered (717,726). It should be noted that reactive arthritis or Reiterõs syndrome may be the Þrst clinical manifestation of HIV infection. Psoriasis is also common in HIV infection occurring in 5Ð20% of patients and is complicated by psoriatic-like arthritis in up to one-half of cases (715,727). Since skin rashes may be extensive in HIV-infected patients with Reiterõs syndrome, it may be difÞcult in some cases to distinguish between Reiterõs syndrome and psoriasis (710).

A less etiologically well-deÞned type of oligoarthritis termed ÒHIV-associated arthritisÓ has been described, which is characterized by subacute painful involvement of the knees and ankles with fewer than 10,000 leukocytes/mm^3 on synovial ßuid analysis (705,706). Proposed

TABLE 13.22. *Guide to therapy of opportunistic infections in HIV-infected patients*

Infection	Speci c Treatment	Alternative Treatment	Maintenance Therapy	References
FUNGAL INFECTIONS				
Oral candidiasis (thrush)	Clotrimazole troches (10 mg) 5 × /day	Nystatin oral suspension 100,000μml, 5 ml QID swish/ swallow;or ketoconazole 200–400 mg/day; or uconazole 100 mg/day PO (may need to increase dose); or itraconazole 200–400 mg/ day PO; or amphotericin B oral solution 1 ml (100 mg) QID	Same as initial therapy but may reduce frequency of administration	557–562
Candida esophagitis	Fluconazole 100 mg/day PO or IV; may need to titrate upward to 400–800 mg if response slow	Ketoconazole 200–400 mg/day PO; or itraconazole 200–400 mg/day PO; or amphotericin B 15–25 mg IV/ day; or caspofungin 50 mg/kg IV daily	Same as initial therapy	557,563–566
Histoplasmosis	Amphotericin B □0.5 mg/kg/ day*; 2 gm total dose	Itraconazole 200 mg BID PO or 200 mg/day IV	Itraconazole 200 mg PO BID; or amphotericin B 1 mg/kg weekly	527,557,567–576
Cryptococcosis	Amphotericin B, 0.7 mg/kg/day plus ucytosine 100 mg/kg/ day × 2 weeks	Amphotericin B □0.5 mg/kg/ day*; or uconazole 400 mg PO or IV/day	Fluconazole 200–400 mg PO/day	539,540,557,576–584
Coccidioidomycosis	Amphotericin B, □0.5 mg/kg/ day*; □1 g total dose	Fluconazole 400 mg/day IV or PO; or itraconazole 200 mg BID PO or 200 mg/day IV	Fluconazole 400 mg PO/day; or itraconazole 200 mg PO BID; or amphotericin B 1 mg/kg IV weekly	557,568,576,585–588
Aspergillosis	Voriconazole 6 mg/kg IV Q12h × 2 doses, then 4 mg/ kg IV Q12h	Amphotericin B, 0.5–1.5 mg/kg IV/day*; or itraconazole 200 mg BID PO or 200 mg/ day IV	Voriconazole 200 mg PO Q12h	568,576,587,589–592
VIRAL INFECTIONS				
Localized herpes zoster	Acyclovir 800 mg PO 5 × 7–10 days × /day	Famciclovir 500 mg PO Q8h × 7–10 days	None	557,593–596
Disseminated varicella zoster	Acyclovir 10 mg/kg IV Q8h × 10 days	Foscarnet 40–60 mg/kg IV Q8h × 10 days	Consider high-dose oral acyclovir if recurrences are seen	557,596–598
Localized, nonhealing herpes simplex virus, nasal labial, genital, or perianal areas	Acyclovir 200 mg PO 5 × /day × ≥10 days	If suspect acyclovir resistance, foscarnet 40–60 mg/kg IV Q8h × □10 days	Acyclovir 200 mg PO TID or 400 mg PO BID; or famciclovir 250 mg PO BID; or valacyclovir 500 mg PO BID	557,599–604

TABLE 13.22. *continued*

Infection	Speci c Treatment	Alternative Treatment	Maintenance Therapy	References
Cytomegalovirus retinitis	Ganciclovir 5 mg/kg IV Q12h × 14–21 days; or valganciclovir 900 mg PO BID × 14–21 days	Foscarnet 60 mg/kg IV Q8h × 14–21 days; or cidofovir 5 mg/kg IV q week × 2 weeks, then every other week (with probenecid)	Ganciclovir 5 mg/kg IV Q24h; or valganciclovir 900 mg PO daily; or foscarnet 90–120 mg/kg IV Q24h; or cidofovir 5 mg/kg IV every other week (with probenecid); or oral ganciclovir 1 g PO Q8h; or ganciclovir ocular implant; or fomivirsen 330 mg by intravitreal injection every four weeks	557,562,605–618
Progressive multifocal leukoencephalopathy (JC virus infection)	None	None	None	619,620
Parvovirus B19	IgG IV 0.4 g/kg × 5 days	None	If relapse occurs within six months after initial treatment, give single day infusions of IgG 0.4 g/kg every four weeks	621
PROTOZOAL INFECTIONS *Pneumocystis carinii* pneumonia	Trimethoprim (15–20 mg/kg/day) with sulfamethoxazole (75–100 mg/kg/day) in 3–4 divided doses IV or PO × 21 days plus a tapering dose of corticosteroids if $PaO_2 < 70$ or A-a gradient > 35 mm Hg. One possible regimen is prednisone 40 mg PO BID × days 1–5, prednisone 40 mg PO daily × days 6–10, prednisone 20 mg daily × days 11–21	Pentamidine 3–4 mg/kg IV daily × 21 days; or trimethoprim 20 mg/kg/day PO in 4 divided doses plus dapsone 100 mg PO daily × 21 days; or atovaquone elixir 750 mg PO BID with meals × 21 days; or trimetrexate 45 mg/m^2 IV daily × 21 days plus folinic acid 20 mg/m^2 IV Q6h × 24 days; or clindamycin 600 mg IV Q6h plus primaquine 15 mg base PO daily × 21 days; plus steroids when indicated, for all the above regimens.	Trimethoprim (160 mg)-sulfamethoxazole (800 mg) PO TIW; or dapsone 100 mg PO daily (dapsone plus pyrimethamine regimens are preferred for toxoplasma antibody + patients, see Table 13.17); or atovaquone 1,500 mg PO/day; or aerosol pentamidine 300 mg once/month by the Respirgard II nebulizer	208,543,557,622–637

TABLE 13.22. *continued*

Infection	Speci c Treatment	Alternative Treatment	Maintenance Therapy	References
Cerebral toxoplasmosis	Pyrimethamine 50–100 mg/day PO with sulfadiazine 1 g PO QID plus folinic acid 5–20 mg/day PO, until resolution or stabilization, with improvement of clinical signs and CT abnormalities	Trimethoprim (10 mg/kg/day)—sulfamethoxazole (50 mg/kg/day) in two to three divided doses; or clindamycin up to 1,200 mg IV Q8h with pyrimethamine 50–100 mg/day PO with folinic acid 5–20 mg PO/day; or pyrimethamine plus folinic acid as above plus one of the following: clarithromycin 1gm PO Q12h; atovaqone elixir 750 mg PO Q12h; azithromycin 1,200–1,500 mg PO/day; dapsone 100 mg PO/day	Pyrimethamine 25 mg/day plus sulfadiazine 1 g/day plus folinic acid 5 mg/day	557,638–644
Microsporidial enteric infection	Albendazole 400 mg PO BID	Fumagillin 20 mg PO TID × 2 weeks (experimental)	? None	645–649
Cryptosporidiosis	Paromomycin 500–750 mg PO QID	Azithromycin 500–1250 mg/day; or nitazoxanide 1,000 to 2,000 mg/day (experimental)	Same as initial therapy	166,557,650,651,651a, 651b
Isospora belli enteric infection	Trimethoprim (160 mg) with sulfamethoxazole (800 mg) PO QID × 10 days	Pyrimethamine 75 mg/day PO and folinic acid 5 mg PO daily × 10 days; or cipro oxacin 500 mg PO BID × 10 days;	Same as initial therapy if response; or trimethroprim (160 mg) sulfamethoxazole (800 mg) TIW; or pyrimethamine 25 mg/day plus folinic acid 5 mg/day	166,652–655
Cyclospora	Trimethroprim (160 mg)—sulfamethoxazole (800 mg) PO QID × 10 days	Cipro oxacin 500 mg PO BID × 10 days	Trimethoprim (160 mg)—sulfamethoxazole (800 mg) PO TIW	166,655–657
HELMINTHIC INFECTION				
Strongyloides stercoralis	Ivermectin 200 r ~ glkg/day × 1–2 days	Thiabendazole 25 mg/kg Q12h x □2 days	None	166,658,659

TABLE 13.22. *continued*

Infection	Speci c Treatment	Alternative Treatment	Maintenance Therapy	References
BACTERIAL INFECTIONS				
Nocardiosis	Sul soxazole 1–2 g PO QID × 6 weeks	Minocyciine 100–200 mg PO BID; or other antimicrobials based on susceptibility testing	Continue therapy at 1 g sul soxazole PO QID	660–664
Salmonella bacteremia	Cipro oxacin 750 mg PO BID × 6 weeks	Amoxicillin 500 mg PO TID; or trimethoprim (160 mg)—sulfamethoxazole (800 mg) PO TID	? None, if cipro oxacin is used as primary therapy; otherwise, continue primary therapy	664–666
Mycobacterium tuberculosis	Isoniazid 300 mg PO daily with pyridoxine 50 mg PO daily, with rifampin 600 mg PO daily, with pyrazinamide 15–30 mg/kg per day PO (once daily or in 3–4 divided doses up to daily maximum of 2 g), with ethambutol 15–25 mg/kg daily; all × 2 months (if multiply resistant strain suspected, use 5 drugs)[D]	Same except may substitute streptomycin 20–40 mg/kg (maximum 1 g) IM daily for ethambutol	Isoniazid 300 mg PO daily withpyridoxine 50 mg PO daily, with rifampin 600 mg PO daily; both × 6 months total course (including initial two months)	160,163,524,667–670
Mycobactenum avium complex	Clarithromycin 500 mg PO BID with ethambutol 15–25 mg/kg PO daily (many experts use three-drug initial regimen)	Add or substitute rifabutin 300–600 mg PO daily, cipro oxacin 500–750 mg PO daily, amikacin 7.5 mg/kg IV Q12–24h	Continue initial therapy	557,671–689
Bacillary epithelioid angiomatosis	Erythromycin 500 mg PO QID x 2–4 weeks	Doxycycline 100 mg PO BID	None, unless relapse occur	690–695
Helicobacter cinaedi cellulitis and/or bacteremia	Doxycycline 100 mg PO or IV Q12h x 2–6 weeks	Gentamicin 3–5 mg/kg/day IV	None	696,697
*Rhodococcus equi*Vancomycin 15 mg/kg IV Q 12h plus rifampin 600 mg PO or IV/day × 4–8 weeks	Macrolide plus rifampin 600 mg IV or PO daily	Macrolide plus rifampin 600 mg PO daily	698–701	

[a] Recent evidence suggests that *Pneumocystis carinii* is a fungus (701a).
[D] Many drug-drug interactions can be expected when either rifampin or rifabutin are used in conjunction with anti-retroviral agents, particularly protease inhibitors and non-nucleoside reverse transcriptase inhibitors, see Table 13.17 for speci c recommendations and references 9, 17 and 163.
Q, every; PO, orally; IV, intravenous; BID, twice daily; TID, three times daily; QID, four times daily; IgG, immunoglobulin G; CT, computed tomography; IM, intramuscularly; CNS, central nervous system.
* If patients unable to tolerate amphotericin B due to nephrotoxicity, consideration should be given to substitution of a lipid preparation of amphotericin B (571,575,576).

TABLE 13.23. *Rheumatic manifestations in HIV infection (705–710,715)*

Arthralgias
Painful articular syndrome
Seronegative spondyloarthropathy
Reiter's syndrome
Reactive arthritis
Psoriatic arthritis
Undifferentiated
HIV-associated arthritis
Connective tissue-like disorders
Myopathies/Myositis
Sjögren's-like syndrome (diffuse in ltrative lymphocytosis syndrome)
Vasculitides
Systemic lupus erythematosus-like disease
Rheumatoid arthritis-like disease
Miscellaneous
Septic arthritis and other septic complications
Avascular bone necrosis
Osteopenia
Osteoporosis
Myalgias
Fibromyalgia

pathophysiologic mechanisms include direct HIV infection of joints (716,728–730), immune complex deposition (716,731,732), and an atypical form of reactive arthritis occurring in the absence of HLA-B27, antecedent genitourinary or enteric infections, and inßammatory synovial ßuid. In general, the process is transient lasting several weeks and is not destructive (710,711), although a polyarticular erosive form has been reported (708,710).

For patients with these forms of arthritis or with Reiter's syndrome, nonsteroidal antiinßammatory agents are the mainstay of treatment. A trial of sulfasalazine may be warranted in instances of sustained inßammation (706), but steroids, other immunosuppressive medications or etretinate are usually reserved for refractory cases (710,717,733).

A wide spectrum of inßammatory and non-inßammatory muscle disorders have been described in association with HIV infection (see Chapter **??**) (708–710). HIV associated polymyositis patients do not exhibit in their sera the autoantibodies described in the idiopathic forms (709).

Almost the entire clinical spectrum of vasculitides have been reported in HIV-infected patients (734–737), including those of the polyarteritis nodosa type (710,738,739). The latter patients present primarily with a peripheral sensory or sensorimotor neuropathy. The etiology for the vasculitis is unknown in most cases, although cytomegalovirus infection is one recognized cause (740,741). Polymyalgia rheumatica and temporal arteritis may also occur more commonly in HIV-infected patients (725).

A syndrome characterized by massive parotid enlargement and xerostomia that superficially resembles Sjögren's syndrome is a well-established but relatively infrequent complication of HIV infection (742,743). In adults the syndrome appears to have an immunogenetic basis and is found principally in blacks who have the DR5 allele (743). It is characterized by markedly elevated numbers of CD8+, CD29+ lymphocytes in blood that infiltrate various glandular and extraglandular tissues (728). Consequently, it has been designated as the "diffuse infiltrative lymphocytosis syndrome." Unlike classic Sjögren's syndrome, autoantibodies are usually absent. Extraglandular involvement may include the liver, lung, gastrointestinal tract, kidney, thymus, and nervous system (744). The possibility of HIV infection should always be considered in an individual with unexplained bilateral parotid enlargement. Anecdotally, we have observed striking resolution of parotid enlargement following irradiation of the gland.

Various other autoimmune phenomena have been associated with HIV infection including the production of autoantibodies (705). Aside from antiplatelet antibodies that may be responsible for thrombocytopenia (745,746), antibodies directed at other targets, including red blood cells, leukocytes, and nuclear and cardiolipin antigens, are usually clinically silent (705). The same can probably be said for the presence of circulating immune complexes (731,732) and cryoglobulins (747).

FUTURE PROSPECTS

Studies of novel chemotherapeutic and immunomodulatory agents to control HIV infection are likely to increase in the coming years. In particular, within the next several years, agents that target parts of the HIV lifecycle other than the reverse transcriptase and protease enzymes will probably become part of the armamentarium for treatment. The role of IL-2 as an immunomodulator will be better defined, and the role if any, of strategic drug interruptions will be defined. Further study will also help to better understand when to start and change antiretroviral therapy. Data from the numerous vaccine trials regarding preventive and therapeutic vaccines will be forthcoming and with hope the results will be promising. Better outcomes that may result from these advances in turn will further alter the natural history of HIV infection.

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Chapter 14

HIV Disease in Women

Lisa Ferrigno and Jack A. DeHovitz

The prevalence of HIV infection in the United States has increased among women, while decreasing among white men such that women now represent an estimated 30% of new HIV infections in the U.S., and an increasing proportion of new AIDS cases.

This chapter will focus on those facets of HIV that may differ between men and women as well as on clinical issues speciÞc to women in order to provide guidelines for their care. It should be emphasized that, except regarding those issues that are sex-speciÞc, treatment algorithms for HIV-infected women do not differ from that of men. Discussions about the changing epidemiology of HIV will provide the clinician with a framework to determine who may be at risk and to inform the application of guidelines to prevent subsequent HIV transmission. Although antiretroviral recommendations currently remain the same for men and women, the review of Þndings regarding early HIV infection and the reported discrepancy in HIV viral load will allow the clinician to interpret forthcoming data appropriately and to address misinformation or misinterpretation of these data by patients. Finally, much of the chapter will deal with sex-speciÞc issues, such as the differential presentation of gynecologic problems in the HIV-infected woman and a brief summary of obstetric considerations.

Importantly, the needs of all women seeking primary care should not be overlooked in the context of HIV infection. While much emphasis has recently been placed on the role of the HIV-experienced provider in providing HIV care, it is also important to insure that patients can obtain womenÕs health care at the same or adjacent settings. An emphasis on providing Òone-stop shoppingÓ for care, will ensure, to the extent possible, both coordinated and high quality care. In addition, the importance to provide an atmosphere of trust and conÞdentiality.

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EPIDEMIOLOGY

It is estimated that approximately 25% of the 800,000 to 900,000 Americans living with HIV infection are women. While AIDS rates in women are lower compared to men (9.3 per 100,000 women vs. 32.4 per 100,000 men in 1999), the cumulative percentage of AIDS cases in women almost tripled from 6.7% in 1986 (1) to 18% in 1999 (2). Women of color represent the majority of AIDS cases in women and their proportion continues to increase. In 1999, for example, African-American women accounted for 63% of newly reported AIDS cases (2). In the same year the case rate for Latinas (14.9 per 100,000) was more than six times the rate for white women. In 1998 HIV was the third leading cause of death among African-American women ages 25Ð44 (3).

The mode of acquisition of HIV in American women has evolved over time. Early in the epidemic, the majority of female AIDS cases were due to injection drug use (IDU). However, by 1995 the proportion of women infected through the heterosexual route surpassed that of IDU (4). As of 1999, the proportion of women who had known sex with an HIV-infected partner or an individual at high risk was approximately 40% as compared to 30% who had used injection drugs. Of note, however, is the fact that the proportion of those with no known risk according to the surveillance deÞnition has increased to slightly greater than 30% in 1999. Studies suggest that at least 50% of these individuals may have acquired HIV heterosexually (5). This is supported by a report by the Centers for Disease Control and Prevention (CDC) which estimates that 81% of those reporting no ascertained risk were infected via heterosexual sex (6). While non-injected drugs are not a vehicle for infection, cohort studies have revealed that much of the heterosexual risk of HIV is associated with use of drugs, such as alcohol and cocaine.

Other important trends characterize the epidemic in women. For example, there is an increasing proportion of AIDS cases occurring in women in the South, perhaps reßecting the dramatic increase in other STDs Þrst seen in that region a decade ago (7). Insurance status also varies by gender. Women are more than twice as likely to be covered by Medicaid, as are men and half as likely to be privately insured (8). These data reinforce the fact that the intersecting epidemics of poverty, drug use, and sexually transmitted diseases will continue to affect the evolution of the HIV epidemic in women in the U.S.

RESEARCH AND WOMEN

Concerns have been raised that research about AIDS in general and studies describing the natural history of HIV infection and the favorable outcomes of HAART utilization in particular, have under-represented women (9). Indeed, most of the initial cohorts (such as the Multicenter AIDS Cohort Study-MACS) assembled to assess the natural history of HIV infection were comprised exclusively of men. As a result, much of the early data supporting the use of biological markers such as CD4 cell count and plasma HIV RNA level (viral load) were developed from exclusively male cohorts. To begin to answer gender speciÞc questions, population based studies were stratiÞed by gender. A prominent example of this was a study of injecting drug users, the AIDS Link to Intravenous Experience (ALIVE) study, which closely examined both behavioral and clinical differences between male and female drug users (10). However, by the early 1990s two large prospective cohort studies were assembled that were comprised exclusively of women with HIV disease and as well as women at risk. These two studies were the HIV Epidemiology Research Study (HERS) (11) funded by the CDC and the WomenÕs Interagency HIV Study (WIHS) (12) funded primarily by the NIH. While the HERS study was discontinued, the WIHS study remains in the Þeld and has recently been expanded to enroll a younger cohort.

Perhaps more difÞcult to examine, however, is the impact of representation of women in clinical trials. Most NIH funded clinical research has included women at rates similar to the proportion of women infected across the country (e.g. by 1998, approximately 20% of ACTG trial participants were women, roughly the proportion of those making up the proportion of women infected). Community based clinical trials, such as the Community Programs for Clinical Research on AIDS (CPCRA) and AmFARÕs community based clinical trials network, as well as industry studies, have as part of their mission to include classically under-represented persons, such as minorities, women, and people with current or history of injection or other drug use. Equal representation, however, does not necessarily translate into ability to detect gender differences in study outcomes (13). Of course, this is not only true for studies of HIV; however, caution is urged in imposing standards for studies to be able to detect gender differences as the number of patients required to demonstrate small differences may be prohibitive (14). While most indications are that women will respond similarly to men for studied regimens, differences found in Òreal practiceÓas compared to the clinical trials setting may be exaggerated in women (15). In the clinical setting implementation of strategies used by clinical trials to encourage follow-up should be initiated.

NATURAL HISTORY OF HIV INFECTION IN WOMAN

Early in the epidemic several studies suggested that women with HIV might have worse outcomes than men (16,17). However, subsequent studies that controlled for access to medical care and other characteristics have not revealed differences in the rate of disease progression (18,19). While the natural history may not vary between men and women, several studies suggest that important surrogate markers such as CD4 + count and viral load may have gender-speciÞc differences.

As CD4 counts may differ by gender even in a healthy population, this may to some degree account for the lack of differences in natural history reported in the studies cited above. For example, it has been documented that immunocompetent women tend to have signiÞcantly higher CD4 counts than men (20) and women infected with HIV maintain a slightly higher CD4 cell count than men (21).

Viral load following HIV seroconversion has been shown to be an independent predictor of the risk of progression to AIDS and death (22). Although conßicting data exist, collective evidence to date indicates that there are differences in viral load, particularly within the Þrst Þve years of infection in women as compared to men. Early studies, such as those published by Bush (23) and Lyles (24) found no signiÞcant difference in the baseline viral loads of men and women. However, in both studies, although non-signiÞcant, the viral loads of women off treatment were approximately one-half that of the men; in BushÕs study, this discrepancy persisted after the participants received therapy. The lack of signiÞcance in these studies might be explained by the small sample sizes.

One of the Þrst studies to show a discrepancy in viral load was a report from the ALIVE cohort, a prospective study following HIV seropositive and seronegative injection drug users. In this cross-sectional study, Farzedegan et al. found that among 421 men and 106 women, baseline viral load in women was almost 40% lower than that of men (25). A more deÞnitive characterization of this phenomenon came from a subsequent prospective study from the same cohort (26). Of 3,380 IDUs, a total of 202 (77% men and 23% women) seroconverted and met inclusion criteria for the analysis. Women were signiÞcantly younger at seroconversion (36.7 years vs. 32.6

years). At the first visit after seroconversion, none was utilizing antiretroviral therapy (ART), whereas proportions utilizing ART were similar at subsequent visits. At the initial visit after seroconversion, it was found that the viral load was significantly lower in women as compared with men. This effect persisted throughout the second year after seroconversion although it became less pronounced several years out. Importantly, there was no difference with regard to time to clinical progression at these visits or subsequently.

Similar results emerged from a comparative study of the WIHS and MACS cohort (26). This study examined the impact of gender on HIV progression. After adjustment for differences in measurement, HIV-1 RNA levels were 32–50% lower in women than men with CD4+ counts of greater than 200 but not at counts lower than that. Women were also noted to have more rapid declines (almost twofold) in CD4+ counts than men. While all studies controlled for possible confounding factors, of note is that the WIHS/MACS comparative study also found that injection drug use was independently associated with a lower viral load, as was being non-white. Furthermore, women tend to be younger at seroconversion than men, which was the case in the ALIVE cohort. In male cohorts, CD4 counts tend to be higher in younger patients, again underscoring the possibility that women may have a larger early decrease.

The basis for differences in viral load response by gender is unknown. It has been suggested that the differing hormonal milieu of women and men may have an effect on viral replication (25). Reproductive hormones have been shown to have an impact on lymphocyte function and cytokine production (28,29). The underlying questions are whether these early differences in immunologic and virologic markers have any significance for eventual clinical outcomes and whether antiretroviral therapy should be initiated at a lower viral load than is currently recommended for men. Several factors need to be considered when addressing these critical questions. First, as noted above, it appears that men and women have a similar rate of progression to AIDS, even in the studies reporting discrepant viral loads for the first several years of infection. Second, a viral load cut-off above which rapid progression to AIDS can be predicted has not yet been identified. Third, differences in viral load appear to be most pronounced during the first few years of infection when the risk of progression to AIDS and death is lowest. Fourth, numerous studies have confirmed that the CD4+ cell count appears to be a better predictor than viral load. Finally, recent treatment recommendations have altered cutoff values for consideration of treatment of HIV disease. As a result, treatment is generally not recommended until later in the disease, a setting where viral load levels of men and women tend to be similar for a given CD4+ count. Thus, at this time, there are no differential therapeutic recommendations for HIV therapy based on gender.

DIFFERENCES IN OPPORTUNISTIC INFECTIONS

Rates of HIV-associated illnesses are generally similar between men and women. Nonetheless, there are some differences. Historically, women have experienced lower rates of Kaposi's sarcoma (KS) (30). Although KS remains rare in women, unusual presentations have been noted, including KS presenting as a vulvar mass and diagnosis by cervical biopsy (31,32). Although rates of KS in women have increased somewhat, they remain substantially below that of men, a phenomenon that is thought to be due to a lower prevalence of HHV-8 the putative etiologic agent of KS. However, HHV-8 is present in women. In a recent study from the WIHS, HHV-8 infection was noted in 15% of HIV-infected women and in 6.3% of those without HIV infection (33). Sexual acquisition of HIV appeared to be the strongest risk factor for serologic reactivity to HHV-8.

Rates of other opportunistic infections (OI) are approximately equal in men and women. Early studies suggested that that esophageal herpes and candidiasis were more common in women (34,35). More recent data, obtained after the widespread availability of HAART, indicate that some differential rates may exist, but may not be solely attributed to gender. Data from the Adult Spectrum of Disease study, the largest database of patients receiving care for HIV disease in the United States, examined rates of opportunistic infections over the time of availability of HAART (36). From 1996 to 1998, it was found that women experienced a higher incidence of esophageal candidiasis, tuberculosis, cryptosporidiosis, and toxoplasmosis. Not noted was whether these incident OIs were presenting conditions for the population studied or whether they occurred at follow-up when the patients were under care. Most important, variations appear to be associated with risk, but not necessarily gender. Injection drug users (particularly active users) may be more likely to experience certain OIs, but these rates are similar between men and women.

GENITAL TRACT INFECTIONS

Gynecologic infections are frequently found in women at risk for HIV, as well as those with HIV infection. The immunosuppressive effects of HIV can alter both their frequency and natural history. Given the risk factors for HIV, sexually transmitted diseases (STDs) represent a significant concern. Nonetheless, available data suggests that new STDs occur with approximately equal frequency in HIV-infected and uninfected women (37,38).

Bacterial Vaginosis

Bacterial vaginosis (BV), although not considered a sexually transmitted disease, is the most frequent cause of

vaginal symptoms among women of childbearing age (39). It is thought to result from a disruption of the normally acidic vaginal environment created by lactobacilli. For reasons that are not clear, disruption of the microßora occur resulting in the overgrowth of such organisms as *Gardnerella vaginalis*, anaerobes and *Mycoplasma hominis*. Subsequent data have borne out important perinatal sequelae of untreated BV, including premature rupture of membranes, preterm delivery and chorioamnionitis.

There is no speciÞc evidence that BV pursues a more aggressive course in HIV-infected women. However, several studies have found that women with BV appear more susceptible to infection with HIV (40Ð43). Sewankambo et al. reported on approximately 4,000 women in Uganda; of those with severe BV, HIV prevalence was 26.7% as compared to 14.2% in those with normal vaginal ßora (40). In a prospective study in Malawi, of 1,159 HIV seronegative women, only one woman with no symptoms associated with microßora changes associated with BV became HIV infected; in the group meeting all criteria for BV, 10 of 274 seroconverted, yielding an odds ratio of 3.72 (41). This increase in susceptibility is thought to be secondary to changes in vaginal ßora, including lack of lactobacilli, leading to a less acidic vaginal environment. More recent work suggests that the organisms that become active in the altered vaginal ßora associated with BV may promote HIV-1 expression through the production of a cytokine-like substance, termed by some as ÒHIV inducing factor (HIF)Ó(42,43). Treatment of BV as delineated by the Centers for Disease Control is outlined in Table 14.1.

Trichomoniasis

Trichomonas is also a frequent cause of vaginitis, and is found in women at high risk for sexually transmitted diseases (44). As with BV, studies have suggested that women with trichomoniasis are at higher risk of sexual acquisition of HIV (45). Trichomoniasis is thought to create an easier portal of entry for HIV by causing inßammatory changes in the vaginal mucosa, such as punctate hemorrhages and local cellular inÞltration. As a result of local inßammation, bidirectional increased risk is thought to occur, leading to an increased chance for infection of the partner of HIV-infected women, thus underscoring the importance of treatment of the patient and her partner expeditiously. As with BV, trichomoniasis may pursue a more aggressive course in the HIV-infected patient. Both patient and sexual partner should be treated with either a single 2-gram dose or a seven-day course (500 mg twice daily) of metronidazole, if infection is identiÞed.

Although STDs causing genital lesions may lead to increased rates of HIV infection, both trichomoniasis and BV may result in more HIV infections because of their much greater prevalence (46).

TABLE 14.1. *Recommended management for bacterial vaginosis*

- Metronidazole 500 mg po twice a day for seven days
- Clindamycin cream 2%, 5 g intravaginally at bedtime for seven days
- Metronidazole gel 0.75%, 5 g intravaginally twice a day for ve days

Note: Clindamycin cream is oil-based and may weaken latex condoms and diaphragms.
Alternative regimens: metronidazole 2 g po × 1 dose, or clindamycin 300 mg po twice a day for 7 days.
Source: CDC, 1998.

Vulvovaginal Candidiasis

Several reports early in the AIDS epidemic indicated that HIV-infected women with mild to moderate immune deÞciency have higher rates of vulvovaginal candidiasis (VVC) and may be more recalcitrant to treatment than their HIV-negative counterparts (47Ð49). The frequency of VVC has been shown to signiÞcantly increased only in those HIV-infected women with a CD4+ count of less than 100/mm^3 (50). However, a more recent study utilizing the HERS cohort, with the beneÞt of physical exam, cultures and a HIV-seronegative control group, did not Þnd that vaginal candidal disease is more frequent in HIV seropositive women (51). Although HIV-positive women were more likely to report a history of VVC (84% vs. 77%), the rate of women with VVC at the time of the cross-sectional examination was identical for the HIV seropositive and negative groups (9%). Although HIV-infected women appear to be more likely to be colonized with candida (37% vs. 21%), these colonization rates were similar regardless of CD4 count; thus, the signiÞcance of this is uncertain. Also, women reporting a history of frequent VVC infections were not more likely to be colonized at time of exam. Of interest, HIV seronegative women were more likely to be colonized with a non-albicans species (most frequently *C. glabrata*), although a common perception holds that the opposite is true, e.g. that HIV-infected women harbor more atypical strains.

Therapy should be initiated with topical azoles and may be more effective if given for at least seven days. Persistent or recurrent infections may be treated with oral azoles, particularly one dose (150 mg) of ßuconazole. In this case, conÞrmation of diagnosis by culture with species identiÞcation and susceptibilities may prove useful, although recent application of antifungals may inhibit growth. Recent data indicate that VVC was decreased in an *in vitro* model with indinavir and ritonavir, which strongly inhibit an enzyme considered to be a virulence factor of pathogenic Candida species (52).

Factors promoting VVC in any patient population should be identiÞed and controlled, if possible. Examples of co-factors would be concomitant diabetes, steroid use,

tight-Þtting clothing, douches and pregnancy. Although ßuconazole, 200 mg po weekly was found to be effective in preventing recurrent VVC and oral candidiasis, it is thought by some to be unnecessary (46). Consideration for prophylaxis might be given if antibiotics are prescribed and the patient has a history of VVC with antibiotic administration.

Ulcerative Diseases

Genital ulceration in the context of HIV infection is perhaps best recognized for its facilitative role in the HIV transmission (53Ð55) and may increase the risk of both acquisition (in the HIV-negative woman) as well as transmission.

In the evaluation of the immunocompromised woman with genital ulceration, a complete history and a full evaluation including cultures and appropriate blood work are appropriate as the presentation may not be classic. Complete examination should include a dark-Þeld examination or direct immunoßuoresence test for *T. pallidum*, HSV culture or antigen tests, as well as consideration of *H. ducreyi* culture. Multiple agents can be present in as many as 20% of patients.

Idiopathic genital ulcers have been described in women with advanced HIV disease (56,57). They present as intractable ulcers that can progress to Þstula formation and have been reported to cause severe bleeding. Clinical responses have been noted following coricosteroid treatment, initiation of antiretroviral therapy, and thalidomide (58).

Herpes Simplex Virus

Ulcer disease caused by HSV is common: data from the National Health and Nutrition Exam Survey (NHANES III) (59) revealed an HSV-2 seroprevalence of 21.9% among 13,000 participants. Additionally, one of the strongest predictors of positive serologic status was female sex. In immunocompetent people genital ulceration tends to be secondary to HSV-2; however, as immunosuppression progresses, HSV-1 also frequently presents as a genital ulcer. Severe herpetic outbreaks may indeed herald the onset of severe immunosuppression. The extent of disease in severity and locale may make it difÞcult to differentiate HSV infection from zoster. HIV-infected women with genital HSV-2 infections are more likely to shed HSV and to have more frequent recurrences than are HIV-negative women. Recurrences have been shown to increase in patients with lower CD4+ counts (60).

Although several antiviral agents newer than acyclovir are on the market, given its low cost, it should be considered the Þrst-line agent. Other agents such as famciclovir or valacyclovir can be considered if the patientÕs pill burden is such that it would preclude adequate adherence. (Treatment with acyclovir requires dosing at three to Þve times per day versus other agents requiring BID dosing.) Similarly, for successful suppressive therapy, the once a day dosing of valacyclovir may be better suited for selected patients than the increased dosing required for the other medications. Treatment and suppressive therapy guidelines are listed in Table 14.2.

Acyclovir resistance is not uncommon and can be observed in 11Ð17% of HIV-infected patients (61).

TABLE 14.2. *Recommended management for HSV*

	Drug	Dose
First clinical episode	Acyclovir	400 mg po three times a day for 7 to 10 days
	Acyclovir	200 mg po five times a day for 7 to 10 days
	Famciclovir	250 mg po three times a day for 7 to 10 days
	Valacyclovir	1 g po twice a day for 7 to 10 days
Recurrent episodes	Acyclovir	400 mg po three times a day for 5 days
	Acyclovir	200 mg po five times a day for 5 days
	Acyclovir	800 mg po twice a day for 5 days
	Famciclovir	125 mg po twice a day for 5 days
	Valacyclovir	500 mg po twice a day for 5 days
Daily suppressive therapy	Acyclovir	400 mg po twice a day
	Famciclovir	250 mg po twice a day
	Valacyclovir	500 mg po once a day
	Valacyclovir	1000 mg po once a day
Severe disease	Acyclovir	5 to 10 mg/kg body weight IV every 8 hr for 5 to 7 days or until clinical resolution is achieved
Acyclovir-resistant HSV	Foscarnet	40 mg/kg body weight IV every 8 hr or 60 mg/kg IV every 12 hr for 3 wk
	Topical Cidofovir gel 1%	Applied to lesions once a day for 5 consecutive days

Source: CDC, 1998.

TABLE 14.3. *Recommended management for syphilis*

Stage	Treatment
Primary and secondary syphilis and early latent	Benzathine penicillin G 2.4 million units IM (single dose); additional treatment recommended by some (i.e. three weekly doses of penicillin)
If penicillin-allergic (nonpregnant patients only)	Doxycycline 100 mg po twice a day for 2 wk, OR tetracycline 500 mg po 4 times a day for 2 wk
Late latent syphilis or syphilis of unknown duration (including tertiary syphilis)	Examination of the CSF must be performed before initiating treatment. If the CSF examination is negative, patients should be treated with 7.2 million units of benzathine penicillin G IM (three weekly doses of 2.4 million units each)
Neurosyphilis	Aqueous crystalline penicillin G 18 to 24 million units a day (administered as 3 to 4 million units IV every 4 hr) for 10 to 14 days. Administration of benzathine penicillin 2.4 million units IM after completion of the IV regimen recommended by some.
	Recommended follow-up
Primary and secondary syphilis	HIV-infected patients require clinical and serologic evaluation for treatment failure at 3, 6, 9, 12, and 24 mo after treatment. Treatment failures necessitate a CSF examination and retreatment (three weekly doses of 2.4 million units of benzathine penicillin G if CSF examination is negative). The latter regimen should also be considered for patients whose titers do not decrease fourfold within 6 to 12 mo.
Latent syphilis	HIV-infected patients require clinical and serologic evaluation at 6, 12, 18, and 24 mo after treatment. The CSF examination should be repeated and appropriate treatment instituted if clinical symptoms develop, titers rise fourfold, or if titers fail to decline fourfold between the evaluations at 12 and 24 mo.
Neurosyphilis	CSF examination should be repeated every 6 mo until the cell count is normal. Retreatment should be considered if the cell count has not decreased after 6 mo or if the CSF is not entirely normal after 2 yr.

CSF, cerebrospinal uid.
Source: CDC, 1998.

HIV-infected women who do not respond to standard therapy should have the diagnosis conÞrmed by culture. In the absence of clinical response, alternatives such as intravenous acyclovir, foscarnet, topical trißuride or topical cidofovir should be considered (62).

Syphilis

Clinical manifestations of syphilis in the HIV-infected patient appear similar to those of HIV-negative women. However, the diagnosis of syphilis may be complicated by a higher rate of false-positive serologic tests, presumably secondary to HIV-induced polyclonal activation (63). The recognition, treatment, and evaluation of syphilis in immunocompromised individuals should be aggressive and consistent with guidelines promulgated by the CDC. Neurosyphilis should always be considered in light of abnormal neurologic Þndings in the HIV-infected person. Also, it is not unusual, particularly in the immunocompromised person with HIV, that symptoms usually attribute to particular stages of syphilitic infection may occur in tandem, not at all, or in a more accelerated fashion.

The CDC has outlined alternative treatment options for individuals with penicillin allergies, which are presented in Table 14.3. If the history indicates mild to moderate penicillin sensitivity, with no history of life-threatening respiratory involvement or Stevens Johnson syndrome, desensitization may be considered. As concern exists about the possibility of higher relapse or non-response rates in HIV-infected persons (64), follow-up after treatment should be vigilant and include clinical and serologic evaluation.

Chancroid and Other Causes of Ulcerative Diseases

Chancroid, although relatively rare, should be considered in the differential diagnosis of ulcer disease. Of 143 men evaluated in an outbreak of genital ulcer disease, Mertz, et al. (65), found that 39% were positive for *Haemophilous ducreyi*, the causative organism of chancroid. The genital ulcer surveillance group found ulcers positive for *H. ducreyi* greater than 10% of the time in Chicago (12%) and Memphis (20%) (65). Chancroid should be suspected as the etiology of genital ulcerative disease in patients with a history of a singular tender

pustule subsequently becoming ulcerated or multiple ulcers with lymphadenopathy when herpes infection has been ruled out. Culture is not universally available; laboratories should be notiÞed if *H. ducreyi* is being considered. The degree of suspicion should be raised if the patient originates from an area where *H. ducreyi* is endemic and as common as gonorrhea, such as parts of Africa and Asia.

Anecdotal reports suggest that patients with HIV and chancroid require more prolonged therapy than HIV-uninfected patients (66). Nonetheless, treatment failure was not associated with HIV seropositivity in a randomized, controlled trial of the treatment of chancroid comparing ciproßoxacin with erythromycin (67).

Both lymphogranuloma venereum (LGV) and granuloma inguinale (donovanosis) are rare conditions in the U.S. In addition to genital ulcerations, LGV presents with painful lymphadenopathy, usually unilateral; additionally, Þstulae or strictures may be present. Diagnosis is through exclusion of other causes and appropriate serology. Treatment is erythromycin or doxycycline for three weeks; however, HIV-positive persons may require extended courses of treatment. Ulcers in donovanoisis tend to be painless and friable and without associated lymphadenopathy. Diagnosis is with site biopsy. Treatment is also for three weeks, with either TMP-SMX or doxycycline; the CDC also recommends adjuvant aminoglycoside in those with HIV infection.

Pelvic Inßammatory Disease

Pelvic inßammatory disease (PID) is a severe complication of STDs. Concomitant HIV infection is frequently seen in patients with PID with rates ranging from 8% in the U.S. to as high as 29% in Africa (68,69). As a result, serologic testing for HIV should be offered to women with a diagnosis of PID.

HIV infection appears to alter the course of PID. For example, in one study of matched autopsy specimens, HIV-infected women had a higher prevalence of endometritis than did HIV-negative patients (70). It also appears that HIV-infected women may be more likely to develop complications of PID such as a tubo-ovarian abscess (TOA). In the PID-HIV infection study, comparing 44 HIV-infected women and 163 non-infected women, rates of sonogram-conÞrmed TOAs were higher (bordering on signiÞcance), with 45.8% of HIV-infected women having TOAs versus 27.1% of the HIV-negative group ($p = 0.07$) (71). Similarly, an earlier study reported that HIV-infected women had a higher rate of TOA (22.6% vs. 13.7%; $p = 0.24$), as well as more frequent surgical interventions (26.6% vs. 8.4%; $p = 0.058$) (72). These and other studies have also noted higher fevers, lower white blood cell counts, and higher erythrocyte sedimentation rates in HIV-infected women, compared to seronegative controls (73).

Although studies such as those cited above suggest a more aggressive course, prospective studies have shown little difference between HIV-positive and HIV-negative women in response to antibiotic therapy. As a result, outpatient therapy has been used with success in HIV-infected women (71,73).

HPV, CERVICAL DYSPLASIA AND CERVICAL CANCER

Cervical dysplasia, a precursor of cervical cancer is strongly associated with HIV infection. The prevalence and incidence of cervical and squamous intraepithelial dysplasia is also strongly associated with declining CD4 count, a higher plasma HIV viral load and the presence of human papillomavirus (HPV) (74,75). Progression to invasive cervical cancer (ICC), is usually associated with oncogenic strains of HPV such as HPV-16 or HPV-18 and progresses in a largely stepwise fashion, with progression through mild atypia, low and high grade dysplasias, *in situ* carcinoma, and Þnally to invasive carcinoma.

Given the incidence and prevalence of other STDs in HIV-infected women, one might expect the rates of HPV to be similar to that of HIV-negative women. As highlighted in this section, rates of HPV incidence, carriage, as well as the prevalence of the spectrum of HPV related disease is disproportionately higher in HIV-infected women. With the publication of studies demonstrating increased rates of pre-cancerous cervical lesions and a small number showing increased rates of ICC, cervical cancer was added to the AIDS case deÞnition in 1993. As will be discussed below, this inclusion may have been somewhat premature. While rates of all HPV-associated lesions are higher in HIV-infected women, invasive cervical cancer rates may not be increased.

Squamous Intraepithelial Lesions/Oncogenic HPV Carriage

Incident and prevalent cervical squamous intraepithelial lesions (SIL) are found more frequently in the HIV-infected woman (76). In one study, which assessed the cervical manifestations, the investigators compared 328 HIV-infected and 325 HIV uninfected women with negative Pap smears at baseline. Using polymerase chain reaction (PCR), 54% of HIV-infected women had HPV detected as compared with 32% of the HIV-negative women. During follow-up, 20% of HIV-infected women showed biopsy conÞrmed SIL compared to 5% of HIV-negative women. This and other studies have found oncogenic types of HPV more frequently in HIV-infected women. Utilizing the same cohort as referred to in the above study, Sun et al. found that rates of oncogenic types of HPV was higher in HIV-infected women (77).

Cancer

Although rates of cervical intraepithelial neoplasia (CIN) have been shown to be higher and more persistent in

HIV-infected women, follow-up studies have not definitively shown higher rates in women with HIV disease. One early study in Brooklyn, New York, showed rates of cervical cancer significantly higher than that expected in that community (78). A subsequent study in Germany found that of 111 HIV-infected women 4% presented with ICC as compared to none of 76 hospitalized injection drug using women and 0.4% of HIV-negative women presenting to an outpatient clinic (79). Several more recent studies (80,81), however, failed to show increased rates of cervical cancer. Possible reasons for this discrepancy include the long length of time for full carcinomatous changes to develop coupled with a relative shorter life expectancy of HIV-infected women. In a study of participants in Cote dÕIvoire, ICC was not found to be associated with HIV-1, but was significantly associated with HIV-2, leading some to speculate that those infected with HIV-1 would die from other AIDS related causes prior to developing cervical cancer in contrast to the slower disease course allowed by HIV-2 (80). An additional study revealed that the rate of abnormalities by Pap smear was 81% when women were HIV-infected and had carriage of an oncogenic HPV type (81). Surveillance in the form of screening at adequate intervals through provider awareness of the inclusion of cervical cancer in the AIDS case definition, may also have largely precluded advancement of potential lesions to the invasive stage. Thus, early fears that fulminate cervical cancers would result from aggressive and accelerated HPV progression have not been borne out.

Extracervical Disease

Areas outside of the cervix, including the vulva and the vagina, have been reported to show abnormal changes associated with HPV in patients with HIV. For example, one study reported HPV-induced changes in extracervical areas (vulvar, vaginal, perianal, or multicentric) as being much more common than in HIV-negative controls (8% vs. 1%, respectively) (82). Of note, most women who developed extracervical disease had a history of CIN.

HPV associated genital warts are also detected more frequently in HIV-infected women. Chirgwin et al. (83), found the prevalence of warts to be 8.7% in HIV-infected women as compared with 1.2% in HIV negatives. Chiasson et al., using a different NYC cohort, also found the difference to be significant between HIV-positive and -negative women (5.6% vs. 0.8%, respectively) (84).

A more recent examination of the cohort reported on by Chirgwin et al. yielded an odds ratio of 9.33 for the incidence of genital warts in HIV-infected women (11.4% vs. 1.4%) as compared to those uninfected (85). Indeed, of all the gynecologic disorders examined by this cohort, those attributable to co-infection with HPV constituted the three highest increased risks (genital warts, OR = 9.33; abnormal Pap, OR = 7.76; presence of oncogenic HPV, OR = 2.0).

Smoking has previously been shown to increase the risk of cervical cancer, presumably via its impact on HPV. Similar findings have been noted in smokers with HIV and genital warts. One study demonstrated rates of genital warts that were three times higher in female smokers for both HIV-positive and -negative women (86). The relative risk was greatly increased (5.2) when adjusted for HIV status and foreign versus American born status.

Persistence

Persistence of HPV carriage is increased, and recurrences of cervical HPV associated lesions occur more frequently in HIV-infected women. The previously mentioned Ellerbrock study (76) showed that among women with HPV detected at baseline, a greater proportion of HIV-infected women persistently shed HPV compared with their HIV-negative counterparts (61% vs 23%, respectively). Recurrences of HPV mediated cervical disease are also more frequent. In a group of women with identified CIN who underwent treatment and follow-up, 69% of the HIV-infected women had subsequent abnormal Pap smears compared to 31% of HIV-negative women (87).

Mechanism

Based on the fact that rates of nonviral STDs are not generally increased in the HIV-infected woman, exposure to new strains of HPV probably only plays a minor role in the increased rates of carriage and HPV disease seen in HIV-infected women. It has been suggested (88), that viral interplay may Ònunmask Ólatent HPV infection; specifically, that the tat region of HIV may upregulate the E6 and E7 regions of HPV. As HIV needs only to be active and replicating for this to happen, this upregulation may occur early in infection, accounting for the increased incidence of HPV shedding earlier, rather than later, in the course of disease.

CD4/VL Association

Rates of HPV carriage, especially of oncogenic types, appear to be associated with decreasing CD4 counts and a higher HIV RNA load (89,90). Luque et al. showed that of 74 women with HIV RNA measurements available, 59% with HIV RNA greater than 10,000 copies/ml had detection of oncogenic HPV types as compared to 19% whose viral load was less than this number (81). Of note, 81% of those in the higher viral load bracket had an abnormal Pap smear compared to 24% of those in the lower category.

Depressed CD4 counts may also predict higher rates of recurrence of disease and persistence of HPV infection.

Fruchter et al. (87) showed that in women with CIN and a CD4 count of less than 200, rates of recurrence were as high as 87%. Sun et al. (91) similarly demonstrated higher rates of persistence of HPV shedding in women with CD4 < 500 as compared to HIV-negative women or HIV-infected women with a CD4 > 500. Interestingly, rates of detection of new oncogenic HPV have been shown to be inversely correlated with CD4 decline; that is, detection of new oncogenic HPV in women with CD4 greater than 500 was 45.3/100 person years as compared to 20.6/100 patient years in those with CD4 less than 200 (90). The authors suggest that ÒnewÓ detections may actually represent reactivation of old infections and that the viral interplay thought to precipitate this reactivation is likely to occur earlier, rather than later in HIV infection. The study by Luque et al. which demonstrated a relationship between viral load and presence of oncogenic HPV, did not reveal a similar relationship to CD4; however, the sample size was smaller and the study was cross-sectional, and therefore unable to comment on incidence or recurrence with time and changing CD4 (81).

Screening

Screening recommendations for HPV-mediated disease are based on recommendations which have been outlined by the U.S. Public Health Service (92). A Pap smear should be included in the initial work-up after HIV infection is conÞrmed. An additional Pap smear in the Þrst year should be obtained regardless of an initial negative result and if the results are normal, annually after that. More frequent Pap smears should be obtained after the evaluation and/or treatment of abnormal results. For patients whose Pap smears are interpreted as atypical squamous cells of undetermined signiÞcance (ASCUS), several management options are available; the choice depends in part on whether the interpretation of ASCUS is qualiÞed by a statement indicating that a neoplastic process is suspected. Follow-up by Pap tests without colposcopy is acceptable, particularly when the diagnosis of ASCUS is not qualiÞed further or the cytopathologist suspects a reactive process. In such situations, Pap tests should be repeated every four to six months for two years until three consecutive smears have been negative. If a second report of ASCUS occurs in the two-year follow-up period, the patient should be considered for colposcopic evaluation.

Patients with Þndings of ASCUS or low grade SIL should have follow-up four to six months after identiÞcation of the initial lesion. Those with higher-grade lesions should receive subsequent Pap smears after treatment at three to four month intervals; if subsequent Pap smears are negative, a reduction in frequency to every six months is appropriate. Colposcopy is warranted after a single Pap demonstrating cytologic atypia and may be warranted after a smear revealing severe inßammation. All women with a diagnosis of SIL should have colposcopy.

Treatment

As with other opportunistic infections in HIV-infected patients there is evidence of improvement in HPV associated lesions with the use of HAART. In one study of 168 women with median follow-up of 17.7 months, women with both high and low grade lesions were found to regress twice as fast if using a PI or NNRTI inclusive regimen (93). Regression to normal was 23.8% for high grade and 14.8% for low grade lesions. HAART was found to be associated with regression when other factors, such as CD4 count and HIV-RNA levels, were controlled for (93). Other studies have presented similar results (94); however, at least one study did not Þnd an associated decrease in progression or increase in regression with the use of HAART (95). Nonetheless, the use of antiretroviral therapy does not modify recommended treatment and surveillance algorithms for HPV mediated disease.

Table 14.4 summarizes treatment and follow-up recommendations. High-grade lesions should always be treated with coninization or loop excision. Cryotherapy has yielded rates of recurrence over 50%, which increases with degree of immunosuppression (96,97). Topical 5-ßououracil (5-FU) has been shown to decrease rates of recurrence following treatment for high-grade lesions (98). Its role as prophylaxis in low-grade lesions remains controversial and is probably not warranted at this time because of irritant effects on vaginal mucosa and the possibility of increased rates of transmission of HIV with administration. Patients with invasive carcinoma are best treated

TABLE 14.4. *Recommended management for abnormal Pap smears*

Pap Smear Result	Management (Based on Histologic Findings)
Severe in ammation	Evaluate for infection; repeat Pap if inadequate
Atypia, atypical squamous cells of undetermined signi cance	Colposcopy, biopsy if indicated; follow with Pap q 6 mo
Low-grade squamous intraepithelial	Colposcopy, biopsy if indicated; follow lesion (CIN 1) with Pap q 6 mo: consider repeat colposcopy annually
High-grade squamous intraepithelial lesion (CIN 2 or 3), carcinoma *in situ*	Colposcopy, biopsy; treat with loop excision or conization
Invasive carcinoma	Colposcopy with biopsy or conization; treat with surgery or radiation (referral to gynecologic oncologist needed)

Source: CDC, 1998.

by gynecologic oncologists; referral should be made appropriately.

SEX-SPECIFIC ISSUES

Menstrual Effects

Chronic diseases that do not directly affect the reproductive tract can result in menstrual dysfunction. Both renal and hepatic diseases have been reported to cause menstrual abnormalities. Early reports suggested that HIV-infected women experienced menstrual abnormalities more frequently than HIV-negative counterparts (99). Subsequent cross-sectional studies using retrospective data were contradictory with one study reporting an association (100) and another reporting no association between the presence of oligomenorrhea, amenorrhea, menorrhagia or dysmenorrhea and HIV serostatus or CD4+ cell counts (101). In a single site study Chirgwin et al. utilized prospectively recorded menstrual calendar data and noted that HIV-infected women were more likely to experience long cycles and amenorrhea (102).

In order to further deÞne the impact of HIV on the menstrual cycle, a prospective study of participants in the HERS and WIHS cohorts was initiated utilizing menstrual diaries. HIV infection was associated with very short cycles (< 18 days) (103), although the odds of this Þnding were of borderline signiÞcance after controlling for multiple factors, including age (OR, 1.45; CI, 1.00Ð2.11). However, women with a CD4 count less than 200 were more likely to have cycles greater than 40 days. Higher viral load was associated with very short cycles, longer mean cycle length and more variable cycles. Thus, these Þndings suggest that menstrual dysfunction is primarily associated with advanced HIV disease. Other determinants, such as age, substance use, and body mass index probably represent more important etiologic factors causing menstrual dysfunction.

Hypermenorrhagia may lead to anemia; if dysmenorrhea is not identiÞed as the source, erroneous decisions may be made regarding the etiology of the anemia with subsequent inappropriate decisions such as changing ART or attributing anemia to disease progression. Although menstrual irregularities may be overlooked as minor, ascertainment of the origin of such changes is warranted. Decreased estrogen levels may predispose women to a number of adverse outcomes and should be regarded similarly as in the HIV-negative woman. Premenopausal, sexually active women should have pregnancy ruled out, and in the case of evidence of anovulatory cycles, the issue of desire for reproductive capability should be addressed. Known disturbances to the hypothalalmic-pituitary axis leading to menstrual changes (particularly oligo- or amenorrhea) should be evaluated. For example, ascertainment of psychological stress or changes with age and addressing those issues may be more appropriate than dismissal of symptoms to advancing HIV disease. Furthermore, female-speciÞc adverse events of certain drugs should be discussed with patients. Megace, a progestin based agent used frequently for wasting, may cause irregular bleeding.

Reports of night sweats in conjunction with irregular or absent menses in the pre- or peri-menopausal woman should prompt the clinician to conÞrm whether the reported symptoms may be an indication of Òhot ßashesÓ rather than assuming night sweats. Estrogen deprivation, especially prematurely, is being investigated as a cause of many adverse outcomes, some of which may mimic or exacerbate HIV-related conditions. The lipodystophy syndrome may promote advanced or early cardiovascular disease, as does a lack of estrogen. Osteoporosis is a concern for all women with decreased estrogen and may be likely as HIV-infected women live longer. It may be secondary to poor nutrition and inability to perform weight-bearing exercise. In prescribing hormone replacement therapy, care must be used in using these agents in combination with HAART, as drug interactions may occur. For example, known interactions exist with some oral contraceptives (OCPs) and ARTs: nevirapine decreases levels of ethinyl estradiol (104), as does ritonavir. Conversely, indinavir may to lead to an increase in the estrogen component of OCPs, although the clinical signiÞcance is unknown (105). These studies suggest that interactions such as those described may alter the levels of endogenous hormones. However, drug therapy has not been speciÞcally linked with the relevant clinical outcomes.

Determinants of HIV Shedding in the Female Genital Tract

As heterosexual transmission is the major worldwide mode of transmission of HIV (106) and most perinatal infection is thought to occur after exposure during delivery (107), determinants of predicting risk of exposure to HIV in the female genital tract are important. As discussed previously, epidemiologic studies have elucidated some determinants of increased risk of transmission, such as diseases leading to inßammation of the genital tract. More recently, investigators have added to these observational data through quantiÞcation of HIV in the female genital tract.

Several studies have demonstrated a positive correlation between HIV-1 RNA plasma levels and those found in cervical, vaginal or lavage specimens (108Ð111). These studies have shown demonstrable viral shedding between 29%Ð62% for vaginal swab or lavage samples and between 32%Ð67% for cervical samples. Some of the variation is attributable to differences in the sensitivity of the assays used. Hart et al. demonstrated that samples taken after initial swabbing have more blood present and

may account for higher viral counts (111). This point may be important as another study (112) demonstrated an increase in viral shedding if blood was present, irrespective of the quantity. This finding may be important in interpretation of data from another study suggesting that transmission of HIV occurs equally in a bi-directional manner between men and women in heterosexual activities (113). Thus, the presence of a minor amount of RBCs, as might be expected to occur during vaginal intercourse, might facilitate the sexual transmission of HIV.

Investigators evaluated Kenyan HIV-infected prostitutes (114) and found that gonococcal and candidal infections were associated with increases in genital HIV detection (OR = 3.1 (1.1–9.8) and 2.6 (1.2–5.4), respectively). Viral shedding was highly correlated with decreased CD4 count and symptomatic HIV disease; however, plasma viral loads were not performed as part of this study. Use of OCPs and depot medroxyprogesterone acetate (DMPA) were also associated with higher rates of viral shedding; this effect was still significant after controlling for CD4 count, as women with a higher CD4 count were more likely to use these contraceptive forms. Aforementioned studies in the United States (112) and elsewhere (109), however, did not document the same effects with the use of hormonal contraceptives. An increase in HIV shedding was also not seen with concomitant STDs when the studies controlled for viral load or CD4 (111).

Examination of the use of antiretrovirals has shown mixed results. In univariate analyses, the use of combination antiretroviral therapy has been associated with decreased viral shedding in some studies (112) but, when controlled for in a multivariate analysis with viral load, use of combination therapy lost its effect. One study evaluated changes in viral shedding when a new antiretroviral regimen was initiated and found that in 10/14 individuals changing therapy, the amount of viral shedding decreased to below the level of detection, which was significant when compared to the group who did not change therapies ($p = 0.006$) (111).

Despite the fact that there is a demonstrable correlation between plasma virus load and genital viral shedding, with presumed infectivity, exceptions do exist. Almost one-third of women with undetectable viremia (lower limit of assay 500 copies/ml) had detectable genital viral RNA (112). This suggests the possibility of a separate micro-environment reservoir for HIV and that eradication of viremia may not correlate with elimination of sexual transmission. Further studies should implement newer assays that allow for quantification of virus at a lower level and more sensitive, standardized assays for genital specimens to facilitate comparisons.

Lipodystrophy Syndrome in HIV-Infected Women

Lipodystrophy first attracted wide attention as a result of case reports describing "buffalo humps" (115) referring to fat accumulation along the upper back, and increased abdominal girth, characterized as "protease paunch" (116) or "Crix belly" (117), referring to increased abdominal girth associated with protease inhibitors (PI) or, for the latter term, indinavir (brand name, *Crixivan*). Associated metabolic abnormalities may include frank diabetes mellitus, hyperinsulinemia, dyslipidemia, (notably hyperlipidemia, with the exception of HDL, which tends to be reduced). Interestingly, although reports of gross redistribution of fat first brought this syndrome to attention, one of the first such published reports was not of truncal or dorsocervical fat pads, but of breast enlargement (118). Hypertrophy of the breasts of women has been described elsewhere and only in women (119–121) suggesting a hormonal influence.

As noted, these changes were first described in association with protease inhibitor use, although associations with nucleoside analogs have been found in some studies (122–125). Additionally, data reported from a retrospective case series prior to the introduction of protease inhibitors suggests the process may be linked to HIV disease and not to antiretroviral drug use, as there were indications of the existence of changes in body composition and metabolic abnormalities (126). Studies investigating the mechanism of these physical and metabolic changes suggest a causative effect of protease inhibitors on the metabolism of retinoic acid, disrupting subsequent steps in the differentiation and function of peripheral adipocytes (127). Hyperlipidemia results, in turn promoting hyperinsulinemia and diabetes in those with a pre-existing propensity; central fat accumulation occurs in some as those fat stores are thought to be more metabolically active and accumulate fat by default.

Initial characterizations of the body composition changes were done by case report, cross-sectional and case control studies. Often, patients were "self-diagnosed" or identification made by clinicians without objective measures, which has led to the large variability in the reported prevalence of these changes (from 2–84%) (128,129). In one prospective study of 494 patients, all of whom were taking a PI throughout the duration of the study period, the syndromes of lipoatrophy, lipodystrophy or mixed were detected by patients and confirmed by study clinicians, although objective measures, such as thigh and/or waist circumference, were not used (130). Seventeen percent of patients were found to experience lipodystrophy. Of the factors examined with regard to risk of developing lipodystrophy, female sex carried one of the largest hazard ratios (1.70, $p = 0.021$ in univariate analysis; 1.87, $p = 0.028$ in multivariate analysis). In a post-marketing report by Merck & Company, Inc., characterizing adverse event reports of lipodystrophy associated with indinavir use, the rate of fat redistribution in women was five times that seen in men (360/100,000 vs. 67/100,000) (131).

One study sought to describe body composition, metabolic parameters and selected sex hormone levels in a group of HIV-infected women in comparison to weight

matched HIV-negative controls (132) in which a large subset of the case patients (59 of 75) were considered to have wasting syndrome (less than 90% of ideal body weight or loss of greater than 10% of body weight prior to infection). Overall body fat percentage and lean body mass was similar between cases and controls; however, cases had signiÞcantly higher truncal fat and trunk to extremity fat ratio with a lower average extremity fat. Interestingly, there was no association with regard to PI use, although a signiÞcant correlation existed between duration of PI use and triglyceride levels. The relatively short duration of PI use (six months) may account for the lack of a statistical Þnding. Average insulin levels were higher in HIV-infected persons, which remained true after age matching with controls and 30% of the HIV-infected women were considered to be hyperinsulinemic. Insulin levels remained signiÞcantly higher in the subgroups analyzed, including those considered to have wasting disease and in those with an abnormal fat distribution (increased trunk-to-extremity fat ratio). Similarly, triglyceride levels in HIV-infected women were higher than those in controls; this effect was also seen in those with wasting as compared with controls.

These authors also examined many of these metabolic and hormone measures in eumenorrheic and amenorrheic case patients (132). Insulin levels were more significantly increased in amenorrheic patients, suggesting a contributing hormonal component, but other measures, including weight, truncal-to-extremity fat mass and testosterone levels were not different between these groups.

A subsequent study by the same authors (133) did demonstrate increased free testosterone in lipodystrophic women with HIV, as compared with either HIV-infected women without lipodystrophy and HIV-negative age and weight matched controls. Additionally, the LH : FSH ratio was increased in a similar manner. Estradiol levels were not signiÞcantly different among groups. Importantly, hormone levels were obtained at a standardized time during the menstrual cycle, in the early follicular phase, in contrast to other studies evaluating these same measures but which did not show differences. The possibility of hormonal contribution to the lipodystrophy syndrome is important to consider as similar metabolic abnormalities are found in women with hormonal disturbances, notably polycystic ovary syndrome (134,135).

This brief review underscores the need for further studies in this area, powered to detect an increased risk of the lipodystrophy syndrome in women. If hormonal mechanisms contribute to this process, this might guide research into interventions. The implications of this syndrome are great: hyperlipidemia, insulin resistance and central fat accumulation are factors known to lead to vascular complications (136,137). Interventions may be possible. In a small study (138) non-diabetic HIV-infected persons with hyperinsulinemia or abnormal glucose tolerance tests showed improvement in several parameters when treated with metformin compared to subjects receiving placebo. Reductions in insulin levels, weight and diastolic blood pressures were seen.

OBSTETRIC ISSUES

A singular milestone in the obstetric care of the HIV-infected woman was the ACTG 076 study reported in 1994, demonstrating a decrease in the rate of transmission of HIV infection from mother to infant from 25.5% in controls to 8.3% in women treated with ZDV during the third trimester and labor, plus administration of ZDV to the newborn (139). Although a challenging regimen, and amidst fears that more widespread use of the regimen would reveal unforeseen fetal outcomes, after the expeditious translation of these results into clinical guidelines, beneÞcial outcomes persisted in various contexts (140Ð142).

When assessing the pregnant woman with HIV infection, it is important to keep in mind that pregnancy itself does not affect progression of HIV disease (143,144). Pregnant women should, in general, receive the same therapy for HIV infection as they would if not pregnant. However, metabolic differences, speciÞc concerns about the teratogenic potential of certain drugs as well as considerations related to the prevention of perinatal transmission, need to be taken into consideration. For example, physiologic changes in pregnancy may affect the pharmacokinetics of antiretroviral drugs. In addition, some antiretrovirals should not be used in pregnancy. Efavirenz, for example, has been reported to cause teratogenic defects in animal studies and should not be used in pregnant women. If a woman becomes pregnant while on this agent, counseling regarding associated risks should be undertaken, and, should the pregnancy be continued, ultrasound performed to rule out anomalies (145). Hydroxyurea may also be teratogenic and should be avoided.

Since the publication of the ACTC 076 study (139), a number of Þndings have helped to deÞne the determinants of perinatal transmission of HIV. For example, while it is known that HIV can be transmitted from mother to child throughout pregnancy, studies suggest that the majority of HIV infected infants are infected during the intrapartum and post-partum periods. Together with data demonstrating a time dependency factor for seroconversion in neonates up until 90 days, it has been suggested that between 40Ð80% of transmission occurs in the peripartum period. This has been supported by a retrospective analysis of short courses of ZDV which indicated that administration of ZDV to the newborn or during the intrapartum period to the mother and subsequently to the neonate conferred rates of transmission lower than if not administered until three days of life (9.3%, 10.0%, and 18.4%, respectively) (140). Nonetheless, it appears that the mother's plasma viral load may be the most powerful predictor of the risk of perinatal transmission. If the viral load can be reduced to an undetectable level, the risk of mother-to-child transmission is extremely low (146,147).

Interpretation of data suggesting a beneÞcial effect on transmission of Cesarean section (C-section) is more complicated. As noted above, perinatal transmission usually occurs during the peripartum period. Several studies have now been published linking an increased period (>4 hours) of rupture of membranes to an increase in transmission rates (148,149). The beneÞts of C-section have now been conÞrmed in several prospective studies and meta-analyses (150Ð153). These studies have demonstrated a reduction in risk of transmission by odds ratios of between 0.2Ð0.45 for C-sections performed electively (prior to the onset of labor and/or rupture of membranes). In women receiving ZDV prophylaxis, this has resulted in transmission rates of 0 to 2%. It should be noted that the beneÞt of C-section has been more consistently seen with elective section. Although HIV-infected women have not been deÞnitively shown to experience perioperative infections at higher rates, the American College of Obstetrics and Gynecology (ACOG) has suggested that prophylactic antibiotics may be useful (154). Because of the multiple timepoints during gestation and delivery that transmission might occur and their relationship to absolute viral load, use of C-section should be considered secondary in importance to maximizing viral load reduction during pregnancy.

Ideally, assessment of maternal HIV serostatus and viral load should be made as early in pregnancy as possible. Current data neither support removing viral suppressive therapy nor denying initial therapy, even early in pregnancy (155), with the exception of efavirenz, which has been found to cause severe anomalies in monkeys with use in early pregnancy. Registries and animal safety data have not led to similar contraindications in any trimester of pregnancy for other antiretroviral agents.

With the utilization of measures adjunctive to the 076 protocol guidelines, such as maximal viral reduction and prudent use of C-section, it is highly likely that perinatal transmission will be dramatically reduced in this country. Although there has been improvement in the identiÞcation of HIV-infected women who are pregnant prior to childbirth since the implementation of ACTG 076 Þndings (70% in 1993 compared to 80% in 1996), progress in the identiÞcation of these women could still be made. The issue may be one primarily related to public health and education since the majority of those not tested prior to delivery have little or no prenatal care (1 prenatal visit) (156). New York State has addressed this issue by mandating that all women have access to knowledge of their HIV serostatus within 48 hours of giving birth. In this way, reduction of perinatal transmission will hopefully continue, even in light of lack of prenatal care and identiÞcation of HIV in the prenatal period.

Other studies of short courses of antiretrovirals are mainly being conducted in the developing world, where drug availability is more limited. Examples of successful regimens include a course of twice daily ZDV beginning at 36 weeks and during labor to nonbreastfeeding women. The rate of perinatal transmission in one such study was reduced by 50% (157). Other agents have also been studied to determine if they could play a role in short-course therapy. One such drug is nevirapine, a nonnucleoside reverse transcriptase inhibitor. In a Ugandan study, the effectiveness of short-course nevirapine (one dose of 200 mg to mother at the onset of labor and one dose of 2 mg/kg to babies within 72 hours of birth) was compared to zidovudine. Resulting transmission rates were 8.1% for nevirapine vs. 10.3% (158) for zidovudine. This study provided hope that an inexpensive and effective alternative to the 076 protocol would be available for the developing countries. Other short-course trials are ongoing. However, the effectiveness of all such regimens in the developing world is reduced because of breastfeeding-mediated transmission.

Breastfeeding

One of the oldest and strongest pieces of evidence for the dangers of breastfeeding comes from a study early in the epidemic in which women who contracted HIV via blood transfusion postpartum, then breastfed, and subsequently transmitted HIV to their infants. The risk of transmission after incident infection was 29% (159). The breast milk of women infected with HIV has been demonstrated to contain HIV (160) and there is clearly a continuing risk. A prospective cohort study in Africa found that in the Þrst six months of life there was a 0.7% per month incidence of HIV transmission (161). As a result of these and other studies, breastfeeding is proscribed in women with HIV infection.

Although this recommendation is a major issue in some developing countries, it should not be an issue in any country with a clean water supply and adequate nutritional supplementation available. Periodic reminders about the risks of breastfeeding, for pediatricians in areas with high rates of HIV as well as for women from areas where formula is not available, may be warranted.

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Chapter 15

Aids Manifestations

Sharon Nachman

In a discussion of human immunodeÞciency virus (HIV) infection in infants, children, and adolescents, one must be cognizant of the fact that information on prevention and transmission, treatment of opportunistic infections, primary therapy, and toxicities from medications is constantly changing and expanding. This chapter will discuss HIV infection in infants, children, and adolescents. Several websites are available including: www.cdc.gov, www.hivatis.org, and www.pedhivaids.org for updating of this knowledge base.

EPIDEMIOLOGY AND TRANSMISSION

The cumulative number of AIDS cases reported by June 2001 to the CDC was 793,026. Adult and adolescent AIDS cases accounted for about 784,000, with the remainder of them (9,000) being children under 13 years of age (1). Of the children with AIDS, 26% were from New York, 16% were from Florida, 9% were from New Jersey and 7% were from California. The estimated total number of HIV-infected children in the U.S. is currently between 10,000 and 20,000. Most of these are between the ages of 7 and 15 (1). With the aging of the perinatally infected population and the increasing number of adolescents infected through adult behaviors, it is anticipated that the number of adolescents with HIV infection will increase over the next decade.

Although fewer than 1% of the current reported cases of AIDS in the U.S. have been among adolescents, the impact of HIV infection in this age group is more serious than this Þgure might suggest. Adolescence is a developmental stage of life normally characterized by experimentation, risk taking, and sexual exploration within the context of feelings of invulnerability, which makes it a uniquely high-risk period for acquisition of HIV. Of all reported AIDS cases in the U.S. through 2000, 18% were among those aged 20Ð29, with almost 4% of the total among those aged 20Ð24. The latency period from acquisition of HIV infection to development of AIDS in adults suggest that a signiÞcant proportion of HIV-infected young adults aged under 29 years will have acquired their infection as teenagers.

Seroprevalence data from the Job Corp in the U.S. indicates that disadvantaged and out-of-school youth are at highest risk for HIV infection (2). The rising rates of other sexually transmitted diseases (STDs) and unplanned pregnancies among adolescents are suggestive of a substantial risk for sexually transmitted HIV infection in this age group. Sexual exposure, including heterosexual, homosexual, bisexual, and sexual abuse, has been a prominent mode of HIV transmission among adolescents with AIDS, with heterosexual transmission the identiÞed risk factor in 33% of adolescent females with AIDS and homosexual transmission in 42% of adolescent males diagnosed with AIDS in 2001. Although IV drug use is relatively uncommon among adolescents, the disinhibiting effects of drug use, particularly alcohol, cocaine, and crack, as well as the cost of dependence result in increased sexual risk taking, including prostitution, and contribute to the further spread of HIV (3).

Approximately 8,000 infants are born to HIV-infected women in the U.S. each year. In 1994 the Pediatric AIDS Clinical Trials Group demonstrated that zidovudine therapy administered to selected HIV-infected pregnant women and their newborn infants reduced the rate of perinatal HIV transmission from 25% to 8% (4). In 1995, the U.S. Public Health Service published the Þrst set of guidelines calling for universal, routine HIV counseling and voluntary HIV testing of pregnant women. The rapid implementation of these guidelines by health care workers (and acceptance by HIV-infected pregnant women) has resulted in a dramatic decrease in perinatal HIV transmission in the past seven years. Prior to the routine use of antiretrovirals in pregnancy, perinatal transmission of HIV occurred in 25% of all deliveries. Since the advent of

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routine use of HAART in these women, transmission now occurs in less than 5% of all pregnancies (5). This interruption of transmission is the single most important development in the care of HIV-infected women and their families. Between 1992 and 1998, perinatally acquired AIDS cases declined 75% in the United States. For further discussion of prevention of perinatal transmission please refer to the current guidelines on the Web @ http://www.hivatis.org.

Postpartum transmission of HIV infection from mother to newborn via breast-feeding has been reported and documented in women who acquired HIV infection after delivery through sexual relations (6) and blood transfusion (7,8). In an Australian cohort of 11 women with documented postpartum acquisition of HIV who were breast-feeding their newborns, three transmitted HIV to their infants, yielding an estimated risk of 27% for breast-feeding during primary maternal HIV infection. These cases may be accounted for by the signiÞcant HIV viremia and presumed increased level of infectivity in the Þrst three to six months after acquisition of HIV infection (9). Whether breastfeeding is a signiÞcant mode of transmission in women who are already HIV-infected during pregnancy and clinically stable was recently elucidated. A metanalysis of studies with varying perinatal transmission rates from different parts of the world suggests that breastfeeding increases the rate of perinatal transmission of HIV by 14% (95% conÞdence interval 7Ð22%) (10). This risk was highest in women who intermittently breastfed their infants. The risk-beneÞt ratio for breast-feeding in HIV-infected women is affected by the infant mortality rate associated with infectious disease or malnutrition in conjunction with the relative risk for bottle-fed infants (11). Where sterile formula is readily available, this ratio clearly favors bottle-feeding, and infected women should bottle-feed rather than breastfeed their infants. For parts of Africa and many other Third World areas, however, the situation is much less clear and favors breastfeeding in areas where the infant mortality attributable to bottle-feeding is greater than one in seven (12).

Iatrogenic acquisition of HIV through transfusions and the use of non-sterile needles are ongoing problems in many countries where blood products are not screened and disposable needles are not used. Reports of hospital-acquired HIV infection in Romania and the former Soviet Union are examples of this problem. The scope of this problem may be even larger than anticipated with many third world countries lacking the resources to obtain sterile needles for routine use in clinics or hospitals. Data on needle stick injuries have not revealed any transmission of HIV to a child from an infected health care provider (13).

More than 200,000 children are sexually abused in the U.S. each year (14), and sexual abuse of infants, children, and adolescents is a documented mode of transmission of HIV infection (15,16). Nonetheless, sexual transmission of HIV is not a reportable category of HIV exposure for children, and many barriers exist to identifying children infected with HIV through sexual abuse (17).

Recent guidelines regarding both needle stick injuries and sexual post exposure prophylaxis are now available on the Web @ http://www.hivatis.org.

DIAGNOSIS

In adolescents, children, and infants older than 18 months, deÞnitive diagnosis of HIV infection is made in the same was as it is in adults, by using the enzyme-linked immunosorbent assay (ELISA) and Western blot assays, which provide serological conÞrmatory evidence of a humoral immune response to HIV by detecting HIV-speciÞc IgG antibodies; however, because maternal IgG antibody is transferred to infants across the placenta, all infants born to HIV-infected women are antibody positive at birth. An IgG antibody response cannot be used to diagnose HIV infection in infants until they are 18 months of age, when maternal antibody is no longer present and the infantÕs own humoral immune response should have been mounted. To determine infection status before age 18 months, several viral detection assays are currently used, including HIV DNA PCR, HIV RNA PCR and HIV viral culture.

HIV DNA PCR is the preferred virologic method for diagnosing HIV infection during infancy. A meta-analysis of published data from 271 HIV-infected children indicated that HIV DNA PCR was sensitive and speciÞc for the diagnosis of HIV infection during the neonatal period. 38% of infected children had positive PCR tests by age 48 hours. The sensitivity of this test increased by the second week to 93% testing positive by 14 days of life (18).

It can be argued that HIV RNA PCR can also be used to identify HIV-infected infants, however, data are limited comparing it to HIV DNA PCR in this age group. Thus a positive HIV RNA PCR is indicative of HIV infection, but a negative test (below the limits of detection = 400 copies /ml) is not indicative of the absence of infection.

The use of these assays for diagnostic purposes has been recommended for all infants born to HIV-infected women (19). A presumptive diagnosis of HIV infection can be made with one positive HIV culture or PCR assay on non-cord blood and a deÞnitive diagnosis made with a conÞrmatory test on a different blood sample. HIV infection can be reasonably excluded with two negative HIV culture or PCR results, one obtained at age 1 month or later, the other at age four months or later (20). Some experienced clinicians are comfortable in excluding HIV infection using these assays at earlier ages. The current standard of care requires a negative HIV ELISA and Western blot at age 18 months to exclude HIV infection deÞnitively.

TABLE 15.1. *Immunologic categories for HIV-infected children based on age-speciÞc CD4 T-lymphocyte counts and percentage of total lymphocytes*

Immunologic Category	Age		
	< 12 Months Cells/mm^3 (%)*	1–5 Years Cells/mm^3 (%)	6–12 Years Cells/mm^3 (%)
No evidence of suppression	> 1,500 (> 25)	> 1,000 (> 25)	> 500 (> 25)
Evidence of moderate suppression	750–1,499 (15–24)	500–999 (15–24)	200–499 (15–24)
Severe suppression	< 750 (< 15)	< 500 (< 15)	< 200 (< 15)

* Percentage of total lymphocytes.

Viral culture is performed on peripheral blood mononuclear cells co-cultured with uninfected mononuclear cells that can support HIV growth and detect latent HIV-infected cells by stimulating viral replication. Evidence of p24 antigen, reverse transcriptase activity, or syncytium formation indicates the presence of HIV in such samples. The sensitivity of this test is age dependent. Results of studies indicate a sensitivity of 24% during the Þrst week of life, 85% at one month, exceeding 90% in infants by two to three months of age, and nearly 100% by six months of age (21,22). In one large cohort study, two negative cultures taken between one and six months of age had a speciÞcity of 99.2Ð100% in deÞning an uninfected infant (22).

HIV culture is more complex and expensive to perform than DNA PCR, and deÞnitive results may not be available for two to four weeks. Although use of standard and immune-complex dissociated p24 antigen tests are highly speciÞc for HIV infection, the sensitivity of these tests is less than the sensitivity of other HIV virologic tests (23). The use of p24 antigen testing alone is not recommended to exclude infection in infants less than one month because of a high frequency of false negative assays during this time.

HIV exposed infants should be tested soon after birth, at age one to two months, and again at three to six months. Some investigators suggest that in addition to testing soon after birth, testing at two weeks should also be included as part of a diagnostic evaluation.

In developing countries, virologic diagnostic assays are unfortunately not universally available to many practitioners, and a presumptive diagnosis frequently requires correlating clinical symptomatology with surrogate laboratory parameters in HIV-exposed infants. In these settings, diagnosis of HIV may include CD4 cell depletion, clinical symptomatology and a positive HIV ELISA and Western Blot.

The 1994 CDC classiÞcation system for HIV infection for infants deÞned an HIV-infected infant using HIV-speciÞc clinical symptomatology in conjunction with laboratory evidence of both cellular and humoral immune dysfunction. The most frequently used laboratory parameter of immune function is the CD4 + lymphocyte count. In infants and children, as in adults, a depressed CD4 + lymphocyte count or reversed CD4/CD8 ratio is indicative of immunocompromise; however, healthy infants and children normally have much higher CD4 cell counts than healthy adults. The median CD4 + lymphocyte count for adults in a sample of uninfected subjects was 1,027, whereas the median CD4 + lymphocyte count for infants aged one to six months in the same study was 3,211 and for those aged seven to twelve months 3,128. Whereas the absolute number of CD4 + lymphocytes is higher in infants and children, the percentage of CD4 + lymphocytes is relatively stable from infancy to adulthood. It is important to be familiar with the normal age-speciÞc lymphocyte counts when evaluating the immune status of infants and children. This classiÞcation system (Table 15.1) includes three immunologic categories of HIV-infected children based on age-adjusted CD4 + cell values (24).

Most infants and children with HIV infection have hypergammaglobulinemia, which is indicative of polyclonal B-cell activation (25,26). Hypergammaglobulinemia has been described as the most common laboratory abnormality in HIV-infected children followed by a reversal of CD4/CD8 ratio (27). Normal immunoglobulin levels in infants and children are also age speciÞc and need to be considered when evaluating a child for hypergammaglobulinemia. Elevated beta-2-microgloublin and neopterin levels have been reported in HIV-infected children. Other laboratory abnormalities commonly seen in pediatric HIV infection (some of them nonspeciÞc) include (1) hypogammaglobulinemia, seen in 3Ð5% of cases; (b) anemia, which is usually secondary to chronic disease and has been associated with disease progression (other causes, such as iron deÞciency, sickle cell, and lead toxicity must be ruled out); (c) thrombocytopenia, seen in about 10Ð20% of HIV-infected children and documented to be associated with antiplatelet antibody in 80% of these (28); and (d) leukopenia. Other clinical manifestations possibly indicative of pediatric HIV infection are discussed below. The laboratory abnormalities seen in adolescent HIV infection are similar to those seen in adults and discussed elsewhere in this volume.

TABLE 15.2. *1994 revised HIV pediatric classiÞcation system clinical categories*

Category N: Not Symptomatic

Children who have no signs or symptoms considered to be the result of HIV infection or who have only one of the conditions listed in Category A

Category A: Mildly Symptomatic

Children with two or more of the following conditions but none of the conditions listed in Categories B and C:

- Lymphadenopathy (> 0.5 cm at more than two sites; bilateral = one site)
- Hepatomegaly
- Splenomegaly
- Dermatitis
- Parotitis
- Recurrent or persistent upper respiratory infection, sinusitis, or otitis media

Category B: Moderately Symptomatic

Children who have symptomatic conditions other than those listed for Categories A or C that are attributed to HIV infection. Examples of conditions in clinical Category B include but are not limited to the following:

- Anemia (< 8 gm/dL), neutropenia (< 1,000 mm^3), or thrombocytopenia (< 100,000 mm^3), persisting > 30 days
- Bacterial meningitis, pneumonia, or sepsis (single episode)
- Candidiasis, oropharyngeal (i.e. thrush) persisting for more than two months in children aged more than six months
- Cardiomyopathy
- Cytomegalovirus infection with onset before age one month
- Diarrhea, recurrent or chronic
- Hepatitis
- Herpes simplex virus (HSV) stomatitis, recurrent (i.e. more than two episodes within one year)
- HSV bronchitis, pneumonitis, or esophagitis with onset before age one month
- Herpes Zoster (i.e. shingles) involving at least two distinct episodes or more than one dermatome
- Leiomyosarcoma
- Lymphoid interstitial pneumonia (LIP) or pulmonary lymphoid hyperplasia complex
- Nephropathy
- Nocardiosis
- Fever lasting more than one month
- Toxoplasmosis with onset before age one month
- Varicella, disseminated (i.e. complicated chickenpox)

Category C: Severely Symptomatic

- Children who have any condition listed in the 1987 surveillance case de nition (51) for acquired immunode ciency syndrome, with the exception of LIP (which is a Category B condition)

CLINICAL MANIFESTATIONS AND PRESENTATIONS

In infants and children, HIV infection is a chronic disease with multiorgan system involvement; indeed, there is probably not an organ system that is not affected by HIV. As in adults, HIV disease presents in infants and children with a broad spectrum of manifestations, some speciÞc to young people. The 1994 CDC classiÞcation system (24) (Table 15.2) of HIV disease in children divides cases into four clinical categories: (a) *N* for no signs or symptoms, (b) *A* for mild signs or symptoms, (c) *B* for moderate signs or symptoms, and (d) *C* for severe signs or symptoms (25). Most of the symptomatic clinical manifestations of pediatric HIV disease are related directly to HIV infection or to the immunosuppression secondary to it. There is a wide range of clinical symptomatology from nonspeciÞc Þndings to severe manifestations of common childhood illnesses, AIDS-deÞning conditions, and end-organ dysfunction.

In general there are two common patterns of presentation of HIV infection in children. One pattern, representing about a third of all perinatally acquired infections, involves early onset of severe disease with rapid progression and, if untreated with antiretrovirals, poor prognosis. Infants in this group usually present with severe opportunistic infections (most often *Pneumocystis carinii* pneumonia (PCP)) or encephalopathy within the Þrst two years of life. Data from the Pediatric Spectrum of Disease Project delineate this group of patients as rapid progressors, i.e. those with early onset of disease manifestations and death before 48 months of age (29). Many of these children become identiÞed as HIV infected because of severe illnesses that arise abruptly. In this group, PCP is seldom insidious; infants may be seen by a physician during one week with some mild general symptoms and have fulminant, life-threatening PCP the next. It is because of the presentation of illness in this group of patients that early PCP prophylaxis is required. These children are rarely seen in the post perinatal prophylaxis era, with most HIV infected women receiving antiretroviral therapy

during pregnancy, labor and delivery and babies receiving ART therapy for six weeks postnatally. When infants are identiÞed as HIV infected during the postnatal period, their virus is often sensitive to most antiretroviral therapies (30). However, most experts agree that it is reasonable to evaluate for presence of resistant virus when designing treatment regimens for these infants (31).

The second presentation pattern of pediatric HIV infection involves later onset of disease symptomatology and is associated with a better prognosis. These children generally present after the Þrst year of life with a more indolent disease course, consisting of a variety of the more general clinical manifestations including failure to thrive, severe varicella (or zoster), or recurrent bacterial infections. Relative to children diagnosed with AIDS in the Þrst year of life, LIP is more common in this group, as are other signs of lymphoproliferation, such as generalized lymphadenopathy and parotitis. It is not unusual for school-aged children to be identiÞed with perinatal HIV infection as a result of this type of presentation; some of these children are not diagnosed with AIDS or HIV infection until they are aged 10 or 11 years. In a cohort of 42 perinatally infected children aged 9Ð16 years followed up at CHAP in NJ, the mean age at diagnosis with HIV infection was 88 months. Although most children in this cohort had HIV-related symptomatology, many with signiÞcant disease, almost a quarter of the children remained asymptomatic with relatively intact immune systems at a mean age of 136 months (32). Children who present later in life may be clinically similar to asymptomatic adults or show only subtle HIV-related signs and symptoms before presenting with more obvious conditions, such as thrush. Recurrent bacterial infections are likely to occur as AIDS-deÞning conditions in both early and late onset presentations of HIV. Renal and cardiac involvement may occur as later manifestations of illness after other signiÞcant HIV-related disease is diagnosed (33).

A number of factors are believed to contribute to the rapidity of clinical progression in some children compared with others. Rapid progressors are suspected to have been infected during the early prenatal period or to have been born to women with more advanced disease. An analysis of 162 HIV-infected infants from the French Prospective Multicenter Cohort revealed that the infantsÕ risk of opportunistic infection (OI) or encephalopathy in the Þrst 18 months of life correlated directly with the degree of maternal HIV-related symptomatology and p24 antigen level, and inversely with maternal CD4+ lymphocyte count at the time of delivery. Fifty percent of infants in this study born to mothers with AIDS developed OIs or encephalopathy by 18 months compared with 14% of infants born to mothers who were either asymptomatic or had generalized lymphadenopathy (34,35,36). Recent data indicate that infants who have positive virologic tests within the Þrst week of life are more likely to progress rapidly to AIDS within the Þrst year (37). A prospective study evaluating RNA PCR levels in HIV-infected infants reported higher levels than in adults, with almost all infants having levels over 100,000 copies/ml (38). Rapidly progressing infants have higher peak plasma HIV RNA copy number, with a peak viral load of 724,000 copies/ml, compared with a peak of 219,000 copies/ml in slower progressors (38,39). Additionally, no infant in that study with a peak HIV RNA copy number of less than 80,000 copies/ml had rapidly progressive disease.

Two major clinical factors affect the prognosis of children with HIV infections: their speciÞc HIV-related diseases and their age at presentation. A study of 172 perinatally infected children treated at a Miami hospital (40), prior to HAART therapy availability, showed median survival rates from diagnosis of one month for those with PCP, Þve months for those with nephropathy, 11 months for those with encephalopathy, 12 months for those with candida esophagitis, 50 months for those with recurrent bacterial infections, and 72 months for those with LIP. Thus, both later presentation and later infection appear to be associated with longer survival.

It is important to note that an AIDS diagnosis in and of itself is not an accurate prognostic indicator for children. The variability in prognosis is a function of the conditions responsible for the AIDS diagnosis. Although OIs, encephalopathy, and recurrent bacterial infections are all AIDS-deÞning conditions, the Þrst two were often associated with a signiÞcantly worse prognosis than the latter. However, this is no longer the case with most children, who, after being identiÞed as HIV infected are treated and despite initial severe depletion can repopulate their CD4 cell populations.

Common signs and symptoms seen in children with HIV infection that are not AIDS deÞning include lymphadenopathy, hepatomegaly, splenomegaly, parotitis, recurrent diarrhea, failure to thrive, and recurrent fevers. It is important to evaluate children for speciÞc infectious etiologies for these conditions, although HIV or the ensuing immunodeÞciency may be the sole cause. Common oropharyngeal signs include persistent thrush, severe painful gingivitis, HIV-speciÞc periodontal disease, recurrent aphthous stomatitis, and recurrent herpetic gingivostomatitis. Some of these conditions are extremely common and, as with adults, lymphoproliferation manifesting as lymphadenopathy may be the Þrst objective sign of disease.

As in other immunosuppressed conditions, children with HIV infection may have severe manifestations of relatively self-limited and usually non-life-threatening conditions common in childhood. There are several childhood illnesses that manifest themselves more seriously in children with HIV infection and include severe recurrent fungal skin and nail infections (tinea, candida), recalcitrant molluscum contagiosum, severe condylomata, recurrent and chronic otitis media and sinusitis, recurrent upper respiratory tract infections, and asthma.

Also included in this group are severe and life-threatening manifestations of varicella and measles. While most HIV-infected children have little problem with varicella, prolonged disease with varicella may be seen in those children with severe immune compromise. In one study of HIV-infected children with varicella, seven of eight children had evidence suggestive of varicella pneumonitis (41). In another study, seven of 17 HIV-infected children with varicella developed chronic, recurrent, or persistent disease (42). Some clinicians suggest treating all HIV-infected children with varicella aggressively with acyclovir as soon as there is evidence of disease. Varicella-exposed severely immune compromised children should be given varicella zoster immune globulin in an attempt to prevent or modify the course of disease. The incidence of zoster in HIV-infected children is close to that of children with leukaemia (42).

Measles can also be life-threatening in the severely immune compromised HIV-infected child (43). Even with appropriate immunization, many HIV-infected children, especially those with low CD4+ lymphocyte counts, do not mount protective antibody responses against most routine childhood vaccinations and continue to be susceptible because of their impaired humoral immune response (44). Any HIV-infected child who is exposed to measles should receive intramuscular immune globulin as prophylaxis. Children already on intravenous gamma globulin (IVIG) therapy may be protected but should receive an additional dose of IVIG if the exposure occurs more than two weeks after their last infusion.

In both adults and children, the OIs related to the immunodeficiency caused by HIV are varied and frequently difficult to treat. In children, AIDS-defining OIs often represent primary infection with the organism rather than the recrudescence that is typically the case in adults, which may be why PCP is a more severe illness in infants than in adults. Adult OIs that are rarely seen in children aged under eight years include toxoplasmosis, cryptococcal disease, and other disseminated fungal infections, such as coccidioidomycosis and histoplasmosis. Their relative scarcity in children probably relates to lack of exposure to the etiologic agents. These infections, however, are seen in adolescents. On the other hand, tuberculosis has become a problem in children born into households in which adults may be infected with both HIV and tuberculosis. HIV-infected children, like adults, are more susceptible to the rapid progression of *Mycobacterium tuberculosis* from infection to disease (tuberculosis) (45). Whereas space limitations preclude a comprehensive review of OIs in children, OI prevention and treatment guidelines are available on the web.

In the pre-HAART era, the most common OI in infants and children was PCP 20. Before widespread use of PCP prophylaxis beginning in 1991–1992, PCP accounted for a higher percentage of reported AIDS indicator diseases and up to 65% of OIs in the pediatric population (40,46,47). Currently, the most common infections seen in HIV-infected infants and children are those of bacterial origin, specifically pneumococcal.

RECURRENT BACTERIAL INFECTIONS

HIV infection is associated with significant abnormalities in B-cell-mediated immune responses. In infants, laboratory-documented B-cell dysfunction usually precedes T-cell abnormalities (48,49), possibly the result of an interference with normal B-cell maturation, as humoral immune responses are incomplete at birth. The normal maturation of B cells, including the ability to produce antigen-specific antibodies, requires lymphokines produced by functioning CD4+ lymphocytes. Most adults with HIV infection were exposed to the common bacterial pathogens before becoming HIV infected; therefore, they tend to have circulating protective antibodies against them and circulating B cells with a retained anamnestic response to these pathogens. Thus, adults tend to get serious bacterial infections with common pathogens only late in the course of disease, when they are severely immunocompromised. With improved survival in a more immunocompromised state, however, the problem of severe bacterial infections in adults is on the rise.

In contrast, HIV-infected children have defective primary and secondary antibody production to T-cell-dependent and independent antigens (48,50); when children are exposed to common bacterial pathogens for the first time, these abnormalities result in severe manifestations of infection early in the course of their HIV infection. Since 1987, recurrent serious bacterial infections have been part of the CDC case definition of AIDS for children (51). The most common infections that meet the case definition are bacteremia and pneumonia. The most common organisms include *Streptococcus pneumoniae, Haemophilus influenzae, Salmonella* spp., and *Staphylococcus aureus* (52,53).

CENTRAL NERVOUS SYSTEM

CNS involvement is a more common manifestation of HIV infection in infants and children than in adults, and although its true incidence is unknown, it is believed to occur in most of those infected. HIV is believed to enter the CNS through HIV-infected macrophages, which can cross the blood-brain barrier. In infants, entry of HIV into the CNS may be facilitated by infection with HIV in utero before establishment of this barrier (53a). It is unclear exactly how HIV causes neurological dysfunction; direct HIV effects and indirect effects through cells of the macrophage lineage and the elaboration of toxic cytokines have been postulated.

There is a broad clinical spectrum of neurological abnormalities seen in pediatric HIV infection (54–58). There may be relatively normal development suddenly

followed by either loss of milestones or failure to attain new milestones. The onset of developmental delay may be followed by periods of relative stability in neurological function or rapid neurodevelopmental deterioration. Pyramidal tract involvement may be seen, with resulting spastic paresis. Hypertonicity and hyperreßexia are common manifestations of motor involvement.

Prior to the widespread use of antiretrovirals, static encephalopathy was seen in about one quarter of children with HIV infection. It is characterized by developmental delay of varying severity without loss of previously attained milestones. Children in this group can have improvement in neurological function with continued acquisition of developmental skills, but usually in a delayed fashion. Progressive encephalopathy, characterized by progressive deterioration in cognitive, motor, or language skills and loss of previously attained developmental milestones, often is seen in patients with severe immune compromise (59). Progressive encephalopathy, which is associated with a poor prognosis, can be characterized by a plateau course without continued loss of milestones, or a rapidly progressive course. Neuroimaging studies of HIV infection in children include Þndings of ventricular enlargement, cerebral atrophy, white matter attenuation, and cerebral and basal ganglia calciÞcation (60). The possibility of CNS lymphoma (61) always must be considered in the child who develops new neurological signs and symptoms.

Antiretroviral therapy improves the neurodevelopmental functioning of infants and children with HIV infection (62Ð64). Children can regain lost motor and developmental milestones with therapy. In some children, this is dramatic, with the reversal of incontinence, gait abnormalities, or lost cognitive milestones after initiation therapy (65,66).

GROWTH

Assessment of growth is an integral part of the care of any pediatric patient. This is especially true in the care of HIV-infected children. Growth delay is common in HIV-infected children and thought to be due to disease progression (67Ð69). The basal metabolism of children with HIV infection is increased when compared to uninfected children, and when stressed, caloric needs increase, on average 12% for each degree Celsius increase in temperature, 25% for acute diarrhea and 60% for sepsis (70Ð72). Growth failure is often the Þrst sign of HIV associated symptoms. However, recent data seem to suggest that antiretrovirals may also play a part in growth delay, as even a cohort of children with undetectable virus in plasma continued to have growth failure (73).

HEMATOLOGIC

Anemia in patients with HIV infection is not uncommon and may be due to many causes. The most common causes include nutritional deÞciencies such as iron, folic acid and B_{12}, and immune hemolysis, hemorrhage, drug toxicities and Þnally bone marrow suppression secondary to HIV infection itself. Other causes of anemia such as parvovirus infection, *Mycobacterium avium* complex infection, CMV and malignancy may also need to be investigated.

Evaluation for anemia typically includes a complete blood and reticulocyte count, iron level, total iron binding capacity and transferrin level. It is recommended to evaluate the erythropoietin level as well. If the erythropoietin level is less than 500 IU/L a trial of erythropoietin may be warranted.

Neutropenia, like anemia, is a common Þnding in HIV-infected children. It is deÞned as an absolute neutrophil count of less than 1500 cells/mL. It can be seen secondary to infection, nutritional deÞciencies or drug toxicity. In some cases neutropenia may respond to initiation or a change in antiretroviral therapy. It is recommended that granulocyte colony stimulating factor be used to treat neutropenia instead of dose modiÞcation of antiretrovirals, because of the narrow therapeutic range of these drugs (74).

Thrombocytopenia may also be seen in HIV-infected children, and this used to be a common presenting sign of HIV infection (75). Intervention is usually not required, especially if the platelet count exceeds 50,000. In some cases the thrombocytopenia responds to initiation of HAART therapy. For those children whose platelet counts do not respond to HAART therapy, RHo (D) immune globulin is suggested. Other treatment options may include IVIG and steroids (see Chapter ??).

PULMONARY

Pulmonary complications from HIV range from chronic lymphocytic inÞltrative disease of the lung (LIP) to bacterial pneumonias to opportunistic infections such as PCP and MAC. The diagnosis of LIP is usually based on a typical chest radiograph with persistent reticulonodular bilateral inÞltrates. Treatment includes HAART therapy and prednisone if hypoxia is present. When evaluating bacterial pneumonias, blood and sputum cultures should be sent. Therapy is directed at the speciÞc pathogens isolated. Unfortunately, these cultures yield an organism in $<30\%$ of cases and therapy must be directed at the usual pathogens involved in community acquired pneumonias. Special care must be taken to consider expanding therapy to cover resistant pneumococci when warranted. Children with HIV infection who have bronchiectasis or frequent episodes of bacterial pneumonia may beneÞt from daily prophylaxis with TMP/SMX. Patients with severe immune depletion may present with PCP or MAC, and bronchoscopy or bronchoalveolar lavage may be needed in order to make the correct diagnosis. Updated guidelines for the prophylaxis and treatment of opportunistic infections can be viewed @ http://www.hivatis.org

RENAL

HIV associated nephropathy in children presents as a spectrum of disease that ranges from mild to moderate proteinuria that is persistent, hematuria, renal tubular acidosis and end-stage renal disease. Because the progression of disease in children is not as rapid as in adults, the first approach is to observe these patients over a period of time, monitoring electrolytes, blood urea nitrogen and creatinine. Drug toxicity often contributes to hematuria and certain antiretroviral and other drugs should be avoided in these patients. When end stage renal disease has developed these patients must be managed in conjunction with the pediatric nephrology team. In the future, decisions regarding renal transplants must be considered, especially in patients with successful long-term suppression of viral load and a normal CD4 cell repertoire.

CARDIAC

Cardiac involvement in HIV infection has been known since 1983. The spectrum of cardiac disease ranges from silent lesions to dilated cardiomyopathy to myocarditis (76,77). Depressed left ventricular function is common in HIV-infected children, and has lead to some experts recommending yearly cardiac echocardiograms in these children. Other factors that could be involved in the pathogenesis of cardiomyopathy include infections such as Coxsackie B, CMV, Epstein-Barr virus, toxoplasma, pulmonary diseases, wasting, nutritional deficiencies (selenium and carnitine), antiretrovirals (and other pharmacological agents such as pentamidine, amphotericin B, or foscarnet), and illicit drug use.

Mild manifestations of congestive heart failure (CHF) are treated with angiotensin-converting enzyme inhibitors. The two most commonly used agents are enalapril and captopril. Renal function, electrolytes and blood pressure must be closely monitored. Antihypertensive and antiarrhythmic agents are prescribed when appropriate in conjunction with a pediatric cardiologist.

ROUTINE EVALUATION

It is suggested that all HIV-infected infants and children be managed by an expert in Pediatric HIV infection. Ideally the pediatric care team should consist of physicians and other experts in pediatrics, social workers, a nutritionist as well as a psychologist. This team should be able to consult with other pediatric subspecialists, as children with HIV often will have multi-organ involvement from their HIV infection.

The following tests and services should be performed for all HIV infected children:

¥ Chest x-ray (78)
  This test identifies mediastinal enlargement, lung lesions and cardiomegaly. Patients with chronic lung disease should have pulse oximetry measured routinely as well.
¥ Baseline brain CT (79)
  This scan may show calcification as well as brain atrophy. It may be more helpful in the perinatally infected adolescent who comes in with new CNS signs and symptoms.
¥ A regular strength tuberculin skin test (78)
¥ Visual screening (78)
¥ Children with immune category 3 may need to be examined every six months, especially if they are seropositive for CMV or toxoplasmosis.
¥ Psychometric testing (80,81)
¥ GYN exam (78)
  Female adolescents should be referred for GYN care. Baseline and annual GYN visits should be provided for all adolescents who are sexually active to evaluate for STDs and for performing cervical PAP smears.
  All adolescents should be aware of their diagnosis and receive counseling regarding transmission of HIV, safe sex practices, birth control and the risk of perinatal transmission during pregnancy.
¥ Baseline antibody titers should be considered to evaluate toxoplasma, CMV, Epstein-Barr virus, varicella-zoster virus and hepatitis viruses (78).
¥ In addition, annual visits to a dentist are also strongly suggested (82-84).

Routine evaluations should occur at three to four month intervals and should include a complete physical exam (including height, weight and vital sign monitoring), and routine labs such as CBC, liver function profile, and HIV RNA viral load and CD4 cell count. Other laboratory tests may include cholesterol, triglyceride and glucose monitoring, as well as urinalysis and electrolyte monitoring. Other non-routine evaluations may include EKG and echocardiograms (78).

PCP Prophylaxis

In children with HIV infection, PCP is associated with a high rate of morbidity and mortality. It often presents acutely with a peak incidence in the first three to six months of life. In March 1991 recommendations for PCP prophylaxis for infants and children were based on age-dependent CD4+ lymphocyte counts (84a), which differ from values in adults. The guidelines called for initiation of PCP prophylaxis for HIV-exposed children aged 1 to 11 months with CD4+ counts below 1,500 cells/ul, for children 12 to 23 months of age with CD4+ lymphocyte counts below 750, for those aged 24 months to five years with CD4+ lymphocyte counts below 500, and for children aged six years or older with CD4+ lymphocyte counts below 200.

In 1994, these guidelines were revised to recommend that all HIV-infected infants be placed on prophylaxis regardless of CD4+ lymphocyte counts, beginning at one month of age (19). Because it was difficult to determine with certainty which infants born to HIV-infected women were themselves infected, and especially those who are uninfected, these new guidelines recommended initiating prophylaxis in all infants born to HIV-infected women at one month of age and continuing prophylaxis until HIV infection can be reasonably excluded. After the first year of life, the use of prophylaxis is recommended in HIV-infected children based on CD4+ lymphocyte values.

A detailed discussion of prophylaxis and treatment of other OIs in infants and children is beyond the scope of this chapter and is covered elsewhere in this volume as they relate to the adult population.

TREATMENT OF HIV

HIV-Specific Treatment

At least 11 of the antiretroviral agents approved by the U.S. Food and Drug administration for treatment of HIV infections are approved for use in children. Two classes of drugs target the reverse transcriptase enzyme, while a third class targets the viral protease enzyme. Unfortunately, more information is still needed regarding optimal dosing in children due to the wide variability of drug absorption and metabolism among children and drug interactions that affect pharmacokinetic parameters.

When to Initiate Therapy

There is much discussion in the literature regarding the best time to start antiretroviral therapy in children. Unfortunately, there is no one correct answer. Most experts agree that treatment should be initiated in children with severe immune suppression or those with clinical disease progression, but for those children with good immune function and no clinical symptoms, no such clear consensus is available.

Antiretroviral therapy has provided substantial clinical benefit to HIV-infected children. Initial clinical trials with monotherapy demonstrated clinical improvement in growth, neurodevelopmental, immunologic and virologic parameters. Subsequent clinical trials have demonstrated combination therapy to be superior to monotherapy. In a longitudinal study which evaluated children in the pre-HAART and post HAART era, HAART therapy has been demonstrated to enhance survival. Mortality was only 1% in 1997/1998 compared to 5% in 1995/1996 (47).

Data from clinical trials of antiretroviral therapy with one and two drug combinations (in antiretroviral children) (84b) and three and four drug combinations (in nucleoside alone exposed) children (84c) showed that initiation of therapy may be able to produce long-term suppression of viral replication and preservation of immune function in some children. However, the potential problems with early therapy include the risk of short-term and long-term adverse effects, and concerns about viral mutation, especially in populations who will be on lifelong therapy.

Over the past several years, members of the Working Group on Antiretroviral Therapy and Medical Management of HIV Infected Children have developed guidelines for initiation and treatment of HIV-infected children. These guidelines are updated frequently, and the reader is advised to look on the web for the most current guidelines at www.hivatis.org. These guidelines take into account clinical and immunologic status as well as virologic results. Available guidelines for adults are applicable only to newly infected adolescents and most children identified after three years of age. However, due to generally higher viral load measurements in neonates and infants, most experts suggest that babies identified as HIV positive in the first year of life be placed on HAART therapy despite normal CD4 markers.

Aggressive antiretroviral therapy with at least three drugs is recommended for initial treatment of infected children because it provides the best opportunity to preserve immune function and delay disease progression. The goal of therapy is to suppress viral replication maximally while preserving immune function and minimizing drug toxicity.

Biologic variation in plasma HIV RNA within a person is well documented and repeated measurements of HIV RNA levels in a clinically stable adult can vary by as much as 0.5 log 10. This biologic variation may be greater in infected infants and young children. In children with perinatal infection, RNA copy number rapidly declines during the first 12–24 months after birth (0.6 log 10 per year), then continues to decline slowly over the next three to four years (0.3 log 10 per year), but persists at higher levels than most infected adults. Thus, only changes greater than 0.7 log 10 in infants aged less than two years and greater than 0.5 log 10 in children over two years of age are biologically real (Table 15.3). However, no change in therapy should be made as a result of HIV copy number unless confirmed by a repeat test.

A few children and adults with HIV infection do well clinically without therapeutic intervention. They are referred to as long-term nonprogressors. It is estimated that they make up 5% of patients with HIV infection. There are many different theories as to the genetic, immunologic and virologic mechanism of host resistance to disease progression, but unfortunately, clinicians are unable to predict accurately which patient will be part of this cohort of patients. There is no plasma RNA threshold below which an individual is likely to experience long-term nonprogression. Most experts therefore agree that early aggressive therapy in children and adults allows for

TABLE 15.3. *Viral load reduction conversions**

Viral Load Reduction From Baseline (Logs)	Viral Load Reduction From Baseline (%)	Viral Load Reduction From Baseline (-Fold)	Remaining Viral Load Number
0.3 log	50.0%	2-fold	50,000 c/mL
0.5 log	75.0%	3-fold	25,000 c/mL
0.7 log	80.0%	5-fold	20,000 c/mL
1.0 log	90.0%	10-fold	10,000 c/mL
1.5 log	96.8%	32-fold	3,200 c/mL
2.0 log	99.0%	100-fold	1,000 c/mL
2.5 log	99.7%	316-fold	300 c/mL
3.0 log	99.9%	1,000-fold	100 c/mL

* In a hypothetical patient with a starting viral load of 100,000 copies/mL.

the best opportunity for long-term nonprogression, preservation of the immune system and minimization of the risk for antiretroviral resistance.

Issues relating to treatment adherence are important in considering when to initiate therapy and what that therapy should be. Lack of adherence to therapy may enhance the development of drug resistance. Participation by the family and child, when appropriate, in the decision making process, is especially important in situations for which deÞnitive data concerning new treatments are not available.

Despite having been treated with agents from all three classes of FDA-approved antiretroviral agents, a growing number of HIV-infected children are not able to sustain viral replication below the level of quantiÞcation. Although incomplete adherence may be a factor in some of these cases, other factors include the previous use of these agents in the child (i.e. sequential mono or dual therapies or addition of new agents without changing the backbone of the regimen), inadequate dosing due to poorly described pharmacokinetic parameters, and toxicity management. In these cases, the pros and cons of continuing a treatment regimen versus switching to an alternative treatment regimen need to be closely considered and discussed with the child and family.

There is no consensus as to the best approach to treat these patients. Choice of the new regimen should be guided by the childÕs antiretroviral drug history and results from resistance assays. If the child has been exposed to all three available groups of antiretroviral agents, it is unlikely that a simple three- or even a four-drug regimen will sustain suppression of virus to undetectable levels. In this setting, viremia alone, in the face of stable clinical and immunologic status, does not require a medication change in a heavily pre-treated child.

Reasonable approaches to these patients include:

¥ Continuing a regimen that allows viral replication at a level that will not cause additional immunologic or clinical deterioration while waiting for newer therapeutic approaches and agents to be developed.

¥ Choosing an aggressive regimen of four to six agents in an attempt to suppress viral replication to undetectable levels with an acceptable level of toxicity. This regimen may contain two nucleoside reverse transcriptase inhibitors, a non-nucleoside reverse transcriptase inhibitor and two to three protease inhibitors.

¥ Opting for a complete drug holiday (months) with the expectation that wild-type virus will once again predominate in the patient. After this interim period, an aggressive regimen can then be instituted. There are limited clinical data on this approach in adults and children, although it is expected that resistant virus is archived in the genome of the patientÕs cells and will reappear in due time.

Which agent(s) should be used as second- and third-line therapy depends on which agents(s) the patient was started on initially, the resistance proÞle of the circulating virus and the availability of other agents. A physician experienced in pediatric HIV disease should be consulted when these decisions are made.

Intravenous Immune Globulin Therapy

Intravenous immune globulin (IVIG) is often standard therapy for children and adults with primary humoral immunodeÞciency disorders such as BrutonÕs agammaglobulinemia. Passive immunotherapy in the form of IVIG provides protection against a wide range of bacterial and viral pathogens. As discussed above, children with HIV infection often have functional abnormalities of their B-cell-mediated immune system that place them at risk for infections that require an intact humoral response system. Although most children with HIV infection have elevated immunoglobulin levels, this is believed to represent nonspeciÞc polyclonal B-cell activation. Many children fail to mount an antibody response to routine childhood immunizations (50), indicating a functional immunodeÞciency.

The National Institute of Child Health and Human Development (NICHD) completed a placebo-controlled

trial using IVIG in HIV-infected children. This trial was completed prior to the availability of HAART therapy. The trial found that IVIG prolonged the time free from serious laboratory-proven bacterial or clinically diagnosed serious or minor bacterial and viral infections in symptomatic HIV-infected children with CD4 counts greater than 200 (85,86). Unfortunately, this trial did not separately evaluate the efficacy in children with documented B-cell defects but looked only at groups based on CD4 counts.

Based on clinical experience in treating children with humoral immunodeficiency and HIV with IVIG and the results of the above reports, a group of pediatric HIV specialists agreed that IVIG should be considered for use in HIV-infected children with the following conditions: (a) evidence of humoral immunodeficiency as defined by severe recurrent bacterial infections, hypogammaglobulinemia, poor functional antibody responses to documented infections or a lack of response to immunizations; (b) thrombocytopenia; or (c) chronic bronchiectasis (87).

Childhood Immunizations

Immunizations represent the cornerstone of preventive medicine for children. The recommended childhood immunization schedule of the Advisory Committee on Immunization Practices and the American Academy of Pediatrics should be used in HIV-infected children with the following alterations. Paralytic poliomyelitis is a potential complication of the oral polio vaccine (OPV) for both the immunocompromised patient and the immunocompromised family members through virus excreted in stool. Use of inactivated polio vaccine (IPV) instead of OPV is recommended for HIV-infected infants and children as well as uninfected infants and children living in households with infected adults. In the early years of the HIV epidemic, infected children in the U.S. received both primary and booster doses of oral polio vaccine without complications, and in developing countries OPV is still considered standard of care for all children.

The measles mumps rubella vaccine is a live attenuated viral vaccine given between 12–15 months of age, with a booster at four to six years of age. In areas where there is a high prevalence of measles it may be given as young as six months of age. There has been one reported case of measles pneumonitis following measles, mumps, and rubella vaccine (MMR) in a severely immunocompromised 20-year-old HIV-infected man. Although the use of MMR is recommended for most HIV-infected children, the CDC currently recommends considering withholding MMR in severely immunocompromised HIV-infected children (88).

The Advisory Committee on Immunization Practices (ACIP) recommends that the varicella vaccine be considered for asymptomatic or mildly symptomatic HIV-infected children, with an age specific CD4 T lymphocyte percentage greater than 25% (89,90). The vaccine should be administered in two doses with a three-month interval between the doses. It is strongly recommended for HIV negative siblings and household members of HIV-infected individuals. Susceptible children with HIV infection exposed to varicella should be considered for passive immunization with varicella-zoster immune globulin. If a child has received IVIG within three weeks before the exposure, then VZIG is not necessary.

A yearly inßuenza vaccine is recommended for infants and children with HIV infection who are over six months of age. Children less than nine years of age who have never received the vaccine should be given two doses, one month apart. For children previously immunized, one dose per year is sufficient.

The newly licensed pneumococcal conjugate vaccine should be given to all HIV-infected infants and young children as per ACIP guidelines. A clinical trial is underway evaluating this vaccine in HIV-infected children over the age of two years. However, current recommendations are to give the pneumococcal polysaccharide vaccine every 5 years in HIV-infected children.

Other vaccines such as hepatitis B, pertussis, tetanus and *H. inßuenzae* type B are the same as for noninfected children. Bacillus Calmette-Guerin (BCG) is a live attenuated vaccine and is contraindicated for children with HIV living in the U.S.. The World Health Organization has continued to recommend this vaccine to be given at birth to children living in countries with a high prevalence of tuberculosis.

NUTRITIONAL ISSUES IN CHILDREN WITH HIV INFECTION

Pediatric HIV infection frequently results in nutritional deficiencies and growth failure. Weight loss and failure to gain weight can occur as early as the first four months of life as well as in children who become symptomatic at an older age. Failure to thrive and malnutrition are due to a number of factors, including (a) decreased intake resulting from oral and gastrointestinal pathology that cause nausea, anorexia, pain, and deceased taste as well as neurological complications that result in ineffective swallowing mechanisms; (b) impaired absorption resulting from HIV-related enteropathy and gastrointestinal infections; (c) increased metabolic requirements secondary to the chronic HIV-related inßammatory illness; and (d) decreased intake resulting from side effects and toxicities (such as nausea, anorexia, vomiting, hepatitis, and pancreatitis) of various medications used to treat HIV infection and its complications. Particularly in children in end-stage disease, it is important for the clinician to consider these adverse reactions within the risk-to-benefit ratio of therapy with a specific agent. These physical complications of HIV infection further contribute to the marginal nutritional balance frequently seen in children living in communities affected by poverty. Besides protein and caloric deficiencies, a number of trace element and vitamin

deÞciencies may complicate the clinical course of HIV infection (91). SpeciÞc nutritional deÞciencies of selenium, iron, zinc, vitamin B6, vitamin A, and vitamin E may result in neurological or cardiac abnormalities and contribute to the rashes and cytopenias commonly seen in HIV infection. In young infants, when CNS growth is still occurring, nutritional deÞciencies may have profound long-term effects.

Based on these factors, there should be an aggressive approach to nutritional support for HIV-infected infants and children that includes (a) proactive nutritional assessment including anthropomorphic measurements and dietary intake history with each physician visit and periodic selected laboratory parameter monitoring; (b) diagnosis and treatment of oral-gastrointestinal disease, with special attention to pain management; and (c) aggressive replacement and nutritional supplementation beginning with oral supplementation and progressing as necessary to nasogastric/gastrostomy tube feeding and in advanced disease with malabsorption to total parenteral hyperalimentation. Nutritional care of HIV-infected children needs to receive the attention of the research community as well as those providing direct care. Recently physicians have started evaluating the use of gastrostomy tubes for administration of both medication and food supplementation (92).

DRUG TOXICITIES

One of the emerging new syndromes arising from HIV and its treatment is that of lipodystrophy (93). This syndrome, initially thought to be exclusively associated with protease inhibitor therapy, can now be seen with all classes of antiretrovirals. Common signs include extremity fat wasting and excessive deposition of fat on back and trunk. Common metabolic abnormalities include insulin resistance, elevated cholesterol, triglycerides, and abnormal bone metabolism (94).

Nucleoside Analogs

Nucleoside analogue drugs are known to induce mitochondrial dysfunction, as these drugs have varying afÞnity for mitochondrial gamma DNA polymerase. This afÞnity can result in interference with mitochondrial replication, resulting in mitochondrial DNA depletion and dysfunction (95). The relative potency of the nucleosides in inhibiting mitochondrial gamma DNA polymerase *in vitro* is highest for zalcitabine (ddC), followed by didanosine (ddI), stavudine (d4T), lamivudine (3TC), zidovudine (ZDV) and abacavir (ABC) (96). Toxicity related to mitochondrial dysfunction has been reported in infected patients receiving long-term treatment with nucleoside analogues, and generally has resolved with discontinuation of the drug or drugs; a possible genetic susceptibility to these toxicities has been suggested (95). These toxicities may be of particular concern for pregnant women and infants with *in utero* exposure to nucleoside analogue drugs.

Clinical disorders linked to mitochondrial toxicity include neuropathy, myopathy, cardiomyopathy, pancreatitis, hepatic steatosis, and lactic acidosis. Among these disorders, symptomatic lactic acidosis and hepatic steatosis may have a female preponderance (97). These syndromes have similarities to the rare but life-threatening syndromes of acute fatty liver of pregnancy, and hemolysis, elevated liver enzymes and low platelets (the HELLP syndrome) that occur during the third trimester of pregnancy. A number of investigators have correlated these pregnancy-related disorders with a recessively-inherited mitochondrial abnormality in the fetus/infant that results in an inability to oxidize fatty acids (98,99). Since the mother would be a heterozygotic carrier of the abnormal gene, there may be an increased risk of liver toxicity due to an inability to properly oxidize both maternal and accumulating fetal fatty acids (100). Lactic acidosis with microvacuolar hepatic steatosis is a toxicity related to nucleoside analogue drugs that is thought to be related to mitochondrial toxicity; it has been reported in infected individuals treated with nucleoside analogue drugs for long periods of time (more than six months). Initially, most cases were associated with ZDV, but subsequently other nucleoside analogue drugs have been associated with the syndrome, particularly d4T. In a report from the FDA Spontaneous Adverse Event Program of 106 individuals with this syndrome (60 patients receiving combination and 46 receiving single nucleoside analogue therapy), typical initial symptoms included one to six weeks of nausea, vomiting, abdominal pain, dyspnea, and weakness (97). Metabolic acidosis with elevated serum lactate and elevated hepatic enzymes was common. Patients in this report were predominantly female gender and high body weight. The incidence of this syndrome may be increasing, possibly due to increased use of combination nucleoside analogue therapy or increased recognition of the syndrome.

PAIN MANAGEMENT

Pain management is an especially important but frequently undertreated clinical problem in HIV-infected infants. The prevalence of pain in adults with AIDS ranges from 40Ð60%, depending on the stage of illness (101). Physicians in general are poorly trained in the control of pain by medication, and many do not appreciate that newborns and infants experience pain. Whereas many fear they will make their patients drug addicts, only 0.4% of patients given narcotics in hospitals ever have a problem with opiate dependence.

A much more aggressive approach must be taken to controlling pain in children. SpeciÞc barriers to management of pain in HIV-infected children include: (a) the

difficulty of assessing pain in young children; (b) the difficulty of assessing pain in children with neurological impairment; (c) parental denial of their children's disease; (d) resistance to the use of narcotics by families who have a history of drug use; and (e) resistance by clinicians to treat pain in children because of myths like the following: Children lie about pain to get attention; if children deny pain or do not complain, they are not in pain; and children who can fall asleep or who can play cannot be in pain. Vigorous and proactive use (preferably before the onset of anticipated pain) of appropriate pain medications using weight-adjust dosages, including aspirin, acetaminophen, codeine, ibuprofen, morphine, and methadone, is essential to the overall quality of life for HIV-infected children. The use of EMLA cream as a local anesthetic for blood drawing can make the difference between an agonizing or enjoyable visit to the doctor for a child with chronic illness.

Nonpharmacological approaches to pain management (including relaxation, hypnosis, play therapy, visualization, and distraction) also should be applied in pain control, especially when the pain is related to procedures.

ADHERENCE

Rates of adherence (102) to antiretroviral therapy have been correlated with virologic success. There is considerable interest in clinical strategies to promote improved adherence (Table 15.4). Interventions have included scripted telephone reminder calls, once daily versus twice daily therapies, directly observed therapy (DOT), and reminders via alarm watches. Unfortunately, none has been remarkably successful. Often individuals with worst adherence discontinue therapy sooner than more adherent patients, resulting in less resistance among poorly compliant patients and increased resistance in partially compliant patients (103). Adherence in young children depends on the child's care provider administering the medications. This can be especially difficult if the medications are bad tasting or smelling, must be given constantly during the day, have large volumes, or cannot be taken with food. This may be compounded by the care providers needs if they are also HIV-infected (104).

TABLE 15.4. *Factors affecting adherence*

- Availability of liquid product vs. pills
- Quantity (volume) of therapy
- Frequency (qd, bid, tid, qid)
- Ability to swallow pills/soft gel caps
- Taken with or without food, acidity
- Storage (e.g. refrigeration)
- Disclosure
- Psychosocial factors
  - Parental health
  - Parental treatment
  - Parental perception of antiretroviral therapy
  - Child's development stage
  - Belief that medications are bene cial
  - Reliability of a parent/guardian
  - Foster care/consent
  - School/day care
  - Reputation of therapy in community
  - Medical provider accessibility

SOCIAL ISSUES IN THE DELIVERY OF CARE

The conditions of poverty, including inadequate housing, may interfere with the delivery of optimal health care. Typically, mothers are the strongest advocates for their children, but this advocacy may be hindered by the fact that mothers of HIV-infected children are often single parents and poor (105). In some cases, symptomatic HIV infection or drug use may interfere with a mother's ability to care properly for her child; more often, however, mothers are assertive in seeking care for their children while neglecting their own needs (106). The general shortage of openings in drug treatment programs is especially severe for women who are HIV infected, pregnant, or have children. All these socioeconomic conditions must be addressed in designing effective health care systems for families with HIV infection.

Families also can benefit from psychosocial support in dealing with many aspects of an HIV diagnosis in a child. The diagnosis may be the first evidence that a parent is infected and may give rise to guilt or anger, leading to further disruption of the family unit. Apparently resolved emotional issues may require periodic reexamination, as, for example, when parents are confronted repeatedly by the differences between a child who is developmentally delayed and healthy peers. Decisions about the disclosure of an HIV diagnosis may arise on multiple occasions as different audiences are encountered such as family, friends, siblings of the infected child, the child himself or herself, day-care workers, school nurses, and teachers (107). Many parents choose to disclose the diagnosis on a need-to-know basis; however, children and their siblings often find it less stressful to know the diagnosis than to be left in the dark about something unnamed but apparent. Counseling may help parents decide whether and how to disclose the diagnosis, which should be done in a developmentally appropriate way. Clinical experience suggests that under the proper circumstances it is beneficial for children with normal cognitive development to have the opportunity to discuss aspects of their illness with trusted adults. The issue of disclosure of diagnosis is particularly pressing because perinatally infected children live into mid and late adolescence. In a cohort of 42 perinatally infected children aged nine through 16 years followed at CHAP, Newark, NJ, fewer than 60% were specifically told their diagnosis. Uninfected but HIV-affected siblings often have mental health needs as well, especially when they face the eventual loss of siblings and one or both parents. A failure to deal successfully with

psychosocial issues may impede families from seeking optimal medical care for their children.

CHALLENGE OF HIV INFECTION IN ADOLESCENTS

Like other HIV-infected persons, adolescents with HIV infection come disproportionately from minority communities. As a group, adolescents are at high risk of acquiring HIV by nature of their developmental stage. The Agency for Health Care Policy and Research guidelines on Evaluation and Management of Early HIV Infection details the unique set of issues in caring for adolescents with HIV infection. These include: (a) differences in the epidemiology of HIV infection among youth; (b) special barriers to youth both in receiving care for their HIV infection and in prevention services, including counseling and testing, resulting from variable laws regarding consent and conÞdentiality for those aged under 18 years; (c) lack of HIV-speciÞc clinical services for adolescents; and (d) the limitation on youths participating in clinical trials. One of the major challenges facing those caring for adolescents is the prevention of HIV transmission, both primary and secondary. In general, preventive programs have relied on incomplete or limited information services not linked to care or programs that teach and encourage behavior change. Successful adolescent HIV programs have employed voluntary, conÞdential, or anonymous counseling and HIV testing with direct linkage to adolescent speciÞc care. Although speciÞcs of medical management of HIV-infected adolescents, in terms of medication use and disease assessment, are similar to those used for adults, many aspects do differ from that which is appropriate for children and adults. The history and physical examination need to be interpreted in the context of age-speciÞc differences and pubertal stage. History-taking from adolescents needs to detail sexual and drug-using behavior, recognizing the psychosocial and cognitive-developmental stage that may inßuence the accuracy of the information obtained, and incorporate counseling and coping mechanisms employed by the adolescents. There should be an awareness of the potential for other sexually transmitted diseases. Issues relating to treatment adherence are especially important in caring for adolescents.

Of interest are recent data suggesting that perinatally infected adolescents may also exhibit delay in pubertal development, not related to clinical or immunologic conditions or antiretroviral therapy (108).

Perhaps most importantly, services for adolescents need to be provided by an experienced physician comfortable in dealing with youth and issues of adolescent sexuality within the context of a developmentally appropriate environment.

DEVELOPING STANDARDS OF CARE FOR CHILDREN WITH HIV

Twenty years ago, 95% of children with leukemia died; today, up to 85% are cured. The intensity of effort put into controlling childhood leukemia should serve as a template for our efforts to treat HIV-infected children. Efforts to improve quality of life while working toward a cure for HIV will require a multidisciplinary approach, calling on the skills of physicians, nurses, social workers, nutritionists, pharmacists, dentists, and developmental specialists. This effort must take the childÕs entire family into consideration, whether it is the family of birth or a foster family.

At most large HIV centers a team approach to the child and the childÕs family has resulted in better communication, compliance and better management of children with HIV infection. Team members may include clinicians, nurse and nurse practitioners, psychologists, social workers, nutritionists and dental hygienists. This family centered team approach may be even more necessary in the future with the aging into adolescence of the majority of perinatally HIV-infected children.

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Chapter 16

Pulmonary Complications of HIV Infection

Krystn R. Wagner and Richard E. Chaisson

Pulmonary diseases are common complications of infection with human immunodeficiency virus (HIV). These include both opportunistic infections, such as *Pneumocystis carinii* pneumonia (PCP), as well as other complications such as Kaposi's sarcoma (KS), non-Hodgkin's lymphoma (NHL) and changes in pulmonary function. Since 1996, the widespread use of highly active antiretroviral therapy (HAART) has had a significant impact on the epidemiology of HIV-associated opportunistic infections (1). Overall, there has been a substantial decline in the incidence of PCP, community acquired pneumonia, and other pulmonary complications of advanced HIV disease in populations with access to HAART. The risk of opportunistic infections among patients with depressed CD4 cell counts, particularly <200 cells/mm^3, however, remains similar to that in the pre-HAART era. As a result, it remains essential to understand the distinctive clinical features, diagnosis and treatment of common pulmonary complications in patients with HIV infection.

The occurrence of opportunistic disease in patients with HIV infection is a function of underlying host immunodeficiency, as well as exposure to potential pathogens, pathogen virulence and antimicrobial susceptibility, as well as other modifying factors such as chemoprophylaxis and cigarette smoking. The epidemiology of pulmonary complications in patients with HIV infection varies among geographic regions. This is a function of environmental conditions, such as exposure to indigenous pathogens, as well behavioral and socioeconomic factors, such as access and adherence to antiretroviral therapy. Due to the lack of universal access to HAART, chemoprophylaxis, or vaccinations, there is a widening gap between the incidence of HIV-associated complications in industrialized versus developing countries. In this chapter, we discuss the pulmonary complications of HIV infection in populations with access to HAART, while acknowledging that the large burden of HIV disease exists in the developing world. With increasing availability of prophylaxis and HAART in currently resource-poor nations, however, the pulmonary and other complications of HIV infection will more closely resemble those seen in developed nations.

Potential respiratory pathogens in patients with HIV infection are listed in Table 16.1. The risk of each complication correlates with the individual's degree of immunosuppression. The absolute CD4 cell count (or CD4

TABLE 16.1. *Common respiratory pathogens and other complications in HIV infection in relation to CD4 count*

Pathogen	CD4 Count
Bacteria	
Streptococcus pneumoniae	Any
Haemophilus inßuenzae	Any
Staphylococcus aureus	<500
Pseudomonas aeruginosa	Any, usually <50
Rhodococcus equi	<100
Nocardia species	<100
Mycobacterium tuberculosis	Any
Nontuberculous mycobacteria	<100
Protozoa	
Toxoplasma gondii	<100
Fungi	
Pneumocystis carinii	<200
Histoplasma capsulatum	<100
Coccidioiodes immitis	<200
Aspergillus species	<100
Viruses	
Cytomegalovirus	<50
In uenza	Any
Tumors	
Kaposi's sarcoma	<500
Non-Hodgkin's lymphoma	<500
Other	
Lymphocytic interstitial pneumonitis	<500

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cell percentage) remains the best surrogate marker for assessing the risk of HIV-associated complications (2Þ4). With CD4 cell counts greater than 500/mm^3, there is an increased risk of infections with more virulent organisms capable of causing disease in immunocompetent persons, such as *Streptococcus pneumoniae* or *Mycobacterium tuberculosis*. When the CD4 cell count falls to less than 200/mm (3), the risk of opportunistic pathogens that rarely cause disease in normal hosts, such as *Pneumocystis carinii*, signiÞcantly increases (5). The HIV plasma RNA (viral load) is predictive of the long-term rate of loss of immune function and is an independent risk factor for opportunistic infections (6). However, the viral load has less prognostic value for the risk for opportunistic infection at a given point in time than the CD4 lymphocyte count.

APPROACH TO DIAGNOSIS

An approach to evaluating the patient with HIV-related pulmonary disease is depicted in the algorithm shown in Fig. 16.1. After conÞrmation of pulmonary disease from abnormalities on a chest radiograph, documented defects in oxygen transport or evidence of parenchymal inßammation by CT scan, secretions from the respiratory tract are usually examined for the presence of pathogens. For patients with expectorated sputum, Gram stain and acid-fast bacilli smears and cultures are appropriate. For patients who do not produce spontaneous sputum, sputum induction is a common procedure with a reasonably high sensitivity for some respiratory pathogens including *P. carinii*. The negative predictive value of sputum induction for PCP is moderately low, however, and infection cannot be excluded by a negative test result. If a speciÞc diagnosis is not established, bronchoalveolar lavage (BAL) is extremely sensitive and speciÞc for identifying HIV-related pulmonary pathogens. Transbronchial biopsy may increase the yield of bronchoscopy for some diagnoses, although complications of this procedure occur in as many as 10% of patients. The use of high resolution CT scanning has improved the noninvasive approach to evaluating patients with pulmonary symptoms, but this technique can only suggest speciÞc etiologies of pulmonary opportunistic diseases on the basis of characteristic Þndings. A

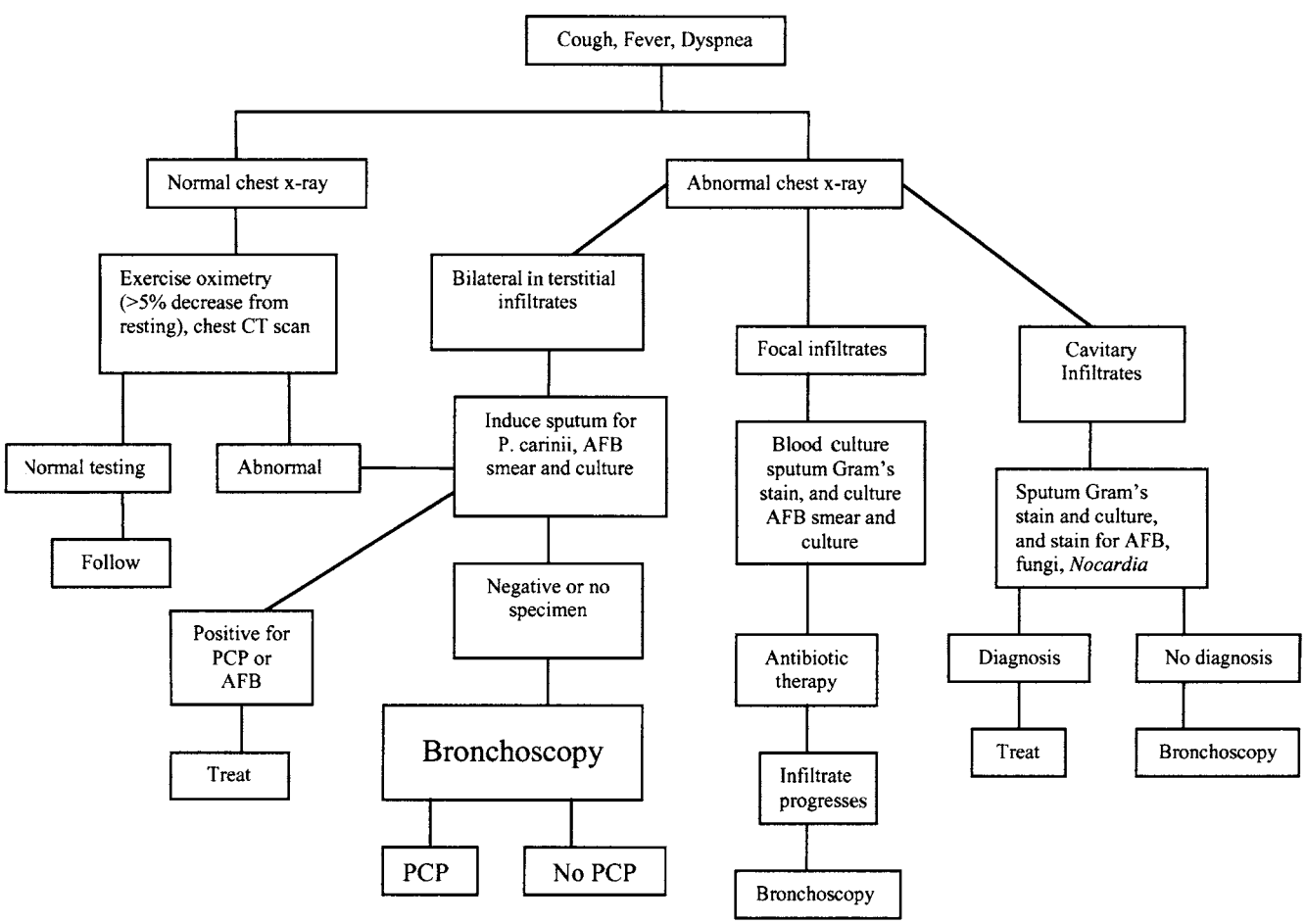

FIG. 16.1. Diagnostic algorithm for the evaluation of pulmonary disease in patients with HIV infection.

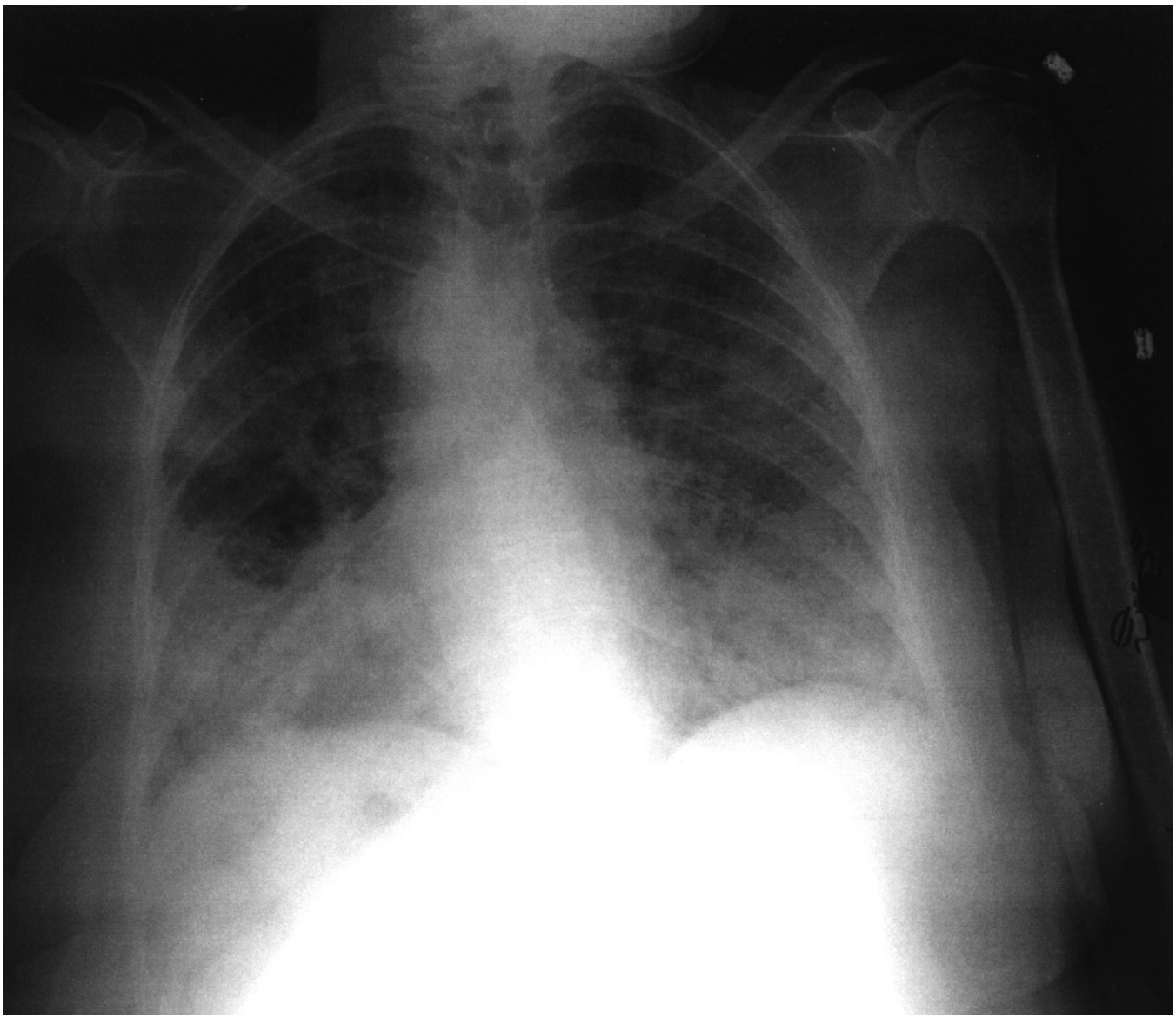

FIG. 16.1a. A 55 year old woman with advanced HIV infection presented with three weeks of dry cough, breathlessness, hypoxemia and fever. Her chest radiograph shows diffuse interstitial in ltrates. Induced sputum revealed *P. carinii*.

speciÞc diagnosis still requires microbiological or histopathological conÞrmation.

Infectious Pulmonary Complications of HIV

Pneumocystis carinii Pneumonia

P. carinii was previously classiÞed as a protozoan based on its morphologic appearance and response to antiprotozoal drugs. Genetic analysis of ribosomal RNA and mitochondrial DNA, however, suggests that *P. carinii* is a fungus. While the environmental reservoir and transmission patterns are unknown, approximately 80Ð90% of children are exposed to *P. carinii* before age three. The organism does not cause disease in immunocompetent persons. Prior to the AIDS epidemic, there were approximately 100 cases of PCP per year in the United States. These cases were typically associated with a variety of conditions accompanied by defects in cell-mediated immunity.

In 1981, clusters of PCP in Los Angeles, San Francisco, and New York heralded the beginning of the AIDS epidemic. Before the widespread use of chemoprophylaxis in the early 1990s, PCP was the presenting manifestation of HIV infection in 60% of AIDS patients. Approximately 95% of patients who present with PCP have CD4 cell counts less than 200/mm^3, and the incidence increases as the CD4 cell count declines further. Since the introduction

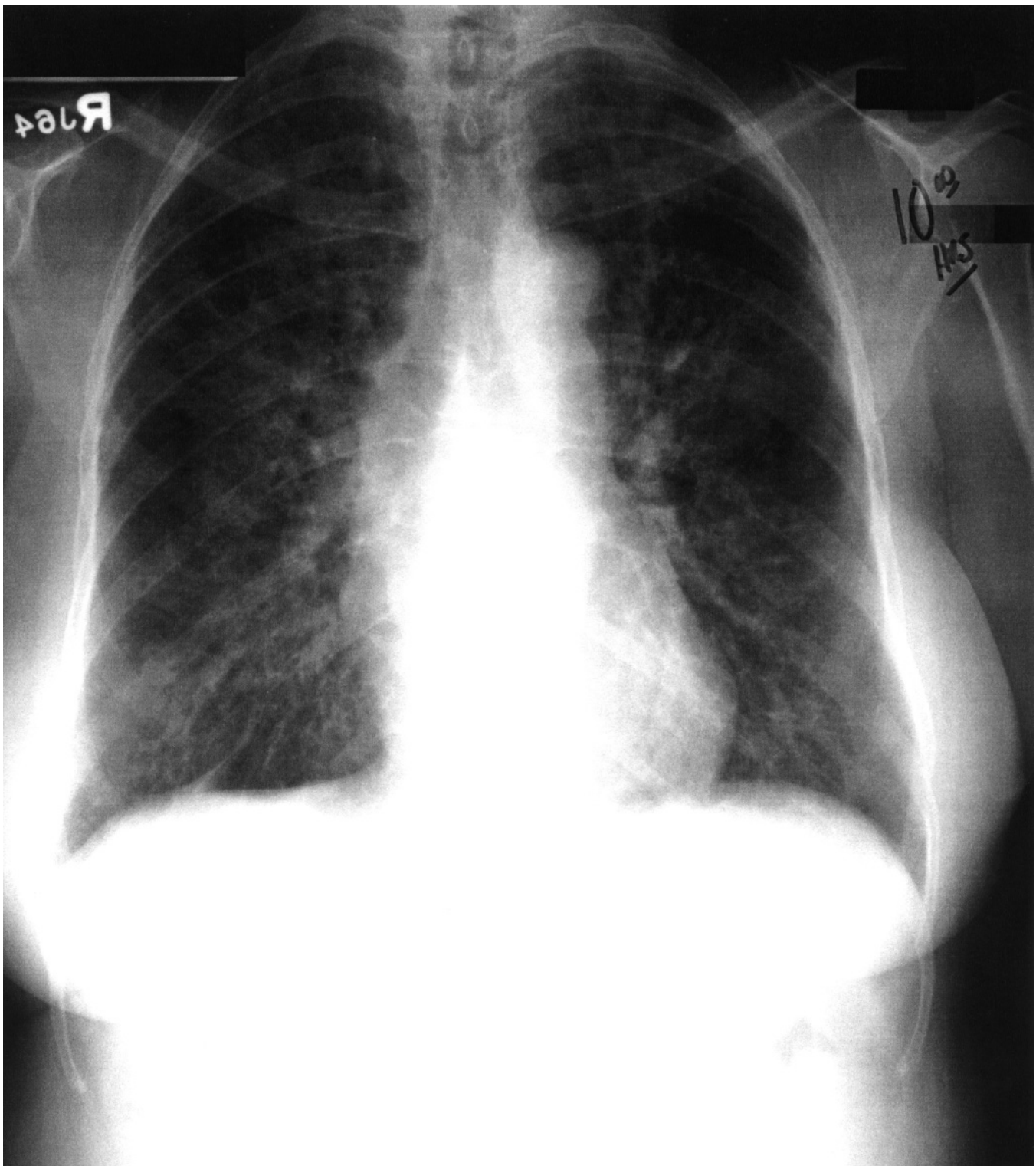

FIG. 16.1b. The same patient ve days later. Despite intravenous cotrimoxazole and oral prednisone, she became progressively hypoxemic and her chest radiograph reveals worsening in ltrates. She subsequently improved without a change in therapy, and did not require mechanical ventilation.

of HAART, the incidence of PCP, along with other opportunistic infections, has fallen dramatically.

Despite these epidemiological changes, the risk of PCP remains essentially the same for individuals with low CD4 cell counts. PCP remains a frequent clinical presentation in patients without a prior diagnosis of HIV infection. In addition, lacks of access to or adherence with prophylaxis and antiretroviral therapy contribute to new cases of PCP.

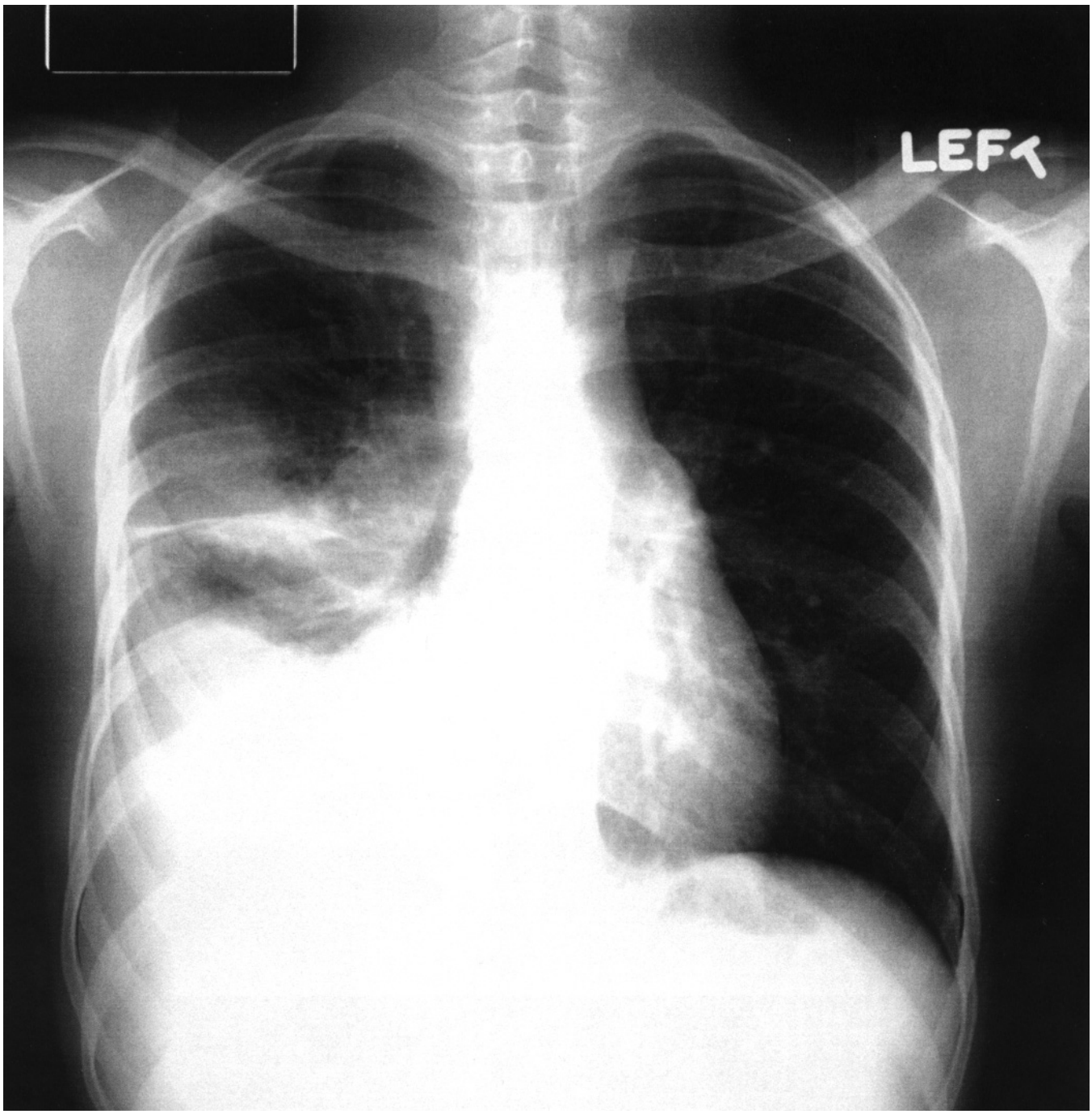

FIG. 16.1c. A 22 year old man admitted to the hospital for fevers, cough, chest pain, cervical adenopathy and weight loss. His chest radiograph shows right paratracheal and hilar adenopathy, a right pleural effusion with loculation and adjacent in ltrate. He was found to have HIV infection with a CD4 cell count of 133 per cubic milliliter. Cultures of sputum, pleural uid and lymph node all grew *M. tuberculosis*. He was treated with standard therapy and responded well, and antiretroviral therapy was initiated after two months without complication.

Clinical and Laboratory Manifestations

In patients with HIV infection, PCP is characterized by a slow, indolent time course with clinical symptoms progressing over weeks to months. Typical manifestations include fever, dyspnea, and nonproductive cough. Other symptoms include fatigue, chest tightness, night sweats, anorexia and weight loss. Presenting symptoms and time course are variable, however, and symptoms may develop over several days, suggesting acute community acquired pneumonia.

The clinical signs of PCP include hypoxia and tachypnea, with a resting $PaO_2 < 80$ mm Hg found in 80% of cases. Desaturation with exercise, such as stair-climbing,

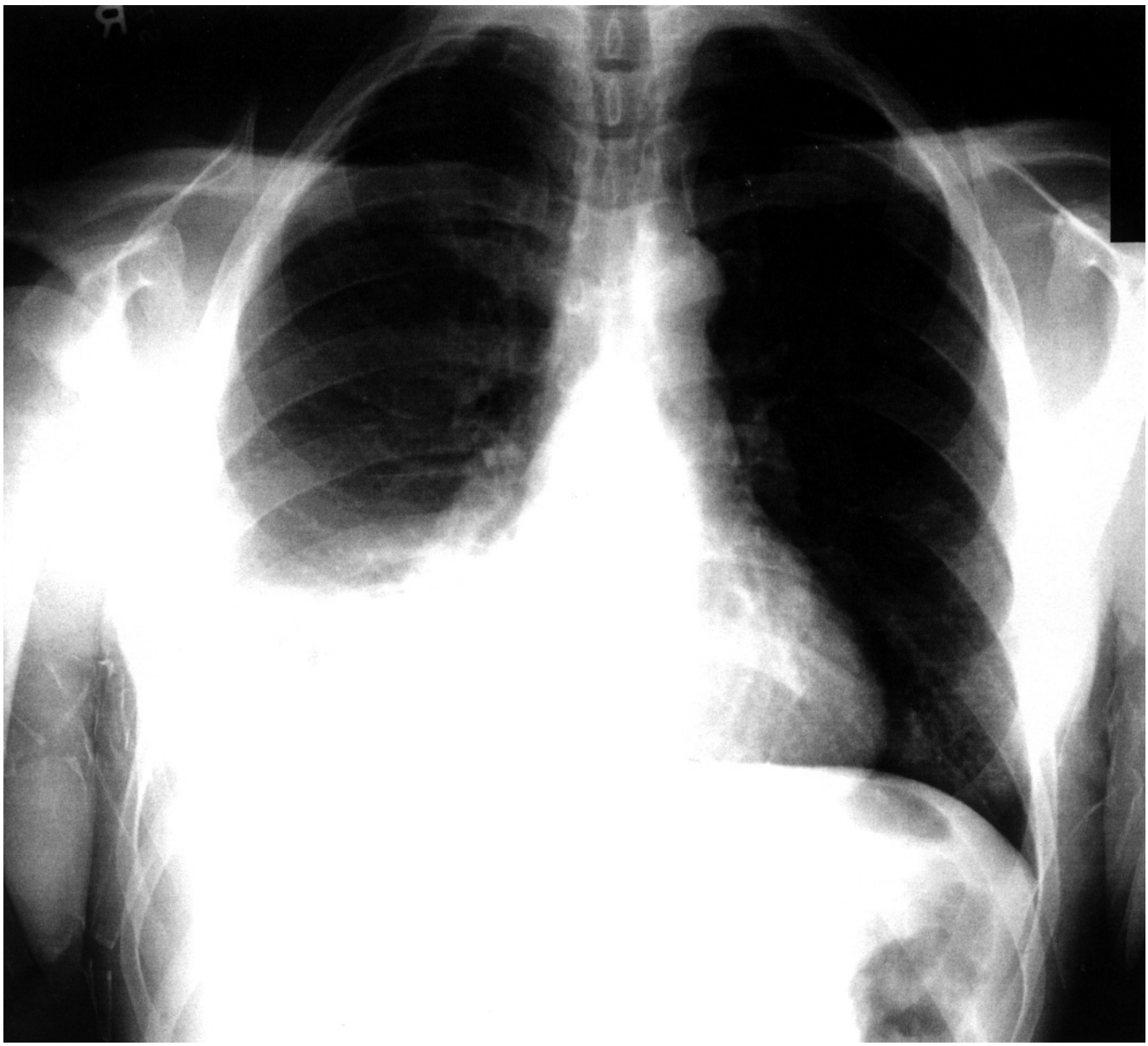

FIG. 16.1d. A 44 year old man with HIV infection and CD4 count of 326 per cubic milliliter presented with a two-day history of fever, productive cough and chest pain. His chest radiograph shows a right middle lobe in ltrate and a large rght pleural effusion. Blood cultures grew *S. pneumoniae*. After drainage of 0.5L of pleural uid and treatment with ceftriaxone, the patient improved.

is a highly sensitive marker of PCP, even if the PaO_2 is normal (7,8). Fever is observed in 80Ð90% of cases. The chest examination is often unremarkable; however, physical diagnosis may reveal other signs of advanced HIV infection, such as oral thrush or oral hairy leukoplakia. The presence of such signs is of particular signiÞcance when evaluating a patient not yet known to be HIV seropositive or with an unknown CD4 cell count. As when evaluating patient symptoms, the clinician should be alert to atypical clinical signs of PCP.

Chest radiographs typically show bilateral diffuse inÞltrates in an interstitial or interstitial-alveolar pattern. In patients receiving aerosolized pentamidine prophylaxis, the inÞltrates may be conÞned to the lung apices, radiographically mimicking pulmonary tuberculosis. The chest X-ray may be normal in as many as 30% of cases, usually in association with mild disease (9). Other radiographic Þndings may include pneumothorax, nodules, cavitation, and patchy or nodular ground glass inÞltrates on computerized tomography scan. Laboratory Þndings in PCP are generally non-speciÞc. The white blood cell count is typically normal or reduced with a marked lymphopenia. Serum lactate dehydrogenase (LDH) level is typically elevated in PCP, with a sensitivity

of 78–100%. However, LDH is not a specific marker for PCP. LDH elevations are a surrogate for radiographic severity and are not useful in differentiating PCP from other illnesses (10). The degree of elevation, however, correlates with disease severity and predicts mortality at diagnosis.

P. carinii cannot be cultured using fungal or other media. Diagnosis depends on cytopathology of induced sputum or BAL. The diagnostic algorithm typically begins with a sample of sputum induced using nebulized hypertonic saline. Expectorated sputums are generally not available from patients with PCP. Traditional stains used to identify PCP include toluidine blue-0 and methenamine silver, which stain the cyst wall. Giemsa stains identify trophozoite forms and have been useful in identifying *P. carinii* in sputum specimens. More recent technology uses monoclonal antibodies for direct and indirect immunofluorescence. The sensitivity of induced sputum for PCP varies greatly among different centers but approaches 90% at some institutions. A positive result, however, obviates the need for bronchoscopy or additional diagnostic evaluation. The cytopathology associated with PCP is not altered by antipneumocystis therapy; therefore, empiric treatment is recommended in patients with suspected PCP prior to diagnostic test results. Future diagnosis of PCP may incorporate the use of polymerase chain reaction (PCR) on respiratory specimens and/or serum. The role of PCR in the diagnostic algorithm for PCP remains to be established.

Given the variable negative predictive value of induced sputum for PCP, early fiberoptic bronchoscopy is recommended for patients with negative induced sputum. BAL has a high sensitivity for diagnosing PCP, with sensitivities typically exceeding 95%. Bilateral and/or site directed BAL improves the yield. In nearly all cases, a negative BAL result for PCP obviates the need for treatment. However, BAL may identify other pathogens or provide other diagnostic information. A review of PCP diagnosis in San Francisco found that induced sputum identified 80% of cases and BAL found *P. carinii* in 32% of patients with negative induced sputa (11). Other pathogens were identified in 24% of patients. Of the 95 patients who stopped antipneumocystis therapy after a negative BAL, only two developed PCP at more than 6 weeks after bronchoscopy.

Empiric PCP treatment without BAL is controversial. A number of authors have argued that for patients with clinical features strongly suggestive of PCP, empiric therapy is warranted and provides effective treatment with minimal morbidity (12). Others have noted that among patients with a "classic" presentation of PCP, more than 25% do not have the disease, and that empiric treatment would delay the diagnosis of other illnesses. In addition, treatment for PCP is associated with potential adverse reactions. BAL may successfully identify other pulmonary complications of HIV that can mimic PCP, such as community acquired bacterial pneumonia, cryptococcal pneumonia, tuberculosis and tracheobronchial Kaposi's sarcoma. Failure to perform early BAL may result in clinical worsening of the patient beyond the point where bronchoscopy may be safely performed.

Treatment

The medical management of PCP includes the decision whether to hospitalize the patient. Indications for the hospitalization of HIV-infected patients with PCP include hypoxia ($PaO_2 < 70$), requirement for parenteral antibiotics, uncertainty regarding the diagnosis, and risk of medical non-compliance. Trimethoprim-sulfamethoxazole (TMP-SMX) is the preferred initial treatment for PCP. The recommended regimen is trimethoprim 15 mg/kg per day plus sulfamethoxazole 75 mg/kg daily for 21 days in three to four divided doses. In patients with severe PCP, initial treatment should be parenteral due to the risk of erratic oral absorption. Mild to moderate PCP may be treated effectively as an outpatient with oral antibiotics. The typical oral regimen is two double-strength tablets of TMP-SMX three times per day.

Patients with AIDS have a high incidence (25–50%) of adverse reactions to trimethoprim-sulfamethoxazole, including fever, skin rash, bone marrow suppression, and elevation of liver enzymes. For patients intolerant of TMP-SMX, there are a number of alternative regimens. In patients with mild to moderate PCP, effective alternatives include trimethoprim (15 mg/kg/day) plus dapsone (100 mg/day) and oral or intravenous clindamycin (300–450 mg po every six hours or 600 mg IV every eight hours) combined with oral primaquine (30 mg base/day). Potential adverse reactions to dapsone include hemolytic anemia in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency, methemoglobinemia and hepatotoxicity. Primaquine may also cause hemolytic anemia in persons with G6PD deficiency, as well as fever, rash, methemoglobinemia, and diarrhea. Atovaquone which may be used for the treatment of mild to moderate PCP (see Chapter 00) is generally well tolerated but has less efficacy and greater cost than TMP/SMX.

TMP/SMX remains the treatment of choice for severe PCP. The addition of corticosteroids is indicated for patients with $PaO_2 < 70$ mm Hg or an A-a gradient > 35 mm Hg (13). The initiation of antipneumocystis therapy often results in a decline in PaO_2 of 10–30 mm Hg. This decline is thought to be due to pulmonary inflammation and edema in response to dying *P. carinii*. Corticosteroids started within 72 hours will prevent or reduce the decline in PaO_2, improving pulmonary function and survival. The recommended regimen is prednisone 40 mg twice daily for five days, then 40 mg daily for five days, followed by 20 mg daily for the remainder of the treatment. Adjuvant corticosteroids for PCP may result in increased mucocutaneous HSV, oral or esophageal candidiasis, acute psychosis and hyperglycemia; however, an increased risk of other opportunistic infections is not apparent.

An alternative regimen for the treatment of severe PCP is intravenous pentamidine 3Ð4 mg/kg daily for 21 days. Pentamidine is also associated with a high incidence of adverse reactions including azotemia, hyperglycemia or hypoglycemia, pancreatitis, hypotension and bone marrow suppression. The clinical response to effective anti-pneumocystis therapy is typically slow. Treatment failure is deÞned as progressive deterioration four to Þve days into therapy or lack of clinical or radiographic improvement after seven to ten days. Secondary processes should be considered, including concurrent infections, congestive heart failure, pulmonary embolus, and other underlying lung diseases. In addition, PCP is associated with the development of pneumothoraces. If a patient with conÞrmed PCP is deteriorating on Þrst-line therapy (TMP/SMX or pentamidine), there are no criteria to guide a switch in therapy. No change is recommended for at least Þve to seven days. If a patient has residual respiratory symptoms or requires supplemental oxygen at the end of 21 days of therapy, the treatment course should be lengthened to four to Þve weeks. Repeat bronchoscopy during or at the completion of therapy frequently demonstrates the presence of *P. carinii*. In patients doing well clinically, this is not an indication for continuation of therapy beyond three weeks.

TMP/SMX resistance in PCP may explain some cases of AIDS patients who fail TMP/SMX prophylaxis and/or treatment. Sulfamethoxazole (SMX) and dapsone act on dihydropteroate synthase (DHPS), a key enzyme in folate metabolism. Point mutations in the *P. carinii* dihydropteroate synthase (DHPS) gene have been associated with the failure of sulfa or sulfone prophylaxis in AIDS patients (14). Kazanjian et. al. ampliÞed and sequenced DHPS genes from 97 AIDS patients with PCP between 1991 and 1999. DHPS mutations were found in 76% of isolates from patients with prior sulfa or sulfone exposure versus 23% of isolates from unexposed individuals ($p = 0.001$) (15). DHPS mutations in *P. carinii* impact response to sulfa/sulfone prophylaxis and treatment as in other bacteria and *Plasmodium* species.

Prevention

In 2002, the U.S. Public Health Service (USPHS) updated the guidelines for the prevention of opportunistic infections in persons with HIV (16). These guidelines include new recommendations for primary and secondary PCP prophylaxis. Chemoprophylaxis against PCP should be initiated when the CD4 cell count is less than 200/ul or there is a history of oropharyngeal candidiasis. In addition, patients with a CD4 cell percentage of less than 14% or another AIDS-deÞning illness should be considered for prophylaxis. TMP-SMX is the preferred prophylactic agent. One double-strength tablet per day is recommended but one single-strength tablet per day or one double-strength tablet three times per week are also effective regimens and may be better tolerated. In a patient who has had an adverse reaction to TMP-SMX, if clinically feasible, it is desirable to continue or reinitate TMP-SMX prophylaxis, sometimes following one of several desensitization protocols or a reduction in drug dose or frequency. Alternative prophylactic regimens include dapsone 100 mg daily, dapsone plus pyrimethamine and leucovorin (if patient is toxoplasma IgG positive), aerosolized pentamidine 300 mg monthly administered via the Respirgard II[a] nebulizer, and atovaquone 1500 mg daily (see Chapter 00).

The updated USPHS/IDSA guidelines recommend that primary pneumocystis prophylaxis should be discontinued in patients who have responded to HAART with an increase in CD4 cells to > 200 cells/mm^3 for at least three months. As the guidelines point out, this recommendation is largely based on observational and randomized trials in which most patients were taking a protease inhibitor and many had sustained virologic suppression below the assayÕs limit of detection. Primary prophylaxis should be reinstituted if the CD4 cell count declines to below 200 cells/mm^3 or the patient otherwise meets criteria for PCP prophylaxis.

Secondary prophylaxis is recommended for patients with a history of PCP, regardless of CD4 cell count, using TMP-SMX or one of the alternative drug options for primary prophylaxis. Secondary prophylaxis should be discontinued when the CD4 cell count exceeds 200/mm^3 for at least three months in response to HAART. If the episode of PCP occurred with CD4 > 200 cells/mm^3, it is currently recommended to continue life-long secondary prophylaxis regardless of recovery in CD4 cell count.

BACTERIAL PNEUMONIA

As a result of defects in both cell-mediated and humoral immunity, persons with HIV infection are more susceptible to bacterial infections, particularly those with encapsulated organisms. Bacterial pneumonia is a common complication of HIV infection and can occur within all CD4 cell strata. Recurrent bacterial pneumonia (≥ 2 episodes in 12 months) is included in the CDC surveillance case deÞnition of AIDS.

In a prospective study of more than 1,200 patients with and without HIV infection, the rate of pneumonia in the HIV-positive group was 5.5 episodes per 100 person-years, as compared to 0.9 per 100 person-years in the HIV-negative group ($p < 0.001$) (17). Among HIV-infected patients, the rate of bacterial pneumonia was inversely related to the baseline CD4 cell count, with the highest risk among patients with a CD4 cell count less than 200/mm^3. Injection drug use was also associated with an increased risk of bacterial pneumonia in all strata of CD4 cell counts. The study also found an increased risk associated with cigarette smoking among patients with CD4 cell counts less than 200/mm^3. Although there has been a signiÞcant

decline in the incidence of bacterial pneumonia since the introduction of HAART, the same risk factors for bacterial pneumonia are assumed to operate.

The most common pathogen causing bacterial pneumonia in patients with HIV infection is *Streptococcus pneumoniae* (18). Persons with HIV have an incidence of pneumococcal bacteremia that is approximately 100 times greater than that found in the general population. Other etiologic agents include *Haemophilus inßuenzae*, *Staphylococcus aureus*, *Klebsiella* species, *Pseudomonas aeruginosa*, *Rhodococcus equi*, and *Nocardia* species. The frequency of pneumonia due to atypical pathogens including *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella* species appears to be extremely low.

Clinical and Laboratory Manifestations

HIV-infected patients with bacterial pneumonia generally have a similar clinical presentation as patients without HIV infection. Common signs and symptoms include fever, rigors, dyspnea, productive cough with purulent sputum, and pleuritic chest pain. Patients typically present with an acute syndrome of less than one weekÕs duration, in contrast to the more indolent course associated with PCP. The physical examination may reveal fever, tachypnea, and evidence of consolidation on chest examination.

Laboratory Þndings associated with bacterial pneumonia often include an elevated leukocyte count with a predominance of neutrophils and immature forms. The absolute leukocyte count may be relatively low, however, in patients with advanced immunodeÞciency.

The chest radiograph is variable, depending on the etiologic agent. Pneumococcal pneumonia is typically associated with radiographic Þndings of focal segmental or lobar consolidation with or without pleural effusion. Nodular or cavitary lesions are found in association with *Staphylococcus aureus*, *Nocardia* species and mycobacterial infections.

The diagnostic algorithm begins with an analysis of the gram-stained sputum and sputum and blood cultures. Prior antibiotics usually preclude culture growth; therefore, sputum and blood samples should be collected prior to the initiation of empiric antibiotic therapy.

Bacteremia is a more frequent complication of pneumonia in patients with HIV infection. The mortality rate with bacteremic pneumococcal pneumonia is 5% to 11%. It is recommended that blood cultures be obtained early in the evaluation of a patient with pneumonia.

Treatment

The sputum Gram stain and local antibiotic resistance patterns will guide the choice of antibiotic. For example, if *S. pneumoniae* is suspected from the sputum analysis, preferred treatment options include a third generation cephalosporin, such as cefotaxime or ceftriaxone, a ßuoroquinolone, or a macrolide. Patients with pneumococcal pneumonia resistant to penicillin or a seriously ill patient in a region with high rates of resistance should be treated with a parenteral third generation cephalosporin or a newer ßuoroquinolone (levoßoxacin, gatißoxacin, moxißoxacin). Empiric antibiotic therapy may be revised based on the sputum culture data and antibiotic susceptibility testing. HIV-infected patients with community acquired bacterial pneumonia usually respond rapidly to treatment. Clinical failure on appropriate antibiotics for likely bacterial pathogens should prompt additional diagnostic tests and expanded therapy for possible co-pathogens or other pulmonary processes. In earlier studies, approximately 5% of patients with bacterial pneumonia also had PCP.

Prevention

Prevention of pneumococcal disease with pneumococcal polysaccharide vaccine has been recommended for patients with HIV infection for some time, though controversy exists regarding vaccine efÞcacy. Several early studies showed poor antibody responses to the vaccine, particularly in patients with advanced HIV disease. A case-control study in the early 1990s found a beneÞcial effect of vaccination for patients with CD4 cell counts above 200, but no beneÞt, and possible harm, for patients with CD4 counts below 200 (19). A subsequent case-control study by the CDC found that the overall efÞcacy of pneumococcal vaccination was 49% (95% CI, 12%Ð70%) (20). However, a prospective, placebo-controlled trial performed in Africa found an *increased* risk of both pneumococcal infections and all bacterial pneumonia in patients randomized to vaccination (21). Studies of the efÞcacy of protein-polysaccharide conjugate pneumococcal vaccines have not been completed, though evidence from *Haemophilus* conjugate vaccine studies suggest that this approach may be more efÞcacious.

Despite the Þndings from the placebo-controlled trial in Uganda, the Infectious Diseases Society of America and the U.S. Public Health Service recommend that the 23-valent pneumococcal polysaccharide should be given to all HIV-infected patients with a CD4 cell count $\geq$200 cells/mm^3 and should be considered for patients with CD4 cell counts $<$200 cells/mm^3. This recommendation is based on the consistency of observational studies and concerns about the applicability of the Ugandan trial to circumstances in the United States.

In persons with HIV infection, the duration of antibody responses to the pneumococcal antigens in the vaccine is unknown. Revaccination is suggested at Þve years, although there is limited clinical data to support this practice. A recent randomized, placebo-controlled trial evaluated the potential risks and beneÞts of reimmunization in patients with HIV infection (22). Antibody levels

measured prior to immunization were similar to those in unimmunized patients. The increase in serum IgG to four pneumococcal capsular polysaccharides at two, six, and twelve weeks achieved statistical signiÞcance in the reimmunized patients compared to baseline values. However, the antibody levels did not differ signiÞcantly between the revaccinated patients versus those receiving saline placebo. This suggests that reimmunization produces only a modest increase in capsule-speciÞc IgG levels which may have little or no clinical beneÞt. At the same time, revaccination produced only minor side effects including soreness, erythema, or swelling at the injection site and fever. No adverse effects on CD4 cell count or plasma HIV-RNA levels were observed.

Patients who receive TMP-SMX for PCP prophylaxis, compared to aerosolized pentamidine, have a lower incidence of bacterial respiratory tract infections, as do patients receiving azithromycin or clarithromycin for prevention of *Mycobacterium avium*-complex infection. The use of prophylactic antibiotics for prevention of bacterial pneumonia *per se* is not recommended, however, since this practice could promote the development of resistant organisms.

NOCARDIA

Nocardia Asteroides

Nocardiosis is described in HIV infection and it is an indicator disease for AIDS. In most cases, disease occurs in patients with advanced immunodeÞciency (ie, CD4 cell count $< 200/mm^3$) (23,24). Patients typically present with an indolent course and nonspeciÞc constitutional complaints. Pulmonary symptoms, such as cough or dyspnea, may develop. As in patients without HIV infection, the lung is the most common site of disease, although dissemination is common in HIV-infected patients. Radiographic Þndings include nodules, cavities, and diffuse or focal inÞltrates (25). A case of pulmonary and disseminated disease due to *N. farcinica* in an HIV-infected patient has been reported (26).

The diagnosis is suspected if Þlamentous, beaded, branching, gram-positive, acid-fast rods are seen on Gram stain or on modiÞed acid-fast stain of sputum or other respiratory specimens. Because of the slow growth of the organism, cultures should be held for four weeks if nocardiosis is suspected. Culturing sputum for mycobacteria and fungi will improve the chances of isolating *Nocardia* sp.

Sulfonamides are the mainstay of therapy for nocardiosis. Although TMP-SMX is synergistic against *N. asteroides in vitro*, the combination is no more effective than any of the sulfonamides alone. Minocycline is active against *Nocardia in vitro* and has been used successfully in HIV-infected and other patients. Other agents demonstrating *in vitro* activity against *Nocardia* include cefotaxime, ceftriaxone, imipenem, and amikacin, though clinical experience with these agents is more limited.

The optimal duration of therapy for nocardiosis has not been determined for immunocompromised patients (27,28). Some investigators recommend treatment for six to twelve months for patients with pulmonary nocardiosis, regardless of their immune status. Others recommend indeÞnite therapy in the setting of immunocompromise in general. The former recommendation (a six to twelve-month course of therapy) may be most appropriate for HIV-infected patients who successfully recover lost immune function with HART. The use of TMP-SMX for prophylaxis of PCP may help to prevent nocardiosis, although data are limited. Nocardiosis has been described in patients who were prescribed TMP-SMX prophylaxis.

Rhodococcus equi

R. equi (formerly *Corynebacterimi equi*) was Þrst reported to cause disease in an AIDS patient in 1986 (29). Since then, several cases have been reported (30Ð35). The organism is a gram-positive, aerobic, nonmotile, non-spore-forming, pleomorphic bacillus. On Gram stain, its appearance varies from cocci to short rods. Infection typically presents as pneumonia, with unilobar, often upper lobe, pulmonary inÞltrates, which progress over several weeks. Multilobar involvement may develop, and cavitation, pleural effusion, empyema, and bacteremia are common. Of 12 HIV-infected patients with *R. equi* infections, one had cavitary disease, and three had culture-positive pleural effusions (33,34).

Resistance to beta-lactam antibiotics develops frequently. Effective antimicrobial agents include erythromycin, vancomycin, clindamycin, chloramphenicol, and TMP-SMX. Rifampin has been used in combination with other agents and is probably synergistic when given with erythromycin. Prolonged courses of parenteral antibiotics are recommended, and surgical intervention is sometimes necessary for persistent abscesses. Recurrence is not uncommon, even after several weeks of antibiotic therapy.

PSEUDOMONAS AND OTHER UNUSUAL ORGANISMS

Nosocomial pneumonia in patients with AIDS is usually caused by gram-negative bacilli, including *P. aeruginosa*, or by *Staphylococcus aureus* (36,37). As with other hospitalized patients, risk factors include neutropenia, the use of broad-spectrum antibiotics, and the presence of central venous catheters. Nosocomial pneumonia has a higher morbidity and mortality than community-acquired pneumonia.

Pneumonia due to *Pseudomonas* is not uncommon among HIV-infected patients and does not always appear

to be hospital acquired or fulminant in its presentation. Baron and Hollander described 16 patients with advanced HIV disease (mean CD4 count, 25/mm^3) and *P. aeruginosa* pneumonia, diagnosed by Gram stain and culture of sputum or bronchoscopic washings (38). In 12 of the 16 patients, the pneumonia was community-acquired, and Pseudomonas was the sole pathogen in 14 cases. Only four patients, two of whom had community-acquired pneumonia, presented with fulminant pneumonia and sepsis. The remaining 12, all with community-acquired pneumonia, had a more indolent presentation, with a mean duration of symptoms of two weeks. Prior hospitalization, an indwelling catheter, neutropenia, and corticosteroid use have all been associated with the development of deep-seated *P. aeruginosa* infections in patients with HIV (39). Relapses of infection are common.

In a prospective study of patients admitted to Johns Hopkins Hospital with community-acquired pneumonia, Mundy and colleagues reported that gram-negative bacilli were a signiÞcantly more common cause of pneumonia in patients with HIV infection than in uninfected patients (9% vs. 3%) (40). Gram-negative bacteremia may originate from a pulmonary infection, or bloodstream infection may secondarily infect the lungs. Risk factors for gram-negative pulmonary infections in HIV-infected patients include recent hospitalization, venous catheters, intubation and other factors known to increase risk in non-HIV-infected populations.

Pneumonia due to *Salmonella typhimurium* has been reported as well (41,42). Other bacterial pathogens reported to cause pneumonia in patients with HIV infection include *Bordetella bronchiseptica*, *Morancella catarrhalis*, group B *Streptococcus*, *Mycoplasma pneumoniae*, and *Streptomyces* species (43Ð47).

Pneumonia due to *Legionella pneumophila* has been reported in patients with HIV infection, but it does not appear to be common outside of epidemic foci in the community. Cavitary pneumonia due to *Legionella micdadei* and *L. pneumophila* has been described (48,49).

TUBERCULOSIS

Tuberculosis is one of the most common AIDS-related opportunistic infections worldwide, and is the leading cause of death from AIDS in sub-Saharan Africa (50). The incidence of tuberculosis in populations of HIV-infected people is a function of the local prevalence of latent tuberculosis infection and the risk of exposure to tuberculosis in the environment. Patients with HIV infection have an enormously elevated risk of progression of recent tuberculosis infection to active disease and of reactivation of latent tuberculosis infection. Among HIV-infected patients with latent tuberculosis, the risk of reactivation is 3Ð15% per year, higher than in any other patient population ever described. People with advanced HIV infection who acquire new tuberculosis infection have a risk of developing active disease that may be as high as 45%. Thus, HIV infection has a dramatic impact on the natural history of *M. tuberculosis* infection and disease.

Clinical and Laboratory Manifestations (see Chapter 00)

The clinical features of tuberculosis in HIV-infected patients vary with the degree of immunosuppression. Fever for greater than seven days along with weight loss as presenting complaints are more predictive of pulmonary TB than of PCP or community-acquired pneumonia due to pyogenic bacteria. In patients with mildly or moderately depressed CD4 counts, the presentation of tuberculosis is similar to that seen in other populations. Most have disease conÞned to the lungs, with upper lobe inÞltrates that are sometimes cavitary on chest radiograph (51,52). In patients who develop tuberculosis at a more advanced stage of HIV infection, often after the development of other opportunistic infections, tuberculosis tends to be more disseminated and clinical features are less typical. In these cases, although pulmonary infection is common, the radiographic Þndings may be atypical or may mimic those of primary tuberculosis. Cavitary disease is unusual in advanced AIDS, but lower lobe inÞltrates or miliary patterns occur frequently. Intrathoracic adenopathy and involvement of extrapulmonary sites are also common. HIV-infected patients with smear-positive pulmonary tuberculosis have been reported to present with normal chest radiographs, though this appears to be unusual (53).

Extrapulmonary tuberculosis is seen in 24% to 48% of HIV-infected patients (54), although in patients with more advanced stages of immunosuppression, it may occur in more than 70% of cases (55). As with tuberculosis in other populations, the lymphatic system is the most common site of extrapulmonary involvement. HIV-infected patients are more likely to have disseminated, genitourinary, intraabdominal, and mediastinal tuberculosis, with or without concomitant pulmonary disease, than patients without HIV infection (55Ð58). Bacteremia occurs in 26% to 42% of HIV-infected patients with tuberculosis. Tuberculous abscesses of the brain parenchyma or spinal cord may occur, appearing as ring-enhancing or hypodense mass lesions. Tuberculous meningitis is more common in HIV-infected patients, and it has been reported in patients with acellular cerebrospinal ßuid.

Diagnosis and Treatment

The Þrst step in the diagnosis of pulmonary tuberculosis is examination of sputum for acid-fast bacilli, followed by mycobacterial culture. The diagnostic yield of the sputum smear is comparable to that found in HIV-negative patients, but in more advanced stages of HIV disease, the sensitivity may be lower. Sputum culture has a yield of

approximately 90%. BAL may increase the sensitivity of smear and culture. Because bacteremia occurs in a substantial proportion of HIV-infected patients, blood cultures for acid-fast bacilli should be performed for all patients in whom tuberculosis is suspected. A positive puriÞed protein derivative tuberculin test may support a diagnosis of tuberculosis, but a negative result of the skin test should never be used to exclude tuberculosis, because patients may become anergic with progression of HIV disease and decline in the CD4 count. Radiographic Þndings vary considerably, especially in patients with advanced immunodeÞciency. Typical Þndings of apical cavitary disease are unusual in these patients. Patients with acid-fast bacilli present on smears or culture of respiratory specimens should usually be treated for presumed tuberculosis until speciation (either by DNA probe testing or by culture) proves a nontuberculous cause. Therapy for tuberculosis is discussed in Chapter 00.

Non-Tuberculous Mycobacteria

Mycobacterium avium complex (MAC) is a common cause of morbidity in advanced HIV disease though disease is nearly always systemic and not conÞned to the lungs. Although it is frequently isolated from sputum or bronchial washings in colonized patients or patients with disseminated infection, it has only rarely been reported to cause lung disease. However, endobronchial lesions containing granulomas, as well as granulomatous disease of the pulmonary parenchyma, have been reported and represent a Þnding requiring systemic MAC therapy (59). The growth of MAC from respiratory specimens without histopathologic evidence of disease is not an indication for treatment although it may predict later dissemination. The same is true for many other nontuberculous mycobacterial species, such as *M. gordonae*, *M. fortuitum*, *M. chelonei*, *M. xenopi*, and *M. haemophilum*, which rarely cause isolated pulmonary disease in HIV-infected patients. Prevention of MAC with azithromycin or clarithromycin therapy is recommended for patients with CD4 cell counts < 50; prophylaxis can be discontinued when the CD4 has increased to > 100 after the initiation of HAART.

M. kansasii is a cause of serious pulmonary disease in AIDS patients with advanced immunodeÞciency. Disseminated M. kansasii infection is an AIDS-deÞning condition, and it occurs as an index AIDS diagnosis in $< 0.5\%$ of cases in highly endemic areas. There are few data on the incidence of pulmonary disease, but it appears to be considerably more common than disseminated infection. In some series, M. kansasii was isolated more frequently than M. tuberculosis in HIV-infected patients.

Of 19 cases of *M. kansasii* infection reviewed at the Johns Hopkins Hospital, 17 patients had pulmonary disease (60). The clinical features and response to therapy was similar to that of patients with pulmonary tuberculosis. The radiographic features included diffuse interstitial or apical inÞltrates, with or without cavities. Thin-walled cavities were common and thought to be an important diagnostic clue in patients with pulmonary disease and advanced HIV infection. No cavities were seen in the 6 patients with pulmonary *M. kansasii* infection in a series at Parkland Hospital (60a). Radiographic Þndings in that series included nodular, interstitial, or diffuse parenchymal inÞltrates, and one patient had a pleural effusion. In patients with HIV infection, *M. kansasii* disease is associated with a greater degree of immunosuppression than tuberculosis. In the Johns Hopkins series, all patients with *M. kansasii* disease had CD4 lymphocyte counts of less than 200 cells/ mm^3, and the median CD4, count was 49 cells/mm^3.

Extrapulmonary disease due to *M. kansasii* also occurs. In the Hopkins series, Þve patients had extrapulmonary infections, and three of whom had concomitant pulmonary infections. Other sites from which *M. kansasii* was isolated included bone, urine, stool, lymph nodes, and blood. In the Parkland series, four of nine patients showed evidence of extrapulmonary dissemination, with isolation from blood, lymph nodes, pleural ßuid, liver, and cerebrospinal ßuid (60a).

A regimen of isoniazid, rifampin, and ethambutol is effective in the treatment of pulmonary *M. kansasii* infection. Many isolates demonstrate *in vitro* resistance to isoniazid, but the clinical signiÞcance of this resistance pattern is not known. The organisms may be susceptible to sulfonamides and the newer macrolides, and clinical responses have been observed in patients treated with TMP-SMX for presumed or concomitant *P. carinii* pneumonia. Therapy with clarithromycin has been suggested for patients who do not respond to standard regimens.

FUNGAL PULMONARY INFECTIONS

In addition to *Pneumocystis carinii* pneumonia, there are several fungal pulmonary infections in HIV infection typically associated with advanced immunodeÞciency. Beginning with inhalation of organisms from the environment, the respiratory tract is the entry site for most fungal infections. For some fungi, such as *Histoplasma capsulatum*, pulmonary disease may occur at the time of primary infection or during reactivation with progressive immunodeÞciency. Treatment of systemic fungal infections in HIV-infected in adults typically consists of an induction phase followed by lifelong maintenance therapy. It is not yet known in all cases whether maintenance therapy can safely be discontinued in a patient with immune reconstitution as a consequence of HAART. In general, antifungal primary prophylaxis is not recommended in patients with advanced HIV infection.

Cryptococcus neoformans

Unlike the endemic mycoses, *Cryptococcus neoformans* is an encapsulated fungus that is widespread in the environment. An immunocompetent host inhales the cryptococcus and, by virtue of the cell-mediated immune system, successfully kills the organism. In HIV-infected persons (typically with CD4 cell counts less than 100 cells/mm^3), the fungus can disseminate widely, most commonly causing subacute meningitis or meningoencephalitis. Pulmonary cryptococcosis typically presents in a chronic or subacute fashion with cough and non-speciÞc symptoms including fever, sweats and fatigue. The chest radiograph most commonly shows diffuse alveolar or interstitial inÞltrates; although nodules, pleural effusions, cavities and lymphadenopathy have also been observed. Diagnosis requires culturing the organism from respiratory specimens using selective fungal media. Histopathologic evidence is also provided by direct visualization of the yeast in clinical specimens with mucicarmine stains. The serum cryptococcal antigen test is usually positive in cryptococcal infections; however, a negative antigen test does not rule out cryptococcal pneumonia. HIV-infected patients with cryptococcosis at any site should have a lumbar puncture to exclude concurrent cryptococcal meningitis.

Treatment of AIDS-associated cryptococcosis with meningitis requires intravenous amphotericin B 0.7Ð1.0 mg/kg daily plus ßucytosine 25 mg/kg every six hours for two weeks, followed by oral ßuconazole 400 mg per day for eight to ten weeks (See Chapter 00). Lifelong suppressive therapy with ßuconazole 200 mg daily is recommended. According to the 2002 USPHS/IDSA guidelines for the prevention of opportunistic infections among HIV-infected persons, discontinuation of secondary prophylaxis may be considered in patients who successfully completed initial therapy for cryptococcosis and remain asymptomatic, and have a sustained increase (e.g. $\geq$6 months) in their CD4 cell count to $>$100Ð200 cells/mm^3 after HAART (16). Some specialists advise a lumbar puncture prior to discontinuing therapy to establish that the cerebrospinal ßuid is culture-negative. In any case, maintenance ßuconazole should be reinitiated if the CD4 cell count falls below 100Ð200 cells/mm^3 (16).

Histoplasma capsulatum

Pulmonary histoplasmosis is common among AIDS patients living in endemic areas, namely the Mississippi and Ohio River valleys. Spores are inhaled into the lung where they convert to the yeast form. Primary infection may be asymptomatic or may present as a mild respiratory illness. Most cases of histoplasmosis in AIDS represent reactivation of previously acquired infection. Disseminated histoplasmosis rarely occurs in immunocompetent persons but is the most common manifestation in HIV-infected persons with advanced immunosuppression.

Clinical manifestations may consist of constitutional symptoms including fever and weight loss. Respiratory symptoms occur in approximately 50% of cases. Chest radiographs are variable and include diffuse nodular inÞltrates, focal inÞltrates, nodules, and cavities. Physical examination may reveal evidence of disseminated disease including lymphadenopathy, mucocutaneous lesions, and hepatosplenomegaly. Laboratory evaluation often reveals pancytopenia, reßecting bone marrow involvement and elevation in liver enzymes. Approximately 10% of patients present with a sepsis-like syndrome with laboratory evidence of disseminated intravascular coagulation or multiorgan failure.

The diagnostic evaluation includes culture of *H. capsulatum* or histopathologic examination of characteristic yeast forms in biopsy specimens or lavage ßuids. The serum and urine *Histoplasma* polysaccharide antigen tests have high sensitivity and speciÞcity in disseminated histoplasmosis. This test is available through MiraVista Diagnostics in Indianapolis, Indiana (317) 407Ð4629.

Treatment of disseminated histoplasmosis in AIDS consists of intravenous amphotericin B 0.7Ð1.0 mg/kg per day for greater than or equal to three to fourteen days. Lifelong suppressive therapy with oral itraconazole is recommended and serum itraconazole levels and drug interactions should be monitored. In cases of mild to moderate histoplasmosis without meningitis, itraconazole may be used as primary therapy (200 mg three times a day for three days, then 200 mg twice daily for twelve weeks, then 200 mg a day for maintenance). At this time, there is insufÞcient data to recommend discontinuation of maintenance therapy following immune reconstitution in response to HAART.

It is recommended that HIV-infected persons avoid activities associated with increased risk of exposure to histoplasmosis, including exploring caves and cleaning chicken coops. A prospective randomized controlled trial demonstrated that itraconazole reduced the frequency of histoplasmosis among patients with advanced HIV infection who lived in an endemic area but provided no survival beneÞt (61). According to the 2002 USPHS/IDSA guidelines, itraconazole prophylaxis for histoplasmosis may be considered in patients with CD4 cell counts less than 100 cell/mm^3 who live in hyperendemic areas (greater than or equal to ten cases per 100 patient-years) or are at high risk of occupational exposures (16).

Coccidioides immitis

Coccidioides immitis is a dimorphic fungus endemic to the semiarid regions of the southwestern United States, including Arizona, New Mexico, southern California and western Texas. Airborne arthroconidia are inhaled, typically resulting in an asymptomatic infection or a self-limited inßuenza-like illness. In HIV-infected patients with impaired cell-mediated immunity, the arthroconidia

develop into multiple endospores within the pulmonary alveoli. These endospores rupture, leading to widespread dissemination. Disease may occur during both primary infection and reactivation from prior exposure. Pulmonary disease is usually present.

The clinical manifestations of coccidioidomycosis are very similar to those of histoplasmosis, including the chronic constitutional symptoms of fever and weight loss. Respiratory symptoms, including cough and dyspnea, are usually present. The chest radiograph typically shows a diffuse reticulonodular inÞltrate but focal inÞltrates, cavities and hilar lymphadenopathy are sometimes present. Physical examination may reveal generalized lymphadenopathy, skin manifestations including nodules or ulcers, and chronic or subacute meningitis.

The diagnostic approach includes culture of sputum, bronchoalveolar lavage ßuids or transbronchial biopsy specimens. Of note, *Coccidioides immitis* is easily transmissible from positive cultures and requires special handling in the microbiology laboratory. Diagnosis is sometimes made by visualization of giant coccidiodal spherules in respiratory secretions or tissue using stains such as methanamine-silver. Coccidioidal serologies may be positive, but false-negatives occur more frequently with advanced immunodeÞciency.

The preferred treatment of coccidioidomycosis in HIV-infected patients with locally severe or extensive disease consists of amphotericin B 1.0 mg/kg per day. Oral ßuconazole 400 to 800 mg daily is preferred for patients with meningitis (62). Intrathecal amphotericin B should be added for coccidioidomycosis meningitis that fails to respond to ßuconazole. Fluconazole 400 mg daily or itraconazole 200 mg twice a day maintenance therapy is required for life (63). Again, there are insufÞcient data to warrant discontinuation of secondary prophylaxis in patients responding to HAART. Prophylactic azole therapy is not routinely recommended.

Aspergillus

Although pulmonary complications from *Aspergillus* are common in other immunocompromised patients, they are relatively infrequent in HIV-infected patients. Most cases of respiratory aspergillosis are caused by *Aspergillus fumigatus*. A nested case-control study within the Pulmonary Complications of HIV Infection Study identiÞed risk factors associated with *Aspergillus* identiÞcation in respiratory specimens. This study conÞrmed that neutropenia, CD4 cell count less than 30 cells/mm^3, chronic corticosteroids, and prior PCP are risk factors for *Aspergillus* respiratory infection (64).

Isolation of aspergillus from respiratory sites may indicate airway colonization and is not synonymous with invasive *Aspergillus* infection. Diagnosis of aspergillosis therefore requires biopsy evidence of tissue invasion. In cases of pulmonary disease, chest radiographs may show diffuse or focal inÞltrates and cavities. Recommended therapy for invasive pulmonary disease is intravenous amphotericin B 0.7Ð1.4 mg/kg daily. Alternative agents include itraconazole, lipid formulations of amphotericin, and caspofungin. In a recent study in non-HIV infected patients with invasive aspergillosis, initial therapy with voriconazole led to better responses and improved survival than initial therapy with amphotericin B (65). Treatment of any predisposing factors (e.g. neutropenia with G-CSF) is also recommended. In the absence of immune reconstitution, the prognosis for invasive aspergillosis is poor.

VIRAL PNEUMONIAS

Cytomegalovirus

Cytomegalovirus (CMV) is a common isolate from respiratory specimens but a rare cause of clinical pneumonia in persons with advanced HIV infection. Patients with CMV pneumonitis typically present with shortness of breath, dyspnea on exertion, a non-productive cough, and hypoxemia. Chest radiographs may show interstitial inÞltrates. Diagnosis of CMV pneumonitis is challenging and generally requires histologic evidence of CMV cytopathic inclusions in lung tissue or BAL macrophages, pulmonary inÞltrates, and the absence of another pathogen. The practical decision to treat CMV pneumonitis is often based on failure to respond to treatment of a copathogen such as *Pneumocystis carinii*. The recommended treatment for CMV pneumonitis is intravenous ganciclovir 5 mg/kg twice a day for at least three weeks. Alternative treatments are foscarnet 60 mg/kg intravenously three times daily or 90 mg/kg twice-daily for the same duration.

Long-term maintenance therapy for CMV pneumonitis is usually not recommended unless there is a relapse or extrapulmonary disease.

Inßuenza

Inßuenza is a common cause of upper respiratory tract infections and bronchitis. Symptoms include fever, mylagias, and a nonproductive cough. Inßuenza pneumonia is rare and HIV-infected adults do not appear to be at increased risk of developing lower respiratory tract involvement. Cases of inßuenza pneumonia may be complicated by bacterial superinfection with *S. pneumoniae*, *S. aureus*, and *H. inßuenzae*. Typical Þndings on chest radiographs include interstitial inÞltrates and bronchopneumonia. Diagnosis can be made by culture of sputum or nasopharyngeal washings, or by a rapid test for inßuenza antigen. Suspected or proven cases should be treated within the Þrst 48 hours of illness. Treatment options for inßuenza A and B include the neuraminidase inhibitors, oseltamivir or zanamivir, or for inßuenza A only, rimantadine or amantadine.

All HIV-infected patients should receive inßuenza vaccination annually, prior to the inßuenza season. An inactivated trivalent inßuenza virus vaccine is administered in one annual dose. Alternative preventive regimens for those patients with hypersensitivity to henÕs eggs (ie precluding inßuenza vaccination) include oseltamivir 75 mg daily for inßuenza A and B or rimantadine/amantadine 100 mg twice daily (or 100 mg daily in patients older than 65 years) for inßuenza A only. A number of studies have demonstrated conßicting results regarding a transient elevation in HIV-plasma viral load in response to inßuenza vaccination. Kolber et. al. evaluated whether a rise in plasma viral load in patients with previously undetectable viral loads could result in the development of new resistance mutations (66). In this study, 34 patients with undetectable viral loads on HAART had repeat plasma viral loads measured at two and four weeks post-vaccination. Seven out of 34 patients had elevations in their viral loads and two of the patients revealed new reverse transcriptase inhibitor or protease inhibitor mutations. Although there is a concern that vaccination may result in low-level viremia and the development of antiretroviral resistance, the same phenomenon may occur with equal or greater frequency during live inßuenza infection.

HIV-ASSOCIATED MALIGNANCIES

KaposiÕs Sarcoma (KS) and non-HodgkinÕs Lymphoma (NHL) are two of the most common HIV-associated malignancies. Patients with disseminated KS and NHL frequently develop pulmonary involvement. In addition, there are case reports of AIDS-related primary pulmonary lymphoma. Some of the tumors, including large cell NHL and KS, have viral associations: Epstein Barr Virus (EBV) and human herpes virus 8 (HHV8) respectively.

KaposiÕs Sarcoma

Pulmonary involvement with KaposiÕs sarcoma generally follows the appearance mucocutaneous lesions and occurs in approximately 20Ð40% of patients with mucocutaneous KS and pulmonary symptoms. The clinical and radiographic presentation is similar to PCP. Symptoms include shortness of breath, dyspnea on exertion, and dry cough, as well as constitutional symptoms such as fevers, night sweats and weight loss. Hemoptysis is a serious potential complication of pulmonary KS. Chest radiograph Þndings include nodular inÞltrates that extend along bronchovascular bundles, interstitial or alveolar inÞltrates, cavitary lesions, hilar adenopathy, and pleural effusions. On Þberoptic bronchoscopy, characteristic red or purple ßat lesions may be seen in the central airways. Biopsies are not routinely performed since the yield is only 20Ð30%, accompanied by a substantial risk of bleeding. The presence of a pulmonary inÞltrate on chest radiograph with a negative gallium scan is highly suggestive of KS.

KaposiÕs sarcoma is associated with human herpes virus 8 (HHV8). HHV8 DNA has been detected in sarcoma tissue and in the bronchoalveolar lavage ßuid of patients with pulmonary KS. A Swiss prospective study determined the speciÞcity, sensitivity, and predictive values of using HHV8 DNA detection in the BAL to diagnose pulmonary KS (67). Seventy-two immunocompromised patients underwent BAL for diagnostic evaluation of fever, respiratory symptoms and/or inÞltrates, including 38 HIV-infected patients. BAL ßuids were analyzed for the presence of HHV8 DNA using a nested polymerase chain reaction technique. On bronchoscopy, four patients had characteristic KS lesions and the corresponding BAL samples were positive for HHV8. One additional BAL sample of an HIV-infected patient with no evidence of KS was HHV8 positive. These results suggest the potential role of HHV8 DNA detection for diagnostic purposes in pulmonary KS.

Systemic chemotherapy is recommended for patients with pulmonary KaposiÕs sarcoma. Treatment options include liposomal daunorubicin or doxorubicin; taxol; or adriamycin, bleomycin and either vincristine or vinblastine. The prognosis is generally poor in patients with advanced HIV infection. Therapy is discussed in more detail elsewhere in this volume.

Non-HodgkinÕs Lymphoma

NHL usually develops at an advanced stage of HIV disease with extensive involvement at extranodal sites. Other common sites of involvement include the gastrointestinal tract, bone marrow, central nervous system, and mucocutaneous sites. Radiographic features may include well-delineated pulmonary masses or nodules, hilar and mediastinal lymphadenopathy, parenchymal inÞltrates, and pleural effusions. Diagnosis generally requires either transthoracic or open lung biopsy. Chemotherapeutic regimens are discussed elsewhere in this volume.

Primary pulmonary lymphoma is rare. In a large French cohort study of 4,700 HIV-infected adults, there were 12 cases of primary pulmonary lymphoma diagnosed between 1986 and 1996 (68). All twelve cases were high-grade NHL and occurred in patients with a CD4 cell count of □200 cells/mm^3. Diagnostic criteria were: (1) histologically proven pulmonary lymphoma; (2) absence of mediastinal and/or hilar adenopathy; and (3) absence of extrathoracic lymphoma. Histologic specimens were obtained by transbronchial biopsy or open lung biopsy. Routine bronchial biopsies were negative. Subacute symptoms included respiratory and constitutional complaints. In contrast to disseminated NHL, LDH levels were rarely and only moderately elevated. All cases of primary pulmonary lymphoma had evidence of latent EBV infection.

LYMPHOCYTIC AND NONSPECIFIC INTERSTITIAL PNEUMONITIS (LIP AND NIP)

Interstitial pneumonias other than PCP are seen in a small proportion of patients with advanced HIV disease. Lymphocytic interstitial pneumonitis is an unusual complication of HIV infection in adults, and is more frequently seen in younger children. It is characterized by reticulonodular pulmonary infiltrates, decreased diffusing capacity, and alveolar lymphocytic hyperplasia. Nonspecific interstitial pneumonitis is seen in adults with clinical features suggesting PCP, though gas exchange defects are usually milder and the CD4 count is often >200. Histologically the syndrome is characterized by mononuclear infiltrates and interstitial edema. The etiology is unknown, and treatment is supportive.

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Chapter 17

Mycobacterial Disease in Patients with HIV Infection

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HIV infection has been one of the principal reasons for the recent increase in incidence of mycobacterial diseases worldwide. Tuberculosis (TB) and HIV have become two of the leading causes of mortality in the world from any single infectious agent. Other non-tuberculous mycobacteria, once considered rare have now become more prevalent. The goal of this chapter is to review important aspects of the interplay of HIV infection with mycobacteria, speciÞcally focusing on features that are clinically relevant.

TUBERCULOSIS AND HIV INFECTION

Understanding TB in patients infected with human HIV is important from many standpoints. First, among tuberculous-infected individuals, immunosuppression because of HIV is the greatest known single risk factor for progression to TB disease (1Ð4). Predictably, the incidence of TB has risen signiÞcantly in areas where HIV and infection with TB is prevalent (i.e. sub-Saharan Africa, parts of tropical Americas, and many urban areas of the United States with large populations of intravenous (IV) drug users and/or socioeconomically depressed minority groups) (1Ð14). Second,TB differs from other HIV-related infections in that it is spread respiratorily from person to person both in normal and immunocompromised hosts. There is, therefore, signiÞcant potential for an aerosol-transmitted disease such as TB to spread rapidly among HIV-infected persons exposed to each other and to/from them to non-HIV infected contacts (e.g. household members, health care personnel). Third, mycobacterial disease in HIV-infected patients often presents with an atypical clinical picture: tuberculin skin anergy is common, the chest radiograph is often atypical, and there is a high incidence of extrapulmonary and disseminated disease, all contributing to confound the diagnosis (1,9,12,14Ð17), which may easily be missed unless these features are appreciated. Thus, there must be a high index of suspicion for TB among persons who are HIV-infected or who belong to Acquired Immune DeÞciency Syndrome (AIDS) risk groups. Fourth, and most importantly, TB is a preventable and curable disease, even in an HIV immunosuppressed population (13,15,18,19).

Controlling TB among HIV-infected populations demands (1) early recognition and preventive treatment for latent tuberculosis infection (LTBI) in persons dually HIV and tuberculous infected (e.g. widespread tuberculin skin testing and preventive therapy programs among HIV risk groups), (2) early recognition and treatment of HIV-infected patients with TB disease (e.g. aggressive TB case Þnding), and (3) strict attention to adherence to anti-TB therapy (often including directly observed therapy). Controlling TB in this population also requires the institution and maintenance of strict administrative and environmental TB control measures in HIV clinics and other physical locations (such as medical wards and health care settings), where HIV immunosuppressed patients at high risk of contracting TB are recurrently exposed to one another (20Ð26). ControllingTB in HIV-infected populations requires dealing with the HIV disease component as well. Efforts must be aimed at prevention of new HIV

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infections through public education, screening the blood supply (which is not universal in some developing countries) (7,8) and through the treatment of HIV disease itself.

Pathogenesis of Tuberculosis

Transmission of Mycobacterium tuberculosis

The likelihood of transmission of *M. tuberculosis* is influenced by the following four factors: (1) the number of organisms entering the air, (2) the concentration of organisms in the air, determined by the size of the space and the adequacy of ventilation, (3) the length of time an exposed person breathes air containing tubercle bacilli, and (4) questionably, the immune status of the exposed individual (26–29). Some believe, though it has not been definitively proven, that HIV-infected persons and others with impaired cell-mediated immunity may be more likely to become infected with *M. tuberculosis* after an exposure than persons with normal immunity. It is well documented, however, that HIV-infected persons and others with impaired cell-mediated immunity are more likely to develop TB disease if they are infected (see below) (27,30–33).

Risk factors for acquiring tuberculosis infection, a prerequisite for the development of disease, are related to the likelihood of contact with a person with active, untreated pulmonary TB disease ("source case"). In the United States, risk factors include: known close contact to an active case of pulmonary tuberculosis, immigration from endemic areas (e.g. Africa, Asia or Latin America), exposure to untreated tuberculosis patients in congregate living facilities (e.g. homeless shelters, correctional facilities, nursing homes or other healthcare facilities), older age, residence in areas with a higher incidence of TB (e.g. inner cities) and travel to foreign endemic areas (34).

Tuberculosis is usually spread person to person, through the air by droplet nuclei, particles 1 to 5 μm in diameter that contain *M. tuberculosis* bacilli (35). *M. tuberculosis* enters the air when patients with active pulmonary tuberculosis disease cough, speak, talk, sneeze, or sing, though coughing remains the most effective method of aerosolization (35–37). Droplet nuclei may also be produced by aerosol treatments, sputum induction, aerosolization during bronchoscopy or through manipulation of lesions during the processing of tissue or secretions in the hospital or laboratory. These droplet nuclei are so small that air currents normally present in an indoor environment can keep them airborne for long periods of time (26,38). Individuals who are in prolonged, close contact with patients with TB disease, especially in environments with poor ventilation are most likely to inhale the organism and become infected with TB (especially in congregate settings such as jails, prisons, shelters and hospitals) (39). However, only approximately one third of all individuals who are in close contact with a person with TB disease for a prolonged period of time become infected (40,41). In countries where TB is endemic, the chances of exposure to a person with TB disease are higher, thus increasing the chances for transmission.

When a person inhales a droplet nucleus, which may contain between 1–400 organisms, it usually is trapped in the upper respiratory tract and then removed by mucociliary clearance (42,43). Organisms deposited on intact mucosa or skin do not invade tissue. Only the smallest droplets, those less than 5 μm, make it to the alveoli.

Response to Inhaled Tubercle Bacilli

Inhaled tubercle bacilli that reach the alveolar spaces are usually ingested by alveolar macrophages, where they may be killed immediately (*natural immunity*) (44,45). If not killed, they may persist or replicate within macrophages, which then may lyse them and infect other alveolar macrophages. From this initial site of deposition in the alveoli, the tubercle bacilli then are transported within macrophages to the regional lymph nodes, and from there may be widely disseminated hematogenously throughout the body. Most primary pulmonary and extrapulmonary lesions remain asymptomatic and are contained due to the development of T-cell immunity (*granulomatous reaction*) during a two- to eight-week period after primary infection. Evidence of primary tuberculosis may be seen on radiographs as a lower lobe pulmonary infiltrate, or later as a focal granuloma which may or may not calcify (Ghon foci) and occasionally with an associated ipsilateral calcified hilar node lesion after dissemination (Ranke complex). Also during this time the tuberculin skin test should become positive, documenting the occurrence of latent tuberculosis infection (LTBI). In general, 5% of persons develop TB disease within the first two years following primary infection, and an additional 5% develop TB disease at some later time during the course of their lives. If a tuberculous-infected person also becomes infected with HIV, they have a much greater likelihood for reactivation TB to occur at some point during the slow progression of HIV-induced immunosuppression. In this situation, the incidence of TB has been shown to be approximately 8% per year in the absence of HIV therapy (46). If a patient is severely HIV immunosuppressed at the time of initial infection with tuberculosis, then rapid progression to TB within weeks to months is expected (19,30–32,47–49).

Macrophages are pivotal in the generation of an immune response against mycobacteria (44,50–53). Macrophages phagocytose and process mycobacterial antigens, which then are presented to antigen-specific T-helper (CD4) lymphocytes within lymph nodes. T-helper lymphocytes with specific receptors recognize the mycobacterial antigens as well as human leukocyte antigen DR locus (HLA-DR or "self") proteins on the macrophage membranes and become activated. T-cell activation also

requires the synthesis and release of interleukin-1 (IL-1), a soluble cytokine, from macrophages. Once T-helper cells are activated, they produce soluble factors (lymphokines) that promote both further clonal T-cell proliferation (IL-2) and increased macrophage antigen presentation, recruitment, and effector reactivity (γ-interferon, macrophage activating factor, macrophage chemotactic factor, and macrophage migration inhibition factor) (54–56).

In response to immunologically competent T lymphocytes in areas of infection with TB, monocytes enter the area and undergo transformation into activated macrophages and subsequently into specialized histiocytic cells, which are organized into granulomas (tubercles). These lymphokine-activated macrophages exhibit increased metabolic and enzymatic activities, including the release of tumor necrosis factor alpha and have an enhanced ability to ingest tubercle bacilli and inhibit their growth. Further multiplication and spread of mycobacteria are usually then arrested within the microscopic granulomas at the initial site of pulmonary infection, in regional lymph nodes, and at distant extrapulmonary sites. A small number of living organisms persist in dormant foci (*latent infection*), often for the life of the patient, and may reactivate at any time if host defenses decline. This cellular immune response not only confers protection against mycobacterial disease but also is intimately linked with cellular hypersensitivity as expressed by a positive tuberculin skin test reaction, formation of tissue granulomas, caseation necrosis, and liquefaction and cavity formation. Although activated macrophages limit replication of mycobacteria, they are also responsible, through the liberation of enzymes, for much of the local tissue damage. Paradoxically, if the immune-mediated tissue damage is too extensive (e.g. caseous necrosis and cavity formation), a favorable environment for the multiplication of mycobacteria may result, leading to classic Þbrocavitary TB in a relatively immune competent host.

Role of HIV

One of the most signiÞcant contributions to the resurgence of TB has been the emergence of HIV (57) and the association between the two organisms which augments each of their deadly potentials. HIV, by destroying the CD4 cells of the hostÕs immune system, allows the TB that may be dormant in the patient to activate and rapidly cause disease. In response to reactivation of TB, the CD4 cells become stimulated and begin to replicate. This activation of CD4 cells further renders these cells vulnerable to infection by HIV and allows the HIV to replicate further within them. This leads to a vicious cycle of increasing viral load causing a further deterioration of the hostÕs immune system and leading to increased vulnerability to infection and neoplasms.

Studies have shown that the one-year mortality rate for HIV-infected patients who have completed therapy for TB ranges from 20–35% despite being cured of TB, and this rate shows little variation between cohorts from industrialized and developing countries.(58–64) Studies have also shown up to a four times greater mortality rate among TB patients who are infected with HIV compared to those who are not coinfected (58,61,64,65). The risk of death for persons with HIV-related TB follows a bimodal distribution, peaking within the Þrst three months of initiation of antituberculosis therapy and then again after one year (62). Although the cause of death in the initial period of therapy may be a result of the TB (61), death after the initial few weeks of antituberculosis therapy usually is attributed to morbidity from the progression of HIV disease, itself enhanced by the TB (60,66,67).

HIV infection causes a profound immune defect by producing a qualitative and progressive quantitative deÞciency of the CD4+ T-lymphocyte population, which in turn results in the impairment of B-cell function, cytotoxic T-cell function, natural killer (NK) cell function and macrophage function, including macrophage antigen presenting capabilities, recruitment, and activation (68–71). The immune defect involving both CD4 cells and macrophages is most crucial in the defense against mycobacterial disease. Without recruitment and activation of macrophages by T-cell secreted lymphokines, there is poor granuloma formation, poor containment of mycobacteria, frequent reactivation of mycobacterial disease with large organism loads, spread to regional lymph nodes, and wide hematogenous dissemination with mycobacteremia. In this setting, caseation necrosis and cavity formation (e.g. in the lung) are less prominent or absent and tuberculin skin tests are often falsely negative. Among persons with mycobacterial disease, the frequency and severity of these features are correlated with the CD4+ peripheral blood count and/or function. As *M. tuberculosis* is more virulent than nontuberculous mycobacteria or other opportunistic pathogens such as *Pneumocystis carinii* (PCP), reactivation TB tends to occur relatively early in the course of HIV immunosuppression and at higher peripheral blood CD4+ lymphocyte counts (e.g. CD4 count often $>200/mm^3$) (66,72–74). There is some evidence both in animals and humans that the ability to generate an immune response to TB has a genetic basis (44,75–79). Therefore, even among HIV-infected populations coinfected with TB, there may be variabilities in the time of onset and severity of TB.

HIV also infects monocytes and macrophages that bear the CD4 surface receptor. These cells are resistant to both the cytolytic and syncytium-forming effects that HIV has on CD4+ lymphocytes. Although not destroyed, the HIV infected monocytes and macrophages are defective in chemotaxis and other cellular functions. These cells may serve as important reservoirs of persistent HIV infection, (80–83). Alveolar macrophages may also be infected with HIV both *in vitro* and *in vivo*. Therefore, it is possible that only a relatively small number of droplet nuclei containing tubercle bacilli may be necessary to infect patients with

HIV infection because of impaired alveolar macrophage function (i.e. impaired natural immunity against inhaled mycobacteria) (68,81,82). If this is true, patients with HIV infection may be at higher risk than HIV-infected persons for becoming infected with TB following exposure to a contagious patient (though this has not yet been deÞnitively shown), in addition to being at higher risk for developing TB disease after infection is established.

In some HIV-infected patients, the plasma HIV RNA level rises substantially with the progression to TB disease, and TB treatment alone may lead to reductions in viral load in these dually infected patients. (84) TB and HIV also interact in the lungs, the site of primary infection with *M. tuberculosis*. In a recently published study of HIV-infected patients with TB, researchers found that the viral load was higher in the bronchoalveolar lavage ßuid from the lung with TB disease versus the other unaffected lung, and correlated with increased levels of tumor necrosis factor in the bronchoalveolar ßuid of the lung with TB disease (85). In addition, the HIV quasispecies from the lung with TB disease differed from those in the plasma of the same patient. These data suggest that pulmonary TB might act as a potent stimulus for the cellular-level replication of HIV, and consequently may contribute to the development of resistant viral strains.

It is still too early to evaluate fully the effects of antiretroviral therapy on tuberculosis in patients infected with HIV. It is hoped that by improving the immunologic status of the host, there may be a reduction in the morbidity and mortality caused by TB. However, given the known potent interactions of HIV and TB and the resultant deleterious effects upon the host, it is of paramount importance to emphasize the need for prevention, early recognition, and effective treatment for both diseases.

Epidemiology of HIV-Related Tuberculosis

In terms of human suffering, the toll exacted by TB on mankind has been and continues to be staggering. Despite the development of an effective cure for this dreadful disease over Þfty years ago, almost eight million new cases arise worldwide each year with as many as three million deaths (86). Almost two billion people, one third of the worldÕs population, are infected with *M. tuberculosis,* and it is from this vast pool of individuals harboring latent infection that most new cases arise.

Tuberculosis has always been a disease that disproportionately affects the poor and disenfranchised, and in the early twenty-Þrst century the developing world is home to the majority of these people. Over 90% of TB cases and 95% of deaths from TB (87) occur in developing countries. Although poverty, attendant malnutrition and lack of health care services certainly are important factors in contributing to the extent of TB in the developing world, something even more insidious has become the preeminent driving force: HIV.

In the centuries-long struggle between man and the tubercle bacillus, nothing has changed the epidemiology of TB as much as HIV. In just twenty years, HIV has caused the reversal of hard earned downward rates of TB in many developed and developing countries. In some countries, health status indices of large segments of the population have been set back by thirty years.

As TB disease can complicate HIV at any level of immune suppression, rates of TB tend to increase shortly after the introduction of HIV into a population where latent TB infection is common. The overlap of these two pandemics is most pronounced in the developing world, but some sub-populations in developed countries are also involved.

Incidence of HIV/TB

The incidence of HIV-related TB depends upon the amount of overlap of the two epidemics in a given population. In the United States the degree of overlap is uncertain, as information regarding HIV infection among TB patients is not always reported. In order to overcome incomplete reporting, health departments from all Þfty states, plus New York City and Puerto Rico, worked with the Centers for Disease Control and Prevention (CDC) to cross-match TB cases from 1993Ð1994 with their respective AIDS/HIV registries (88). The analysis found that, overall, 14% (6,863) of the 49,938 cases of TB reported during that time period were found in both registries. There was a marked disparity of HIV-infection rates among TB patients found between reporting areas with a range of 0% to 31% of TB cases being concomitantly listed on AIDS/HIV registries. Six states (California, Texas, Florida, Illinois, Georgia and New Jersey) and New York City accounted for 80% of all TB/HIV cases reported during 1993Ð1994 (88). Although HIV has been implicated as a major reason for the recent resurgence of TB in the United States, the relative contribution of HIV to the TB epidemic depends, as mentioned above, on geographic location. In a multi-center cohort study, Markowitz et al. found a marked geographic discrepancy in the incidence of HIV-related TB. Residence in the eastern United States was the strongest demographic risk factor. The authors found a higher percentage of positive PPDs at baseline (12% in the east versus 3.5% in other locations) and a higher PPD conversion rate among eastern U.S. patients with HIV, which suggested a higher degree of exposure to TB in the east (89).

Epidemiological Trend in the United States

Some of the highest rates of TB among HIV-infected patients were recorded at a time when TB rates were substantially higher than they are at the time of this writing. Since a signiÞcant proportion of cases of TB

among HIV-infected individuals are the result of recent transmission (90,91), the rates and percentage of cases among HIV-infected individuals would be expected to drop as TB services improve and infectious individuals receive proper, prompt treatment. However, certain demographic groups such as the homeless and drug abusers still have higher rates of co-infection and disease. The foreign-born, a well-described risk group for TB, is one that is relatively under-represented among demographic groups with HIV/TB. Our experience in Florida is one exception where certain Haitian groups have very high rates of co-infection (92). With regard to the homeless, a study (91) in San Francisco found the incidence of TB to be 270 per 100,000 persons. The HIV-infected PPD positive individuals in this prospectively followed cohort had a TB incidence rate of 4.5% per person-year. Eighty-eight per cent of the HIV seropositive individuals had clustered restriction fragment length polymorphisms of the *M. tuberculosis* isolates indicating recent transmission.

Despite the persistence of HIV related TB in increasingly well-deÞned demographic groups, TB rates have been declining in the United States for most of the last decade. The major contributing factor to this has been the improvement of TB services around the country after years of neglect. Highly active antiretroviral theropy (HAART) promises to reduce TB related morbidity further among HIV-infected individuals. Although TB is unusual among opportunistic infections in that it can occur at any level of immune suppression, the incidence is greatest at lower CD4 levels (57,89). Reduction in viral loads and improved T-cell counts should result in lower rates of TB disease in this population. Although prospective data are scarce, data from Europe indicates that HAART is at least partially responsible for the decrease in HIV/TB rates now being seen in industrialized countries (93).

Global Aspects of the HIV/TB Epidemic

Although the HIV epidemic has left an indelible mark on the epidemiology of TB in developed countries, the convergence of these two epidemics has been most devastating in the developing world. Rates of TB around the world are illustrated in Fig. 17.1 (86). The developing world is home to the vast majority of individuals with latent TB infection, and as HIV spreads in these populations, TB rates climb inexorably. Nowhere is this more evident than in sub-Saharan Africa. Global regional TB and HIV co-infection information is shown in Table 17.1 (86).

Even before the AIDS epidemic, TB was a major cause of morbidity and mortality in Africa with a case rate that in some places exceeded 200 per 100,000 (94). This Þgure was over twenty times that found in the United States at that time. In little over a decade, HIV seropositivity rates among TB patients in sub-Saharan Africa rose from 20% to 70% (95), with a 300Ð400% increase in the number of

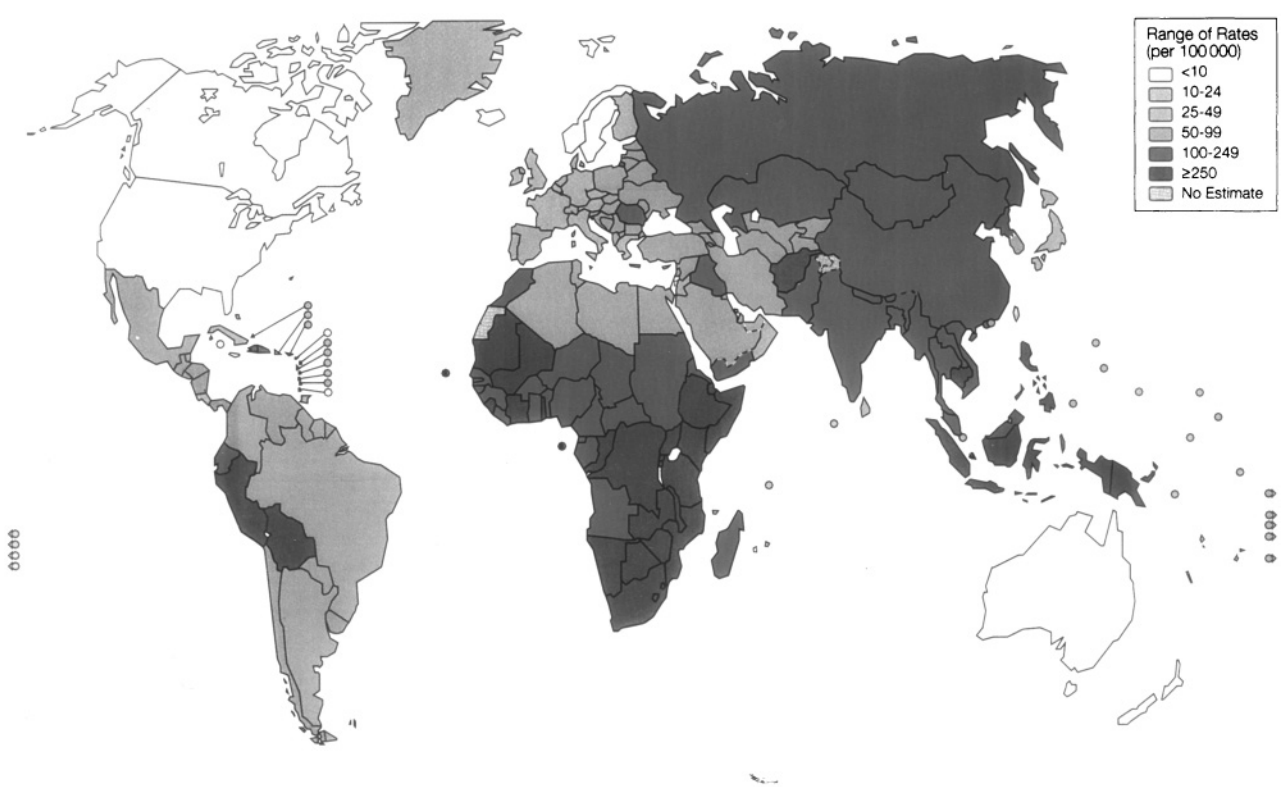

FIG. 17.1. Estimated per capita incidence rates of tuberculosis (all forms) by country in 1997 (86). Reprinted with permission form JAMA.

TABLE 17.1. *Estimates of TB burden in the 22 highest-incidence countries**

Rank	Country	WHO Region	Population Thousands	Rates†										
				Incidence	SS + Incidence	Prevalence	SS + Prevalence	Prevalence, %	Infection Death Rate	TB CFR %	Cases, %	HIV-Positive TB/ HIV	CDR All %	CDR SS + %
1	India	SEAR	960,178	187	84	505	227	44	46	24	3	188	63	34
2	China	WPR	1,243,738	113	51	219	91	36	21	18	0	12	30	30
3	Indonesia	SEAR	204,323	285	128	786	350	49	68	24	1	12	4	7
4	Bangladesh	SEAR	122,013	246	111	508	221	46	55	23	0	8	21	25
5	Pakistan	EMR	143,831	181	81	405	180	40	44	25	1	18	0	0
6	Nigeria	AFR	118,369	214	93	383	166	36	58	27	14	702	7	10
7	Philippines	WPR	70,724	314	141	693	310	47	68	22	0	16	94	83
8	South Africa	AFR	43,336	392	159	604	263	38	166	42	45	2,540	62	80
9	Russian Federation	EUR	147,708	106	48	163	73	18	17	16	1	5	78	60
10	Ethiopia	AFR	60,148	260	109	367	161	36	82	31	30	1,543	37	24
11	Vietnam	WPR	76,548	189	85	289	102	44	26	14	1	50	59	82
12	Democratic Republic of China	AFR	48,040	269	114	397	175	36	81	30	25	706	0	0
13	Brazil	AMR	163,132	75	33	115	51	25	11	15	5	91	68	80
14	Tanzania	AFR	31,507	308	127	396	173	23	99	32	37	1,026	48	55
15	Kenya	AFR	28,414	297	122	371	161	36	99	33	40	2,013	47	55
16	Thailand	SEAR	59,159	142	63	305	135	43	29	21	10	561	36	36
17	Myanmar	SEAR	46,765	171	77	348	146	41	40	24	5	384	21	27
18	Afghanistan	EMR	22,132	333	150	753	342	34	104	31	5	0	2	2
19	Uganda	AFR	20,791	320	128	451	195	34	146	46	50	1,532	42	65
20	Peru	AMR	24,367	365	119	288	129	44	30	11	2	131	65	95
21	Zimbabwe	AFR	11,682	538	207	626	264	36	283	53	65	4,603	70	60
22	Cambodia	WPR	10,516	539	241	963	426	64	90	17	3	792	28	50
		Total	3,657,421	174	77	375	164	39	41	24	7	213	41	34

* These countries are ranked by number of cases and are positioned in a list of the 22 highest-incidence countries on the basis of numbers of new cases. TB indicates tuberculosis; WHO, World Health Organization; incidence, new cases; SS + , sputum smear-positive; prevalence, all forms new and existing; infection prevalence, percentage of population infected with *Mycobacterium tuberculosis* (MTB); CFR, case fatality rate among TB cases; TB/H1V, MTB/H1V co-infection; CDR all, all forms TB case detection rate (all-forms TB case noti cation/ estimated all forms incident TB); CDR SS + , smear positive case detection rate (SS + case noti cations/ estimated SS + incident TB); SEAR, Southeast Asian region; WPR, Western Paci c region; EMR, Eastern Mediterranean region; AFR, African region; EUR, European region; and AMR, American region.

patients developing TB (96). One study found that approximately one-third of patients diagnosed with HIV had TB disease at presentation (97). High rates of HIV and TB co-infection have also been observed among the children of AIDS patients. Bhat et al. showed that the co-infection rate among children may range from 10–40% (98). Even in areas with very efficient TB programs, rates of TB continue to rise in the wake of the HIV epidemic. Tanzania, with a model TB program, saw a doubling of TB cases in the early 1990s with two-thirds of the increase in smear positive cases being directly attributable to HIV infection (99). Mortality rates among HIV/TB patients is exceptionally high with almost half of all patients dead within 2.5 years, double the rate of non-HIV infected TB patients (100).

The impact of HIV in Asia has been less when compared to Africa, but it is expected to increase. This is of great concern because 60% of individuals in the world with latent TB infection live in Asia (101,102). Also of concern is the possibility that a third wave of TB consisting of multi-drug resistant TB may emerge with even greater force in this region because of significant rates of TB drug resistance in Asia.

Of the nearly five million cases of TB in Asia that occurred in 1990, only 85,000 of them were felt to be related to HIV (103).

Perhaps more significantly, in a hospital at the National AIDS Reasearch Institute in Pune, India, researchers found that the HIV seroprevalence rate among newly diagnosed TB patients rose from 3.2% in 1991 to over 20% in 1996 (104). Rates of HIV-related TB vary throughout the Indian subcontinent, with co-infection rates of 2–20% (104,105). Perhaps more significantly, in a hospital at the National AIDS Research Institute in Pune, India, researchers found that HIV seroprevalence rate among newly diagnosed TB patients rose from 3.2% in 1991 to over 20% in 1996 (104).

Similarly in Chiang Rai, Thailand, the seroprevalence rate among TB patients was 1.5% in 1990 compared with 45.5% in 1994 (107). Due to the high background rate of latent TB infection, TB has quickly become the most common HIV-related opportunistic infection in some Asian countries (108,109).

While countries in Europe have had variable rates of HIV/TB co-infection, Russia is on the verge of an epidemic of TB/HIV that could potentially compromise worldwide efforts to control the disease. Although Russian prisons have been at the epicenter of their emerging epidemic for some years, it is now clear that there is a parallel epidemic in the civilian population as well. National figures in Russia from 1997 indicated TB notification rates for incarcerated individuals and the civilian population of 4,000 and 81.3 per 100,000 respectively (110). HIV is not as yet a major contributing force to the epidemic in Russia; however, data indicate that HIV is spreading rapidly among intravenous drug users. In the first quarter of 2000, the number of newly HIV-infected individuals in Russia increased 4.6-fold over the same time period in 1999 (111).

Throughout Latin America and the Caribbean, accurate assessment of the extent of HIV/TB is hampered by incomplete reporting of HIV status (112). Co-infection rates range from less than 2% (113) to over 50% (114). Studies utilizing restriction fragment length polymorphism analysis have shown that in areas endemic for TB, most cases of TB disease are the result of reactivation of latent disease (115–117). Ferazoli and colleagues working in Brazil found that 38% of HIV positive and 25% of HIV negative TB cases had strains that appeared in clusters indicating recent transmission (115). Other workers have utilized molecular techniques to document nosocomial outbreaks (23).

Future Epidemiologic Issues of HIV/TB

The epidemiology of HIV/TB is rapidly evolving as HIV continues its march across the globe. The impact of HAART on the course of the converged epidemics in developed countries with access to these medications is unknown, but it is likely the impact will be favorable. Whether or not these drugs will become accessible to developing nations is currently an issue of intense debate. Furthermore, TB is often the sentinel infection of AIDS (even in developed countries) and appears before HAART is even considered. In developing countries, an important factor will be whether or not HIV transmission of HIV can be controlled through behavior modification education as has been reported in some areas (118,119). This is particularly relevant in areas such as Asia and Russia with higher levels of TB drug resistance and more crowded urban settings that would further foster the spread of TB. With the prospects of an effective vaccine for TB and HIV being at best many years away (120,121), action is necessary before TB and HIV become irrevocably entrenched and TB becomes untreatable because of multidrug resistance.

Clinical and Diagnostic Aspects of Tuberculosis

General

Tuberculosis disease develop at any level of CD4 count and not uncommonly heralds the diagnosis of HIV infection itself (66,74,122–126). Symptoms of TB in HIV-seropositive patients are nonspecific, commonly occur in other conditions associated with AIDS, and often are present for many weeks before the diagnosis of TB is made or even considered. In contrast to pyogenic pneumonias in which the onset is abrupt over one or two days, the onset of TB is frequently insidious. Patients often present with constitutional symptoms of fever, night sweats, fatigue, malaise, and weight loss. Patients with pulmonary

TB may have cough, sputum production, hemoptysis, pleuritic chest pain, or dyspnea. Signs and symptoms of extrapulmonary or disseminated TB depend on the organ and tissue affected (e.g. regional or generalized lymphadenopathy, hepatosplenomegaly, or CNS abnormalities).

M. tuberculosis is a more virulent organism than most other opportunistic infections that deÞne AIDS, and among tuberculous-infected patients, reactivation TB would be expected to occur at an earlier stage of HIV-induced immunosuppression, at a rate up to 8% per year (46). Since TB may be a sentinel infection in patients with HIV disease, HIV serology should always be obtained when TB is diagnosed or suspected and inquiries about clinical signs and symptoms of HIV infection should be elicited (e.g. persistent fever, chronic diarrhea, weight loss) (34).

Conversely, as both the general systemic and local organ symptoms of tuberculosis are nonspeciÞc and commonly occur in other HIV (and non-HIV) related conditions, there should be a high index of suspicion for TB among patients with known HIV infection or in those at high risk for HIV infection such as intravenous drug users. Clinicians should routinely screen for signs and symptoms of tuberculosis in patients with HIV infection, including routine, periodic tuberculin skin test (TST)s. (19,127). There is increasing evidence that patients with severe immunosuppression, and false negative TSTs, may regain their ability to react on TST when started on effective antiretroviral therapy, as part of an immune reconstitution. Thus skin testing previously anergic patients after successful HAART may be a useful diagnostic tool (19,128).

In the past, conventional theory held that TB disease was generally a result of reactivation (129,130) of a previous infection. While that still may hold true, especially in patients with higher CD4 counts, recent studies have shown that a substantial proportion of cases of tuberculosis among HIV-infected patients may be a result of recent exposure and infection (31,90,131Ð135). Using restriction-fragment-length-polymorphism analysis, it was found that nearly one-third of all TB cases in San Francisco reported during 1991 and 1992 were the result of recent infection and, among other factors, this was associated with a diagnosis of AIDS. Furthermore, few of these cases were identiÞed by conventional epidemiologic contact tracing (131).

The clinician should also bear in mind that tuberculosis, whether pulmonary or extrapulmonary, may present concurrently with one or more other opportunistic infections or tumors, either in the same or different organ or tissue, Therefore, clinical specimens should be sent for mycobacteriological testing regardless of other clinical diagnoses or suspicions.

Pulmonary Tuberculosis

Symptoms of pulmonary tuberculosis in the HIV-coinfected patient may run the gamut from those typically associated with pulmonary disease to atypical, nonspeciÞc presentations. Patients may present with any or all of the ÔclassicÕ constitutional symptoms of tuberculosis, such as fever, night sweats, fatigue, weight loss and malaise. These symptoms are not pathognomonic for tuberculosis, and may be associated with a number of HIV-related conditions; however, their occurrence should raise the suspicion for tuberculosis in the differential diagnosis. These constitutional symptoms are rarely of the acute nature seen with other bacterial, fungal or viral infections, and may be present for weeks or months prior to the diagnosis.

Pulmonary involvement occurs in 70Ð93% of HIV-infected patients with TB (34). Pulmonary-related symptoms of TB might be cough, sputum production, pleuritic chest pain, hemoptysis or dyspnea. In the absence of severe immunodeÞciency, deÞned by a CD4 count of $< 200/mm^3$, the radiographic picture may resemble that of an HIV negative patient (136Ð138), displaying inÞltrates (often upper lobe, consistent with reactivation) and even cavities. However, more commonly in advanced HIV disease, the chest radiograph is atypical (16,136Ð140). Hilar and mediastinal lymphadenopathy, paratracheal lymphadenopathy, lower lobe inÞltrates, and diffuse alveolar, linear, or miliary inÞltrates are relatively common in more severely immunosuppressed patients with HIV infection. Severe immune deÞciency often leads to a chest radiographic pattern resembling primary TB (i.e. hilar or mediastinal lymphadenopathy with or without non-cavitating pulmonary inÞltrates (Figs. 17.2 and 17.3). Unilateral or bilateral pleural effusions may occur. An enlarged cardiac silhouette should raise the possibility of tuberculous pericarditis and/or pericardial effusion. With appropriate treatment, the radiographs tend to clear. When they worsen, other pulmonary infections or etiologies should be pursued (141). However, some authorites have noted a transient worsening in chest radiographs in TB patients on appropriate TB therapy, after the initiation of antiretroviral therapy. Diagnostic evaluations in these patients may not reveal another etiology, and it is thought that these Þndings are due to immune reconstitution with an inßammatory response, which causes the new radiographic Þndings. (See section on ÒParadoxical Worsening of TB After Initiation of HAARTÓ) (142).

Extrapulmonary Tuberculosis

Extrapulmonary disease occurs in 34% to 72% of HIV-infected tuberculous patients (143Ð148), with lymphatic and disseminated forms (miliary TB or two or more noncontiguous tuberculous sites) predominating. Bone marrow, gastrointestinal tract, and the CNS are also common extrapulmonary sites among HIV-immunosuppressed patients, but TB can affect any organ system in the body and unusual clinical presentations occur frequently in this setting (66,125). The rate of disseminated tuberculosis is high in HIV coinfected patients, and clinicians

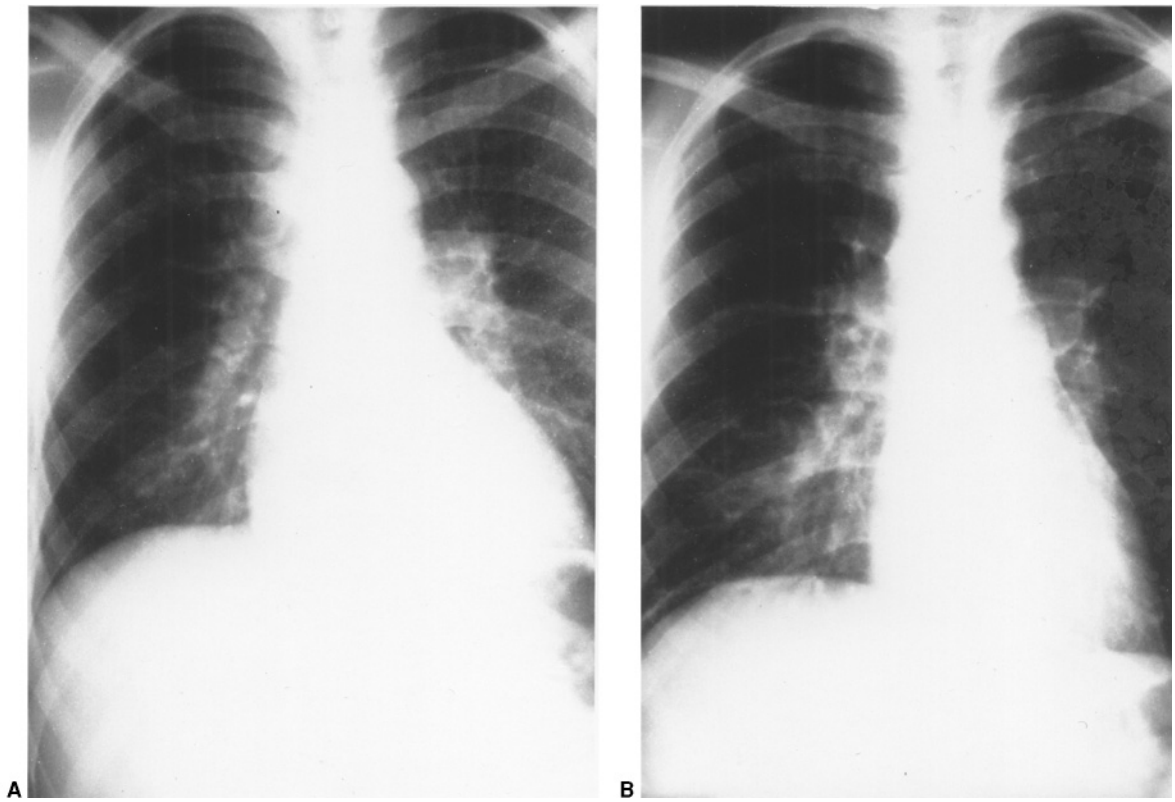

FIG. 17.2. A: Chest radiograph in a patient with tuberculosis and HIV infection. There is right paratracheal adenopathy (arrow) with clear lung fields. Sputum culture was positive for *M. tuberculosis*. B: The radiograph abnormality resolved (as did the patient's symptoms) following several months of antituberculosis drug treatment. (From ref. 1, with permission.)

should remember that pulmonary and extrapulmonary TB often present in the same patient, especially in those with advanced immunodeficiency.

Patients with extrapulmonary TB may present with the same constitutional symptoms of fever, night sweats, fatigue, weight loss or malaise as found in pulmonary tuberculosis and/or with more specific signs or symptoms related to the organs or systems involved (e.g. regional or generalized lymphadenopathy, hepatosplenomegaly or CNS abnormalities). There may in addition be symptoms of pulmonary TB along with those of extrapulmonary disease.

Diagnosis

A high index of suspicion for TB and an aggressive diagnostic approach are required to avoid missing a contagious and highly treatable disease.

Both the generalized symptoms of TB (fatigue, malaise, weight loss, fever, and night sweats) and the local symptoms of TB (cough or other local symptoms, depending on the particular organ affected) are nonspecific. In patients with known or suspected HIV infection, there should be a particularly high index of suspicion for TB in intravenous drug users, individuals born in countries with a high prevalence of tuberculosis, persons with a known past history of a significant tuberculin skin test reaction, patients with chest radiographs suggestive of old TB (e.g. apical scarring), and persons with a history of recent TB exposure (149).

Conversely, in patients with proven TB, the physician should have a high index of suspicion for concomitant HIV infection if the patient is in an AIDS risk group, has "unusual" or extrapulmonary TB (especially disseminated and lymphatic), or has any symptoms or signs that are common in HIV infection but uncommon in TB (e.g. unexplained persistent diarrhea, thrush, hairy leukoplakia of the tongue, dysphagia, or generalized lymphadenopathy).

Pulmonary Tuberculosis

Once the suspicion of tuberculosis is raised, an evaluation should be begun immediately and infection control procedures put in place. It should be emphasized that recent studies have well documented transmission of TB in healthcare settings (e.g. HIV clinics) (21,23–25, 30,33,48,150,151). If the patient is in a hospital, jail, prison or other congregate facility, s/he should immediately be placed in respiratory isolation in a negative pressure room, a chest radiograph should be obtained, if not already done, and sputum sent for mycobacteriological

testing (acid fast bacilli (AFB) smear and culture, and nucleic acid amplification (NAA) testing) (see Bacteriology Section). It should be noted that diognostic testing for tuberculosis does not have to be performed as an inpatient. In situations where the patient is living in a "safe" environment, (nobody in the household is immunosuppressed (e.g. with HIV infection or under the age of one year old)), testing can proceed as an outpatient as long as the patient understands the importance of following infection control precautions (e.g. staying in the household until being told it is safe to leave). If adequate specimens cannot be obtained, sputums may be induced (using either aerosolized hypertonic or hypotonic saline) in a negative pressure or outdoor environment. Comprehensive respiratory infection control procedures should be in place in any area that employs cough-inducing procedures (e.g. bronchoscopy, sputum induction) (see Environmental Control Section) (152).

If sputum is not obtainable, and there is evidence of pulmonary parenchymal disease (either by abnormal chest radiograph, CT or other means), then sputum collection via fiberoptic bronchcoscopy with bronchoalveolar lavage and possible transbronchial biopsy may be indicated. The specimens should again be sent for AFB smear, culture and NAA testing. Sputum induction may be as effective in making the diagnosis of tuberculosis as bronchoscopy, but if other etiologies such as malignancy are suspected, bronchoscopy may have greater utility (153–157). Smears and cultures of (post)-bronchcoscopy sputums and lavage fluid may be positive for TB bacilli even when the chest radiograph appears "normal" (16,158). Mediastinal lymph nodes may be biopsied during bronchcoscopy or at mediastinoscopy, when indicated.

CT scan results will vary depending on the degree of immunosuppression. Less immunosuppressed patients may show "classic" upper lobe densities and nodules, with or without cavitation, while patients with advanced HIV disease may only show hilar, mediastinal and/ or paratracheal lymphadenopathy. Mediastinal gas may be seen on CT scans from fistulas between tuberculous mediastinal lymph nodes and the esophagus or tracheobronchial tree (159,160).

In patients with pleural effusions, either unilateral or bilateral, a thoracocentesis with or without pleural biopsy may be indicated (obtaining pleural tissue has been shown to increase the yield of diagnosis to approximately 80–90% as opposed to approximately 50–60% with pleural fluid culture alone (161,162)). It should be noted

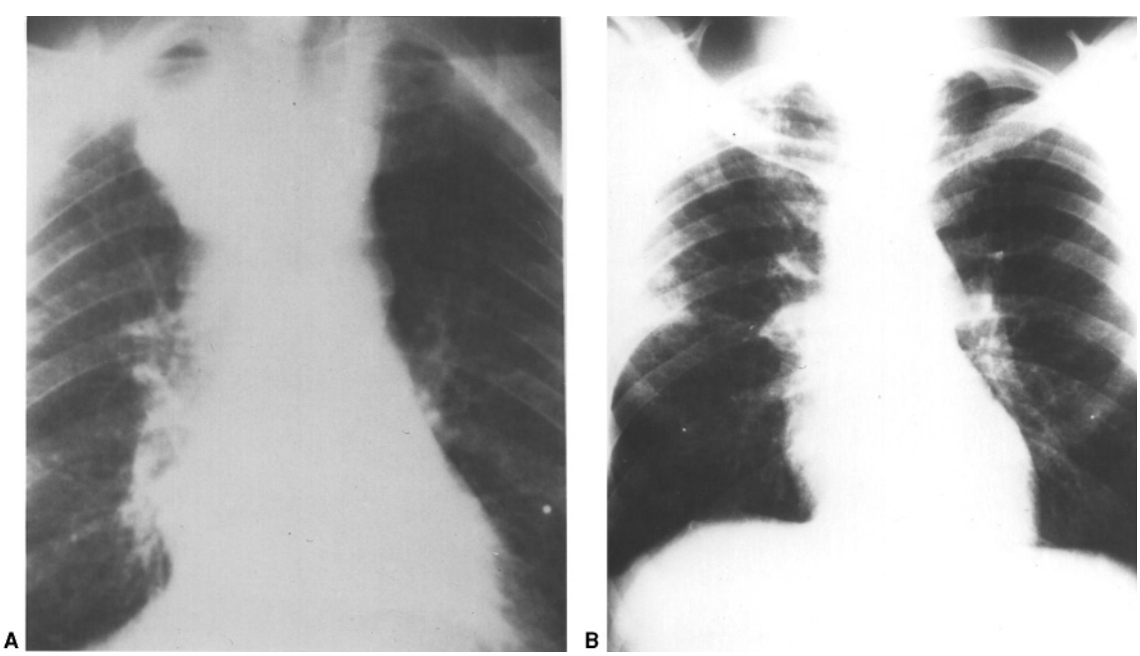

FIG. 17.3. A: Chest radiograph in a patient with tuberculosis and HIV infection. There is right paratracheal adenopathy with clear lung fields. The patient complained of dysphagia. Esophagoscopy with biopsy revealed granulomatous esophogitis with a positive tissue culture for *M tuberculosis*. A CT scan (not shown) revealed air in the mediastinum; an esophogram (not shown) revealed extravasation of barium, consistent with a tuberculous mediastinal lymph node-esophogeal fistula. The patient's symptoms and radiographic abnormalities resolved following anti-tuberculous drug treatment. (The CT scan and esophogram were previously reproduced elsewhere (1).) B: Chest radiograph in a patient with tuberculosis and HIV infection. Right paratracheal adenopathy and a right upper lobe infiltrate are present. Sputum culture was positive for *M. tuberculosis* and the patient responded to anti-tuberculous drug treatment. Although the chest radiograph resembles primary tuberculosis (i.e. noncavitary pulmonary infiltrate with ipsilateral hilar lymphadenopathy), the patient was documented to have a strongly positive PPD-tuberculin skin reaction several years previously. (He never received therapy for latent tuberculosis infection.) The chest radiograph therefore probably represents an atypical pattern of reactivation TB due to HIV-induced immunosuppression. (Part (A) from ref. 1, with permission; part (B) from Pitchenik AE, Fertel D. Tuberculosis and nontuberculous mycobacterial disease in HIV infected patients. *Med Clin North Am* 1992;76:121–171, with permission.)

however that the usual pleural ßuid Þndings of lymphocytic predominance with a lack of mesothelial cells may not always be present (163Ð166).

It should be remembered, however, that approximately 10% of the cases of pulmonary tuberculosis will turn out to be culture negative; e.g. the patient has clinical evidence of TB (symptoms, chest radiograph Þndings) and negative laboratory results (e.g. smear, culture), but has a signiÞcant clinical response to anti-TB therapy. These patients should still be considered to be cases of tuberculosis (27,167).

Patients may also present with another pulmonary infection such as PCP, with diffuse linear interstitial lung inÞltrates, which may obscure the radiographic diagnosis of concurrent pulmonary TB (168,169). Intrathoracic lymphadenopathy, diffuse miliary inÞltrates, and pleural effusions are chest radiographic features that are diagnostically helpful when they occur in the setting of HIV disease as they are typical of TB and highly atypical for PCP. Classic tuberculous upper lobe inÞltrates and cavitary lesions are also diagnostically helpful when they occur but are uncommon. Poor T-cell immune response in HIV immunosuppressed patients explains the reduced frequency of tuberculous-induced cavity formation and Þbrosis and the increased frequency of miliary inÞltrates and intrathoracic and extra thoracic lymphadenopathy representing regional and distant spread of tubercle bacilli.

Extrapulmonary Tuberculosis

Diagnosing extrapulmonary tuberculosis requires a high index of suspicion. In suspected disseminated tuberculosis, especially in advanced HIV disease, specimens from blood, urine, stool, bone marrow and liver biopsy may be sent for mycobacterial testing, when clinically indicated. Generally, *M. tuberculosis* is not cultured from blood unless the CD4 count is below 200 cells/mm^3 (170Ð173). The best yield from urine is the Þrst morning specimen, when urine and sediment have been collecting overnight (174,175). While stool cultures may reßect local disease, they may also yield positive results from sputum swallowed into the GI tract, and a pulmonary site should not be overlooked (176).

When there is evidence of local, or organ speciÞc disease, specimens should be obtained from these sites, (e.g. cerebrospinal ßuid, urine, pleural ßuid, bone marrow, liver, superÞcial cervical, intrathoracic and intraabdominal lymph nodes, pericardium and pericardial ßuid, brain, stool, seminal ßuid, skin, soft tissues, abscesses) and sent for testing. It may be prudent to remind the clinician performing the tissue biopsy not to place the specimen into formalin until tissue has been obtained for all cultures, AFB and otherwise. Formalin will preclude culture growth, but if this has already been done and repeat specimens are impossible, check with the laboratory about the possibility of NAA testing in order to attempt to conÞrm the diagnosis.

Aside from AFB results, analysis of certain ßuid specimens may give added information. CSF in suspected tuberculomas and tuberculous brain abscesses may show a pattern typical of tuberculous meningitis with pleocytosis, increased protein and decreased glucose, or may have only minor or no abnormalities (3,177). TB meningitis may present with typical CSF Þndings, or show minor or nonspeciÞc abnormalities, the latter usually in severely immunosuppressed patients. Pleural ßuid may show typical Þndings of an exudate with lymphocytic predominance and no or rare mesothelial cells (178,179). Recent studies, however, have shown that tuberculous pleural ßuid in HIV-infected patients may contain signiÞcantly fewer monocytes (180) and have higher mesothelial cell counts than in HIV uninfected patients (163).

Certain imaging studies, such as CT scans of the head, chest, spine and abdomen, MRIs of the brain, spinal cord and soft tissues, and gallium scans may help in localizing and deÞning the extent of TB disease and in directing biopsies (1,159,160,177,181Ð188). On abdominal CT scans, the presence of focal lesions in the liver, spleen, kidneys, pancreas, gastrointestinal tract or other viscera and enlarged lymph nodes with central or diffuse low attenuation suggests disseminated mycobacterial disease, particularly TB (189).

Although generalized lymphadenopathy is common in HIV disease, usually associated with HIV-related lymphoid hyperplasia, neither hilar nor mediastinal lymphadenopathy is common for HIV related lymphoid hyperplasia alone. The likelihood that a patientÕs lymphadenopathy represents a mycobacterial or another speciÞc disease increases markedly when chest concomitant radiographic abnormalities or fever are present, or when lymph nodes are tender, ßuctuant, matted, disproportionately large, or growing in a regional area (e.g. the neck or the abdomen). A lymph node aspiration may be done, followed, if necessary, by an excisional biopsy. Incision and drainage of a ßuctuant suspected TB lymph node is generally not recommended as it may lead to Þstula formation with ongoing drainage. Granulomas are usually seen on biopsy and acid-fast smears positive in 67Ð90% of cases (72,190Ð192). When hilar or mediastinal lymphadenopathy is present, neoplastic, fungal and especially mycobacterial disease should be considered.

The presence of increased adenosine deaminase activity (ADA) (an enzyme found at the cell surface of lymphocytes and macrophages that has been found to be especially elevated in ßuid which contains lymphocytes that have been activated by TB), has been reported as a useful (but at times non-speciÞc) test in the diagnosis of TB in certain body ßuids, such as cesetrospinal ßuid (193,194), and possibly pleural effusions. It also may be helpful in diagnosing TB in patients with HIV infection (195Ð200).

Bacteriology

There are conßicting reports about whether HIV-infected patients with culture-proven pulmonary TB are less likely to have positive AFB sputum smears than non-HIV infected patients with pulmonary TB. The AFB smear has been shown to be positive in 44% to 65% of HIV-infected patients (60,72,201,202). The sensitivity of acid-fast sputum smears tends to be greater in HIV-infected TB patients who are less immunosuppressed and have chest radiographs typical of reactivation TB compared with those who are more severely immunodeÞcient and have chest radiographs typical of primary or miliary TB (201Ð203). In New York City, among Þrst-time diagnosed patients with AFB positive sputum smears, only 50% are caused by MTB and 50% caused by non-tuberculous mycobacteria (NTM) (204). In patient populations with substantial smear-positivity caused by NTM, it is necessary to rapidly differentiate between TB and NTM in order to ensure proper case management and save scarce healthcare resources (i.e. utilization of negative pressure rooms and initiation of contact investigations).

Culture techniques are regularly utilized to diagnose mycobacterial infections. Recovery and identiÞcation of mycobacteria from clinical specimens and the availability of drug susceptibility results are signiÞcantly more rapid by use of BACTEC 460TB radiometric broth (BD Biosciences, Inc. Sparks, MD) (205Ð207). A combination of broth and solid medium (i.e. egg-based Lowenstein Jensen or agar-based Middlebrook 7H 10), in addition to acid-fast stains, provides a rapid and sensitive method for detecting mycobacteria. The current commercially available continuous-monitoring systems, such as the BACTEC 900MB and BACTEC MGIT (BD Biosciences, Inc.) (208), MB/BacT (Organon-Teknica, Inc., Durham, NC) (209), and ESPII (Accumed, Inc., Chicago, IL) (210) allow for more rapid turnaround time.

Nucleic acid probe kits have provided a major advance in the rapid identiÞcation of *M. tuberculosis* complex, *M. avium* complex, *M. avium, M. intracellulare, M. kansasii*, and *M. gordonae* from culture inasmuch as results are available within two hours (211). Probe technology has a sensitivity and speciÞcity of nearly 100% (212) when at least 10^5 organisms are present. Thus, these probes are not sensitive enough to be used on sputum; more helpful are NAA tests (see below).

Susceptibility testing is recommended on all initial isolates of *M. tuberculosis* from patients. (27) Results of susceptibility testing utilizing liquid broth methods may take one to two additional weeks after culture results are available and at times longer when solid culture methods are utilized. Rapid detection of rifampin resistance can signiÞcantly assist in proper patient care, as rifampin resistance will dictate longer and more complicated treatment to prevent negative outcomes (213). Somoskovi et al. (214) recently reviewed the mechanism of action and the molecular basis of resistance to rifampin and other Þrst-line anti-tuberculosis drugs. The mechanism of action of rifampin is to inhibit mycobacterial transcription by targeting DNA-dependent RNA polymerase. The development of resistance to rifampin is due to mutations in a well-deÞned 81-bp (27-codon) central region of the gene encoding the beta-subunit of RNA polymerase (*rpoB*). More than 96% of rifampin-resistant strains contain a mutation in this 81-bp region of *rpoB*, thus allowing for a straightforward molecular approach to detect rapidly rifampin resistance and/or multi-drug resistance. However, at this time, radiometric susceptibility testing for Þrst-line drugs with conÞrmation of drug resistant strains on Middlebrook 7H10 with an expanded number of compounds and drug concentrations, is still considered the gold standard (205).

Effective treatment regimens for tuberculosis are difÞcult to assess because of the slow growth rate of *M. tuberculosis* in culture and its protracted clearance from sputum. Desjardin et al. (215) measured levels of *M. tuberculosis* 85B (alpha antigen) messenger RNA, 16S ribosomal RNA, and IS*6110* DNA in patientsÕ sputum specimens as potential surrogate markers of response to anti-tuberculosis chemotherapy. Sputum specimens were sequentially collected for up to one year from 19 smear-positive pulmonary tuberculosis patients under adequate treatment. Results showed that levels of 85B mRNA declined after initiation of therapy, as did viable *M. tuberculosis* colony counts, with 90% of patients becoming negative for both markers after two months of treatment. The rapid disappearance of *M. tuberculosis* mRNA from sputum suggests that it is a good indicator of microbial viability. However, additional studies are warranted to prove the usefulness of this new Þnding, especially as a marker for rapid assessment of response to chemotherapy under program conditions.

Although not common practice before the AIDS epidemic, physicians should now routinely send blood cultures for the diagnosis of mycobacterial disease in HIV-infected patients (216Ð218). In one report, 7 (26%) of 27 HIV-infected patients with TB had positive blood cultures for *M. tuberculosis,* and in several other studies this proportion was even higher (up to 42%) (3,190,191,219). Mycobacterial blood cultures should be obtained in HIV-infected patients whenever they have unexplained fever (220Ð223). The diagnostic yield is highest in patients with fever higher than 39.5¡C, a miliary pattern on chest radiograph, and an elevated serum alkaline phosphatase or lactate dehydrogenase value (219).

Given the severe immunodeÞciency that many co-infected patients with HIV have rapid TB diagnosis is essential to decrease transmission, as well as morbidity and mortality. The modern mycobacteriology laboratory must respond to this Ònneed for speedÓ by providing studies that may hasten the diagnosis.

NAA is the newest tool available to diagnose rapidly and more speciÞcally tuberculosis disease. Results of

NAA testing may be available within four to six hours of beginning the test. Compared to smear microscopy, it has higher sensitivity and speciÞcity (224,225). NAA assays can detect tuberculosis even when AFB sputum smear microscopy is negative. Furthermore, NAA, in contrast to smear microscopy, can differentiate between *M. tuberculosis* complex and NTM, whereas AFB stains detect only the presence or absence of acid-fast bacilli.

The appropriate number of specimens to test with NAA will vary depending on the clinical situation, the prevalence of TB, the prevalence of NTM, and laboratory proÞciency (226,227). Based on available information, the following algorithm, recommended by the CDC, is a reasonable approach to NAA testing of respiratory specimens from patients with signs or symptoms of active pulmonary TB disease for whom a presumed diagnosis has not been established.

Algorithm (228):

1. Collect sputum specimens on three different days for AFB smear and mycobacterial culture.
2. Perform NAA test on the Þrst sputum specimen collected, the Þrst smear-positive sputum specimen, and additional sputum specimens as indicated below.
   a. If the Þrst sputum specimen is smear-positive and NAA-positive, the patient can be presumed to have TB without additional NAA testing. However, unless concern exists about the presence of NTM, the NAA test adds little to the diagnostic work-up.
   b. If the Þrst sputum is smear-positive and NAA-negative, a test for inhibitors should be done.
      i. If inhibitors are not detected, additional specimens (not to exceed a total of three) should be tested. The patient can be presumed to have NTM if a second sputum specimen is smear-positive, NAA-negative, and has no inhibitors detected.
      ii. If inhibitors are detected, the NAA test is of no diagnostic help. Additional specimens (not to exceed a total of three) can be tested with NAA.
   c. If sputum is smear-negative and NAA-positive, additional specimens (not to exceed three) should be tested with NAA. The patient can be presumed to have TB if a subsequent specimen is NAA-positive. This test is currently FDA-approved for negative smear respiratory specimens using transcription mediated ampliÞcation only.
   d. If sputum is smear-negative and NAA-negative, an additional specimen should be tested with NAA. The patient can be presumed not to be infectious if all smear and NAA results are negative. The clinician must rely on clinical judgement in decisions regarding the need for anti-TB therapy and further diagnostic work-up because negative NAA results do not exclude the possibility of active pulmonary TB disease.
3. If the indicated repeat NAA testing fails to verify initial NAA test results, the clinician must rely on clinical judgement in decisions regarding the need for anti-TB therapy, further diagnostic work-up, and isolation.
4. Ultimately, the patientÕs response to therapy and culture results are used to conÞrm or refute a diagnosis of TB.

NAA tests can enhance diagnostic certainty, but they do not replace AFB smear or mycobacterial culture, and they do not replace clinical judgment. Clinicians should interpret these tests based on the clinical situation. NAA tests often remain positive after cultures become negative during therapy and can remain positive even after completion of therapy (229).

Few studies have addressed the use of NAA assays for samples of non-respiratory origin (224,230,231,232). Paradoxically, it is precisely situations like TB meningitis for which a rapid and accurate laboratory diagnosis is of prime importance, since often the smear is negative and cultures grow *M. tuberculosis* only after several weeks, if at all. TB of the central nervous system remains among the most devastating forms of human TB. It causes high rates of death and neurologic disability and is often very difÞcult to diagnose (233,234). Smears of CSF are positive in less than 10% of patients in some reports (235,236). Even though culturing of CSF is also an unreliable diagnostic technique, a positive mycobacterial culture remains the gold standard of diagnosis in TB meningitis (237,238).

Recently Caws et al. reported data from a national molecular diagnostic service for TB meningitis in the United Kingdom (239). An IS*6110* targeted PCR had a sensitivity of 75% when compared to a positive culture, and 39% when compared to a Þnal clinical diagnosis of TB meningitis. In contrast, culture was positive in only 17% of clinically diognosed cases. NAA is currently the most rapid diagnostic test for TB meningitis, providing a better sensitivity compared to culture, though it is still not optimal. CSF specimens from patients suspected of having TB meningitis should be processed immediately. When a Lumbar puncture is performed at least 5ml of CSF should be submitted to the mycobacteriology laboratory as quickly as possible for growth detection and NAA testing.

1. Laboratory test results should always be correlated with the patientÕs clinical presentation, and the clinician should notify the laboratory when results are not consistent. An established and ongoing professional relationship between clinicians and laboratory personnal enables recognition of inaccurate results earlier, and therefore may minimize the potential harm to the patient. False-positive cultures of TB and other mycobacteria as a result of contaminated laboratory reagents or other technical laboratory problems have occurred. Studies (240,241) have demonstrated the occurrence of about 3Ð4% false-positive TB cultures. In studies analyzing drug-resistant TB (242) or specimens from patients with negative AFB smears and only one positive culture (243), erroneous results were reported

in 13% and 56%, respectively. However, one needs to be aware of the possibility that the error may have occurred in the pre-testing phase, such as using a nonsterile bronchoscope for specimen collection (244) or through detection of residual amplifiable *M. tuberculosis* DNA that might have remained on a sterile bronchoscope (245,246).

Laboratory results alone (i.e. positive culture, drug resistance) are not enough to dictate a particular strategy in a patientÕs care; careful clinical correlation is necessary in making the correct diagnosis.

Clinical Pathology of Tuberculosis in HIV-infected Patients

All tissue and fluid specimens from HIV-infected patients should routinely be tested for mycobacteria (smear, culture and possible, nucleic acid amplification) regardless of histologic or other microbiologic findings. Specimens from HIV-immunosuppressed patients may reveal mycobacteria on culture even though there are no AFB on smear, no granulomas are seen on histology, and evidence of coexisting nonmycobacterial infections or neoplasms are present (247).

The pathologist should have a high index of suspicion for mycobacterial disease when examining tissue from HIV-infected patients, and therefore information regarding the patientsÕHIV status and level of immunodeficiency should be considered when interpreting a result. The extent of granulomatous tissue reaction among HIV-infected patients with mycobacterial disease may depend upon their level of immune function. Although some HIV-infected patients with TB have no granulomas in infected tissue, most do (Fig. 17.4), in contrast to disseminated MAC where granuloma formation in infected tissues is usually poor to nonexistent (1) (Fig. 17.5). *M. tuberculosis* generally produces caseating granulomas, although noncaseating granulomas may also be seen.

In patients with tuberculosis, the histologic picture and frequency of granulomas will vary by the stage of tuberculosis and the site and type of biopsy. Primary pulmonary infection will result in small patches of caseous bronchopneumonia predominantly in subpleural areas in lower and middle lung fields, and in hilar lymph nodes. Miliary tuberculosis (named after the small Òmillet seedÓ appearance of lesions) occurs after hematogenous spread,

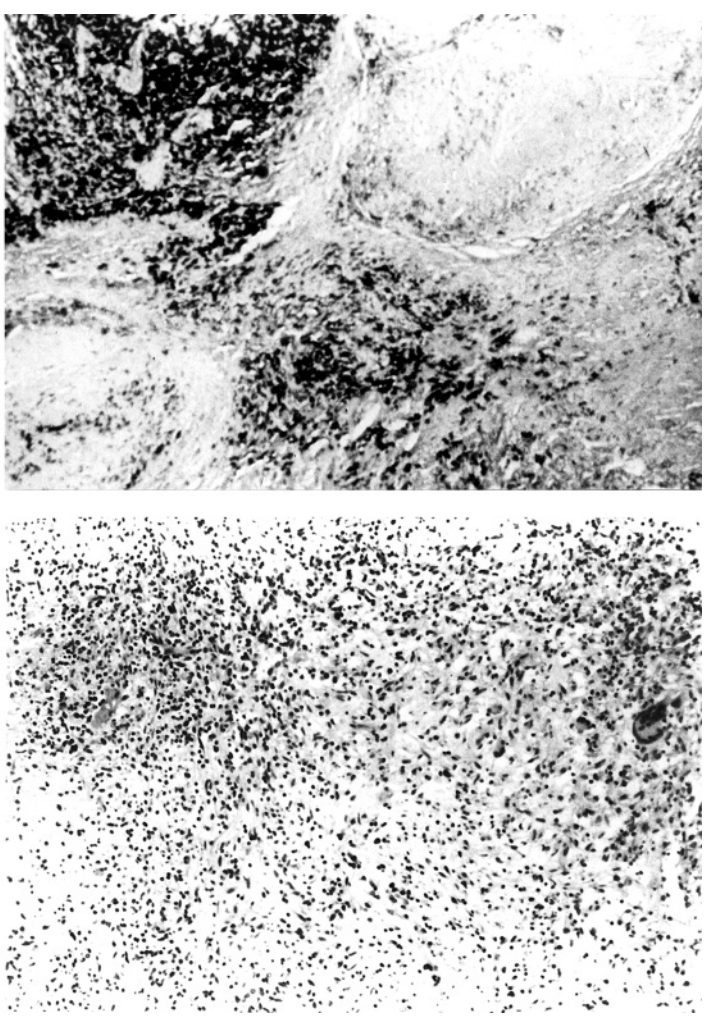

FIG. 17.4. Tuberculosis. A: Lymph node section (hematoxylin and eosin, $\times 50$) from a patient with tuberculous lymphadenitis presenting as his first AIDS-related infection. Well-formed tuberculous granulomas are seen (arrows). B: Bone marrow section (hematoxylin and eosin, $\times 700$) from a patient with disseminated tuberculosis, revealing a multinucleated giant cell (thin arrow) and caseating necrosis (thick arrow). This is in contrast to the MAC-infected tissue seen in Fig. 17.5 in which granulomas and necrosis are absent. (Part (A) from ref. 1, with permission; part (B) from Pitchenik AE, Fertel D. Tuberculosis and nontuberculous mycobacterial disease in HIV infected patients. *Med Clin North Am* 1992;76:121–171, with permission.)

but the term is often used to describe cases with typical miliary radiographic Þndings and an acute progressive course. There may be evidence of diffuse alveolar damage, and microscopically the miliary lesions are seen as small areas of necrotizing granulomatous inßammation Þlling four to Þve alveolar spaces (248). There may be seen, however, a wide spectrum of morphology, ranging from well-formed granulomas with giant cells, to suppurative necrosis with foam cells. Sites most often involved, according to one large autopsy study, in descending order,

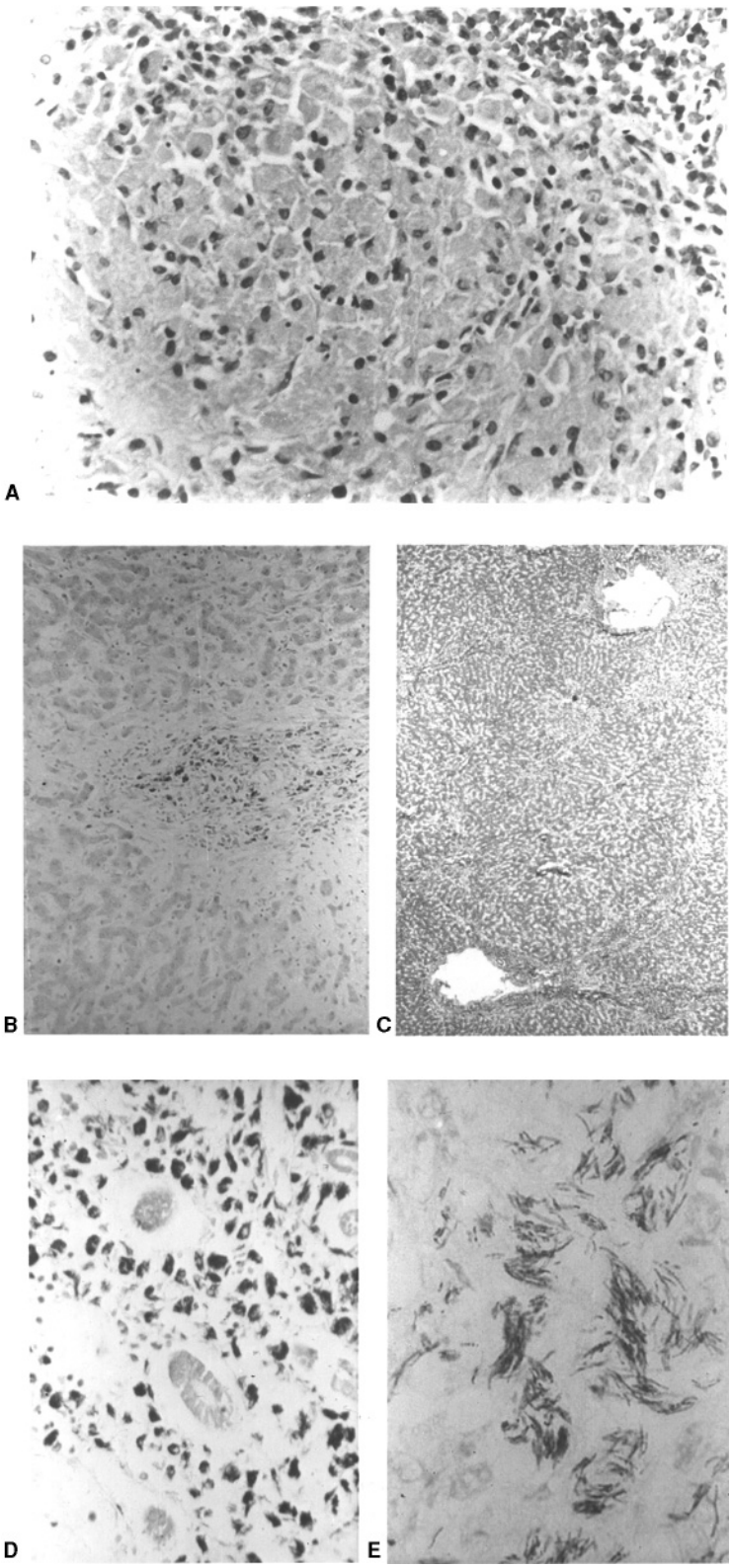

FIG. 17.5. MAC disease. A: Lymph node section (hematoxylin and eosin, ×700) from a patient with disseminated MAC presenting late in AIDS. Large histiocytes are present with abundant foamy cytoplasm. There are no formed granulomas or tissue necrosis, indicative of severe T-cell immunodeficiency. B–E: Liver, intestine, and lymph node from another patient with disseminated MAC disease and AIDS. (B) Liver section (hematoxylin and eosin, ×200) reveals no granulomas and appears essentially within normal limits. (C) Liver section (Ziehl-Neelsen, ×200) reveals numerous acidfast bacilli (arrows) proved on culture to be MAC. (D) Intestine section (Zichl-Neelsen, ×800) and (E) lymph node section (Zichl-Neelsen, ×1500) also reveals numerous acid-fast bacilli without granulomatous reaction. In (E), the acid-fast bacilli are seen, literally packing several macrophages (arrows). (Such macrophages appear large and foamy on hematoxylin and eosin staining as shown in (A).) [Parts (A) and (E) from Pitchenik AE, Fertel D. Tuberculosis and non-tuberculous mycobacterial disease in HIV infected patients. *Med Clin North Am* 1992;76:121–171, with permission; parts (B-D) from ref. 1, with permission.)

are the spleen, lungs, bone marrow and liver, with lesser involvement of skin, kidney, pancreas and joints (248). Grossly visible miliary lesions in lung tissue are not pathognomonic for tuberculosis, and may be seen in other infections (e.g. disseminated fungal infections such as histoplasmosis) or carcinoma.

ÒReactivationÓpulmonary tuberculosis is usually found in the upper lobes, and grossly appears as an acute necrotizing, pneumonic process with evidence of liquefaction and bronchial involvement and intrabronchial spread to contiguous lobes. Cavities, when present, have a wall consisting of granulation tissue, covered by a layer of Þbrous tissue. The immunologic mechanisms underlying cavity formation require a speciÞc degree of intact cellular immunity; therefore cavities are not often seen in patients with advanced HIV disease.

In one study of 11 HIV-seropositive patients who had culture-proven extrapulmonary TB (lymphatic, pleural, bone marrow, liver, and skin), biopsies from the tuberculous site revealed well-formed granulomas in ten (91%). In contrast, granulomas were found in the tissue specimens of only three (33%) of nine patients with culture-proven pulmonary TB who had transbronchial lung biopsies performed by Þberoptic bronchoscopy (72). Similar Þndings were reported by Sunderam et al. (125). This might be explained by the relatively small (millimeter) specimen size obtained by a transbronchial lung biopsy. Among HIV infected patients with disseminated TB presenting as fever of unknown origin, liver and bone marrow biopsies may furnish a rapid clue to diagnosis because of the presence of AFB or granulomas in the specimens.

Although blood cultures also represent a good diagnostic source for suspected dissemination, they generally are not positive unless the patientÕs CD4 count is < 200 cells/mm^3 (170Ð173).

Reasons for Delayed or Missed Diagnosis

Kramer et al. found that no or delayed treatment of tuberculosis in HIV-infected patients was common (48% of 52 patients) and was related more to errors in management (84% of times) than to atypical manifestations of TB (191). Of those in whom diagnosis was delayed, 45% died of TB. In most cases, delays could have been avoided if at least three sputum samples for acid-fast smear and mycobacterial culture had been obtained and if empiric therapy for TB had been given to symptomatic patients in whom chest radiographic Þndings were suggestive of mycobacterial disease.

Among HIV-infected patients with TB, acid-fast smears of sputum and bronchoscopic specimens are often negative and granulomatous reaction is frequently missed in the small transbronchial lung biopsy specimens (72,125,191). Therefore, after appropriate specimens have been obtained for mycobacterial culture, empiric anti-TB therapy should be instituted in all HIV-infected patients in whom chest radiographic abnormalities suggestive of TB are not explained by other causes (3,191). The tuberculin skin test is also seriously underused in symptomatic HIV-infected patients (249). Although anergy is common in HIV-infected patients with TB (44% to 89%), a considerable number (up to 56%) still have signiÞcant tuberculin skin reactions, which may be useful in directing care. For example, if an HIV-infected, tuberculin-reactive patient is symptomatic (e.g. fever of unknown origin) and TB disease cannot be excluded initially, anti-TB therapy should be instituted.

Treatment

Despite their immunosuppressed state, HIV-infected patients with TB who comply with a regimen of standard anti-TB drugs, including both isoniazrd?? (INH) and rifampin (RIF), have rapid sterilization of sputum, radiographic and clinical improvement and low relapse rates similar to that of TB patients who are not HIV-infected (72,123,250Ð254). Treatment for TB should be initiated promptly whenever a positive AFB smear is found in a patient with proven or suspected HIV infection. It should not be withheld on the presumption that the AFB may represent nontuberculous mycobacteria such as *M. avium* complex (MAC) (if this is a question then nucleic acid ampliÞcation testing can be performed to distinguish between TB and NTM). Initial treatment must be directed against the more virulent, contagious, and treatable *M. tuberculosis* until TB is ruled out. If the culture later reveals a nontuberculous mycobacteria, then treatment can be changed or discontinued at the physicianÕs discretion (158,255,256). Even if AFB smears are negative, empiric therapy for TB should be initiated (after specimens are obtained) pending culture results in symptomatic patients in whom chest radiographic Þndings are suggestive of TB (191).

Guidelines for the treatment of TB in the setting of HIV infection have been published by the American Thoracic Society, the CDC, and others (Table 17.2) (15,19,149,167, 257,258). Recommended treatment for TB in HIV-infected patients is now essentially the same as that for non-HIV infected patients (149): a six-month total regimen including INH, RIF, and pyrazinamide (PZA) given for two months, followed by INH and RIF alone for four more months. This is the preferred treatment for patients with fully susceptible organisms who adhere to treatment. Ethambutol (EMB) is usually included in the initial regimen until the results of drug susceptibility studies are available but may be withheld initially if it is known that there is less than 4% primary resistance to INH in the community, and the patient has had no previous treatment with anti-TB drugs, is not from a country with a high prevalence of drug resistance, and has had no known exposure to a drug-resistant case.

TABLE 17.2. *Treatment regimens for human immunodeÞciency virus (HIV) related tuberculosis (TB)*

Six-month rifamycin-based therapy for TB susceptible to INH, RIF and PZA*

Rating[a] (Evidence)[Δ]	Induction Phase Drugs	Induction Phase Interval and Duration	Continuation Phase Drugs	Continuation Phase Interval and Duration	Consideration for HIV Therapy	Comments
A(II)	• INH • RIF or RFB • PZA • EMB (or SM)‡	Daily for two months or Daily for two weeks then two times/ week for six weeks. (Twice weekly therapy should be administered by DOT.)	• INH • RIF or RFB	Daily or two times/week for four months. (Twice weekly therapy should be administered by DOT.)	See Table 17.4 for Coadministration with Antiretroviral Therapy	

Six-Month Rifamycin-based therapy for TB susceptible to INH, RIF and PZA*

Rating[a] (Evidence)[Δ]	Induction Phase Drugs	Induction Phase Interval and Duration	Continuation Phase Drugs	Continuation Phase Interval and Duration	Consideration for HIV Therapy	Comments
B(II)	• INH • SM • PZA • EMB	Daily for two months (eight weeks)	• INH • SM • PZA	Two to three times/week for seven months	See Table 17.4 for Coadministration with Antiretroviral Therapy	SM is coniraindicated for pregnant women SM should be used for the total duration of therapy. When SM can not be used, EMB and INH should be used for at least 12 months

EMB = ethambutol; INH = isoniazid; PZA = pyrazinamide; RFB = rtfabutin; RIF = rifampin; SM = streptomycin.
* Duration of therapy should be prolonged for patients with delayed response to therapy If still culture positve on a sputum collected after two months of therapy, patient should be assessed for non-adherence, malabsorption or drug resistance and therapy should be prolonged for at least four months after cultures become negative CONSULTATION WITH A SPECIALIST IS STRONGLY ADVISED IN THESE CASES.
‡ EMB or SM may be stopped a er susceptibility test results indicate susceptibilty to INH and RIF.
[a] A = preferred; B = acceptable alternative; C = offer when A and B cannot be given.
[Δ] I = randomized clinical trial data; II = data from clinical trials that are not randomized or were conducted in other populations; III = expert opinion.
Adapted from American Thoracic Society/Centers for Disease Control. Treatment of Tuberculosis and Tuberculosis Infection in Adults and Children. *Am J Respir Crit Care Med* 1994;149: 1359-1374.

It should be emphasized that in the presence of HIV infection it is essential to assess the clinical and bacteriologic response. If there is evidence of a slow or optimal sub response (e.g. sputum cultures have not become negative after two months of treatment), therapy should be prolonged as judged on a case-by-case basis (19). Furthermore, DOT is strongly recommended for all HIV-infected TB patients and is mandatory when therapy is administered intermittently on a twice or thrice weekly schedule rather than on a daily basis (19,149). DOT is *routinely* recommended for all HIV-infected TB patients treated in Florida and is considered to be the *community standard* (259). For two weeks, INH, RIF, PZA, and EMB are given daily, followed by twice weekly administration of the same drugs for six weeks, and subsequently twice weekly administration of INH and RIF for 16 weeks, for a total of six months of therapy. Alternatively, INH, RIF, PZA, and EMB can be given three times weekly for six months for patients with fully susceptible tubercle bacilli. Recommended drug dosages for INH, RIF, PZA, and EMB when administered daily, two times per week, or three times per week are outlined in Table 17.3 (19,149).

If public health resources are strained (e.g. in developing countries or rural communities) and insufficient to provide for DOT when needed, responsible family members or other lay persons (e.g. former TB patients known to be responsible) might be recruited for this purpose, perhaps on a paid, "per patient cured" basis (260,261). If DOT is not possible, the use of fixed drug combinations (i.e. INH-RIF-PZA (Rifater); INH-RIF (Rifamate)) should be used routinely, which enhances patient adherence and reduces the risk of inappropriate monotherapy and drug resistance (149,262).

Drug susceptibility tests should be performed routinely on all initial isolates and the treatment regimen revised if there is resistance to any of the drugs being used. When isolated INH resistance is documented, INH should be discontinued (though this has been argued not to be necessary by some (263)) and RIF, EMB, and PZA continued for at least the entire six-month duration of therapy (149). (Some authorities have recommended extending therapy with RIF, EMB, and PZA for at least nine to twelve months in HIV-infected TB patients with INH-resistant organisms (264,265).) Institutions that are experiencing outbreaks of TB resistant to both INH and RIF may need to begin five- or six-drug regimens as initial therapy pending drug-susceptibility test results. If there is resistance to both INH and RIF demonstrated with or without resistance to other first-line anti-TB drugs, treatment becomes less effective, more complicated, and more expensive and requires consultation with a TB expert (266). In general, therapy for multidrug resistant disease (resistant to at least INH and rifampin) should include at least two or three oral drugs to which the organism is susceptible. These drugs should be continued for 18–24 months following sputum conversion to negative. In addition, an injectable drug to which the organism is susceptible (e.g. aminoglycoside) is included for the first four to six months as tolerated (149,267,268). When resistance occurs to the first line agents, second line agents must be included in the treatment regimen. Second line agents are generally less effective and more toxic than first line agents. Second line agents include ethionamide, cycloserine, para-aminosalicylic acid (PAS), capreomycin and kanamycin (or amikacin). The fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin, moxifloxocin, and sparfloxacin) are newer additions to the treatment arsenal and have been shown to be both effective and well tolerated. (149,269–271). Two new rifamycins, rifabutin and rifapentine, have been introduced and offer alternatives to rifampin. Rifabutin has a longer half-life (45 hours) (272) than rifampin (four hours) and has about one-third of the effect on the hepatic CYP450 enzymes (273). Rifapentine has a pharmacokinetic profile midway between rifampin and rifabutin. Resistance to rifampin usually confers resistance to both rifabutin and rifapentine (274). At least one study using once weekly INH and rifapentine showed an increased rate of resistance to rifapentine in the HIV+ study arm, and it is currently recommended that rifapentine not be used in HIV+ patients (275,276). All patients with drug-resistant TB should have DOT whether or not they are HIV-infected.

The more common adverse effects (Table 17.3) of INH include hepatitis and peripheral neuropathy. Vitamin B6 (25–50 mg per day) is often given with INH in order to prevent or treat the peripheral neuropathy (routinely given to HIV-infected individuals). One study showed that the incidence of drug induced hepatitis during treatment with INH was increased five-fold in the presence of hepatitis C virus infection and was increased four-fold when the patient was infected with HIV. If the patient was infected with both hepatitis C and HIV, the risk of drug-induced hepatitis was increased 14-fold (277). Rifampin treatment is associated with rash, pruritis, muscle stiffness, arthralgias, hepatitis and thrombocytopenia. PZA causes an increase in serum uric acid levels that can lead to a gouty arthritis, and it also maybe associated with hepatitis. Ethambutol (EMB) can cause an optic neuritis with loss of red-green color discrimination and visual acuity that can lead to blindness if not detected early. The aminoglycosides are associated with otic, vestibular, and nephrotoxicities. The dosages of EMB, cycloserine, ofloxacin, levofloxacin, and the aminoglycosides should be reduced in renal insufficiency (274).

Anti-TB drug toxicity (e.g. fever or hepatic, hematologic, or dermatological reactions) may be difficult to monitor in HIV-infected patients because of multiple and recurrent nontuberculous infections, non-TB drugs used concurrently, and the fact TB itself may produce similar abnormalities. However, with some exceptions (60,66), most studies show that anti-TB drugs are reasonably well tolerated, and because they are vital, they should not be discontinued because of mild symptoms or mild laboratory abnormalities (73,123,125,278). It has been suggested, and

it has been our experience as well, that signiÞcant adverse reactions to anti-TB drugs (especially to RIF) are more frequent among the more immunosuppressed patients with TB (i.e. those who contract TB in the later stages of AIDS) (279). In one retrospective study, 90% of adverse reactions (mostly to RIF) were seen during the Þrst two months of treatment (190). Concurrent therapy with various antiretroviral and anti-TB medications seems to be well tolerated (258,280).

In most cases, mild adverse effects can be treated symptomatically with prokinetic agents such as metoclopramide for GI distress, non-steroidal antiinßammatory

TABLE 17.3. *Medications for the treatment of Tuberculosis (TB) and latent TB infection*

Drug	Dose in mg/kg (Maximum Dose) Daily children	Daily adults	2 Times/week* children	2 Times/week* adults	3 Times/week* children	3 Times/week* adults	Adverse Reactions	Monitoring	Comments
INH	10–20 (300mg)	5 (300mg)	20–40 (900mg)	15 (900mg)	20–40 (900mg)	15 (900mg)	Hepatic enzyme elevation Hepatitis Peripheral neuropathy Mild effects on central nervous system Drug Interactions	Baseline measurements of hepatic enzymes for adults Repeat measurements: • if baseline results are abnormal • if patient is at high risk for adverse reactions • if patient has symptoms of adverse reactions	Hepatitis risk increases with age and alcohol consumption Pyridoxine can prevent peripheral neuropathy
RIF	10–20 (600mg)	10 (600mg)	10–20 (600mg)	10 (600mg)	10–20 (600mg)	10 (600mg)	GI upset Drug Interactions Hepatitis Bleeding problems Flu-like symptoms Rash	Baseline CBC measurements for adults • CBC and platelets • hepatic enzymes Repeat measurements: • if baseline results are abnormal • if patient has symptoms of adverse reactions Monitor for decreased antiretroviral activity if taken with ART	See Table 17.4 when administered with ART Signi cant interactions with: • methadone • birth control pills • many other drugs Colors body uids orange May permanently discolor soft contact lenses
RFB	5 (300mg)	—	5 (300mg)	—	5 (300mg)	—	Rash Hepatitis Fever Thrombocytopenia Orange coloured body uids With increased levels of rifabutin: • Severe arthralgias • Uvetitis • Leukopenia	Baseline CBC measurements for adults • CBC and platelets • hepatic enzymes Repeat measurements: • if baseline results are abnormal • if patient has symptoms of adverse reactions Monitor for decreased antiretroviral activity if taken with ART	See Table 17.4 when administered with ART Signi cant interactions with: • methadone • birth control pills • many other drugs Colors body uids orange May permanently discolor soft contact lenses
PZA	15–30 (2g)	15–30 (2g)	50–70 (4g)	50–70 (4g)	50–70 (3g)	50–70 (3g)	Hepatitis Rash GI Upset Joint aches Hyperuncemia Gout (rare)	Baseline measurements for adults • uric acid • hepatic enzymes Repeat measurements: • if baseline results are abnormal • if patient has symptoms of adverse reactions	Treat hyperuricemia only if patient has symptoms
EMB	15–25	15–25	50	50	25–30	25–30	Optic neuritis	Baseline and monthly tests • visual ability • color vision	Generally not recommended for children too young to be monitored for changes in vision unless TB is dnug resistant
SM	20–40 (1g)	15 (1g)	25–30 (1.5g)	25–30 (1.5g)	25–30 (1.5g)	25–30 (1.5g)	Ototoxicity (hearing loss or vestibular dysfunction) Renal toxicity	Baseline and repeat as needed • hearing • kidney function	Avoid or reduce dose in adults > 60 years old or in individuals with renal disease

Note: Children □ 12 years old. Adjust weight-based dosages as weight changes.
* All regimens administered two to three times a week should be used with DOT.
EMB = ethambutol; INH = isoniazid; PZA = pyrazinamide; RFB = rifabutin; RIF = rifampin; SM = streptomycin; ART = antiretroviral therapy; DOT = Directly Observed Therapy.
Source: Adapted from American Thoracic Society/Centers for Disease Control. Treatment of Tuberculosis and Tuberculosis Infection in Adults and Children. *Am J Respir Crit Care Med* 1994;149:1359-1374 and American Thoracic Society/Centers for Disease Control. Targeted Tuberculin Testing and Treatmentof Latent TB Infection. *Am J of Resp Crit Care Med* 2000;161:S221–S247.

agents for muscle and joint pain, and with antihistamines (diphenhydramine, hydroxyzine) for itching or rash. Desensitization can be tried in the event that an allergy occurs to one of the agents in the treatment regimen (281,282). It may be necessary to discontinue INH, RIF, and/or PZA if clinically signiÞcant hepatitis (development of signs and/or symptoms of hepatitis at any transaminase level, or transaminase levels above Þve times the upper limit of normal) occurs. In this event, treatment may continue with EMB, streptomycin, and either oßoxacin or levoßoxacin. Once the hepatitis is resolved, INH, RIF, and PZA can be reintroduced, one at a time, as the patient tolerates. If RIF cannot be used, or if PZA cannot be used for the Þrst two months of therapy, the duration of treatment must be extended (see Table 17.2). Patients who cannot tolerate RIF may be able to tolerate twice weekly RBT. If there is underlying infection with hepatits B or C and elevated transaminases occur, treatment for hepatitis B and/or C, may permit a safe reintroduction of INH, RIF, or PZA for the duration of TB treatment (277).

Drug interactions can be problematic in the TB-HIV coinfected patient. Ketoconazole inhibits the absorption of RIF and can result in failure of TB treatment if the drugs are taken together (283). In some patients, INH and RIF can reduce serum ketoconazole and ßuconazole concentrations, resulting in ineffective antifungal therapy (283,284). RIF is well known as an inducer of CYP450 enzymes resulting in low serum drug levels of many other drugs. On the other hand, INH is an inhibitor of the CYP450 enzymes and can cause an increase in serum levels of certain drugs. Among the the drugs most affected by INH and RIF include phenytoin, theophylline, warfarin, hormone-based contraceptives, opiates, and sulfonylureas as well as the HIV nonnucleoside reverse transcriptase inhibitors used and protease inhibitors (274). It has been shown that protease inhibitors in the treatment of HIV infection cause rifamycin levels (e.g. Rifampin) to be increased and rifamycins cause protease inhibitor levels to be decreased, leading to rifamycin toxicity and HIV resistance to protease inhibitors. To address this problem, the CDC in a consensus statement, recommended three options for the concomitant treatment of HIV infection and TB (19,285,286). Rifabutin may be used as an alternative to rifampin when patients are receiving nonnucleoside reverse transcriptase inhibitors or protease inhibitors. Rifabutin has less of an effect on the CYP450 system than does rifampin and although similar drug interactions occur, they are of a lesser magnitude. It has been our experience that rifabutin can be safely used with most of the currently available anti-retroviral agents when doses are adjusted to correct for the interactions. (19,258,287). Possible exceptions for the safe co-administration of HIV and TB drugs might be saquinavir (except when boosted by ritonavir) and full dose ritonavir. It has been our experience that nelÞnavir can be used with rifabutin (300 mg twice a week) with no need for dosage modiÞcation of the nelÞnavir. However, when rifabutin is used with indinavir, the dose of indinavir must be increased from 800 mg to 1200 mg every eight hours, but no dose change is required for rifabutin (258,280). When rifabutin is used with efavirenz, the dose of efavirenz appears to be unaffected while the dose of rifabutin frequently needs to be increased to 450 or 600 mg. Preliminary studies with a lopinavir/ritonavir combination product indicate that the dose of rifabutin should be decreased to 150 to 300 mg and given not more often than three times a week. Because of the variability of these interactions among patients as well as the large variety of different combinations of anti-retrovirals, we feel that serum drug levels may need to be measured for both rifabutin (and possibly INH) and the anti-retrovirals when combination therapy is used. It should be kept in mind that the dosage of both rifabutin and the retroviral drugs may need to be adjusted to avoid either sub-therapeutic or toxic levels (Table 17.4) (258,280,286,288).

Although HIV-infected patients rarely die from TB when it is treated appropriately, subsequent death from disease caused by nontuberculous organisms may occur within one to two years if the underlying immunosuppression is not corrected (19,60,66,72,73,125,289). Furthermore, although TB in HIV-infected patients usually responds to therapy, relapses, although uncommon, occasionally occur (60,64,72,250,254,290–293). Therefore, HIV-infected patients with TB should have regular follow-ups for life (e.g. every three to six months), and mycobacterial examinations should be repeated as clinically indicated. In addition, some consultants have suggested the use of indeÞnite continuation of INH after completion of a course of anti-TB therapy, especially if the patient is in an environment where TB exposure is common (i.e. also serves as prevention for reinfection) (294).

Clinical Response to TB Therapy

Patients need to be monitored for effectiveness of treatment and toxicity. Soon after starting therapy, patients should experience an improvement in symptoms such as cough, fevers, night sweats and weight gain. Defervesence occurs in 60–90% of patients within three weeks of starting therapy (295–297). Cough also has been shown to rapidly improve within two weeks of therapy (298). Radiographic resolution is slow and lags behind patient well-being as well as sputum smear and culture conversion. Improvement in chest radiographs may take one to three months after initiation of therapy. Thus, chest radiographs to monitor response to therapy is not routinely recommended (299). The number of bacilli seen on sputum smear has been shown to rapidly decline within two weeks of starting therapy in most patients (300,301). Cultures become negative in over 85% of patients on therapy for more than two months (149,302).

TABLE 17.4. *Recommendations for coadministering different antiretroviral drugs with the antimycobacterial drugs rifabutin and rifampin*

Antiretroviral	Use in combination with rifabutin	Use in combination with rifampin	Comments
Saquinavif			
Hard-gel capsules (HGC)	Possibly† if antiretroviral regimen also includes ritonavir	Possibly† if antiretroviral regimen also includes ritonavir	Co-administration of saquinavir SGC with usual-dose rifabutin (300 mg daily or two or three times per week) is a possibility. However, the pharmacokinetic data and clinical experience for this combination are limited.
Soft-gel capsules (SGC)	Probably§	Possibly, if antiretroviral regimen also includes ritonavir	The combination of saquinavir SGC or saquinavir HGC and ritonavir, co-administered with (1) usual-dose rifampin (600 mg daily or two or three times per week), or (2) reduced-dose rifabutin (150 mg two or three times per week) is a possibility. However, the pharmacokinetic data and clinical experience for these combinations is limited. Co-administration of sequinavir HGC or saquinavir SGC with rifampin (in the absence of ritonavir) is not recommended because rifampin markedly decreases concentrations of saquinavir.
Ritonavir	Probably	Probably	If the combination of ritonavir and rifabutin is used, then a substantially reduced-dose rifabutin regimen (150 mg two or three times per week) is recommended. Co-administration of ritonavir with usual-dose rifampin (600 mg daily or two or three times per week) is a possibility, though phammacokinetic data and clinical experience are limited.
Indinavir	Yes	No	There is limited, but favorable, clinical experience with co-administration of indinavir‡ with a reduced daily dose of rifabutin (150mg) or with the usual dose of rifabutin (300mg two or three times per week). Co-administration of indinavir with nfampin is not recommended because rifampin markedly decreases concentrations of indinavir.
Nel navir	Yes	No	There is limited, but favorable, clinical experience with co-administration of nel navif** with a reduced daily dose of rifabutin (150mg) or with the usual dose of rifabutin (300mg two or three times per week). Co-administration of nelenavir with rifampin is not recommended because rifampin markedly decreases concentrations of nel navir.
Amprenavir	Yes	No	Co-administration of amprenavir with a reduced daily dose of rifabutin (150mg) or with the usual dose of rifabutin (300mg two or three times per week) is a possibility but there is no published clinical experience. Co-administration of amprenavir with rifampin is not recommended because rifampin markedly decreases concentrations of amprenavir.
Nevirapine	Yes	Possibly	Co-administration of nevirapine with usual-dose rifabutin (300mg daily or two or three times per week) is a possibility based on pharmacokinetic study data. However, there is no published clinical experience for this combination. Data are insuf cient to assess whether dose ad)ustments are necessary when rifampin is co-administered with nevirapine Therefore, rifampin and nevirapine should be used only in combination if clearly indicated and with careful monitoring.
Detavirdine	No	No	Contraindicated because of the marked decrease in concentrations of delavirdine when administered with either rifabutin or rifampin.
Efavirenz	Probably	Probably	Co-administration of efavirenz with increased-dose rifabutin (450mg or 600mg daily, or 600mg two or three times per week) is a possibility, though there is no published clinical experience. Co-administration of efavirenz†† with usual-dose rifampin (600mg daily, or two or three times per week) is a possibility, though there is no published clinical experience.

* Usual recommended doses are 400mg two times per day for each of these protease inhibitors and 400mg of ritonavir.
† Despite limited data and clinical experience, the use of this combination is potentially successful.
§ Based on available data and clinical experience, the successful use of this combination is likely.
‡ Usual recommended dose is 800mg every eight hours. Some experts recommend increasing the indinavir dose to 1,000-1,200mg every eight hours if indinavir is used in combination with rifabutin.
** Usual recommended dose is 750mg three times per day or 1,250mg twice daily. Some experts recommend increasing the nel navir dose to 1,000mg if the three times-per-day dosing is used and nel navir is used in combination with rifabutin.
†† Usual recommended dose is 600mg daily. Some experts recommend increasing the efavirenz dose to 800mg daily if efavirenz is used in combination with rifampin.
Source: Center for Disease Control and Prevention. Updated Guidelines for the use of Rifabutin or Rifampin for the Treatment and Prevention of Tuberculosis among HIV-infected Patients Taking Protease Inhibitors or Non-nucleoside Reverse Transcriptase Inhibitors. *MMWR* 2000;49(9)1B5-189.

Reasons for Failure of TB Therapy

If a patient continues to be culture positive for tuberculosis despite "adequate" therapy, the clinician should consider one or more of the following four etiologies as a possible cause: (1) non adherence, (2) the presence of drug resistance, (3) poor penetration into the affected area (e.g. thick pleural thickening not allowing drug penetration-a rare etiology), and/or (4) lower than expected drug levels. Of the above four etiologies, in our experience, non-adherence is the most common reason for failure of TB therapy. The importance of utilizing DOT to try to minimize this risk cannot be overstated. However, despite the use of DOT, non-adherence may occur and can be difficult to detect (303). If a patient is culture positive for TB despite over two months of therapy, an evaluation searching for etiologies for this failure, including confirmation of drug susceptibilities and therapeutic drug monitoring, should be considered.

Paradoxical Worsening of TB after Initiation of HAART

Paradoxical worsening of TB symptoms, signs, and/or radiographic manifestations have been reported shortly after initiation of highly active antiretroviral therapy (HAART) (128,304–307). The proposed pathogenesis of this phenomenon is an inflammatory response to TB antigens associated with immune reconstitution caused by HAART. Paradoxical responses have been seen months after the initiation of TB chemotherapy, suggesting that TB antigens, without the existence of live TB organisms, could trigger this inflammatory response. Interestingly, PPD reactions "converting" from negative to significant induration have been observed in those patients with paradoxical responses. Our recent observations reveal that 41 of 175 (23%) HIV-infected TB patients experienced paradoxical responses, at a median of 15 days (range, 2–60 days) after the initiation of HAART. Manifestations include fever (60%), cervical and/or intrathoracic lymphadenopathies (40%), bilateral diffuse pulmonary infiltrates (usually military pattern), pleural effusion, and CNS lesions (308). When the patient's clinical course deteriorates during treatment in an HIV-infected TB individual, it is imperative to ensure that this is not due to TB treatment failure or the development of another pathological process, such as an opportunistic infection or a drug reaction. In general, paradoxical reactions are transient and no specific treatment is required. However, when severe manifestations are seen (e.g. airway or vascular compromise, CNS lesions, pericardial effusion causing cardiac tamponade), the administration of immunomodulators, such as systemic corticosteroids, should be considered along with possible discontinuation of HAART.

Primary Drug-resistant TB Acquired Late in AIDS

Immunosuppressed patients exposed to TB can be newly re-infected and contract TB despite old latent tuberculosis infection or disease that was adequately treated in the past (20,47,131,309–311), confirming that these individuals are susceptible to reinfection from subsequent TB exposure. Outbreaks of multidrug-resistant TB among HIV-infected persons have been documented in many different settings, including hospitals and prisons (23,24,32,48,312). These occurrences underscore the importance of strict adherence by institutions serving HIV-infected populations to recommended infection control procedures (26).

Value of HIV Serologic Testing in Patients with TB

All persons with TB should be questioned about risk factors for HIV infection and whether or not risk factors are elicited, urged to have an HIV serology done (314). The finding that a person with TB is HIV-infected (1) alters plans for management of the TB (choice of drugs may be different (e.g. rifampin vs. rifabutin in patients on HAART); DOT is strongly recommended, and lifetime follow-up is indicated); (2) forewarns the physician of additional nontuberculous opportunistic infections that require treatment in their own right, may confound the evaluation of anti-TB therapy, and are often responsible for a fatal outcome within two years of TB diagnosis (19,60,65,72); (3) alerts the physician to the possible need for anti-HIV therapy and PCP chemoprophylaxis; (4) establishes the diagnosis of AIDS (an AIDS diagnosis often establishes eligibility for certain social, medical, and economic benefits); and (5) often affords the earliest opportunity to counsel the patient about notification of sexual partners and risk of transmitting HIV infection to others.

Prevention of Tuberculosis in HIV-Infected Individuals

For public health, there is a hierarchy of interventions in preventing TB, especially among individuals with HIV infection. First, persons with TB disease must be found and treated before they infect others. Second, persons exposed to patients with pulmonary TB (close contacts) must be found and treated to prevent rapid progression to TB disease. Lastly, persons with a history of TB infection must be found and treated to prevent reactivation TB.

Infection with HIV is the strongest risk factor for the development of reactivation tuberculosis and for progression to TB disease after recent infection, with some studies suggesting a rate of up to 8% per year (46,315,316). In addition, other research has suggested one year mortality rates of 20–35% for HIV-infected patients with active TB

disease, even if the TB is cured (317). For these reasons, treatment of latent TB infection among HIV-infected individuals is one of the highest priorities for those programs with adequate resources (19).

Although other tests will likely be available soon (318,319), the TST (PPD) is the most well established test for diagnosing latent TB infection. Limitations of the PPD are well known and include variances in placement of the test, subsequent interpretation of the results, and cross-reactivity with non-tuberculous mycobacteria. Positive PPD testing is dependent upon an intact cell-mediated immune response. Anergy is therefore common in HIV/TB co-infected persons. However, the beneÞts of anergy testing for HIV-infected persons who are tuberculin negative have not been demonstrated, and anergy testing is not recommended (320). Newer tests involving the *in vitro* measurement of gamma interferon production from patientsÕT cells exposed to tuberculin have been developed, studied and approved for limited use for the diagnosis of latent tuberculosis infection, but have not been thorougly evaluated in patients with HIV infection (318,319).

NTM can also cause a positive tuberculin skin reaction thereby decreasing the speciÞcity of PPD testing. The prevalence of TB in various populations will also have an effect on the speciÞcity of the skin test. For these reasons various cut-off points have been established to deÞne a positive PPD reaction: 5mm or greater of induration, 10mm or greater of induration, and 15mm or greater of induration (127) (Table 17.5). The speciÞcity of the test is increased at the expense of sensitivity by increasing the amount of induration required for a positive test (321).

For those individuals with the highest risk of progressing from TB infection to active TB disease, 5mm or greater of induration is used as the cut-off for a positive test. This would include HIV-infected individuals, recent close contacts to an active case of TB disease, patients on immunosuppressive medications such as transplant recipients, and those with chest X-rays showing scarring consistent with previous TB disease that was untreated.

For other individuals at risk for progressing to active TB disease or who have a substantial risk of infection with TB as opposed to other mycobacteria, 10mm or greater is used as the cut-off. Included in this group would be recent immigrants from high-incidence countries (especially within the last Þve years), intravenous drug users, healthcare workers, those in congregate living facilities such as prisoners or nursing home residents, mycobacteriology laboratory workers, and children under four years of age who are exposed to high-risk adults. Certain

TABLE 17.5. *Criteria for tuberculin positivity, by risk group*

Reaction □□5mm of induration	Reaction □10mm of induration	Reaction □15 mm of induration
Human immunode ciency virus (HIV)-positive persons	Recent immigrants (i.e. within the last ve years) from high prevalence countries	Persons with no risk factors for TB
Recent contacts of tuberculosis (TB) case patients	Injection drug users	
Fibrotic changes on chest radiograph consistent with prior TB	Residents and employees[t] of the following high-risk congregate settings: prisons and jails, nursing homes and other long-term facilities for the elderly, hospitals and other health care facilities, residential facilities for patients with acquired immunode ciency (AIDS), and homeless shelters	
Patients with organ transplants and other immunosuppressed patients (receiving the equivalent of □□15 mg/d of prednisone for 1 mo or more)*	Mycobacterial laboratory personnel	
	Persons with the following clinical conditions that place them at high risk: silicosis, diabetes mellitus, chronic renal failure, some hematologic disorders (e.g. ieukemias and lymphomas), other speci c malignancies (e.g. carcinoma of the head or neck or lung), weight loss of > 10% of ideal body weight, gastrectomy, and jejunoileal bypass	
	Children younger than four years of age or infants, children, and adolescents exposed to adults at high-risk	

* Risk of TB in patients treated with corticosteroids increases with higher dose and longer duration.
[t] For persons who are otherwise at low risk and are tested at the start of employment, a reaction of □15 mm induration is considered positive.
Source: Centers for Disease Control and Prevention. Targeted tuberculin testing and treatment of latent tuberculosis infection. American Thoracic Society. *MMWR Morb Mortal Wkly Rep* 2000;49:1-51.

clinical conditions increase the risk for active TB disease, and patients with these conditions would also be considered positive at 10 mm or greater. These conditions include diabetes, silicosis, renal failure, partial or total gastrectomy, intestinal bypass, reticuloendothelial malignancies, carcinoma of the head, neck or lung, and individuals who have lost more than 10% or more of their ideal body weight. All persons with any of the above criteria should be tested by tuberculin (PPD) skin testing. Those individuals without any of the risks listed above should not be routinely tested for TB infection unless TB skin testing is required (e.g. healthcare workers). If an individual without known risk factors is tested, 15mm or greater should be considered positive (Table 17.5).

In the United States, treatment of latent TB infection with isoniazid has been an important, if not underutilized strategy for over 30 years (322). The reported efficacy of INH in non-HIV infected individuals varies considerably in the literature, between 25% to almost 100%, but seems to be most dependent upon compliance (323-326). In a study conducted by the IUATLD in eastern Europe, researchers found that daily INH for one year reduced the incidence of TB by 75%, but when completion rates were factored in, the reduction was 93%, (327) and was maintained over five years of follow-up. Another study conducted among Alaskan villagers showed that the efficacy of INH was maintained for nineteen years (328).

When HIV burst onto the world stage, questions arose once again regarding the effectiveness of latent TB infection treatment. The first regimens studied were those that used INH. Pape et al, in a study conducted in Haiti, randomized 118 subjects to either 12 months of daily INH or placebo. During a mean follow-up of approximately three years, the incidence of tuberculosis was lower in those who received INH compared to those who received placebo (2.2 vs. 7.5 per 100 person-years) (329). When only those who were PPD positive were included, the difference in the two groups was more pronounced with a rate of 1.7 for the INH group and 10.0 for the placebo group. In another study, HIV-infected individuals in a methadone program were followed prospectively to determine the effectiveness of INH in preventing active TB disease. In 53.4 person-years of follow-up, HIV-positive tuberculin reactors who did not receive INH developed TB at a rate of 9.7 per 100 person-years. There were no cases of TB among the tuberculin reactors who took daily INH for 12 months; (18) other studies have shown similar results (330).

What long-term effects are there from treatment of latent TB infection with INH and is there any impact upon survival? It is difficult to answer these questions, as there are many areas with a potentially high incidence of re-infection to strains to which the individual may be exposed. Nonetheless, intriguing results have been reported. Pape and colleagues found that treatment with INH resulted in slower progression of asymptomatic HIV infection with a prolonged survival (329). Moreno et. al found similar improvements in survival among HIV-infected individuals, most significantly in tuberculin reactors, (330), although the impact on mortality is less clear in other studies (331,332). At a clinic in Haiti that specializes in the care of HIV-infected poor urban residents, patients who are HIV positive and tuberculin positive routinely receive INH treatment for latent TB infection for between six months to three years at the discretion of the physician and patient. In a retrospective review of patients attending this clinic, researchers found that 1.4% of 1,005 patients who completed a course of INH subsequently developed TB; however, importantly there was evidence of a post-prophylaxis effect of INH that appeared to be dependent upon the duration of INH therapy. Patients who took only six months of INH developed TB in a median interval time of only eight months, whereas those who received INH for 24-36 months developed TB in a median of 40 months (333).

Previous data had suggested that anergic individuals were at increased risk for the development of active TB disease (18,334). However, data from a trial in Uganda indicated that anergic subjects that completed six months of INH only had a statistically insignificant reduction of 17% in the rate of TB compared to those who received placebo (332). The cost effectiveness and efficacy of treating anergic individuals, particularly in low TB incidence areas, is questionable at best (335). Some experts, however, maintain that the epidemiological context needs to be taken into account and that care should be individualized whenever possible (336).

Despite the sustained efficacy of INH therapy in preventing the development of active tuberculosis in both HIV- and non-HIV-infected individuals, toxicity and compliance continue to thwart the attainment of its full potential as an effective public health intervention. In addition, growing concern over the expansion of INH resistance has eroded confidence in the effectiveness of INH in some populations (337). Due to its potential limitations, researchers have sought viable alternative regimens that include rifampin. A study conducted in a murine model compared the efficacy of various combinations of rifampin, pyrazinamide and INH in sterilizing the spleens of mice infected with *M. tuberculosis*. After two months of daily rifampin and pyrazinamide, 100% of the mice had negative spleen cultures, whereas only 60% of those mice treated with six months of INH had sterile spleen cultures (338). Retrospective and observational studies in non-HIV infected human populations provided further evidence that rifampin was effective in reducing the incidence of TB in at-risk individuals (339,340).

Several studies looked at the use of rifampin in HIV-infected individuals (Table 17.6). The regimens used all focused on short-course therapy to address the issue of compliance, although none of the four protocols used directly observed therapy for the whole treatment. In the largest of these studies, daily INH for 12 months was compared to daily rifampin and pyrazinamide for two

TABLE 17.6. *Results of short-course regimens to treat leatent infection in persons with HIV infection*

Setting and Author	Regimen	PPD Status	Rate of TB per 100 person years	Comments
Uganda Whalen et al. 1997	Placebo (n = 464)	POS	3.4	Showed there was no bene t in treating anergic patients
	6 months INH daily (n = 536)	POS	1.1	
	3 months INH, RIF daily (n = 556)	POS	1.3	
	3 months INH, RIF, PZA daily (n = 462)	POS	1.7	
	Placebo (n = 323)	NEG	3.1	
	6 months INH daily (n = 395)	NEG	2.5	
Haiti Halsey et al. 1998	6 months INH bi-weekly (n = 370)	POS	1.7	Only partial observation of biweekly doses
	2 months RIF, PZA biweekly (n = 380)	POS	1.8	
United States, Haiti, Mexico, and Brazil Gordin et al, 2000	12 months INH daily (n = 792)	POS	1.2	Ef cacy and safety were equal in the two groups.
	2 months RIF, PZA daily (n = 791)	POS	1.2	
Zambia Quigley et al. 2001	6 months INH biweekly (n = 52)	POS	2.3	No directly observed therapy. Long-term follow up showed that effect of therapy lasted at least two and one half years
	3 months RIF, PZA biweekly (n = 49)	POS	2.7	
	6 months Placebo biweekly (n = 60)	POS	9.8	
	6 months INH biweekly (n = 178)	NEG	2.5	
	3 months RIF, PZA biweekly (n = 173)	NEG	3.8	
	6 months Placebo biweekly (n = 166)	NEG	3.1	

Adapted from: Centers for Disease Control and Prevention. Prevention and Treatment of Tuberculosis Among Patients Infected with Human Immunode ciency Virus: Principles of Therapy and Revised Recommendations. *MMWR* 1998;47(No. RR-20):19.

months. After a mean follow-up of over three years, there was no statistical difference between the INH and rifampin/pyrazinamide groups with rates of active TB disease of 0.8 and 1.1 per 100 person-years respectively (342). There was also no statistical difference between the regimens in reducing mortality, toxicity, or HIV progression (342). The regimens were generally well-tolerated except for one arm of one of the studies which utilized a three drug regimen including INH, rifampin, and pyrazinamide which found an increased rate of side effects (332).

In the studies performed in Zambia and Haiti, medications were given bi-weekly, either self-administered (343) or partially supervised (341). No difference was noted between the groups receiving INH or rifampin/pyrazinamide. The Haitian study demonstrated a rate of TB in participants in both arms of the study that was similar to the results reported by Pape in a nearby community with similar patients (329). However, because the intermittent regimens utilizing rifampin and pyrazinamide were not compared to a more prolonged course of INH, experts convened by the Centers for Disease Control have not felt that there was sufficient data to make a definitive statement on the biweekly administration of rifampin and pyrazinamide for treatment of latent TB infection in HIV-infected individuals in the U.S. (19). However, Florida has used biweekly rifampin and pyrazinamide since 1999 with toxicity levels that are equivalent to INH. This regimen has been used exclusively in the context of directly observed therapy and has resulted in a significant improvement in treatment completion rates for latent TB infection (344,345). HIV infected individuals started on treatment for latent TB infection should be on DOT whenever possible, especially when short-course therapy is used.

As short-course regimens become utilized to a wider extent, it is to be expected that toxicity will become more evident. In early 2001, the first fatal cases of hepatotoxicity from rifampin and pyrazinamide used as a short course regimen were reported (346). In a further analysis, an additional 21 cases of liver injury were reported occurring in individuals on short course regimens containing RIF/PZA (347). Although clear predisposing factors were not present in all of the cases, many patients who developed severe hepatitis continued to take the medications after the development of symptoms, a factor also found in INH-related hepatotoxicity (348). Of note, all of the reported patients who developed hepatotoxicity were HIV negative.

Based on these experiences, the following recommendations were made for the utilization of short course treatment with RIF/PZA (347):

1. The two-month RIF-PZA treatment regimen for latent TB infection should be used with caution, especially in patients concurrently taking other medications associated with liver injury, and those with alcoholism, even if alcohol use is discontinued during treatment. RIF-

PZA is not recommended for persons with underlying liver disease or for those who have had INH-associated liver injury. Persons being considered for treatment with RIF-PZA should be informed of potential hepatotoxicity and asked whether they have had liver disease or adverse effects from INH.

2. For persons not infected with HIV, nine months of daily INH remains the preferred treatment for latent TB infection; four months of daily RIF is an acceptable alternative. Two months of daily RIF-PZA may be useful when completion of longer treatment courses is unlikely and when the patient can be monitored closely.
3. Available data do not suggest excessive risk for severe hepatitis associated with RIF-PZA treatment among HIV-infected persons.
4. No more than a two-weeks supply of RIF-PZA (with a PZA dose 20 mg/kg/d and a maximum of 2 gm/d) should be dispensed at a time to facilitate periodic clinical assessments. A health-care provider should reassess the patient in person at two, four, and six weeks of treatment for adherence, tolerance and adverse effects, and at eight weeks to document treatment completion. At each visit, health-care providers conversant in the patientsÕ language should instruct patients to stop taking RIF-PZA immediately and seek medical consultation if abdominal pain, emesis, jaundice or other symptoms of hepatitis develop. Provider continuity is recommended for monitoring.
5. Serum aminotransferase levels (AT) and bilirubin should be measured at baseline and at two, four, and six weeks of treatment in patients taking RIF-PZA, as some side effects may occur in the second month of treatment. Patients need to be monitored throughout the entire course of treatment. Asymptomatic serum AT increases are expected, and usually do not require that treatment be stopped. However, treatment should be discontinued and not resumed for any of the following Þndings: AT greater than Þve times the upper limit of normal range in an asymptomatic person, AT greater than normal range when accompanied by symptoms of hepatitis, or a serum bilirubin greater than normal range.

Initiating Treatment of Latent TB infection and Screening Contacts

Active TB disease must be ruled out prior to starting a patient on treatment for latent TB infection. This can be a clinical challenge due to the atypical and protean manifestations of TB in those with immunosuppression.

Another important and often overlooked factor in the prevention of TB in HIV-infected individuals is HIV testing of contacts of active cases of TB disease. Investigators collected data from 11 urban areas in the United States over a six month period, and of 6,225 close contacts that were identiÞed, HIV status was unknown for 87%. A quarter of those that were known to be HIV positive did not receive adequate screening for the presence of TB infection (a follow up TST or chest radiograph). Of those HIV-infected contacts of an active case of TB, only a third started treatment for latent TB infection and only a sixth completed this treatment (349). In a separate study in New York, researchers found that almost 90% of patients who developed TB were diagnosed before their Þrst visit to an HIV clinic, thereby severely limiting the efÞcacy of any intervention which solely relies on tuberculin testing in HIV clinics for preventing TB (350). Both of these studies imply that more widespread HIV testing of those at risk for the development of TB may have a signiÞcant impact on TB in these groups.

Current Recommendations for Treatment of Latent TB infection

In 1997 the CDC convened a panel of experts to revise the guidelines for the prevention and treatment of TB among patients with HIV. One of the principles emphasized in the guidelines published by that panel (19) was that all HIV-infected individuals need to be screened for TB infection with tuberculin skin tests, and if positive, treated for latent TB. Once active TB disease is ruled out and there are no contraindications to therapy, the most appropriate regimen for latent TB infection should be selected. The various regimens and mode of administration are summarized in Table 17.7. The regimen selected should take into account the individual speciÞcs of the patient, the likelihood of adherence, and programmatic limitations. Monitoring, both clinically and biochemically, is important in ensuring patient safety. Recommendations for monitoring are outlined in Table 17.7. Directly observed therapy, when applied appropriately, can also aid in monitoring for toxicity since patients are being seen at least bi-weekly and many times daily by healthcare professionals.

If the patient is receiving any other medications that are metabolized by the liver, including anti-retrovirals, monthly symptom evaluation supplemented by monthly liver function studies is recommended.

Persistent clinical monitoring throughout therapy is a key to avoiding complications (127). If symptoms of toxicity develop, *medications should be discontinued immediately* and medical evaluation carried out promptly.

Treatment of Latent TB Infection in Developing Countries

Screening and subsequent treatment for latent TB infection is a key element of TB programs in developed countries with adequate resources for TB control. In

TABLE 17.7. *Regimens for treatment of latent Tuberlculosis (TB) infection for adults with HIV infection*

Rating[Δ] (Evidence)[δ]	Drug (See Table 17.2 for Dosages)	Interval and Duration	Monitoring	Comments
A(II)	INH[‡,§]	Daily for nine months	Clinical monitoring monthly Liver function tests† at baseline in selected cases* and repeat measurements if: • Baseline results are abnormal • Patient is pregnant, in the immediate postpartum period, or at high risk for adverse reactions • Patient has symptoms of adverse reactions	Rule out Active Disease before beginning therapy for Latent infection In HIV infected patients, INH may be administered concurrently with nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors, or non-nucleoside reverse transcriptase inhibitors (NNRTIs) Hepatitis risk increases with age and alcohol consumption. Pyridoxine (vitamin B_6 10–25mg/d) might prevent peripheral neuropathy and central nervous system effects. Directly Observed Therapy (DOT) should be used when twice-weekly dosing is used.
B(II)	INH[‡,§]	Two times/week for nine months		
A(I) C(I)	Rifampin** plus pyrazinamide Rifampin** plus pyrazinamide	Daily for two months Twice weekly for two to three months	Clinical monitoring at Weeks 2, 4 and 8 when pyrazinamide is given Complete blood count, platelets, and liver function tests† at baseline in selected cases* and repeat measurements if: • Baseline results are abnormal • Patient has symptoms of adverse reactions	Rule out Active Disease before beginning therapy for Latent Infection See note below (under **) on use of this regimen with certain NNRTIs or protease inhibitors May also be offered to persons who are contacts of patients with INH resistant, rifampin susceptible TB Decreased level of many other drugs Might permanently discolor contact lenses DOT should be used with twice-weekly dosing
B(II)	Rifampin**	Daily for four months	Clinical monitoring monthly Complete blood count, platelets, and liver function tests† at baseline in seiected cases* and repeat measurements if: • Baseline results are abnormal • Patient has symptoms of adverse reactions	Rule out Active Disease before beginning therapy for Latent Infection See note below (under **) on use of this regimen with certain NNRTIs or protease inhibitors May also be offered to persons who are contacts of patients with INH resistant, rifampin susceptible TB who can not tolerate pyrazinamide Decreased level of many other drugs Might permanently discolor contact lenses

† Recommended regimen for children younger than 18 years of age.
§ Recommended regimen for pregnant women. Some experts would use rifampin and pyrazinamide for two months as an alternative regimen in HIV-infected pregnant women, although pyrazinamide ahould be avoided during the rst trimester.
† AST or ALT and serum bilirubin.
* HIV infection, history of liver disease, alcoholism, and pregnancy.
δ A = preferred; B = acceptable alternative; C = offer when A and B cannot be given.
Δ I = randomized clinical trial data; II = data from clinical trials that are not randomized or were conducted in other populations; III = expert opinion.
** Protease Inhibitors or NNRTIs should generally not be administered concurrently with rifampin; rifabutin can be used as an alternative for patients treated with these medications—see Table 17.4 for full details.
Source: Adapted from American Thoracic Society/Centers for Disease Control. Targeted Tuberculin Testing and Treatment of Latent TB Infection. *Am J of Resp Crit Care Med* 2000;161:S221-S247.

contrast, developing countries have historically used their limited resources to Þnd and treat active, usually smear positive, cases of TB disease. In light of the limited resources available, this may be the more feasible and cost-effective intervention for them. However, the HIV epidemic and the resultant rise of TB has raised the question of also treating latent TB infection in developing countries (351).

The World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATLD) in 1994 recommended preventive treatment for PPD positive HIV-infected people who have had active TB disease ruled out (351). Despite these guidelines, there are numerous difÞculties in the widespread implementation of screening and treatment programs especially in the context of signiÞcant resistance to INH in many parts of the world.

Treatment of latent TB infection is most effective in preventing reactivation TB disease. In developing countries with a high rate of TB and HIV, recent transmission accounts for a large percentage of cases, sometimes up to 40% (352). The effect of treating latent TB in these populations is usually short lived and wanes over time (343). In certain instances, interruption of transmission is of signiÞcantly greater importance than preventing reactivation.

Many concerns exist from an operational point of view. The protean clinical manifestations of TB in the setting of HIV co-infection are well known, but will nonetheless provide formidable challenges to clinicians practicing with limited diagnostic resources at their disposal. If active TB disease is not adequately ruled out prior to starting treatment for latent TB infection, an already serious global drug resistance problem could be substantially worsened (353). Limited resources in developing countries can also be a barrier to ensuring the safety and effectiveness of therapy. Once therapy is initiated, monitoring and evaluating potential complications in large numbers of individuals will be necessary. The costs associated with this may be prohibitive for some programs.

Adherence to therapy is perhaps the biggest challenge to effective latent TB infection programs in the United States, and developing countries have problems which are similar or worse. Although feasibility studies have shown rates of compliance similar to those in the U.S. (354), some studies have shown that as many as 61Ð84% of HIV-infected patients do not even start treatment (355,356). Coordinating with the voluntary counseling and testing centers run by national AIDS programs has been proposed as a method of increasing compliance, since those voluntarily tested for HIV maybe more likely to be compliant with treatment (337).

BCG and Prospects for Other TB Vaccines

Vaccination with *M. bovis* BCG (Bacille-Calmette-Guerin) is an important part of the TB control strategy in most developing countries. BCG is a live attenuated strain of *M. bovis*, an organism closely related to *M. tuberculosis*, and is of questionable efÞcacy in all individuals and of uncertain efÞcacy and safety in HIV-infected persons. A meta analysis evaluating the efÞcacy of BCG in non-HIV infected populations showed minimal reductions in adult cases of TB, with this effect even less pronounced in tropical areas (357). The debate regarding the efÞcacy of BCG may be the result of a variety of factors, including the use of different BCG strains, the background rate of infection with atypical mycobacteria and other operational factors.

Populations infected with HIV have not been studied extensively to determine the efÞcacy of BCG in preventing TB in this group. However, a study from Malawi did show a 69% increase in active TB disease among HIV-infected persons who received a second BCG vaccine as compared to placebo (358). Dissemination of BCG in children born to HIV-infected mothers has also been described (359). A decision analysis done by Sterling and colleagues concluded that tuberculin skin testing, followed by isoniazid preventive therapy would be more effective than BCG vaccination in preventing TB in HIV-infected persons (360). In the absence of supportive data, it is difÞcult to think of BCG as an important element in the effort to reduce the incidence of TB in HIV-infected persons. BCG should not be given to any patient who is symptomatically immunosuppressed (361,362). Based on the available data, treatment of latent TB infection seems a more promising strategy than BCG vaccination; however, for most countries, BCG may be the only option despite its shortcomings. In the United States, BCG is not recommended for the general population (363).

Recent advances have increased the possibility of a new TB vaccine. Perhaps the most signiÞcant of these advances is the complete sequencing of the genome of *M. tuberculosis* (364). The DNA sequences of the 4,000 TB genes may help to identify antigens that confer protective immunity and thus lead to various types of vaccine prospects. Despite these advances, little is known about the immunologic mechanisms that confer resistance to TB.

Of the different types of potential vaccines, DNA vaccines hold the most promise. A gene encoding a protective antigen is inserted into an expression plasmid, plasmid DNA is ampliÞed in transformed bacteria, and the plasmid DNA encoding the antigen is injected into the host. The plasmid directly transfects a living cell leading to the host becoming immunized against a heterologous protein produced by his own cells.(365) Various studies utilizing a murine model have shown that DNA plasmid vaccination can lead to speciÞc T-cell responses that contain TB infection (366Ð369). The duration of this effect, however, is unknown, as are questions regarding the safety of DNA vaccines. Several concerns regard the integration of plasmid DNA into the host genome, tolerance induction and auto-immunity (370).

Despite the optimism fostered by recent scientiÞc advances, a TB vaccine that will have an impact on the epidemiology of this disease is still probably years away from becoming a reality. In addition, ethical issues will need to be addressed before vaccine trials can begin, especially as these trials will require testing in developing countries with limited resources.

Environmental Control

Recent studies have used DNA-Þngerprinting to uncover and call attention to environmental issues in the transmission of tuberculosis in HIV health care settings (371Ð376). There is a well-documented history of nosocomial outbreaks of tuberculosis among HIV-infected patients in hospitals, HIV clinics, prisons, jails, homeless shelters, and residential care facilities, and there have been hospital outbreaks of nosocomial transmission of MDR-TB in the mid 1980s and early 1990s (20,30Ð32,47Ð49, 312,377Ð383). During these outbreaks, many health care workers became infected with tuberculosis; some developed active TB disease and a few died (150,384Ð386). All these outbreaks have served to underscore the need for good environmental controls and infection control policies in order to prevent the spread of disease, both to patients and health care workers.

Upon investigation of the causes of these outbreaks, some common underlying etiologies involving programmatic and environmental factors were elucidated: (1) inadequate supervision of TB therapy and adherence, resulting in acquired drug resistance and prolonged infectiousness, (2) hospitalizing, housing or seeing patients with tuberculosis in the same care facility/area as non-tuberculous HIV-infected immunosuppressed patients, and (3) poor administrative, infection control and environmental TB control measures in those facilities. This last factor was responsible for delays in diagnosis and treatment, inadequate respiratory isolation and aerosolization of infectious material during sputum induction, all of which served to transmit tuberculosis (26).

On the basis of a comprehensive review of programmatic and environmental issues, guidelines were issued for prevention of transmission of tuberculosis in health care settings (26). These guidelines address important elements of infection control: (1) early identiÞcation and treatment of latent tuberculosis infection and active TB disease; (2) isolation rooms with negative pressure, at least 6 air exchanges per hour, non-recirculated ventilation vented to the outside, highly efÞcient particulate air (HEPA) Þlters, and ultraviolet lights in both inpatient and outpatient HIV facilities and during cough-inducing procedures; and (3) fully supervised therapy (DOT). The recent outbreaks of TB in HIV facilities support the use of strict environmental control not only in rooms speciÞcally designated for TB patient isolation and cough-inducing procedures (sputum induction, aerosol pentamidine therapy, bronchoscopy, pulmonary function testing), but also in waiting rooms, other clinic areas, and entire wards designated for HIV-immunosuppressed patients. Specialized booths, occupied only by the patient, can be used for sputum induction and aerosol pentamidine therapy (387).

Health care workers who are exposed to patients during cough-inducing procedures should wear properly Þtted N95 facemasks that can Þlter droplet nuclei (388). If it is necessary to administer aerosolized pentamidine therapy, the patient should be evaluated for the possibility of TB before therapy is initiated and again before each treatment (3). In addition, if HIV-infected TB patients are to be treated in HIV clinics of general hospitals (rather than in public health TB clinics), hospital nurses should be designated with the speciÞc responsibility for closely following these patients, as a safeguard for public health. At each scheduled appointment, it should be documented that the appointment is kept, that patients are compliant with therapy, and that they are receiving DOT as required. Surveillance of employees working in HIV areas for tuberculin skin test conversions (e.g. every six months) will identify those who need preventive therapy and also identify possible lapses in TB control.

Hospitals and in-patient facilities need to follow proper infection control procedures regarding respiratory isolation, currently recommended ventilation characteristics and sputum specimen collection. Each facility should have a written policy for initiating respiratory isolation, specimen collection, respiratory isolation practices, and criteria for discontinuing respiratory isolation. In general, however, patients with positive AFB sputum specimens should remain in respiratory isolation, if on therapy, until there is clinical improvement (e.g. reduction in cough, fever) and until three sequential sputum AFB smears on different days are negative (26,389,390). There is no clear time at which to remove suspected TB patients with negative smears from respiratory isolation; however, a clinical response to therapy and at least three days on anti-TB medications should be adequate. The role of nucleic acid ampliÞcation testing in determining when patients can be removed from respiratory isolation has not been fully elucidated, but recent recommendations suggest that individuals with two sputum specimens negative for AFB on smear and nucleic acid ampliÞcation may be considered non-infectious (228).

It should be emphasized that a hospitalized patient need not have negative AFB smears before being discharged from the hospital. If a patient is returning to an environment where there are not Òat-riskÓ individuals (e.g. a home without HIV-infected or other immunosuppressed persons, or very young children), and the health department is doing contact investigations, then AFB negative sputa are not necessary. However, if the patient is to go or return to a congregate living facility or other environment with Òat-riskÓ persons, then three AFB negative sputum smears (if previously positive) and 14 days on anti-TB therapy with a positive clinical response is warranted. In

some rare instances, patients may need to be kept in a hospital for a "fear of flight," possibly resulting in the inability to complete the necessary course of therapy. In these cases, the health department needs to coordinate a treatment plan where upon the completion of therapy can be assured.

Engineering controls should be followed regarding ventilation systems for general areas (e.g. waiting-room and emergency departments), as well as TB isolation rooms. Health-care facilities caring for populations with a high prevalence of HIV and/or TB may need to consider additional environmental methods to aid in the prevention of transmission, such as HEPA filtration or UVGI (ultraviolet lamps), or other in-room filtration (26). Respiratory protection should be available to, and used by, personnel caring for or dealing with patients with known or suspected infectious tuberculosis, and should meet criteria standards (26,388).

The Responsibility of Public Health and its Role in the Future of TB in the United States

Health departments in all fifty states are legally charged by their respective state legislative assemblies with responsibility to control TB in their communities (391). In order to do this, health departments and state TB programs must ensure the following: (1) all patients with active TB disease are diagnosed quickly and appropriately and placed on effective therapy as soon as possible in order to prevent ongoing transmission in the community, (2) all patients remain on an effective regimen until considered cured, and (3) patients with latent TB infection are identified through targeted testing of high risk groups, including contacts of active TB disease cases, and placed on effective therapy to prevent the development of active TB disease.

Health departments achieve these goals through a variety of interventions. Surveillance and the prompt reporting of cases by health care providers and laboratories to the local health department lead to the early initiation of contact investigations and the identification of other persons at risk. Diagnostic assistance is provided to community physicians either in the form of direct laboratory services (392) and/or by consultation with TB experts. Treatment is provided directly by many TB programs with medical, nursing, and outreach interventions all contributing to the cure of patients with TB. Some programs provide only nurse case management and directly observed therapy, both of which are cornerstones of treatment interventions in TB control. These interventions, along with education and consultation, are key elements to achieving the goal of controlling TB in the community. The public health infrastructure that implements these strategies is responsible for the declining rates of TB now being realized in the United States (393).

Now, in the early twenty-first century, the TB epidemic is clearly evolving. On the one hand, the United States is experiencing the lowest rates of TB ever seen, a direct result of the increased investment and commitment to TB control efforts. On the other hand, TB control may become the victim of its own success. As the incidence of a disease decreases, funding for control also decreases, resulting in an increase in the disease; this phenomenon has been referred to by Reichman as the U-shape of concern (394).

Specific recommendations on maintaining the commitment to TB control and steps necessary to achieve its eventual elimination were outlined by a report from the Institute of Medicine in 2000 (395). These recommendations fall into five main categories: (1) maintain control of TB, while adapting to a declining incidence of disease and changing systems of health care financing and management, (2) hasten the decline of TB towards its elimination through increased efforts aimed at targeted testing and the treatment of latent infection, (3) develop tools needed for the ultimate elimination of TB; new diagnostic tests, particularly for diagnosis of infection; new treatments and an effective vaccine, (4) increase U.S. involvement in global efforts to control TB, and (5) mobilize support for TB elimination and regularly measure progress toward that goal. If these recommendations are applied, it is possible that TB will cease to be a significant public health issue by the later part of this century.

MYCOBACTERIUM AVIUM COMPLEX AND HIV INFECTION

Before the AIDS epidemic, MAC disease classically presented as a slowly progressive chronic fibrocavitary pulmonary disease in middle-aged persons (men > women, rural > urban), with underlying predisposing lung conditions characterized by poor lung drainage (e.g. chronic bronchitis, emphysema, bronchiectasis, pneumoconiosis). Extrapulmonary disease occurred primarily in the form of lymphadenitis in pre-school children, and disseminated disease was rare (396,397). From 1940 to 1984, only 37 cases of disseminated MAC were described in non-AIDS patients, most of whom had other underlying immunosuppressive diseases (396). Therefore, even among immunosuppressed patients, disseminated MAC was rare before the AIDS epidemic.

With the development of the AIDS epidemic, before the widespread use of highly active antiretroviral therapy, MAC disease (particularly the disseminated form) had become common, diagnosed in 14% to 30% of HIV-infected patients during their lifetime (398–403) and in up to 50% or more of autopsy cases (399,400,402,404–409). With the increased utilization of effective therapy for HIV, associated with the concomitant improvement in immune function, rates of disseminated MAC in AIDS patients have seen a significant decline (93,410–413). In a study by Kaplan et al., in the United States, the incidence of MAC fell 39.9% in the years 1996–1998, compared to 1992–1995 (412). However, in the United States and other

developed countries, MAC is still by far the most common *Mycobacterium* species isolated from patients with AIDS and is one of the most frequent opportunistic infections reported in patients infected with HIV (398,402,414–417). (In Africa and other developing countries, *M. tuberculosis* is more frequently isolated (418–420).) Even if the HIV status is unknown, culture-proven MAC (as well as *M. kansasii)* disease, at a site other than the lungs, skin, or cervical or hilar lymph nodes, meets the CDC surveillance case deÞnition for AIDS (provided there is no other obvious cause of immunosuppression) (421,422).

In contrast to TB, MAC disease is not believed to be transmitted from person to person, is seen uniformly among AIDS risk groups, occurs late among AIDS-related opportunistic infections, and in the setting of AIDS, is manifested histologically by poorly formed granulomas and macrophages that teem with AFB (1) (see Figs. 17.4 and 17.5). Among HIV-infected patients with MAC disease (in contrast to those with TB), chest radiographs are less speciÞc for mycobacterial disease, pleuritis is uncommon, and positive stool cultures usually represent primary gastrointestinal invasion rather than infected swallowed sputum (203,423). Compared with TB, therapy for MAC disease tends to be less effective (1).

Epidemiology and Pathogenesis

MAC Disease in Patients Without HIV Disease

The true prevalence of MAC disease in the United States (among HIV and non-HIV patients) is unknown because positive MAC cultures are not routinely reported to public health departments, and the isolation of MAC (especially from sputum) may represent colonization rather than disease (424). Nevertheless, before the HIV epidemic, large nationwide skin test surveys (using PPD-S and PPD-Battey) conducted by the U.S. Navy and Public Health Service on recruits suggested that MAC infection was common (10% to 70% prevalence depending on region), occurred most frequently in the southeast United States (~70% prevalence), was more frequent in rural areas, and (in contrast to latent tuberculosis infection) was more common at younger ages (425–427). A skin test survey conducted on medical students and hospital employees in New Orleans revealed similar Þndings (i.e. PPD-Battey skin reactivity was common in this area and, among young adults, was much more common than PPD-S reactivity) (428). A nationwide survey of nontuberculous mycobacterial isolates in 1979 and 1980 revealed that MAC accounted for 60% of isolates and that MAC isolation rates were highest in the Southeast, supporting the skin test survey results (429).

In contrast to TB, MAC is not transmitted from person to person but is acquired from the environment by means not well understood. Antibodies to MAC protein antigens were detected in 70–80% of a population that did not have a prior history of exposure to infected persons (430). MAC is the most ubiquitous nontuberculous mycobacteria in the environment. It is found in domestic animals, soil, dust, dried plants, and water, especially in fresh and brackish water in warmer climates, such as in estuaries and rivers along the southeast coast of the United States (397,425, 429,431,432). There is evidence that MAC-infected aerosols are produced from these waters and that those MAC strains that are preferentially aerosolized are those more commonly isolated from persons with disease (433).

These studies considered together, and the facts that MAC disease before the HIV epidemic was most common in the southeast United States and was predominantly pulmonary, have given support to the hypothesis of airborne transmission of MAC infection from the environment to humans, at least in HIV negative patients.

MAC Disease in Patients with HIV Disease

In HIV-infected patients with disseminated MAC disease, autopsies have shown massive involvement of the small and large intestinal mucosa and submucosa and adjacent lymph nodes, disproportionate to pulmonary involvement (434,435). These patients tended to have had more gastrointestinal symptoms than pulmonary symptoms and their chest radiographs were often normal (436,437). This strongly suggests that among HIV-infected patients, at least in part infection is acquired from environmental sources via the oral route (i.e. primary gastrointestinal infection from ingesting food or water) (414,434,435). One study implicated showers in a Veteranõ3 Administration hospital as the source of disseminated MAC in HIV-infected patients (438). Other studies, however, prospectively examining environmental sources as the etiology of disseminated MAC in HIV infected patients with CD4 counts below 50 cells/mm^3, were unable to identify either food or water as a source, but suggested MAC strains residing in potted soil may be associated (439,440).

Plasmid-containing MAC strains are associated with increased virulence in animal models of disease, are usually present in isolates associated with human disease, and are much more common in aerosols than in soil or dust (425,441). Furthermore, in HIV-infected patients the prevalence of MAC disease is similar in most areas of the United States, in all HIV risk groups regardless of socioeconomic status, in males and females, and in non-Hispanic whites and blacks (only Hispanics with HIV infection have a lower prevalence) (442). This Þnding also supports the hypothesis that MAC infection is not spread from person to person but from the environment where the organism is ubiquitous (e.g. in water and/or soil) and exposure is unavoidable and common to most, if not all, groups. The similar rate of disseminated MAC among all HIV risk groups does not support homosexual intercourse as a means of transmission, as had been previously proposed (434).

Although the prevalence of MAC disease in HIV-infected patients does not seem to vary by location, race, sex, or HIV risk group, it does vary signiÞcantly by age: Higher rates are seen among younger age groups (442), suggesting that MAC frequently infects young persons, including infants and young children, and that disease results from recent infection rather than reactivation of latent infection (442). The lack of antibody response to MAC in patients with HIV disease and disseminated MAC also suggests recent primary infection, as HIV infected patients lack the ability to mount antibody responses to other primary infections, but not to reactivation infections (416,443,444). A third line of evidence suggesting primary disease from recent environmental exposure is that asymptomatic MAC colonization of respiratory secretions and stool commonly precedes MAC bacteremia among patients with HIV disease (399,407,445Ð447). In the normal host, asymptomatic MAC colonization may also occur, but without dissemination (416). It is thought that immune defenses in ÒnormalÓ human hosts are adequate to protect against MAC infection, whereas in HIV-immunosuppressed persons, MAC acts as an opportunistic pathogen.

Differences in MAC Isolates from HIV and Non-HIV Patients

In addition to biochemical and DNA probe methods used to differentiate MAC from the other nontuberculous mycobacteria, 28 types of MAC can be recognized by seroagglutination. This method has been helpful in epidemiologic studies as only a few serovars predominate in human disease. Serovars 1Ð6, 8Ð11, and 21 are *M. avium*, and serovars 7, 12Ð20, and 25 are *M. intracellulare*. (448,449). A predominance (more than 90%) of serovars 1, 4, and 8 is seen in patients with HIV disease (403,439,450,451). Serovar 4 is the most common in New York and San Francisco, while serovar 8 is the most common in Los Angeles (452). Approximately 40% of MAC isolates from non-HIV patients are *M. avium,* with the rest being *M. intracellulare,* as opposed to 98% *M. avium* among patients with HIV (453). Furthermore, the MAC isolates from patients with and without HIV differ in various ways. MAC isolates from patients with HIV disease, as compared with isolates from non-HIV patients, are more likely to be plasmid-containing, more virulent *in vitro* and in animal models, susceptible to ethionamide and cycloserine, and resistant to kanamycin and rifampin (407,416,453Ð456). Whether these differences in MAC isolates from HIV and non-HIV patients are due to different virulence factors relative to different host susceptibilities, different routes of infection, or distinct geographic preponderances of different MAC serovars remains to be determined.

Prevalence of Disseminated MAC Disease in HIV Disease

Data from the CDC revealed that between 1992 and 1997, the incidence of MAC disease was 76.6 per 1,000 person-years, whereas the incidence of disseminated *Mycobacterium* other than either *M. tuberculosis* or MAC was 6.6 per 1,000 person-years (417). This Þnding differs markedly from the distribution of disseminated nontuberculous mycobacteria before the HIV epidemic (38% MAC, 33% *M. kansasii*, 14% *M. fortuitum* complex, 13% scotochromogens) (397,442). Even in areas of the midwestern United States, where *M. kansasii* disease was relatively high before the HIV epidemic, disseminated MAC accounted for more than 90% of disseminated nontuberculous mycobacteria (442).

The cumulative incidence of disseminated MAC and HIV reported to the CDC, representing approximately 7% of all reported AIDS cases, is certainly an underestimate, (416) as disseminated MAC tends to occur in the later stages of HIV immunosuppression, a mean of 7Ð15 months after other AIDS-deÞning conditions have been diagnosed and reported to CDC. In North America, clinical studies have shown disseminated MAC in 14% to 30% of HIV-infected patients, though these may be underestimates, as autopsy series have shown disseminated MAC in as high as 50% of HIV-infected patients studied (398Ð409).

With the increased use of prophylaxis for MAC, as well as effective therapy for HIV resulting in improved immune function, rates of disseminated MAC have seen a signiÞcant decline (93,410Ð413). The CDC, in surveillance of AIDS deÞning illnesses, noted that the incidence of MAC disease decreased signiÞcantly, from 101.4 per 1,000 person-years in 1992 to 15.6 per 1,000 person-years in 1997 (417). Disseminated MAC is insidious in onset and presents late in the course of HIV disease; therefore, the diagnosis is often missed during life, and clinical studies can underestimate its true prevalence. On the other hand, autopsy data can overestimate the proportion of HIV-infected patients who develop disseminated MAC due to a bias toward patients who die with unexplained clinical syndromes (416).

In summary, MAC disease in HIV-infected appears markedly different than in HIV negative patients without AIDS (407,425,434,435,442,453Ð456): (1) it is much more common, (2) it constitutes a much higher proportion of all nontuberculous mycobacterial cases, (3) it is much more likely to be disseminated rather than a chronic Þbrocavitary pulmonary disease superimposed upon other chronic pulmonary diseases which are characterized by poor lung drainage, (4) the route of infection seems to be mainly gastrointestinal rather than respiratory, and dissemination appears to originate predominantly from the gastrointestinal source, (5) it is most common in younger age groups and its incidence appears to decrease with increasing age, rather than occurring predominantly in

those middle aged or older, (6) the geographic distribution is fairly uniform throughout the United States rather than predominantly in the southeast, and (7) the serotypes, DNA sequence, prevalence of plasmids, virulence, and drug susceptibility patterns of MAC isolates from HIV-infected patients are different from those isolated from HIV negative patients. Although the precise reasons for these differences are unclear, it is likely that HIV-infected patients have an immune defect that makes them more susceptible to MAC infection and disease, especially via the gastrointestinal route. The ability to bind to and enter intestinal epithelial cells has been demonstrated with some strains of MAC *in vitro*, a property that may contribute to the relative virulence of MAC in HIV-infected patients via the gastrointestinal tract (457–459). It is likely that once invasion of the gut epithelium has occurred, the profound immune defect in advanced HIV disease permits widespread dissemination. It is also possible that specific strains of MAC are uniquely virulent in this setting. It is clear that with improvement of the immune function in patients on HAART, the incidence of MAC is dramatically decreasing.

Clinical Features of MAC Disease

Disseminated MAC Disease

Unlike TB, disseminated MAC disease usually occurs in the later stages of HIV immunosuppression. The clinical signs and symptoms of MAC infection are nonspecific and therefore difficult to distinguish from other HIV-related conditions or drug toxicities. Nevertheless, numerous studies have revealed fairly consistent information regarding the clinical picture of MAC disease in patients with HIV disease (399–403,418,423,436,437, 458–474). Common symptoms include fever, drenching night sweats, fatigue, anorexia, malaise, and weight loss, all of which are often present for several months before the diagnosis is made (e.g. patients present with a chronic wasting syndrome). Abdominal pain and diarrhea may be prominent symptoms in patients with MAC infection of the bowel. Involvement of the small bowel can produce an illness mimicking Whipple disease, with malabsorption and similar intestinal histologic findings (399,435,474–476). Enlarged infected periportal lymph nodes may cause extra hepatic biliary obstructive jaundice (399). Despite frequent pulmonary involvement, only 9% of 114 patients with disseminated MAC disease had pulmonary symptoms and signs suggestive of pulmonary MAC (477). Diagnoses other than MAC disease should be excluded in a patient with respiratory symptoms (436,437).

On physical examination, patients are often febrile, cachectic, and may or may not have chest findings, peripheral or bulky intraabdominal lymphadenopathy, or hepatosplenomegaly. Subclinical hepatitis, thrombocytopenia, marked leukopenia, and progressive anemia including red cell hypoplasia (478) may occur, suggesting involvement of bone marrow and liver. Anemia and elevated alkaline phosphatase may be relatively more common in disseminated MAC disease compared with other HIV related opportunistic infections.

Among patients with HIV disease, MAC has also presented as localized pneumonia (447), mediastinal-endobronchial fistulas appearing as endobronchial mass lesions on bronchoscopy (479,480), mediastinal-esophageal fistulas with esophagitis (481), terminal ileitis mimicking Crohn's disease (482), pericarditis (483), meningitis (484), endophthalmitis (485), septic arthritis and osteomyelitis (486), cutaneous abscesses (487), and infections of skin, lymph node, and rectal mucosa in association with Kaposi's sarcoma (399,488). Localized disease is relatively uncommon and usually occurs in patients with higher peripheral blood CD4 counts.

MAC Colonization vs. Pathogen in HIV Disease

The approach to the patient with nontuberculous mycobacteria isolated from the airway specimen is challenging. Patients with HIV found to have nontuberculous mycobacteria in sputum or bronchoalveolar lavage with little evidence of pulmonary disease (e.g. no respiratory symptoms or radiographic parenchymal involvement) are usually felt to be without clinical significance (424). MAC rarely causes isolated pulmonary disease in patients with HIV disease. Rigsby et. al reported that 46 of 650 AIDS patients had positive sputum cultures for MAC but only two were diagnosed confidently as having pulmonary MAC disease (489).

Of note, cases of focal lymphadenitis and fever, not associated with bacteremia or other organ involvement, have been reported in patients whose CD4 count increased after the initiation of HAART; this has been considered to be due to immune reconstitution with an inflammatory response (490).

Diagnosis of MAC Disease

Disseminated MAC infection should be suspected in any HIV immunosuppressed patient who presents with unexplained systemic symptoms. The great majority of patients will have fever, debilitation, and weight loss with a peripheral blood CD4 count of less than 100/mm^3 (399,400,423,491). Of 55 patients with MAC disease, Modilevsky et al. found that 48 (87%) had another AIDS-defining illness diagnosed 2 to 37 months (mean, 7.8 months) before MAC disease was diagnosed (423). Of 50 patients with MAC disease diagnosed before death, Hawkins et al. found that 48 (96%) had an AIDS defining illness 1 to 24 months (mean, 9.3 months) before MAC disease was diagnosed (399). In two studies of patients with MAC infection, the concentration of peripheral blood

CD4 lymphocytes averaged 52 cells/mm^3 and 70 cells/mm^3 (400,423), in contrast to HIV-infected patients with TB in whom the mean or median peripheral blood CD4 lymphocyte concentration was 170 cells/mm^3 in one study and 326 cells/mm^3 in another (201,423).

Blood cultures remain the most common method of diagnosing disseminated MAC disease. One retrospective study suggested that two blood cultures will detect most cases (95%) of disseminated MAC infection (492). Laboratory testing has recently become more sensitive for mycobacterium with the BACTEC TB460, which has yielded a higher number of MAC isolates (493,494).

Although pulmonary involvement with disseminated MAC is frequent in HIV-infected patients, prominent pulmonary symptoms are rare (423,436,437). Chest radiographs may show a nodular, diffuse, or patchy inÞltrate with or without hilar or mediastinal adenopathy; commonly, they may appear normal despite the presence of disseminated disease (465). Cavitary disease, pleural effusions, and a miliary pattern are uncommon (465). Although the radiographic Þndings are nonspeciÞc, they may lead to sputum studies, bronchoscopic washings, or lung biopsy specimens.

Abdominal CT scans may show marked hepatosplenomegaly, diffuse jejunal wall thickening, and large retroperitoneal and mesenteric lymph nodes of homogeneous soft-tissue density, which are quite suggestive of disseminated MAC in this setting (495,496). Lymph nodes with central or diffuse low attenuation also may be seen in HIV patients with disseminated MAC on CT scanning, but are less common and more typical of TB, in which caseation necrosis is more frequent (495). Gallium scans may also be useful to locate infected sites (e.g. lung, lymph node, bone marrow, skin) and to direct biopsies (487,497Ð499).

Liver biopsy with acid-fast staining might be of use in HIV-infected patients with elevated liver related enzymes in order to make the diagnosis of disseminated MAC, and mycobacteria may be visible on pathologic studies. On the other hand, the diagnostic yield of a liver biopsy is very limited in patients with suspected disseminated MAC disease who have negative blood cultures and normal liver-related enzymes (500).

Microbiology

The genus *Mycobacterium* consists of close to 100 different species (230), all of which are similar in acid-fast staining. Many of these may be isolated from humans, though many are found in the environment. A specialized laboratory should be able to provide a precise species identiÞcation of most AFB isolated from humans. Clinical suspicion for a fastidious mycobacterium, such as *M. haemophilum* or *M. genevense*, needs to be conveyed to the laboratory, which in turn can inoculate special media and utilize appropriate incubation conditions. In contrast, the distinction between pathogen and saprophyte is not always clear-cut for each individualÕs isolates. Each mycobacterial isolate, like each patient, must be evaluated individually as to its potential to cause disease (424).

Among patients with disseminated MAC, the organism has been cultured from almost every body Þuid and tissue, including blood, stool, urine, sputum, CSF, lung, bone marrow, liver, lymph node, spleen, pancreas, tongue, esophagus, stomach, intestine, heart, adrenals, kidney, eye, and skin (2,73,399Ð401,407,423,431,434,446,501Ð503). A positive culture from a normally sterile site, such as blood, bone marrow, liver, or lymph node (the most commonly involved and accessible sterile sites), is diagnostic of invasive and (usually) disseminated disease. However, false-positive cultures of MAC and other mycobacteria as a result of contaminated laboratory reagents or other technical laboratory problems have occurred (504).

Blood culture is a particularly good noninvasive test for disseminated MAC in HIV-immunosuppressed patients and is the diagnostic procedure of choice in this setting; MAC bacteremia tends to be persistent and blood cultures, using a lysis-centrifugation technique (Dupont Co., Wilmington, DE, U.S.A.), are positive in the great majority of cases (e.g. 70% to 98%) (399,401,403,407,423,501,502, 505). As previously stated, one retrospective study suggested that two blood cultures will detect most cases (95%) of disseminated MAC infection (492). As MAC usually is not found free in plasma (506), a lysis-centrifugation technique is employed to increase the sensitivity of mycobacterial blood cultures and is accomplished by lysis of peripheral blood leukocytes, which releases viable intracellular mycobacteria which are then concentrated by centrifugation. The concentrate then can be plated on conventional media (eg. Lowenstein Jensen slants, Middlebrook 7H10 or 7H11 agar). Culture on agar takes 15Ð20 days. Plating on the solid agar, however, allows quantitative colony counts in blood, a useful means of determining both the magnitude of infection and the response to treatment (especially useful in clinical drug trials) (403,407,501,502,507,508).

Unprocessed blood (5 ml) also can be inoculated directly into BACTEC TB460 broth (avoiding the tedious process of lysis and centrifugation). The radiometric BACTEC 460TB system allows more rapid diagnosis by detecting growth of mycobacteria within 6 to 12 days; however, this technique precludes quantitative measurement of the bacteremia. Use of a lysis centrifugation system followed by inoculation of a BACTEC 460TB radiometric broth medium may be inhibitory and is not recommended. DNA probes for MAC can offer a rapid two-hour species identiÞcation once mycobacterial growth in BACTEC 460TB broth or on solid culture media is achieved (509,510). Auramine Þuorochrome staining of uncultured peripheral blood concentrate for AFB is insensitive for detecting mycobacteremia and generally is not done. Buffy coat smears for AFB are also insensitive

but have been reported to detect mycobacteremia in patients with AIDS (511–513).

Stool culture and AFB smear are simple noninvasive tests that may be used to investigate for invasive MAC disease if the patient has constitutional symptoms (399). Although there is some evidence that a positive AFB smear of stool (subsequently identified as MAC) is indicative of disseminated MAC disease, the predictive value of a negative AFB stool smear or a positive stool culture is poor (514). Positive MAC cultures from sputum or bronchial washings are common and raise suspicion for, but are also not diagnostic of, invasive or disseminated disease (399,409,446,465). If MAC is found in pulmonary or stool specimens, other specimens from sterile sites (e.g. blood, bone marrow) should be cultured for MAC to search for proof of disseminated disease. Positive cultures from respiratory secretions and stool commonly precede bacteremia; therefore, close clinical follow-up of patients with these findings is necessary (399,445–447). Horsburg et al. found that 8 (33%) of 24 patients colonized with MAC in sputum or stool progressed to disseminated disease with the same species of MAC with which they were colonized, whereas none of 16 patients colonized with other nontuberculous mycobacterial species progressed to dissemination with the colonizing species ($p = 0.01$). (Mean follow-up was 5.2 months for patients colonized with MAC and 7.3 months for patients colonized with other mycobacteria) (445). Disseminated MAC frequently occurs in the absence of prior detectable respiratory or gastrointestinal colonization and routine screening for colonization is felt to be impractical (515).

Currently, uniform agreement concerning the indications for susceptibility testing of clinically significant isolates of MAC does not exist (424). However, investigators who have extensively studied MAC have recommended that susceptibility testing be performed in the following situations:

i. clinically significant isolates from patients on prior macrolide therapy
ii. isolates from patients who develop bacteremia while on macrolide prophylaxis
iii. isolates from patients who relapse while on macrolide therapy
iv. initial isolates from blood or tissue (patients with disseminated disease) or from clinically significant respiratory samples to establish baseline values.

For susceptibility testing of other nontuberculous mycobacteria, the reader is referred to the Tentative Standard (M24-T2)-*Susceptibility Testing of Mycobacteria, Nocardia, and Other Aerobic Actinomyces*, of the National Committee of Clinical Laboratory Standards (516).

Pathology

Disseminated MAC in patients with HIV disease primarily involves the reticuloendothelial system (bone marrow, liver, spleen, and lymph nodes) and gastrointestinal tract. Massive MAC involvement in the intestinal Peyer's patch and regional lymph nodes, along with intestinal erosions, is common. The most common gastrointestinal site is the duodenum; the esophagus and large intestine are less frequently affected (517).

The distinctive pathologic feature of MAC disease in patients with HIV disease (on hematoxylin and eosin staining of tissue specimens) is a poorly defined granuloma consisting of pale-blue striated histiocytes filled with mycobacteria (467,473,518–521). The striations within the histiocytes represent large numbers of AFB as demonstrated on Ziehl-Neelsen staining (see Fig. 17.5). The features often described in typical granulomatous inflammation, such as lymphocytic infiltrates, caseation necrosis, epithelioid histiocytes, and Langhans giant cells, are present in only a few cases. Occasionally, there is no suggestive tissue reaction at all, despite positive AFB smears and positive cultures for MAC (423,460) (Fig. 17.5B, C). Tissue culture may be positive when the AFB smear is negative. Therefore, all specimens from HIV-immunosuppressed patients must be stained for AFB and cultured for mycobacteria, regardless of tissue reaction, AFB smear results, or concomitant nonmycobacterial pathology. Bone marrow is easily biopsied and frequently yields diagnostic specimens, as do biopsies from lung, lymph node, liver, and the gastrointestinal tract. On gross inspection, involved organs may appear yellow because of the pigmentation of the organisms (419).

When disseminated MAC is suspected, the best specimens for culture are blood, stool, bone marrow, and, if necessary, liver. Taken together, the yield should be almost 100% (399). Cultures of respiratory, stool, and sterile site specimens are frequently positive when the smear of the specimen is negative (501).

Assessment of Morbidity and Mortality from Disseminated MAC Disease in HIV Disease

In patients with HIV disease, MAC infection disseminates widely and involves many tissues and organs (eg. blood, bone marrow, liver, spleen, lymph node, and gastrointestinal tract), but the organism has relatively low virulence. Autopsy series suggest that it does not usually cause significant tissue damage or organ failure (408,522). Nevertheless, the bacterial load in disseminated MAC is formidable (as high as 10^4–10^5 colony forming units of MAC/ml blood within circulating monocytes and 10^9–10^{10} bacilli/g of tissue within fixed macrophages of the reticuloendothelial system), and clinical studies suggest that disseminated infection contributes significantly to fever and debilitation among patients in the late stages of HIV disease (436,505,523,524).

It is uncertain whether patients with disseminated MAC die of this disease or multiple other coexisting infections (eg. PCP or cytomegalovirus) (464,525). In one study from

1989, the median survival for HIV-infected patients with disseminated nontuberculous mycobacteria (almost all MAC) was 7.4 months, whereas the median survival for HIV-infected patients without MAC was 13.3 months ($p < 0.0001$) (442). The survival analyses for patients in this study, however, were challenged (525). A second similar case-controlled study calculated the median survival in these two groups as 3.5 and 9.1 months, respectively (526). In a third study involving 1,044 patients with AIDS or HIV-related condition who were started on zidovudine, 12.4% developed MAC disease during two years of follow-up, and this was a strong independent predictor of death (527). It has been suggested that the shortened survival of AIDS patients with MAC disease (which frequently involves the intestine) results from inanition (400,518,528). The preponderance of evidence suggests that MAC disease does contribute (directly or indirectly) to the morbidity and mortality of HIV disease in its late stages.

Treatment for MAC Disease

Challenges of Treatment for MAC Disease in HIV-infected Patients

Pulmonary or disseminated MAC disease has traditionally been difficult to treat in patients without HIV because of the relative drug resistance of the organism. MAC disease in patients with HIV disease responded even more poorly to antimycobacterial chemotherapy (396,529,530), primarily due to severe immunosuppression, the large number of organisms, and possibly because MAC isolates from HIV-infected patients have more virulence or different drug susceptibility patterns from those of non-HIV isolates (407,454,456). Unlike the treatment for TB, it is less clear whether various drug regimens for disseminated MAC in HIV patients have a major impact on morbidity and mortality, which drug regimens and what drug doses are most effective, and whether *in vitro* drug susceptibility results (e.g. testing single drugs or drug combinations) predict bacteriologic and clinical response to therapy (523,531). *In vitro* drug concentrations that are easily achievable as serum levels *in vivo* and that predict clinical usefulness of the drug are well established for TB but are not well defined to guide treatment for MAC disease. In MAC disease, clinical response or lack of response to a drug is often poorly predicted by the results of standard qualitative drug susceptibility tests. The *in vitro* critical drug concentrations used in these qualitative tests were originally developed and standardized for *M. tuberculosis,* not for MAC (532).

An exception may be clarithromycin. A correlation between minimal inhibitory concentration (MIC) and the microbiologic response to therapy was established in a clinical trial of clarithromycin monotherapy for disseminated MAC disease (533,534). More than 90% of patients had a significant decrease in bacteremia, and most patients became culture negative when their initial MAC isolates had an MIC of clarithromycin of 2 mcg/ml or less using the BACTEC TB460 susceptibility test system. In contrast, patients had a clinical and bacteriologic relapse when the MIC increased to 32 mcg/ml or higher. Therefore, MAC strains can be classified for accuracy and clarity as either susceptible or resistant to clarithromycin if the MIC is 2 mcg/ml, or 32 mcg/ml or greater, respectively. The clinical implications of MIC between 4 and 16 mcg/ml are unclear (535). With clarithromycin as a possible exception, conventional qualitative drug susceptibility testing (as used for *M. tuberculosis)* should not be applied to MAC (424,535); however, some centers with extensive experience believe that quantitative and combination drug susceptibility testing on MAC isolates may furnish information that facilitates the choice of drugs, drug dosages, and drug combinations that are more efficacious than standard regimens (536).

Often MAC is resistant to two of the standard first-line TB drugs, isoniazid and pyrazinamide, but shows variable susceptibilities *in vitro* to rifampin, rifabutin, ethambutol, clofazimine, ciprofloxacin, ofloxacin, levofluxacin, imipenem/cilastatin, rifapentine, cycloserine, amikacin, clarithromycin, and azithromycin (403,523,531,532, 537–541). The effects of drugs in combination are often additive and occasionally synergistic and bactericidal (542,543). Nevertheless, there have been conflicting reports on the clinical efficacy of regimens that include drugs to which MAC shows some susceptibility (399,400, 403,414,466,470,523,540,544–553). Initial reports indicated a lack of success using drug regimens that included rifabutin and clofazimine (399,551). Subsequently, several reports showed that similar drug regimens, including rifabutin and clofazimine, with or without amikacin, resulted in defervescence, a resolution of night sweats, malaise, and (less frequently) diarrhea, a decline in mycobacteremia and, in some cases, the sterilization of blood cultures without bacteremic relapses (505,528, 546–549).

Symptomatic and bacteriologic responses also have been shown using a drug regimen without rifabutin (i.e. ethambutol, clofazimine, ciprofloxacin, and rifampin) (554) and without rifabutin and clofazimine (i.e. ethambutol, rifampin and ciprofloxacin, with or without amikacin) (555,556). The more favorable results in these therapeutic studies may be explained by the use of more drugs to which MAC is susceptible (i.e. four or five), an earlier start of therapy after the onset of symptoms (i.e. within four weeks) and therapy that is continued longer (i.e. more than three months) before assessing the response to therapy (528).

Clarithromycin, azithromycin, and ethambutol (as opposed to agents such as rifampin, sparfloxacin, and clofazimine, which also have *in vitro* MAC activity) demonstrated microbiologic activity in clinical trials of short duration when used as single agents. Reduced

numbers of MAC colony forming units were found in the blood after four to six weeks of therapy, and many patients with reductions in MAC bacteremia had considerable resolution of fever and weight loss (533Ð536,557,558). Clinical and bacteriologic improvement was not sustained despite continued therapy, however, suggesting that monotherapy with these agents at the doses given, does not eradicate MAC and that resistance can develop, perhaps similar to the acquired drug resistance that occurs when monotherapy is used in the treatment of TB.

More recent studies utilizing macrolide antibiotics (e.g. clarithromycin or azithromycin) have shown signiÞcantly improved success. A randomized trial showed that the combination of rifabutin (300mg to 600mg/day), clarithromycin (1,000 mg twice a day), and ethambutol (15mg/kg/day) was superior to the combination of rifampin, clofazimine, ciproßoxacin, and ethambutol, with more rapid resolution of bacteremia and increased survival time (559). In this study, the higher dose of rifabutin was more effective, but frequently caused uveitis (559). For clarithromycin, a prospective randomized trial suggested that the maxmum dose of clarithromycin should be 500 mg twice a day, because the regimen with clarithromycin 1,000 mg twice a day had excess mortality (relative risk of 2.43) (560). Other studies have shown that initial regimens containing a macrolide (clarithromycin, azithromycin) are better than those without a macrolide, and that both clarithromycin and azithromycin are effective (561,562). At least one study has shown that cloÞzimine does not add to the efÞcacy of macrolide regimens and may actually result in excess mortality (558,563).

Probably the best results of therapy for disseminated MAC disease have been seen in patients treated with both HAART regimens and antimycobacterial agents (564). However, given the signiÞcant pharmacokinetic interactions, treatment with both classes of agents must be carefully dosed and monitored (see below).

In contrast to TB, there is no evidence that MAC is communicable to the general population, and MAC disease is less often fatal than TB when left untreated (423,565,566). Therefore, when AFB are found in any specimen from a patient with HIV disease or suspected HIV, our approach, pending culture results, is to institute promptly standard anti-TB drugs as outlined in the section on TB, though this may change with the availability of rapid nucleic acid ampliÞcation testing for TB. With rapid culture methods and speciÞc DNA probes, mycobacteria are now routinely identiÞed within one to three weeks (403,501,502,510,523). If cultures reveal MAC, which is felt to account (or probably account) for the patientÕs signiÞcant symptoms (eg. in the absence of other identiÞed opportunistic infections), the patient has reasonably intact renal and liver functions, and the patient is willing to cooperate in a multidrug treatment plan, an anti-MAC drug regimen may be initiated on a trial (e.g. three-month) basis.

The U.S. Public Health Service (USPHS) Task Force has recently published the following guidelines for the treatment and prophylaxis of disseminated MAC disease in AIDS patients which was recently revised (535,567).

1. Every regimen should include either azithromycin (500 mg daily) or clarithromycin (500 mg twice daily) with one or more other drugs. (At least two agents should be used.)
2. EMB (15 mg/kg) should be used as a second drug with the addition of one or more of the following as a third or fourth agent, especially for HIV-infected patients with severe symptoms and/or extensive disease: either rifabutin (300mg daily), or RIF (600 mg daily), ciproßoxacin (750 mg twice daily), and in some situations amikacin (7.5Ð15 mg/kg daily); INH and PZA are not used.
3. The same treatment regimen should be used for patients on rifabutin or macrolide prophylaxis for MAC who developed disseminated MAC disease despite prophylaxis. A majority of HIV-infected patients who develop disseminated MAC disease despite prophylaxis have isolates susceptible to these prior prophylactic drugs (568).
4. Therapy should continue for the duration of the patientÕs life if clinical and microbiologic improvement is observed. If CD4 counts increase to more than 100 cells/mm^3 following 6Ð12 months of HAART (minimum of 12 mo of MAC treatment should be received), discontinuation of MAC therapy could be considered if there are negative blood cultures and minimal symptoms, but available data are insufÞcient to warrant a recommendation at this time.
5. The usefulness of drug-susceptibility testing to guide the initial selection of drugs is not known.
6. Initiation of HAART regimens should be considered, if not already begun. The choice of regimen should take into account potential drug interactions and medication dosages appropriately adjusted (see TB Section).

From experience with TB-HIV coinfected patients, when these protease inhibitors are used, indinavir or nelÞnavir may be used in combination with rifabutin in order to minimize the incidence of drug interactions (see TB Section).

Clinical manifestations of disseminated MAC, such as fever, weight loss, and night sweats, should be monitored routinely during the initial weeks of therapy. The use of follow-up blood cultures every four weeks during initial therapy may also be helpful in assessing the efÞcacy of the drug regimen. Most patients who respond will show signs of clinical improvement within the Þrst four to six weeks of therapy, with the attainment of sterile blood cultures taking four to twelve weeks (535). If no clinical or bacteriologic response is noted within three months of therapy, discontinuation of the therapy should be considered at this point.

In cases of a bacteremic relapse after an initial clinical and microbiologic response, determining the MIC of azithromycin or clarithromycin is useful to indicate whether these agents will be useful in subsequent therapeutic regimens (535). There are no long-term studies to help in determining the optimal maintenance therapy regimen for disseminated MAC. The aim of treatment is to reduce the bacterial load and thereby to ameliorate symptoms and improve quality of life. These objectives, even if achievable, must be weighed against the signiÞcant toxicity of the multidrug regimen. In one multidrug clinical trial, adverse reactions requiring the discontinuation of a drug to treat MAC disease occurred in 46% of patients (554).

Overall, the eventual successful treatment and prevention of MAC will probably depend on improving immunity utilizing HAART and other interventions that encourage immune reconstitution.

Drug Toxicity and Interactions

Evidence suggests that macrolides (clarithromycin and azithromycin) are the single best agents to include in the multidrug regimen for disseminated MAC. They result in rapid sterilization of blood cultures and appear to be fairly well tolerated in patients with HIV disease. Clairithromycin and azithromycin have similar side effect proÞles, the more common being nausea, vomiting, anorexia, diarrhea, abdominal pain, headache, ototoxicity, an unpleasant bitter taste, and rarely, cholestatic jaundice. Clairthromycin, however, may be more prone to drug interactions, especially with the HIV protease inhibitors and rifabutin due to their effect on CYP450 enzymes; azithromycin has little effect on the CYP450 system. The dose of clarithromycin may need to be reduced in severe renal insufÞciency (535,569,570).

The most common adverse effects of clofazimine are pink to brownish black discoloration of skin and various other tissues and ßuids, skin dryness, rash, and gastrointestinal symptoms including nausea, vomiting, abdominal pain, diarrhea, and anorexia (531,571). Peripheral neuropathy and rarely ocular changes can also occur (535). The addition of clofazimine to a clarithromycin-containing regimen does not improve its efÞcacy and may increase mortality (563,572).

The adverse effects of quinolones include occasional nausea, vomiting, abdominal pain, diarrhea, rash, headache, and lightheadedness. The quinolones have also been associated with tendon rupture (573) and severe photosensitivity reactions have occurred. Children under the age of 18 should generally not receive quinolones because of the potential for premature closure of the epiphiseal plate resulting in diminished growth (574,575). Concurrent administration with food or magnesium or aluminum containing products such as antacids, sucralfate, or ddI will markedly diminish the absorption of ßuoroquinolones. Ciproßoxacin is an inhibitor of the CYP450 system and may increase the toxicity of many drugs such as theophylline, coumadin, and phenytoin. Newer quinolones such as levoßoxacin can be used instead of ciproßoxacin and generally have fewer and less signiÞcant drug interactions, but similar efÞcacy and adverse effects. Depending on the drug, the dose may need to be reduced in either hepatic disease or renal insufÞciency (569,570).

The major adverse effects of amikacin are nephrotoxicity and ototoxicity. Ototoxicity is frequent after eight weeks of therapy and is sometimes irreversible (528,576). The dose of amikacin should be reduced in the presence of advanced age or renal insufÞciency. (570).

The toxicities of the conventional antimycobacterial drugs rifampin and EMB are described in the ÒTreatment of TBÓsection (149,535).

Rifabutin is generally well tolerated (at least as well as rifampin), and the types of adverse reactions have been similar to those reported for rifampin. The most common adverse effects are mild elevations of liver enzymes, gastrointestinal distress (nausea, vomiting, diarrhea), and hypersensitivity reactions such as rash and fever. Reversible bone marrow suppression with leukopenia and thrombocytopenia has also been observed (577). Rifabutin used for MAC prophylaxis in HIV-infected patients has been associated with uveitis and arthritis (578). However, this has generally been at higher doses or when given concomitantly with clarithromycin and/or azole antifungal agents. (559,560,579) It has been shown that 80Ð100% of MAC strains are susceptible to rifabutin at concentrations of 1.0 mcg/ml (531). Although plasma levels of rifabutin do not reach this concentration at conventional doses, higher tissue concentrations do occur (580). Compared with rifampin, rifabutin is a weaker enzyme inducer and may be less likely to interfere with the action of multiple drugs (e.g. coumadin, oral contraceptives, methadone, oral hypoglycemics, digitoxin, quinidine, disopyramide, dapsone, ketoconazole, corticosteroids, HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors) (569Ð571,581,582). Rifabutin is excreted partly through the biliary route and partly through the kidney. In contrast to rifampin, the dose must be lowered in renal insufÞciency (577). Interestingly, rifabutin inhibits the replication of HIV, although this is not of clinical signiÞcance (583). Rifapentine, another derivative of rifampin, may also be effective against MAC. Its pharmacokinetic proÞle appears to be midway between rifampin and rifabutin; however, at least one study comparing rifampin to rifapentine in TB patients demonstrated an increased rate of relapse with mono-resistance to rifamycins occurring in HIV-infected patients receiving rifapentine and INH once a week in the continuation phase (584).

Monitoring for toxicity of antimycobacterial drugs in patients with HIV disease is often confounded by the occurrence of gastrointestinal, hepatic, hematologic, and dermatologic abnormalities from other drugs, other concommitant illnesses, or the mycobacterial disease itself.

The presence of such abnormalities is therefore not an absolute contraindication to the use of antimycobacterial agents. The physician, however, must be alert to true anti-MAC drug toxicity and to the drug interactions that occur among the multiple concomitant medications these patients take. It has been shown that patients can tolerate concurrent therapy with anti-retrovirals and conventional anti-mycobacterial drugs without unacceptable toxicity (450).

An additional problem is that of possible malabsorption of drugs. Gordon et al. demonstrated that most of the 27 patients receiving oral RIF, EMB, ciproßoxacin, and clofazimine for disseminated MAC disease had probable impairment in the absorption of some or all of their antimycobacterial agents. Most patients did not achieve the usual serum concentrations with all four drugs. Whether adjusting dosages on the basis of serum drug levels will be of clinical beneÞt needs to be further studied (585,586).

Prevention

As MAC is ubiquitous within the environment, it seems impossible to prevent exposure. Measures to prevent MAC disease in HIV-infected patients include the use of antiretroviral therapy to prevent and reverse immunosuppression, and the early preventive use of a macrolide or rifabutin when the T-helper cell count falls below 50/mm^3 (567). In two large, prospective, randomized, double blind, multi-center studies, rifabutin (300 mg/day taken orally) reduced the frequency of MAC bacteremia by half during the course of treatment (mean, 218 days). The beneÞt was limited to those patients with CD4 counts below 100/mm^3 at entry. Rifabutin also seemed linked to a clinical beneÞt (fever, fatigue, Kamofsky score, anemia, elevated serum alkaline phosphatase, and need for hospital admission). Discontinuation of rifabutin or placebo because of apparent toxicity (rash, gastrointestinal intolerance, and neutropenia) totaled 16% in the rifabutin group versus 8% in the placebo group. When MAC bacteremias occurred during rifabutin prophylaxis, the prophylaxis did not appear to have selected out for isolates with increased resistance. Although not statistically signiÞcant, there was a trend toward increased survival in the rifabutin group (587). More recently, clarithromycin (588) or azithromycin (568) have become the preferred prophylactic agents due to less frequent dosing, minimal interaction with protease inhibitors, and proven efÞcacy (improved survival in the treated group (567)).

The following are the guidelines for MAC prophylaxis from the USPHS/IDSA, published in 1999 (567).

1. MAC prophylaxis should be considered for all patients with CD4 cell counts below 50/mm^3.
2. Therapy should be initiated with clarithromycin 500mg PO twice daily or azithromycin 1200 mg PO weekly, and continued through the patientÕs lifetime unless, with HAART, a CD4 count rises above 100/mm^3 for a sustained period (e.g. more than three to six months) and there is sustained suppression of HIV plasma RNA for a similar period.
3. If clarithromycin or azithromycin cannot be tolerated, rifabutin (300 mg PO daily) is an alternative prophylactic agent.
4. Before starting preventive therapy, patients should be screened for preexisting mycobacterial disease. Screening may include a chest radiograph, tuberculin skin test, and mycobacterial blood culture.

Future Prospects

Variables such as drug-tissue concentrations and the activity of agents within macrophages inßuence the relevance of serum drug concentrations and *in vivo* drug efÞcacy (523). For example, some beta-lactam antibiotics, such as ampicillin, have promising *in vitro* activity against MAC but are not effective against intracellular organisms, thus limiting their clinical effectiveness (531). Liposome encapsulation of drugs or other carrier technology might be used in the future to enhance delivery of drugs to intracellular sites and thereby increase drug activity against intracellular mycobacteria (523,589). Liposome encapsulation of amikacin improves its activity as compared to free amikacin in the beige mouse model of MAC infection (531). Further studies are needed to determine the efÞcacy and safety of higher drug doses on an intermittent dosing schedule, and to determine the clinical relevance of obtaining serum drug levels and quantitative drug susceptibility tests for single drugs or drug combinations (590,591). As new anti-HIV and immunostimulating drugs are developed, new and existing chemotherapy regimens for MAC may become more effective, or even unnecessary, in the setting of HIV disease and may be reevaluated.

Immune Regulation and Modulation in MAC Disease

The abnormal susceptibility of HIV-infected patients to mycobacteria has been attributed in part to deÞciencies in T-lymphocyte-produced cytokines that activate macrophages. Facilitating anti-mycobacterial macrophage activity and delivering effective drugs into the macrophage appear to be critical as tissue macrophages in HIV-infected patients with disseminated MAC are literally packed with these bacilli (531) (Figs. 5CÐE). Nevertheless, the precise mechanism by which the macrophage defends against MAC is unknown.

Recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) has been shown to inhibit the growth of or kill MAC (592,593). Tumor necrosis factor (TNF) also has been shown to enhance *in vitro* killing of MAC in human macrophages, and the bactericidal effect was augmented when used in combination with antimicrobial agents (594). Furthermore, treatment of macrophages *in vitro* with TNF plus IL-2 reduces intracellular replication of MAC (593).

Bermudez and Young, and Toba et al. found that gamma-interferon does not have macrophage-activating factor activity for *M. avium* infection of human monocytes (595,596).

Nevertheless, Holland et al. (597) found that interferon-gamma in combination with conventional therapy was effective for treating cases of refractory disseminated nontuberculous mycobacterial infection, mainly MAC. These patients may have shared a common defect resulting in altered interferon-gamma production and an inability to kill mycobacteria. Not all of these patients were HIV-infected, and they probably represent only a small subset of patients with MAC disease.

Schnittman et al. (524) showed that high titered anti-MAC immune serum signiÞcantly enhanced the killing of MAC by monocytes from both HIV-infected patients and healthy controls, and also prevented the outgrowth of surviving MAC. One way microorganisms may survive intracellular killing is by inhibition of fusion of lysosomes with the phagosomes containing the organisms, thus avoiding exposure to hydrolytic enzymes (598). Schnittman et al. hypothesized that the anti-MAC antibody may alter the outer capsule of the MAC bacillus, thereby overcoming the inhibition of fusion of the phagosome with the lysosome (524).

Winter et al. found that patients with HIV disease and disseminated MAC infection had anti-MAC antibody levels similar to those of uninfected HIV patients and normal controls, suggesting an inability to mount an anti-MAC antibody response in HIV disease (444). Crowle et al. found that normal human serum inhibits the growth of *M. avium* in normal human macrophages and that sera from HIV-infected patients were deÞcient in this inhibitory property. Furthermore, macrophages from HIV-infected patients were unresponsive to the inhibitor in normal serum (599). Further studies on how normal macrophages respond to the serum inhibitor in order to suppress *M. avium* infection and studies on the clinical efÞcacy of hyperimmune (anti-MAC) gamma globulin in HIV-infected patients with disseminated MAC are needed.

The precise roles of monocyte-T-cell interactions and various cytokines and antibody-dependent mechanisms in enhancing macrophage killing of MAC and other mycobacteria are still unknown. Even though MAC and *M. tuberculosis* are both intracellular pathogens, reside in monocytes and macrophages, and presumably are controlled by cellular immune responses, they may present different challenges to the immune system and therefore may need to be handled differently.

For example, as stated above, HIV-infected patients with disseminated MAC do not produce antibodies in response to infection, and there is some evidence that humoral immunity may play a role in the defense against this organism (444,524,599). In contrast, HIV-infected patients with reactivation TB commonly have antibodies against *M. tuberculosis,* but humoral immunity seems unimportant in controlling the disease (443). Understanding the normal immune defense against MAC infection and understanding the basis of the speciÞc vulnerability of patients with HIV disease to MAC will provide insight for the development of new preventive and therapeutic strategies. In the Þnal analysis, prevention of immunosuppression or restoration of immune function will have the greatest impact on the overall morbidity and mortality of HIV-infected patients and remains the ultimate goal of therapy in this disease.

Future of Mycobacterium Avium Complex and HIV

There is still much to learn about MAC infection and disease in both AIDS and non-AIDS patients. What is the primary route of infection (pulmonary or gastrointestinal)? Do the infecting strains of MAC and the route of infection differ in AIDS and non-AIDS patients, and, if so, why? Why is the geographic distribution of MAC disease among AIDS patients different from that of non-AIDS patients? Is MAC disease usually the result of reactivation of a persistent latent infection (as in TB) or the result of direct progression of primary infection following recent exposure? What is the normal immune response to MAC infection? Why is MAC disease so common in AIDS, and why in this setting does it occur so much more frequently than other nontuberculous mycobacterial disease? Even in Midwestern United States, where *M. kansasii* infection was thought to be more prevalent (pre-AIDS epidemic), disseminated MAC is tenfold more common than disseminated *M. kansasii* in AIDS patients (425,442). Similar Þndings have been reported from England and Wales (600). Furthermore, among HIV-infected persons, colonization of stool or sputum with MAC appears much more likely to predispose to dissemination than does colonization of stool or sputum with other nontuberculous mycobacteria (445). What is the speciÞc immune defect in AIDS that speciÞcally favors disease from MAC (possibly even favoring only a few MAC serovars), and can the selective immune defect be altered? Does disseminated MAC in AIDS patients contribute to their morbidity and mortality, and does treatment make a difference? What are the best drugs, drug combinations, and dosages to use in

treatment? Is drug susceptibility testing helpful in selecting drug regimens?

Other Nontuberculous Mycobacterial Infections in HIV Disease

Other mycobacteria have been isolated much less frequently from patients with HIV infection and include *M. fortuitum*, *M. kansasi*, *M. gordonae*, *M. xenopi*, *M. chelonae*, *M simiae*, *M. haemophilum*, *M. bovis* (BCG), *M. genavense*, *M. malmoense*, *M. scrofulaceum*, *M. celatum*, *M. szulgai*, *M. marinum*, *M. βavescens*, *M. ulcerans* and *M. asiaticum* (442,503,544,601Ð611). The Þrst twelve mycobacteria listed have been reported to cause disseminated disease in patients with HIV disease. Furthermore, even patients with localized NTM disease, such as pulmonary or skin, often have positive blood cultures. Although all these organisms are potentially pathogenic in HIV-infected patients, with the exception of *M. kansasii*, there are no established guidelines for their treatment.

Pulmonary Disease

Pulmonary manifestations of NTM disease in HIV disease merit attention. A retrospective review of 19 HIV-infected patients with *M. kansasii* disease showed 14 patients (74%) had pulmonary disease exclusively with either focal upper lobe inÞltrates or diffuse interstitial inÞltrates; thin-walled cavitary lung lesions occurred in nine patients (612). The disease occurred late in the course of HIV immunosuppression; most patients (84%) had a previous diagnosis of AIDS, and the median CD4+ lymphocyte count was 49 cells/mm^3 (range, 0Ð198 cells/mm^3). Treatment with conventional therapy resulted in the resolution of fever, symptoms, and radiographic inÞltrates, and in a bacteriologic response without relapse while the patients were on therapy. In another study, treatment outcomes in HIV-positive patients were found to be similar when compared to HIV-negative patients (613). Furthermore, a Þve-year population-based study from California showed that 91% of *M. kansasii* disease was pulmonary and estimated that there were 115 *M. kansasii* disease cases per 100,000 HIV-positive persons per year, and 647 cases per 100,000 AIDS patients per year (614). The recommended treatment for *M. kansasii* disease consists of isoniazid, rifampin and ethambutol for a minimum of 18 months and for at least 15 months after culture conversion (544).

Skin Disease

Nontuberculous mycobacteria, especially *M. marinum*, *M. szulgai*, *M. chelonae*, *M. fortuitum*, *M. abscessus*, and *M. haemophilum* may cause skin disease, such as nodules, ulcers, erythema, pustules, abscesses, and panniculitis. *M. abscessus* and *M. chelonae* are typically associated with surgical wounds. *M. haemophilum* usually presents with cutaneous nodular or ulcerating lesions, joint effusions or osteomyelitis. Blood cultures are often positive in nontuberculous skin disease. Skin biopsies and blood cultures establish the diagnosis (424).

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Chapter 18

Neurologic Complications of HIV and AIDS

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Twenty years have passed since the Þrst reports of neurologic illness in patients with AIDS or HIV infection and much progress has been made in treatment (1Ð3). Shortly after identifying the opportunistic nature of infections of the nervous system in patients whose immune system was no longer functional, it was realized that there could be a direct destructive effect of the virus on the neuraxis. Principles borrowed from the care of immunosuppressed transplant and cancer patients, and later from virology, have worked well in the management of the neurologic complications of this disease. Now that methods of prevention and treatment of opportunistic infection and malignancy are in place, new challenges face the clinician. Less common, more resistant, opportunistic organisms are responsible for infections. To treat or prevent opportunistic disease, physicians must be familiar with the predominant infections of an immigrantÕs original country or sites visited by a traveler. In addition, patients who are fortunate enough to reconstitute their immune system will request guidelines for termination of prophylaxis or maintenance treatment of opportunistic infection (4Ð6). At the same time, clinicians of all specialties must be able to recognize late complications in patients who had no access to therapy (7) or were never aware of their HIV infection. For example, 40% of 2,223 patients newly diagnosed with HIV in Baltimore clinics already suffered from AIDS (8).

In treating HIV there needs to be an awareness of the potential neurologic side effects of certain antiretroviral agents. The importance of the degree of central nervous system (CNS) penetration of these drugs remains an important question (9), as does the role of drug resistance of the strains of HIV within the CNS (10).

This chapter will Þrst review the conditions that present with symptoms of neurologic dysfunction as a direct consequence of viral infection, discuss when these conditions can be expected to arise in the course of infection, and the relationship of certain neurologic syndromes such as dementia, myelopathy and peripheral neuropathy to the degree of immunosuppression. Diagnostic tests relevant to CNS involvement, including the use of cerebrospinal ßuid (CSF) as a proxy for brain tissue (15Ð17), as well as other functional investigations of the nervous system will be reviewed. Currently recommended treatment strategies and neurologic side effects of the antiretroviral medications will be outlined (7). In the second section, neurologic manifestations of dysregulation of the immune system will be described, including such Òauto-immuneÓ diseases as chronic inßammatory demyelinating polyneuropathy or Guillian-BarrŽ syndrome, mononeuritis multiplex, and possibly cerebellar degeneration (18). Finally the vast range of opportunistic infections and malignancies will be outlined. The various areas in which the nervous system can be injured or dysfunctional in HIV will be explained, using knowledge of neuroanatomy to envision the characteristic features of cerebral lesions, meningitis, myelopathy, neuropathy and myopathy. Understanding the predilection for various sites of the neuraxis of infectious agents and tumors, layered on the HIV-induced disruption of the nervous system (7), will facilitate a logical approach to diagnosis. Protocols that have proven useful in differentiating various opportunistic conditions will be given (7,19,20) (see Fig. 18.1 and Table 18.1).

NEUROLOGIC SYNDROMES DUE TO DIRECT EFFECT OF HIV

Early Infection

Primary HIV infection may develop as early as two weeks after exposure to the virus (21). Most patients who recognize symptoms associated with primary infection complain of mononucleosis-like symptoms including

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Symptom		Features	Cause
Weakness →	Proximal	↑ Reflexes, Babinski	Myelopathy
		↓ Reflexes	Myopathy
	Isolated	↓ Reflexes	Mononeuritis multiplex
		Proximal or distal	
		May be asymmetric	
	Distal	↓ NCV slowed	Neuropathy (inflammatory) (HIV related)
		↓ Amplitude, NCV normal or slow	
Pain →	Bandlike	Usually thoracic	Myelopathy (compressive mass)
	Patchy	Radicular, can involve cranial nerves	Carcinomatous meningitis
	Cramps	Exertional	Myopathy
		↑ CPK	
Numbness	Distal feet>hands	Burning, numb, tingling	Neuropathy (HIV related)
	Distal (stocking/glove)	↓ All modalities	Neuropathy (HIV related)
		↓ Amplitude	
		↓ Velocity on NCV	
	Spinal level	Usually Thoracic	Myelopathy (cord compression)
Urination change	Urgency/Frequency	↓ Pin, touch	Myelopathy, rarely hydrocephalus
		Culture negative, constipation	
	Incontinence, dribbling	Flaccid bladder	CMV sacral radiculitis or cauda equina mass
Pathologic reflexes	Bilateral Babinski		Myelopathy
	Unilateral Babinski		Brain (mass or PML)
	Bilateral Babinski with		Brain (dementia, usually HIV)
	Snout, grasp, glabellar		

CMV, cytomegalovirus; NCV, nerve conduction velocity.

FIG. 18.1. Localization of neurologic symptoms.

fever, myalgia, arthralgia, a macular rash on the trunk, lymphadenopathy and headache. This headache may be nonspeciÞcally related to fever or due to aseptic meningitis, in which case it is accompanied by meningeal signs such as stiff neck, photophobia, mild confusion or lethargy (22,23). However, not all symptoms are present in a given individual (24,25); in a prospective study by Wallace (24) the distribution of associated symptoms (other than fever which was present in all) included malaise (found in 69%), pharyngitis (69%), adenopathy (59%), myalgias or headache (41%), oral lesions or rash (10%). More severe cases of meningoencephalitis may present with confusion, disorientation and lethargy rarely progressing to coma (26), facial palsy or other cranial nerve dysfunction (1,3,27) or seizures (28,29). Less than 10% of patients present with encephalopathy (23,30), polyradiculopathy or Guillian-BarrŽ(31,32), brachial plexitis (33), ganglioneuronitis (34), myelopathy (35) or myelitis (36,37), neuropathy (38), dyskinesias (39) or myositis with myoglobinuria (40). Most symptoms resolve in about one month, although chronic headache and meningitis have been noted (30). Aseptic meningitis (often asymptomatic) may last for many years based on serial lumbar punctures that show chronic CSF pleocytosis (41).

Laboratory abnormalities in the CSF that may be found in primary HIV infection include include a moderate pleocytosis of mononuclear cells and mild protein elevation with a normal glucose level; the virus may be grown or detected by elevated RNA (7,42,43). Raised levels of HIV RNA and β2 microglobulin in CSF after primary infection respond favorably to antiviral treatment (44). Electroencephalography may be normal or mildly slow, and imaging is normal. Those patients with symptomatic primary infection, without speciÞc CNS involvement, are likely to develop neurocognitive impairment at a more rapid rate. Whether this is due to an initial higher HIV load causing both a more obvious initial illness and more rapid depletion of CD4 or that symptoms of conversion correlate with a more neurotrophic or neurotoxic strain of virus is not known (24).

It is still unclear what causes the neurologic symptoms described above (45Ð48). They appear extremely early but transiently in the course of HIV infection. It has even been proposed that early neurologic complications are salutary, being a function of an effective immune reaction to viral invasion which subsequently lowers the chance of developing AIDS dementia complex or neuropathy (49).

HIV-1-Associated Cognitive/Motor Complex (HIV or AIDS Dementia Complex (ADC))

Despite the excellent response when antiretroviral therapy is effective, AIDS dementia remains a signiÞcant problem, eventually developing in up to 20% of patients with advanced HIV infection (50). Dementia emerges both in patients who have not had access to therapy, and in

TABLE 18.1. *Features of common opportunistic infections and tumors*

Complication	Occurs at CD4	Clinical Signs*	Radiographic Signs	Ancillary tests
HIV Dementia Myelopathy Neuropathy	< 200	Apathy, memory, cognition, judgment, psychomotor slowing	Atrophy on CT, periventricular hypodensity MR	CSF cytokines, PCR
Toxoplasma encephalitis	< 100–200	Focal de cit(s), seizures, depressed mental status, meningeal signs	Single or multiple ring enhancing lesions, more lesions on MRI than CT Thallium uptake on SPECT	Serum IgG titers > 1:256, brain biopsy Improvement on treatment
Cryptococcal meningitis or cryptococcoma	< 50–100	Headache and meningitis, bizarre behavior, lethargy, rare seizures, focal signs, ↑ ICP	Meningeal enhancement, hydrocephalous Enhancing lesions	Ag titer in Serum or CSF + CSF culture, + India Ink
Progressive Multifocal Leukoencephalopathy	< 100	Subacute progressive focal de cit especially posterior fossa,/parietal occipital, vision	Non-enhancing lesion(s) in white matter Hypointense on T1, Hyperintense on T2	+ PCR in CSF for JC virus, brain biopsy
Primary Lymphoma	< 100	Headache, focal signs, seizures, meningitis with random cranial nerve or root dysfunction	Single or multiple homogeneous or ring enhancing lesions, especially deep; Thallium Uptake on SPECT	EBV PCR in CSF, brain biopsy, Improvement with steroids
Herpes zoster	< 100	Paresthesias, painful rash in dermatomal distribution, rarely stroke	None	+ PCR in CSF
Syphilis	Any	Meningovascular (Stroke, Meningeal signs) Tabes dorsalis:sensory loss due to post columns Generalized Paresis Gumma (Mass lesion)	Stroke, enhancing meninges; Spinal cord atrophy Brain atrophy Mass with minimal edema	+ Serum or CSF VDRL or FTA + Monocytes, nl glucose, Pro ↑
CMV	< 50–100	Lumbosacral plexus dysfunction (Sacral root pain, accid bladder) Encephalopathy with brainstem signs	Enhancement on nerve roots or periventricular regions, especially near IV ventricle (MR)	> 500 PMNs in CSF, Glucose + PCR
Tuberculosis	Any	Meningitis, cranial nerve palsies, stroke, mental status changes Cord compression due to spine involvement	Meningeal enhancement especially at basilar cisterns, enhancing mass lesions, may calcify Vertebral body or diskitis	CSF Glu ↓ ↑ lymphocytes, ↑ protein, + PCR or probes, + Acid fast smear or culture
M. avium complex	< 50	Silent meningitis or coinfection	+ PPD	

* In order of frequency. Fever is not discriminating in any of these conditions.

those whose treatment has failed. Treatment failures may occur in patients whose antiviral medications poorly cross the blood brain barrier (51), or in those with inadequate adherence to their drug regimen (48,52) as well as in those whose viral strain became resistant or mutated to a more neurotoxic type (48,53). The exact mechanism of brain damage is still being debated (54Ð81) (see Chapter 00), and it is still not clear if minor HIV-related cognitive dysfunction, which consists of more subtle cognitive, psychiatric and motor symptoms similar to those of metabolic encephalopathy, is always a precursor of dementia (54,55).

The location of HIV within the brain correlates with entry through the endothelium and choroid plexus of the

ventricles, as deeper areas such as the basal ganglia and hippocampi are more affected (55), but indirect effects of the virus, either through infected monocytes or the cytokines they secrete, must be involved to explain the end stage symptomatology.

The normal supportive function of astrocytes, such as producing the neurotrophic factors NGF, IL-6 and leukocyte inhibitory factor, maintaining the blood brain barrier, holding microglia in a dormant, non inßammatory state and detoxifying excess excitatory amino acids such as glutamate, are all interrupted after HIV infection (79). Astrocyte failure also contributes to the remote (temporally as well as spatially) neuronal dysfunction even after successful viral suppression. Gliosis or scarring, though a nonspeciÞc response to injury, is seen early in simian immunodeÞciency virus (SIV) models and must contribute to disrupted function (56).

Clinical Course

After recovery from the neurologic complaints that may be seen during primary HIV infection, patients generally remain free of neurologic symptoms for many years, especially on therapy (other than those attributed to immune dysregulation, such as chronic inßammatory demyelinating polyneuropathy, mononeuritis multiplex, inßammatory polymyopathy, aseptic meningitis, or to antiviral drug toxicity such as mitochondrial myopathy or nucleoside neuropathy). Once the CD4+ T-cell count falls below 200/µl, patients with early dementia demonstrate motor abnormalities resembling signs seen in metabolic encephalopathy such as tremor, asterixis, ataxia or simply psychomotor slowing.

There have been many names for the symptom complex caused by brain involvement of HIV, the most commonly used being AIDS Dementia Complex (ADC). HIV-1 Associated Dementia (HAD) is popular among neurologists. Pathologic terms such as HIV encephalitis are less useful, as the neuropathologic Þndings of multinucleated giant cells, atrophy and white matter pallor can be seen in many patients who are cognitively intact. Similarly, the term HIV encephalopathy, although useful in its implication of a ßuctuating or reversible condition, is confusing in that the ßuctuations are generally due to unmasking of dementia by an unrelated condition such as meningitis, hypoxia, or even metabolic failure. The American Academy of Neurology developed staging criteria (82), along with the term HIV-1 associated minor motor/cognitive impairment, to describe AIDS patients with some symptoms of cognitive dysfunction but preserved activities of daily living or ability to work (83), but outside of longitudinal studies using sophisticated neuropsychological and motor testing, few patients meet this criteria (54). However, the importance of functional measures cannot be underestimated, as this aspect of cognitive impairment has the most direct impact on patientsÕlives. Therefore, while new scales or instruments are being tested for clinical relevance (84), discussion of ADC must take into account that symptom degree is variable. The mildest stage may represent a different condition known as HIV cognitive/motor impairment (85) that does not necessarily progress to severe ADC (86).

ADC is still found in up to 15% of patients with AIDS (50,87), although some series report a drop in incidence to under 2% (50). ADC is presenting at a higher CD4 count (170 compared to 70/mm^3 in the pre-HAART era) (88). Ironically, the incidence of ADC as the AIDS- deÞning illness is gradually going up, from 3% (89) in the early days of the epidemic to 6.5% of a series from Australia presenting in 1997 (88). If mild patients are included, about 25% of those with advanced AIDS will suffer from ADC (48,57). After diagnosis of severe ADC, even those patients receiving HAART generally still survive only six months, especially if they are injecting drug users or already have another AIDS-deÞning illness. However, in some patients, HAART may prolong survival and improve dementia, even if viral load remains elevated in plasma (7,19,90) or CSF (91).

ADC starts in the subcortical regions (55,58,92) for reasons related to the path of viral entry, which may explain the early prominence of motor, mood and behavioral abnormalities with later complaints referable to memory (93). Mood disorders more commonly found in symptomatic HIV infection correlate with slowing on quantitative electroencephalography (94). It has long been appreciated that HIV dementia resembles the subcortical dementia of ParkinsonÕs disease more than AlzheimerÕs (95). Patients describe difÞculty concentrating, sequencing or completing a task, needing to keep lists or other cues to perform previously automatic activities, and slowed thinking. Complaints such as loss of libido, loss of motivation to perform complex tasks at work or hobbies and eventually overall apathy overshadow those of memory or spatial orientation. Use of illicit drugs, especially stimulants, can accelerate the process of cognitive decline (96Ð98), and even alcohol has been shown to interfere with working memory function (99). Rare instances of agitation or mania are described (100,101). Neuropsychologic testing at this stage will display problems with retrieval of memory (not registration), manipulation of acquired knowledge such as word lists and general slowing of thought processes and psychomotor task performance. Spared are tasks measuring attention, (although one study of 29 women of low socio-economic status did Þnd impaired performance on selective attention) (102), language ability and calculation. When only cognitive symptoms such as these are complained of (that is no motor signs are present), depression must be suspected (103), as most patients with ADC simultaneously suffer earlier from motor dysfunction. These patients complain of clumsiness, difÞculty walking despite preserved strength and sensation in the legs, tremor, changed handwriting and inability to do Þne motor tasks such as

typing or playing music. Bradykinesia and bradyphrenia cause inability to perform tasks at previous speeds (104). The latter symptom manifests in performance of tasks that are time-sensitive, such as driving in fast-moving traffic. Although psychomotor slowing interferes with performance of tasks of daily living, the patientÕs apathy may make him or her an unreliable self-reporter of these difficulties.

Patients who formerly complied with follow-up appointments who begin to miss scheduled visits may be beginning to suffer from difficulty with planning associated with dementia. In patients whose viral load begins to increase there may be noncompliance, rather than mutations leading to resistance, for similar reasons.

Disruption of normal sleep patterns, including daytime drowsiness, possibly due to the somnogenic properties of cytokines or dysregulation of nighttime growth hormone secretion, can contribute to cognitive disability and feelings of malaise (105,106). Psychiatric conditions like depression, anxiety or Òdual diagnosesÓ that include alcohol and substance abuse or recreational use of marijuana (19) exacerbate these deficits. Rarely, frank psychotic symptoms such as hallucinations, paranoia or mania may occur. Both generalized and partial seizure activity have been reported (28,107).

Clinical signs that correspond to these symptoms include motor system abnormalities, such as shuffling or unsteady gait with inability to perform tandem walking, asterixis, myoclonus, action or sustension tremor (39,108) and a positive Romberg (ie. swaying after closing the eyes, with the feet in a comfortable stance). Extrapyramidal symptoms are occasionally unmasked by use of neuroleptics for control of nausea or psychiatric symptoms (109Ð111), but can be due to HIV itself (112Ð114). Frontal (primitive) reflexes such as snout, root, grasp, Babinski, palmomental and glabellar signs correlate with advancing dementia, except for the glabellar response (104,115). Saccadic eye movements may be slow (116) and not responsive to antiviral treatment (117). Bradykinesia can be extreme, and in advanced cases the face becomes expressionless. Hyperreflexia is seen in the legs, except when peripheral neuropathy causes depressed ankle jerks. Tone is increased in the legs. Often urinary frequency or incontinence is reported. Eventually patients become abulic, mute, paraplegic or quadraparetic. Mental status screening with the commonly used Mini-Mental State Examination, which is weighted to test memory for following or detecting AlzheimerÕs disease does not uncover the deficits of ADC patients. Other performance driven scales such as the Karnofsky scale (often used with tumor patients), or the Washington Clinical Dementia Rating Scale, modified by Price and Brew to use cognitive measures such as memory, orientation, judgement, problem solving, plus community affairs, home and hobbies and personal care (as motor features are unduly influenced by basal ganglia and spinal cord dysfunction), have been able to measure adequately patientsÕfunctional ability and response to treatment (7). The latter scale, going from 0 to 4, reflects Òreal lifeÓ function as well as neurologic examination results (7,86).

A scale employed by researchers in the field of neuropsychology and HIV is shown in Fig. 18.2. The ACTG (AIDS Clinical Trials Group) uses the following neuropsychological and motor tests to screen for ADC: timed gait, Trail Making Tests A and B, finger tapping (both hands), grooved pegboard and digit symbols. The Multicenter AIDS Cohort Study uses in addition to the Trail Making and symbol digit tests, the Rey Auditory Verbal Learning Test, Rey-Osterreith Complex Figure, and choice reaction times. As ADC advances, patients do worst on tests that assess motor skills such as the grooved pegboard or Trail Making Part A tests. They also have difficulty with information processing as tested by the selective reminding task, and executive function as tested with Trail Making Part B. However, motor abnormalities do not explain all of the difficulties on neuropsychological testing, as an attempt to correlate HIV myelopathy (confirmed by somatosensory evoked potentials) with cognitive impairment (confirmed on several tests) found preserved motor speed, despite the observation that 84% of patients were impaired on tests such as the Grooved Peg Board, Finger Tapping, Trail Making A and B, WAIS-R Digit Symbol, Rey Auditory Verbal Learning and FAS Verbal Fluency (118). Recognition (recall) memory is spared; more problems are seen encoding information or using Òworking memoryÓ (119). Visuospatial performance as measured by copying the Rey-Osterreith figure, WAIS-R block design and construction of figures are better preserved than tests of executive function. Of course, scores on these tests do not always represent neuropsychological abilities (120). Confounding variables that affect performance of all these tests include patient education (less education correlates with more cognitive impairment) (121,122), history of head injury (123), and fatigue and intoxication (124). Although most studies report poorer cognitive function with a history of use of illicit substances such as stimulants (124) or heroin (125), a recent Italian survey found more patients who had acquired HIV by sexual contact were at higher risk for cognitive impairment (121). They proposed that a higher mortality rate or reduced survival time among injecting drug users because of less access to HAART or complications of ongoing illicit drug use, contributes to a smaller pool of cases, accounting for this paradoxical finding (121). Evidence of advanced systemic disease (anemia or wasting) also correlates with poor performance on neuropsychological tests (126).

Ancillary tests used in ADC fall into three major categories: (1) structural: magnetic resonance imaging (MRI) or computed tomography (CT), (2) functional: single photon emission computed tomography, positron emission tomography, magnetic resonance spectroscopy, functional MRI, and electroencephalography and (3) chemical: cerebrospinal fluid.

Symptom		Features	Cause
Weakness	Proximal	↑ Reflexes, Babinski ↓ Reflexes	Myelopathy Myopathy
	Isolated	↓ Reflexes Proximal or distal May be asymmetric	Mononeuritis multiplex
	Distal	↓ NCV[a] slowed ↓ Amplitude, NCV nl or slow	Neuropathy (inflammatory) (HIV related)
Pain	Bandlike	Usually thoracic	Myelopathy (compressive mass)
	Patchy	Radicular, can involve cranial nerves	Carcinomatous meningitis
	Cramps	Exertional ↑ CPK	Myopathy
	Distal feet > hands	Burning, numb, tingling	Neuropathy (HIV related)
Numbness	Distal (stocking/glove)	↓ All modalities ↓ Amplitude ↓ Velocity on NCV[a]	Neuropathy (HIV related)
	Spinal level	Usually thoracic ↓ Pin, touch	Myelopathy (cord compression)
Urination change	Urgency, frequency	Culture negative, constipation	Myelopathy, rarely hydrocephalous
	Incontinence, dribbling	Flaccid bladder	CMV sacral radiculitis or cauda equina mass
Pathologic reflexes	Bilateral Babinski		Myelopathy
	Unilateral Babinski		Brain (mass or PML)
	Bilateral Babinski with snout, grasp, glabellar		Brain (dementia, usually HIV)

[a] NCV, nerve conduction velocity.
[b] CMV, cytomegalovirus.

FIG. 18.2. Dementia scale used in evaluation of cognitive function in HIV (with permission).

Radiographic Imaging: CT and MRI

Atrophy when present on CT (Fig. 18.3) and MRI (Fig. 18.4) does not necessarily mean a patient is demented; however the degree of atrophy does correlate with stage of ADC (127). Atrophy is most marked in the basal ganglia. MRI, which is more sensitive to changes in water, often reveals periventricular abnormalities of patchy or diffuse increased signal on T2-weighted images. Initially these were attributed to demyelination (128,129). More recently, using delayed imaging with contrast enhancement, breakdown of the blood-brain-barrier has been raised as an explanation as well (67,130), especially as the degree of enhancement has been shown to correlate with the degree of dementia (58). Improvement with HAART in white matter signal abnormalities despite progression of cerebral atrophy has been demonstrated in four patients studied by Thurnher et al, supporting the proposition that water ßux, rather than demyelination, is responsible for the white matter changes (131). Imaging, though not conÞrmatory of ADC, is of course required to rule out other causes of dementia, such as mass lesion(s), meningitis or ventricular dilatation.

Radiographic Imaging: Nuclear studies, MR Spectroscopy, fMRI

Functional brain imaging, such as nuclear scan (PET or SPECT) (Fig. 18.5), has shown both patchy defects (132) even in unimpaired subjects, and global decreased perfusion that correlates with motor performance on neuropsychologic tests (133). Possible causes of focal

abnormalities include macrophage or viral-induced neuronal damage in patches, diaschisis from subcortical damage and decreased perfusion due to inßammation of the endothelial cells of the vasculature (132). In patients with only minor HIV-1 associated motor deÞcits or no abnormality, PET scans revealed frontomesial hypometabolism in nine of 19 patients and hypermetabolism of the basal ganglia in seven. As slowing worsened clinically, this changed to hypometabolism in the basal ganglia, prestaging dementia (32). In asymptomatic patients, the increased brain activation may indicate that early injury has necessitated compensatory usage of brain reserve to maintain normal cognitive function. PET scanning has shown hypometabolic areas in the basal ganglia in late ADC and hypermetabolism in early stages (134). These decreases in basal ganglia and thalamic metabolic activity correlate with quantitative EEG changes (ie. generalized slowing in the theta range), suggesting subcortical dysfunction is responsible for symptoms of dementia (135). Due to the expense of nuclear studies, PET scans are not often used.

Alternative functional studies include MR spectroscopy (MRS) and functional MRI (fMRI). MR spectroscopy measures N-acetyl aspartate (NAA) as a surrogate marker of neuronal function, and has shown it to be decreased in frontal white matter, while choline (Cho) and myo-inositol (mI), markers for inßammation and glial activation, are elevated (136). Similar Þndings using MRSI (magnetic resonance spectroscopy imaging), which measures the distribution of choline and NAA, have found changes in the thalamus, frontal and occipital white matter (137). A decreased ratio of NAA/Cho has been found in the basal ganglia of children with HIV-encephalopathy (138). By serially monitoring changes such as increasing Cho and mI to measure inßammation and decreased NAA to measure neuronal loss, the sequence of CNS involvement in HIV infection may be observed *in vivo* (139).

Another physiologic technique, functional MRI (fMRI), determines regions of brain activation. A task known to localize to a speciÞc brain region is performed while the patient undergoes MRI. The blood oxygenation level dependent (BOLD) contrast at rest is subtracted from a fast gradient MRI done while repeatedly performing a task and thereby determines the regions of brain activation. In ChangÕs study of attention and working memory, patients with HIV showed activation in the parietal lobes for simple tasks and greater activation in additionally recruited frontal lobe regions during complex tasks. Reaction times were slower but accuracy was preserved, suggesting greater brain reserves are used to compensate for neural circuit disruptions caused by the virus or its toxins. The increased attentional modulation represented

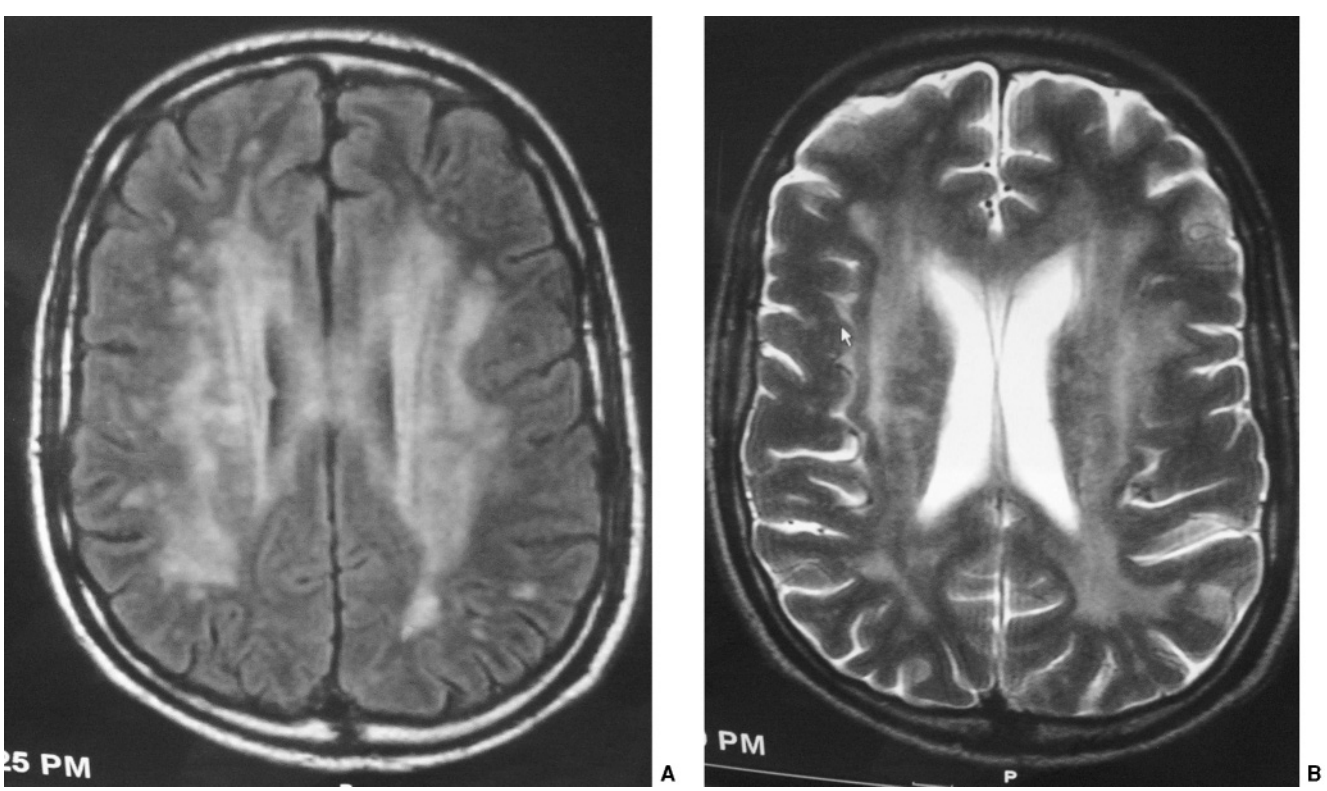


FIG. 18.3. A. ADC (Aids dementia complex): Brain MRI, Flair, of a 32 year old mildly cognitively impaired man, showing periventricular increased signal.
B. Same patient, MRI, T2-weighted, showing ventricular hypertrophy and sulcal dilatation as well as increased periventricular signal.

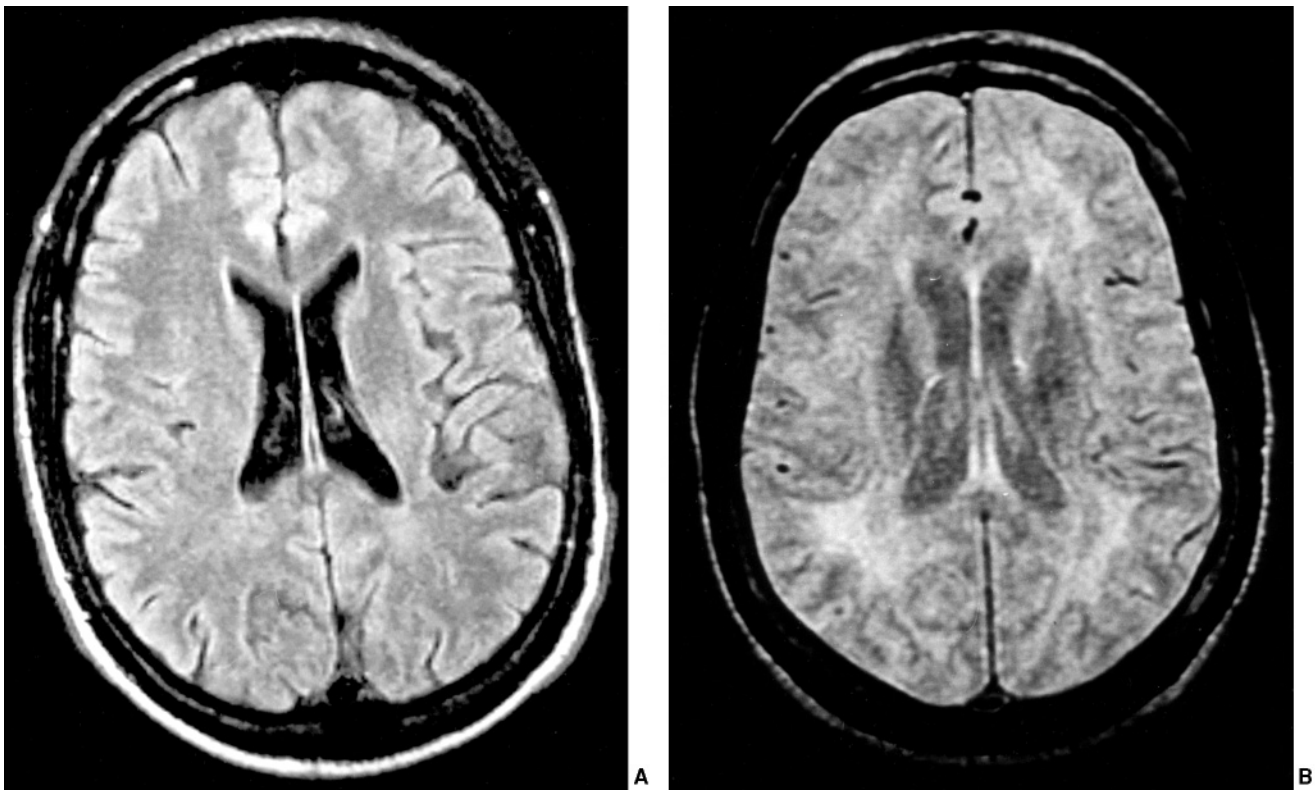


FIG. 18.4. ADC: Brain MRI, T1-weighted with gadolinium, showing increased white matter signal in the periventricular region with no enhancement.

by increased activation of the frontal areas may be due to abnormal frontostriatal connections, with little room for the additional energy required by complex tasks. This correlates with lack of initiation or enthusiasm for non-routine activities of daily living in patients with ADC.

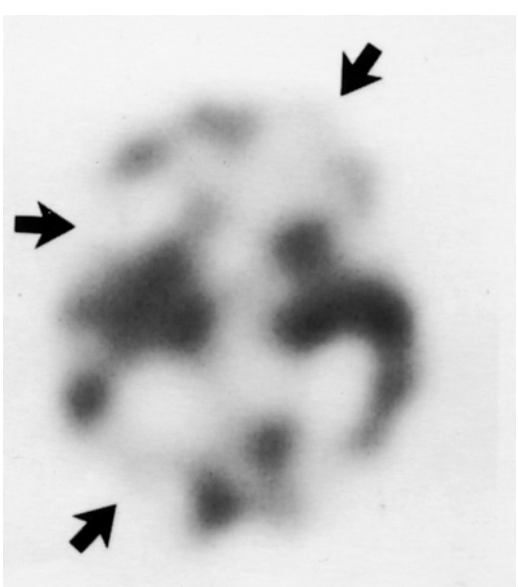


FIG. 18.5. ADC: Multifocal perfusion defects marked by arrows in a patient with dementia (courtesy of Dr Joseph Masdeu).

Functional Studies: Electroencephalography, Evoked Potentials

Neurophysiologic investigation includes electroencephalography (EEG) (Fig. 18.6), which is Òlow and slowÓin late ADC but normal in more than half of patients (140Ð143).

Quantitative electroencephalography is more sensitive but not speciÞc (144) and is not widely available. Abnormalities of spectral powers were suggestive of decreased arousal and increased fatigue in one study of HIV-infected patients (145). In another study, marked changes on quantitative EEG correlated with psychiatric symptoms in otherwise asymptomatic HIV-infected patients, thereby establishing the organic nature of the psychosis (94). In more advanced patients with CD4 < 350, abnormal EEG correlated well with poor performance on neuropsychological testing, abnormal MRI and relaxometry and spectroscopy on MRS (146), as well as with abnormalities on PET scan (135). Evoked potentials, including long-latency event-related potentials are often abnormal even in asymptomatic (non-demented) patients, so their clinical usefulness is not established (147).

Cerebrospinal Fluid Analysis

CSF must be studied, not to diagnose ADC, but to rule out conditions *not* due to ADC, such as carcinomatous,

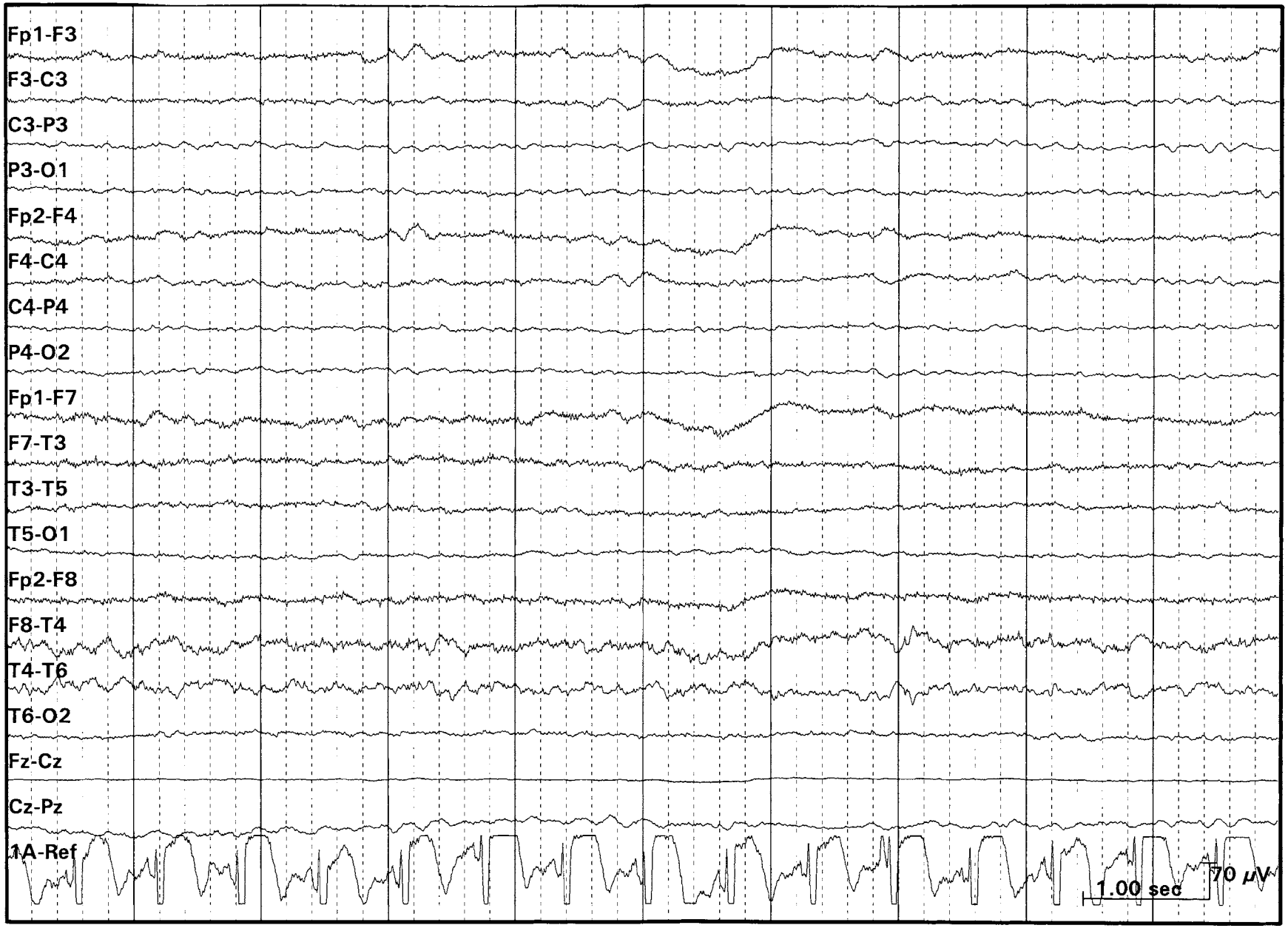


FIG. 18.6. ADC: Electroencephalogram of a 41 year old demented man showing diffuse low-voltage slow background with loss of normal faster frequencies (courtesy of Dr Cynthia Harden).

bacterial (including treponemal or mycobacterial) or fungal meningitis (148). (A few mononuclear cells persist throughout all stages of HIV infection and are not indicative of meningitis or ADC) (15,41,149). Once these treatable conditions are ruled out, the CSF can reveal other useful information in the diagnosis of ADC, such as HIV RNA copies (viral load) (150–152), which correlates better with response to treatment than plasma levels (91). However, the correlation between virus levels present in CSF and in brain parenchyma is not exact, although levels of each correlate with the degree of dementia (153). Although a positive viral culture alone does not provide useful information, especially as the strain present in the brain parenchyma may differ from that in CSF, it is still helpful to determine resistance patterns and concentrations of antiretroviral drugs to modify the choice of therapy (10,48,120,126,154). Comparison of the plasma to CSF gradient of viral load varies with the degree of immunosuppression (the lower the CD4 count the lower the ratio of plasma to CSF viral copies). This ratio, normally 2:1 plasma: CSF in both impaired and unimpaired patients, becomes 5.1:3.8 in impaired patients with CD4 < 200, suggesting that these patients have an independent growth of virus in the CNS (155). CSF viral count is also increased when there is pleocytosis (> 4 WBCs/μL in the CSF), suggesting that leukocytes bring the virus in from the periphery (153,156). Signs of immune activation, such as oligoclonal bands, increased levels of neopterin, β-2 microglobulin and quinolinic acid, correlate with stage of ADC (15,55,157,158) or degree of inßammation. The presence of matrix metalloproteinases (MMPs) correlates with disruption of the blood-brain-barrier (159). The amount of chemotactic cytokine MCP-1 (monocyte-chemotactic protein) also correlates with neurologic disease, including opportunistic infections (91). Indirect measures of HIV-induced excitotoxicity such as glutamate levels in the CSF are used in research (80,160).

VACUOLAR MYELOPATHY AND MYELITIS DUE TO HIV

Approximately 10% of patients with advanced AIDS (CD4 < 50 cells/μl) and 30% of patients with ADC suffer from a syndrome of ataxic, spastic gait disorder with impaired proprioception. In the series reported by Banks et al., symptoms began with motor dysfunction in 41.7%, urinary dysfunction (urgency and frequency) in 29.6% and another 30% had combinations of sensory symptoms, impotence and constipation. Progression to inability to walk occurred in seven months and to incontinence in nine months, regardless of antiviral treatment (161). Many patients also suffer from distal sensory neuropathy (162) and many are also demented by the time myelopathy advances to paraparesis (163). Pathologic examination reveals vacuoles in the posterior and lateral columns of the spinal cord with macrophages carrying off the lipid from the myelin (Fig. 18.7); the thoracic cord is most often involved. No viral particles are seen (164). In more severely involved cases axonal damage is also found (165). This closely resembles the degeneration of combined systems disease due to B12 deficiency. On pathologic examination this spinal cord picture is present in up to 55% of adults with end stage AIDS, but some selection of patients for neurologic disease contributed to this high percentage (166). Children rarely report symptoms consistent with this myelopathy (167).

HIV myelitis, a separate pathologic entity occurring in 8% of spinal cords examined, occurs more commonly in drug users (168). Patients with myelitis are more likely to exhibit a sensory level at any level of the cord. Virus can be cultured from the cord or CSF of patients with myelitis.

MRI of the spinal cord should be done to rule out cord compression or intramedullary infection (169–171), tumor (3,172) or vasculitis (173). If vacuolar myelopathy is present there is increased signal in the white matter columns on T2 in most cases, and atrophy of the spinal cord is seen in up to 70% of cases. CSF studies can rule

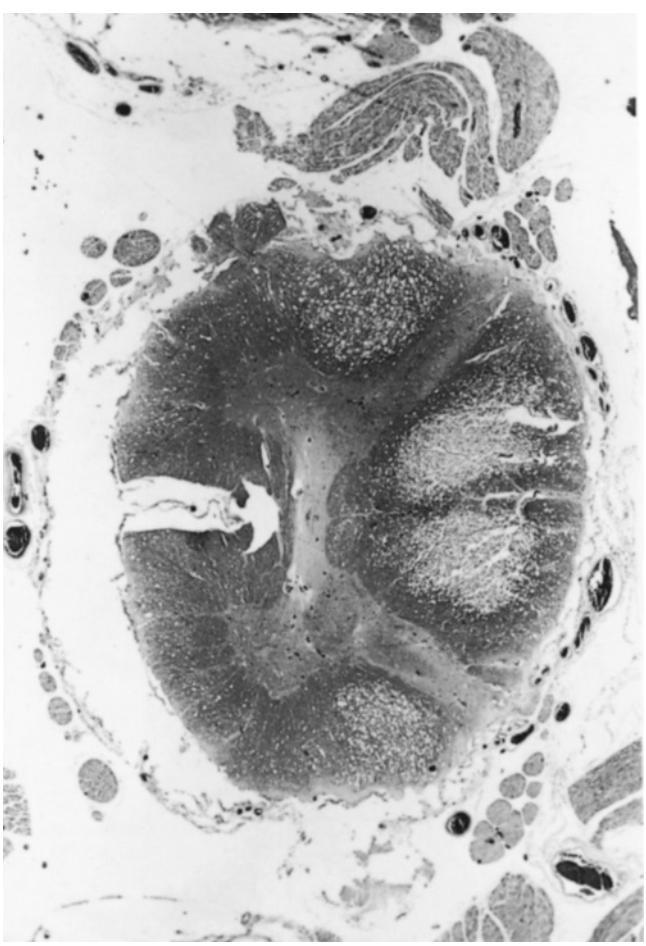


FIG. 18.7. HIV Myelopathy: Spinal cord cross section stained with hematoxylin and eosin and luxol fast blue for myelin, showing vacuolar changes in the posterior and lateral columns (courtesy of Dr Seymour Levine).

out viral causes such as HTLV-I, cytomegalovirus, herpes simplex or zoster, but CSF is normal in vacuolar myelopathy (174–176). In myelitis PCR or culture may be positive for HIV, but the diagnosis is usually made postmortem (162).

Somatosensory evoked potentials may be used to document the extent of dysfunction (177) or eventually may be helpful in predicting treatment response, if they can be shown to normalize before a clinical response is evident.

Since B12 levels are usually normal, despite the pathologic resemblance to combined systems disease, more complex deficiencies of cofactors found in the methylation sequence, such as S-adenosyl methionine, have been proposed (163,178). Even in patients with low B12, supplementation fails to improve the myelopathy (129). For example, deficiency in methylation due to mutations in genes of methionine synthetase and methylenetetrahydrofolate reductase was found in African-American patients who are genetically susceptible to myelopathy in HIV infection (179). Lack of donors for the methyl transfer cycle of oxidation can contribute to the process. Eventual depletion of S-adenosyl methionine causes failure of the trophic activity of the cord that sustains normal function. This may prevent repair of myelin that has been invoked by cytokines, free radicals or other viral products. This would account for the failure of antiretroviral treatment alone to reverse signs of myelopathy (161), with most success occurring in cases of HIV myelitis (180). However, since myelitis is rarely the sole cause of spinal cord disease, being accompanied by vacuolar myelopathy in 92% of autopies, and is basically a pathologic diagnosis (its only differentiating clinical features being a more prominent sensory level and less loss of vibration and proprioception (7)), HAART does not play a significant role in therapy of spinal cord disease (161). Intravenous immunoglobulin produced encouraging results in an open label trial (Simpson, personal communication) as has plasmapheresis (19), supplementation of methionine (161,178,180,181), but this is still under investigation.

HIV NEUROPATHY

As described in the central nervous system, the symptoms of peripheral nervous system involvement vary with the stage of HIV infection and degree of immunosuppression. In the early period of infection, inflammatory demyelinating polyneuropathy may be present in patients who are unaware of their HIV infection. This will be discussed with other "autoimmune" aspects of HIV in the next section.

By far the most common peripheral nerve involvement in HIV is distal symmetrical polyneuropathy (DSPN), which occurs in the later stages of HIV infection. One-third of patients with advanced AIDS complain of burning of the soles of the feet, especially when walking, but even light contact with the soles may trigger it. Often there are painless paresthesias and numbness, present symmetrically (182). Although referred to as a "stocking-glove" neuropathy due to the distribution, as the feet are always involved first; the "glove" or hand aspect is found only in severe cases. The painful burning is accompanied by other AIDS diagnoses or disorders of mood in many cases (183). Weakness occurs only very late in the course of neuropathy but proximal weakness and spasticity due to myelopathy may confuse the exam. The gait in neuropathy, rather than being spastic or ataxic, is usually gingerly in an effort to minimize contact between the painful soles and the floor. In 70% of Tagliati's cases, pin and temperature sensation were decreased and in 60% vibratory sensation was decreased with only 19% experiencing loss of position sense in the feet (182). In 66%, ankle jerks were diminished or lost. Electrical studies reveal an even higher prevalence of neuropathy. Nerve conduction velocities are slow, with reduced sural sensory nerve action potential amplitude (consistent with axonal loss) and decreased compound muscle action potential amplitudes on nerve conduction stimulation (182). However, there is not always a direct correlation between electrophysiologic results and clinical symptoms, as 28% of patients displayed abnormal nerve conductions without clinical signs of neuropathy, while 19% of patients with complaints had normal results on nerve conductions. One explanation for this lies in the failure of small nerve fibers, which carry painful sensation, to be easily studied electrically (182). In addition, the dorsal root ganglia or outer lamina of the spinal cord may be responsible for some of the pain in the legs (184). Electromyography may show chronic denervation but since sensory complaints overshadow motor, electromyography is often omitted due to fear of pain or risk of infection. Correlated conditions include weight loss, malnutrition, low albumin and hemoglobin, older age, and lower CD4 counts (< 100 in 66% of Tagliati's series of 251 patients) (182). Obviously other conditions that can cause neuropathy such as diabetes, renal failure or alcoholism, can act synergistically with HIV. Although numbness and paresthesias also can occur as a side effect of antiretroviral drugs, (didanosine, zalcitabine and stavudine), only 23% of patients with neuropathy in a recent series had used didanosine, and its use did not correlate with degree of symptomatology (183). The combination of these antivirals with hydroxyurea (185,186) or isoniazid (187) is particularly neurotoxic. Care must be used with chemotherapeutic agents such as vincristine, cisplatin and taxol, antibiotics such as dapsone, ethionamide, isoniazid (even with B6 supplementation), metronidazole, and streptomycin, thalidomide or the anticonvulsant phenytoin (185,188–190). Nerve biopsies are not particularly helpful, although skin biopsies have shown loss of nerve fibers (191). C fibers have been shown to be decreased in non-HIV related cases of peripheral neuropathy (192) and are

beginning to be studied in HIV (183,193). On autopsy, almost all patients have evidence of distal axonopathy (194). Loss of unmyelinated C Þbers is most likely responsible for the pain of neuropathy (182).

Isolated nerve involvement such as mononeuritis multiplex represents a separate form of HIV related neuropathy presumably due to inßammatory causes (195). The rare hyperlymphocytosis with CD8 syndrome (DILS: diffuse inÞltrative lymphocytosis syndrome) (57,196,197) also presents with nerve dysfunction and requires immunosuppressive therapy such as steroids in addition to antiviral therapy.

Autonomic neuropathy causes dizziness especially on rising, syncope, palpitations, anhidrosis, sexual and bladder dysfunction, and diarrhea or constipation due to gastroparesis (198Ð201). Autonomic dysfunction is present in up to 50% of HIV-infected patients when measured quantitatively using holter monitoring, tilt tables or nerve conduction studies (200,202). Parasympathetic and sympathetic systems are both involved, causing opposite symptoms in individual patients. Endocrine dysfunction, as measured by low levels of the adrenal hormone DHEA (dehydroepiandrosterone), can accompany autonomic failure (68). As in DSP, the differential diagnosis includes diabetes, alcoholism, vitamin B12 (203,182) or B6 deÞciency and malnutrition (202). These can act synergistically with HIV as well.

Pathologic studies of peripheral nerve biopsies occasionally show inßammatory reactions and rarely HIV particles are seen using immunostaining (7). In animal models the gp120 section of the HIV-1 envelope, introduced epineurally around the sciatic nerve, produced immediate signs of neuropathic pain and transient swelling with increased TNF-α, followed within 30 days by spinal cord gliosis (204). This may help explain why neuropathy is so common, even in patients who successfully respond to HAART, as the spinal cord pathology persists beyond viral exposure. Where direct evidence is lacking for viral presence, cytokines such as TNF-α and interleukin 1, and other inßammatory mediators are proposed as provoking neuropathy (194,205). Since many patients experience neuropathy after weight loss, with low albumin or hemoglobin (182), malnutrition is assumed to contribute to the neuropathy. Treatment is directed at lowering viral load, while minimizing the use of neurotoxic antiretroviral drugs.

Amyotrophic lateral sclerosis or motor neuron disease has been associated with HIV (206,207); antiretroviral treatment caused symptom reversal in a few cases (208,209).

Lumbosacral radiculopathy due to HIV has been described in four patients either at seroconversion or during early infection. It presented with profound weakness isolated to the legs, no sensory or sphincter complaints (unlike CMV), elevated CSF protein and pleocytosis. Electrical abnormalities, which include Þbrillations characteristic of denervation on electromyography and decreased amplitude characteristic of mild axonal neuropathy with normal F waves, differentiate this from the demylination characteristic of Guillian-BarrŽ. All recovered rapidly and almost completely, without treatment (32). Plexopathy with a relatively benign course has also been described later in infection (210).

Standard treatments for neuropathic pain are sometimes useful in DSP, including topical creams such as capsaicin, which depletes Substance P, the main transmitter of the unmyelinated C Þbers which carry painful stimuli, and lidocaine gel, a local anesthetic (211,212). Oral lidocaine (mexiletene) (213) and peptide T have failed to control symptoms (214). Recombinant nerve growth factor may help pain but not numbness, as the nerve Þbers fail to regenerate (191,215). Central pain is addressed by the use of tricyclic antidepressants such as amitriptyline (213,216) or nortriptyline in doses below 50 mg nightly (182). Newer antidepressants, such as venlafaxine, that work on norepinephrine and dopamine reuptake in addition to serotonin (217) may work as well. Wellbutrin has been shown to relieve diabetic neuropathy pain but has not yet been studied in HIV neuropathy (218). The purer serotonin reuptake inhibitors (SSRIs) such as ßuoxetine and sertraline have little effect on pain. Anticonvulsants such as gabapentin (219), valproic acid (7), phenytoin, carbamazepine or more recently lamotrigine (220) have proven useful. Lamotrigine is even more effective in patients with neuropathy due to use of neurotoxic antiretroviral drugs (221). In addition to class-associated dose-related toxicities such as drowsiness or poor concentration, or speciÞc side effects such as tremor from valproic acid, care must always be taken to respect drug interactions with other medications in the antiviral regimen. This especially applies to most anticonvulsants which increase cytochrome P activity in the liver, thereby increasing metabolism and decreasing plasma levels of antiviral medications such as protease inhibitors (14,222). In addition, some drugs such as valproic acid cause stimulation of HIV growth *in vitro* (14,222,223) but analysis of this and other anticonvulsant medications using other cell lines failed to demonstrate stimulation of HIV replication or transactivation of HIV-LTR (98). Carbamazepine has been shown to be protective against neuronal death due to Tat protein *in vitro* (98).

Acupuncture has been studied (216) and techniques of biofeedback or relaxation exercises (224) may also help avoid medication use. Orthostatic hypotension is treated by encouraging increased salt intake, modifying treatment with antidepressants with hypotension as a side effect, or drugs such as ßuorocortisone or midodrine. Osmotic agents such as lactulose or polyethylene glycol can treat constipation, as dopamine blockers such as metoclopramide, which directly stimulate intestinal nerve plexi, can cause extrapyramidal side effects such as tremor and cogwheel rigidity. Cisapride, another gastric stimulant, has been withdrawn due to cardiac side effects. Antiarrhythmics or pacemakers are rarely needed. Tricyclic

antidepressants with anticholinergic effects help relieve sialorrhea, urinary frequency and diarrhea but may exacerbate orthostatic hypotension or arrhythmias. Opiates may be used for diarrhea as well as resins such as cholestyramine and electrolyte modiÞers such as loperamide. SildenaÞl may alleviate impotence but the dose must be lowered in patients using protease inhibitors.

HIV Myopathy

Myalgias or cramps are common during the seroconversion illness of primary HIV infection, but muscle damage, as manifested by weakness, pain or exertional cramps, CPK elevation, and rhabdomyolysis, is rare (40,225). Although muscle involvement from HIV is often due to inßammatory processes, mitochondrial abnormalities may contribute, even in patients without exposure to reverse transcriptase inhibitors such as zidovudine (226Ð229). Inclusion bodies have been seen on biopsy, and nuclear scans using technetium-99m MDP have shown uptake in the muscles (230), suggesting that HIV myopathy is a form of inßammatory polymyositis (231). Because it is in the autoimmune family, this form of myopathy occurs early in the course of HIV infection, or after reconstitution, with CD4 cell counts in the range of 200Ð500/µl. Proximal muscle weakness causes difÞculty reaching up, combing hair, climbing stairs or rising out of a chair. Dysphagia (which is not painful) and neck weakness may be present (although this is more common in zidovudine-induced myopathy than HIV-myositis). EMG shows characteristic small motor unit potentials with full interference patterns on recruitment (giving effort while the muscle is being tested by needle electromyography). Fibrillation potentials occur spontaneously. Biopsy reveals necrosis, occasional inßammatory inÞltrates or inclusion bodies. The muscle breakdown product creatine phosphokinase (CPK) is raised. Nuclear imaging of the body has shown multiple extra-osseous accumulations consistent with skeletal muscle uptake (230).

Treatment with immunosuppressive agents has been successful in about half of cases (232,233). Theoretically, corticosteroids, in addition to the usual complications of hyperglycemia, osteoporosis, adrenal suppression, may accelerate the immune system changes of HIV (234), but they have been safely used at early stages of infection (235). Prolonged use of steroids can cause proximal myopathy and should be avoided. Intravenous immunoglobulin (IVIG) might not be helpful, as it has failed to improve non-HIV related polymyositis (236). Since its effects are immediately known, IVIG might still be worth trying, but its cost (approximately $1,000.00 per treatment) is high.

Drugs that exacerbate myopathy include the protease inhibitors, which gradually produce lipodystrophy that causes limb muscle wasting without much weakness, and the cholesterol-lowering statins, which are often given to offset the hyperlipidemia caused by the protease inhibitors. The symptoms of statin-induced myalgias and weakness may be helped by using the supplement coenzyme Q (50Ð130 units daily) (7).

Therapy of Neurologic Complications of HIV (Dementia, Myelopathy and Neuropathy)

Ancillary Therapy

Novel therapies, in addition to standard antiviral drugs, will become important in the future and will be discussed Þrst. Although the calcium channel blocker nimodipine failed *in vivo* (70,237), when combined with antiviral therapy it seems to slow dementia progression (238). Calcium dysregulation is still suspected to play an important role in the Þnal stage of cell destruction (16). Therefore, when calcium blockade proved unhelpful, investigators turned to triggers of the process that stimulates fatal calcium inßux, such as the excitatory transmitter glutamate (80). However, trials of the N-methyl D-aspartate receptor blocker memantine gave inconclusive results (70,239,240). The calcium channel blockers, such as nimodipine and NMDA receptor blockers, such as memantine, are too costly and cause too many adverse effects to allow continuous use, so the ideal time of use or patient who would beneÞt the most from neuroprotection needs to be determined before efÞcacy can be established.

Because inßammation plays an important role in ADC, work is being done in changing the balance of pro- and anti-inßammatory modulators, such as IFN-γ vs. IL-4 (61), or TNF-α vs. insulin-like growth factor (ILGF) (66,241). After demonstration of reduced inßammation in mouse models (242), platelet-activating factor (PAF) antagonists, such as lexipafant, and TNFα inhibitors are in clinical trials (243,244). COX-2 inhibitors are under investigation in other viral infections for their effects on prostaglandinE_2 and may display anti-inßammatory properties that will help in ADC (245). Although the weak TNFα inhibitor pentoxifylline failed to improve the clinical status of patients (246), another antagonist of TNFα, CPI-1189, has reduced apoptosis induced by gp120, TNFα and quinolinic acid in tissue culture (247) and therefore merits further investigation. Inhibition of the caspase enzymes which contribute to the apoptosis induced by gp120 may help prevent neuronal degeneration (248). Other cytokine blockers with potential beneÞt in dementia are currently being studied for systemic (nonCNS) HIV infection, including thalidomide, peptide T and nordihydroguiaretic acid (246).

The complex role of chronic oxidative stress related to depletion of endogenous antioxidants and increased production of reactive oxygen species, lucidly outlined by

Mollace and colleagues (75), may lead to treatment using free radical scavengers. Mn-SOD (Manganese superoxide dismutase) has been shown in neuronal cell culture to protect from apoptosis signaled by NF-B, in turn stimulated by the protein, TNFR-1-associated death domain (TRADD) (249). Problems of transport across the blood-brain-barrier are being addresed by macrocyclic complexes with SOD enzymic activity. Polyamine modiÞcation of superoxide dismutase also allow better access to cerebral tissue (75). Manipulation of glutathione (GSH) may also reduce oxidative stress (75). Antioxidants such as thioctic acid and selegeline are somewhat neuroprotective (239,244,250,251), and may work by reducing mitochondrial toxicity.

Growth factors have been studied indirectly by measuring the presence of Þbroblast growth factor-1 in relationship to HIV encephalitis and neurodegeneration; these pathologic studies support the idea that growth factors can protect from the neurotoxicity of HIV infection (252).

Because patients often suffer the constellation of dementia, neuropathy and myelopathy along with low serum albumin and weight loss, the importance of nutritional co-factors in these conditions is being investigated (163,182). Methyl donors such as SAMe (221,253) or cobalamin (254) are postulated to restore myelination. Other nutritional co-factors include selenium, a mineral required for glutathione production, which has decreased β2-microglobulin levels when supplemented with doses of 40Ð80 μg/kg/day (12,255). For depletion of zinc, which is associated with immunosuppression and depression (256), supplementation with 2.5 to 4.0 mg/kg/day has been recommended. Substance abusers are at increased risk of vitamin A deÞciency (257), alcoholics of thiamine deÞciency and both populations are at higher risk of general malnutrition (12), warranting special attention to these supplements in patients with the above habits. A recent case report of a 71 year old with focal dystonia and hemichorea (though not in AIDS) that improved with treatment of B_{12} deÞciency lends support to screening for B_{12} deÞciency and cyanocobalamin replacement if levels are low in the many ADC patients with asterixis, tremor and other movement disorders (258).

Ancillary treatments also include the stimulant methylphenidate for cognitive ÒslowingÓ (259), clonidine (100) or methylphenidate for hyperactivity (260) and antidepressants (261) (especially selective serotonin reuptake inhibitors) and relaxation exercises or biofeedback (224). Attention to the cognitive and extrapyramidal side effects of medications chronically used for psychosis (58,262, 263), pain (213), seizures (222), anxiety and sleep will help minimize symptoms of dementia. Prior use of illicit stimulants such as cocaine and methamphetamine may also contribute to Parkinsonism and dementia even when HAART is working to lower systemic viral load (97). Management of addiction to substances including cocaine, heroin, amphetamines and other drugs is therefore important.

Antiviral Treatment

Early treatment may be the best way to eradicate the virus, so providers (including neurologists) must be on the alert for the very Þrst symptoms, such as aseptic meningitis or myalgia (24) and test for viral antigen, rather than wait for the development of a positive serology.

With the extended life expectancy of AIDS patients, other neurologic conditions can develop, such as Alzheimer's dementia or malignancies that are unrelated to AIDS but whose treatment is affected by the virus. Conversely, older patients who present with dementia from HIV can mistakenly be assumed to have Alzheimer's, or HIV-related movement disorders can masquerade as Parkinson's disease. Rarely, a neurologic disease with unknown etiology, such as amyotrophic lateral sclerosis (208,209), may respond to antiretroviral therapy, making it seem worthwhile to perform almost universal HIV screening.

Although a dose-dependent decrease in symptoms of ADC treated with zidovudine as monotherapy has been established since the drug's introduction (264), this improvement lasted less than a year in one-third of patients (265). The high doses of zidovudine required to reach the brain parenchyma are also difÞcult to tolerate due to anemia and myopathy. Therefore, combination therapy with highly active antiretroviral drugs has revolutionized the treatment of HIV in general and CNS infection as well (126,266). A 52% decline in the incidence of dementia was seen after the introduction of HAART to participants of the Multicenter AIDS Cohort study, made up primarily of men who had acquired the virus by sexual contact, with a 43% decline in injection drug user's incidence of ADC (17,266). Use of protease inhibitors, which do not enter the brain easily, have been associated with declines in HIV dementia (50,88,267). In the HAART era, ADC has begun to occur at higher CD4 counts (201Ð350), meaning the clinician should maintain a higher index of suspicion in symptomatic patients (266). The complex pathophysiology responsible for dementia described in Chapter 00 is entirely driven by the presence of HIV or HIV-infected macrophages or microglia. Infected endothelial cells allow traffÞcking of the virus through the choroid plexus (17,48,79,86). Therefore antiviral therapy constitutes the mainstay of treatment for ADC: by decreasing systemic viral load there subsequently is less CNS seeding. If medication gains access to the brain it can also prevent the CNS from acting as a safe haven for viral replication. Failure to lower the number of copies of HIV-1 RNA in the CSF correlates with the number of drugs that penetrate that achieve adequate concentrations in CSF, and with initial higher viral load in CSF (91). However, although an increased CSF viral load has been shown to correlate with

TABLE 18.2. *Relative concentrations of antiretroviral medications in CSF (CSF/plasma ratio)*

Nucleoside reverse transcriptase inhibitors (NRTIs)

Zidovudine (0.3–1.35) > Stavudine (0.16–0.97) > Abacavir (0.3–0.42) >
Lamivudine (0.04–0.47) > Zalcitabine (0.09–0.37) > Didanosine (0.16–0.19)

Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

Nevirapine (0.45) > Delavirdine (0.02) > Efavirenz (0.01)

Nucleotide reverse transcriptase inhibitors

Tenofovir (?) (not highly protein bound so CSF levels should be high)

Protease inhibitors

Indinavir(0.02–0.06) > Saquinavir = Nel navir = Ritonavir = Amprenavir = (< 0.05)

Combination medications

Abacavir/Zidovudine/Lamivudine = Zidovudine/Lamivudine > Lopinavir/Ritonavir
(Trizivir) (Combivir) (Kaletra)

Caveats:
Ratios were derived from measurement of samples in patients with variable intactness of blood-brain-barrier. CSF levels do not always re ect levels in brain parenchyma.

Levels are affected by ef ux as well as in ux of medication; this is in uenced by the presence of other medications and transporters such as P. glycoprotein.

cognitive impairment (120,246), it has not yet been shown that a decrease in CSF viral load improves cognitive performance. Pathologic studies continue to show the same manifestations in recently autopsied brains as did the initial studies (268), and virus that is latent in astrocytes can conceivably be reactivated by proinßammatory cytokines (269). Because up to half of patients fail to maintain a response to HAART, the risk of recurrence of dementia is high (270).

Including combinations, at least 18 medications are currently in use and more drugs, targeting additional sites of viral replication, are constantly being developed; a summary of web sites offering trials or therapy updates exist, such as www.neuro.wustl.edu/narc, www.clinicaltrials.gov, www.hivatis and www.actis.org/indep.html (57).

There are no speciÞc CNS antiviral medications, but the Þrst antiviral, zidovudine, was proven in a dose dependent manner (271) to slow or prevent ADC (272Ð274). Zidovudine remains the leader in CSF penetration in its class of nucleoside reverse transcriptase inhibitors (NRTI) (275), followed by stavudine and abacavir. Protease inhibitors (PI) were introduced in 1996 and non-nucleoside reverse transcriptase inhibitors (NNRTI) joined the medication armamentarium in 1998 (276,277). Results of treatment are measured by decreases in viral load and increases in CD4 cell counts, as discussed elsewhere in the book, but there is no simple measurement of the effect of new treatment protocols on ADC, myelopathy or neuropathy. Choice of appropriate therapy to treat initial infection involving the CNS, and to prevent dementia or treat its symptoms, may rest on knowledge of drug interactions, viral resistance patterns within the CNS, efÞcacy and side effect proÞle, and ability of patients with compromised mental function to comply with the regimen (9,276). In addition, the ability of a drug to cross the blood-brain-barrier (which varies with the drugÕs lipophilicity and the intactness of the blood brain barrier, presumably becoming more open as dementia advances) is a factor in choosing the best regimen to prevent neuropathy and ADC (67,278) (Table 18.2).

Other NRTIs that are effective in the CNS include stavudine (275,279) and lamivudine (280). The NRTI, abacavir, achieves excellent CNS penetration (levels up to 42% of those in plasma) (57,281). Abacavir has been shown to be effective in ADC (7). Mutations conferring resistance to antiretroviral drugs may vary between those present in CSF and those observed in plasma (10). The more discordant the viral resistance pattern in CSF and plasma, the worse the cognitive impairment, suggesting the need for appropriate testing for optimal treatment in patients with severe dementia (10,155). This problem mandates CSF analysis when patients fail to respond clinically to therapy.

Protease inhibitors have poor penetration into the CNS, but in advanced cases with presumed breakdown of the blood-brain-barrier, clinical response of ADC has been seen (282Ð284). Because it is the least protein bound, indinavir should have the best entry into the CNS (284). However, active transport systems exist that cause efßux out of the brain, such as the P-glycoprotein transporter (283). Larger amounts of protease inhibitors may gain access to the brain than predicted when these reverse

transport systems are affected by drug interactions or in the case of ritonavir, by the drug itself (285).

The NNRTIÕs nevirapine (7,280) and efavirenz (7) have been shown to improve dementia. Judgment of neurologic efÞcacy with efavirinez is limited by its common neurologic side effects of confusion, sleepiness and abnormal dreaming, but these tend to be transient (286,287).

The ribonucleotide reductase inhibitor hydroxyurea has also shown some efÞcacy in treating ADC (44,288) but not when used alone.

In addition to directly decreasing viral load, thereby deactivating macrophages that promote destruction through cytokines and halting apoptosis of astrocytes, HAART works by primarily maintaining or restoring the immune system (19,289). This is especially true in the Þrst stage of infection, when HAART can boost CD4 function and possibly eliminate viral penetration into the brain (290).

Measurement of efÞcacy of HAART has been indirect. MRI improvement was documented in four patients (131). Improvement, especially in timed motor function, has been demonstrated using neuropsychological assessment (121,274,291Ð293), including a response to protease inhibitors (293). Sensitive scales such as the Memorial Sloan Kettering and HIV-associated dementia scales showed improvement in 60% of patients treated with HAART. (The failures tended to be injection drug users by history (294).) Functional imaging with PET has not been used lately (295), but fMRI and MR spectroscopy hold promise as non-invasive methods to monitor response to therapy (137,139,296). Using the patientÕs CSF to measure mitochondrial dysfunction in neuronal cell culture may provide a functional bioassay eventually (251), and a reduction in immune activation markers such as neopterin and β_2-microglobulin may offer evidence of clinical improvement (297). Neuropathologic studies have shown decreased multinucleated giant cells after zidovudine treatment (289,298).

Myelopathy can be considered a remote effect of HIV and therefore its symptoms often fail to respond to antiretroviral treatment alone. Therefore, although it is logical to use antiviral therapy to lower the amount of virus available to decrease cytokine production by macrophages, at most the myelopathy may stabilize (161). However, cases associated with myelitis respond well to antiviral treatment (180). As is the case with dementia, agents with better penetration are recommended, but controlled trials are not yet available.

HAART has also been useful in treating the peripheral neuropathy of late infection (190), although an indirect mechanism through decreasing macrophage activation and cytokine release is probably responsible (190,300). Agents with good CNS penetration presumably also reach the peripheral nerve better, so zidovudine, lamivudine, indinavir and nevirapine are recommended. The NRTIs, including stavudine, didanosine and zalcitabine, that are responsible for causing peripheral neuropathy obviously should be avoided. Neuropathies occurring at earlier stages of infection, such as CIDP (chronic inßammatory demyelinating polyneuropathy) (190) or DILS (diffuse inÞltrative lymphocytosis syndrome), completely recover in two-thirds of cases (301). Because these are only indirectly related to the virus, the drug that is chosen does not need to cross the blood brain barrier. Mononeuritis muiltiplex is also autoimmune mediated so antivirals are only useful if the viral load is high (190).

Side Effects of HIV Medication

Along the long course of HIV infection, many direct and indirect treatment-related problems may emerge (Table 18.3). Access to virus sequestered in the central nervous system, across a blood brain barrier that has recovered to an intact state, may become difÞcult (272,275). Cumulative exposure to the mitochondrial toxicity of nucleoside reverse transcriptase inhibitors can lead to myopathy (228) or even dementia (302) in addition to the more well known peripheral neuropathy associated with didanosine and stavudine (303), especially when augmented by hydroxyurea (186). Indirect neurotoxic effects of medication used for symptomatic relief of HIV-induced complications complicate the use of drugs such as antidepressants (used for pain management as well as mood disorders), antipsychotics or dopamine blocking antiemetics (112,262,304) and anticonvulsants, some of which also promote viral replication (220,222). Cognitively impaired patients often have difÞculty adhering to complex treatment regimens, mandating the use of neuropsychologic screening and implementation of measures to insure compliance, such as directly observed therapy.

Side effects of HAART are generally systemic, but speciÞc neurologic problems are seen. The Þrst reported neurotoxicity from antiviral therapy was myopathy, quickly determined to be due to zidovudineÕs inhibition of γ-DNA polymerase in the muscleÕs mitochondrial system (228,302). Carnitine levels may also become reduced by zidovudine use (305). In early studies using high doses, myopathy was present in up to 18% of patients after about a year (306). Pain is found in half of patients, which manifests as cramps on exertion (307). Fatigue and weakness of the proximal muscles of the legs and buttocks are usually present, with eventual wasting of muscles (303). Creatine phosphokinase (CK) is markedly elevated (308), but the degree does not correlate with symptoms. Lactate may be increased at rest (303). MRS in patientsÕ muscles demonstrates phosphocreatine depletion, due to impaired oxidative phosphorylation (303). Electromyography reveals myopathic pattern, with polyphasic units, Þbrillation and positive sharp waves (307,308). Although the lower doses of zidovudine currently used are less likely to produce myopathy, the effect of combination therapy with more than one NRTI and NNRTI, or concurrent use of statins (the need for which is increased by protease

TABLE 18.3. *Neurologic side effects of antiretroviral drugs and other medications*

Type	Medication Antivirals, Other Classes	Symptom
Cognitive	Efavirenz	Dizziness, Insomnia, Vivid Dreams Depression, Confusion
	Zidovudine	Encephalopathy
	Abacavir	Seizures Profound lethargy and weakness, hypersensitivity reaction
	Antiepileptics	Sedation, Dizziness
	Benzodiazapines	Encephalopathy
Muscle	Zidovudine (Combivir,Trizivir)	Muscle cramps, Weakness (proximal) ↑ CPK
	Protease Inhibitors	Lipoatrophy
	Statins (HMG–CoA reductase inhibitors)	Myalgias, Weakness
Nerve	Didanosine, Zalcitabine, Stavudine, Lamivudine ± Hydroxyurea Thalidomide, Isoniazid, Vincristine, Taxol	Distal numbness, toes > ngers, burning paresthesias, dysesthesias, rare weakness, slapping gait lost ankle jerks. Symptoms can progress after drug stopped
	Protease inhibitors	
	Ritonavir, Amprenavir	Carpal tunnel syndrome
	Indinavir	Alteration of taste
Muscle? Nerve?	Stavudine, Didanosine Abacavir	Severe ascending paralysis, lactic acidosis, EMG c/w axonal neuropathy or myopathy
Extrapyramidal Movement Disorders	Antipsychotics Antinausea or Intestinal Motility stimulants	Tremor (resting and action), cogwheel rigidity, ↑ potential for Neuroleptic Malignant Syndrome

inhibitors inducing hyperlipidemia), is not yet known (302,309,310). Management of myopathy usually requires stopping zidovudine, as lowering of the dose usually does not result in complete improvement (311,312). Carnitine supplementation has not been systematically evaluated in patients (302,313).

The next antiviral drug introduced, didanosine (ddI), caused an irreversible peripheral neuropathy in 15% of patients (314), especially those already suffering from HIV-related DISP. The neuropathy is duration and dose related (193) and inßuenced by the degree of immunodeÞciency as well (193,315,316). B12 deÞciency contributes (203,317), as do other neurotoxic drugs such as isoniazid (even with B6 supplementation), ethambutol (190), thalidomide (188), vincristine and taxol (189,318). Newer agents, such as zalcitabine, stavudine and lamivudine also produce neuropathy (in up to 40% of patients using zalcitabine) (319), the severity of which worsens with combination regimens or when either is combined with hydroxyurea (185,186). Symptoms of numbness and paresthesias in the feet and ankles usually emerge after four to Þve months (314,315,319). Weakness is found only in severe cases (320), but ankle jerks are usually depressed or lost. Proprioception is preserved in most cases (314). After cessation of medication use, the symptoms of neuropathy may worsen temporarily, plateau for about eight weeks and then gradually improve. Nerve conduction studies are consistent with axonal neuropathy (decreased amplitude on stimulation, or lowered sural nerve sensory action potential), but may be normal (320,321). Elevated lactate has been found in 70% of patients with toxic neuropathy, but a pathogenic role is unclear (7). Stavudine especially has been responsible for production of hyperlactatemia (322), though not necessarily accompanied by neuropathy (323), but it has been described in patients using abacavir, didanosine, lamivudine and zidovudine, with or without protease inhibitors.

The pathogenic mechanism of neurotoxicity is postulated to be due to the interference with mitochondrial function. The azido group contained by NRTIs competes with natural thymidine triphosphate as a substrate for pol-γ in the synthesis of mitochondrial (mt) DNA. This mitochondrial toxicity has been demonstrated using a rat model of isolated dorsal root ganglia (324) and by measurement of a deÞciency of mtDNA on skin biopsy (325). DeÞciency of acetyl carnitine, required for mitochondrial function, also has been proposed as a factor contributing to nucleoside neurotoxicity (302,313). Patients with family members (on the maternal side) who suffer from mitochondrial diseases such as MELAS or MERFF are at increased risk of mitochondrial toxicity

from these medications. Future work using the ratio of mitochondrial to nuclear DNA may be able to predict this toxicity and govern therapy with nucleosides in patients with HIV infection (322). Screening with lactic acid levels is not helpful as these levels vary and are not predictive of future problems (283).

Hydroxyurea also decreases the intracellular dATP pool, which contributes to its additive neurotoxic effect as well as its efficacy (185).

Protease inhibitors, through their metabolic effects causing glucose intolerance or diabetes in over half of patients, or through their contribution to weight gain, have been suspected of contributing to carpal tunnel syndrome (326).

A severe, rapidly progressive, ascending paralysis resembling Guillian-Barrž has been described in patients using HAART. Stavudine (d4T) was implicated in 35 patients (of 70,000 exposures) while eight others had no specific pattern of antiviral therapy (327,328). Its mechanism is unclear and although it appears to resemble a severe primary axonal neuropathy, or myopathy, it is probably unrelated to the toxic neuropathy described above. This neuromuscular failure may be accompanied by lactic acidosis. Electrophysiology is consistent with primary axonal neuropathy or myopathy. Unlike in Guillian-Barrž, symptoms do not respond to immunotherapy such as IVIG or plasmapheresis, and the condition was fatal in six of 18 patients whose antiretrovirals were not stopped, and one of seven whose stavudine was stopped. Supplements involved with the respiratory chain of mitochondria are being tested, such as coenzyme Q, L-carnitine and riboflavin.

Profound weakness and lethargy has also been reported with abacavir as part of a hypersensitivity reaction (212).

Headache has been attributed to zidovudine in up to 35% of patients, saquinavir in up to 9% and amprenavir in 6% (212), as well as in patients taking stavudine (7).

Encephalopathy has been described in patients using the NNRTI efavirenz, both alone and in combination (277,286,287). Symptoms include dizziness, restlessness, depression, inability to concentrate, insomnia and abnormally vivid or bizarre dreaming. Sixteen of 111 patients in a private practice in California stopped the medication due to these symptoms, despite treatment for depression in 13. In another series, 18% of patients discontinued efavirenz, most due to these side effects (286). Since there is no binding of the drug to any CNS neurotransmitters, it is unclear how efavirenz causes these symptoms. Insomnia has also been reported with zidovudine and in up to 29% of patients using stavudine (212). Wernicke's encephalopathy attributed to thiamine deficiency has also been described with zidovudine (329).

Seizures have been described in patients on zidovudine (333) or didanosine (320,331), although in these early series, zidovudine may have been blamed before the relatively high frequency of recurrent seizures due to HIV dementia was appreciated. Ritonavir, amprenavir and indinavir have rarely caused alteration of taste (212).

SYNDROMES OF IMMUNE DYSREGULATION IN THE NERVOUS SYSTEM (DURING EARLY OR RECOVERING HIV INFECTION)

Viral infections have often triggered neurologic autoimmune disease (332). Immune dysregulation can lead to "attacks" on many parts of the nervous system; proposed pathophysiologic mechanisms include a general activation of B cells leading to production of antibodies that attack myelin or other parts of the nervous system, upregulation of MHC antigen expression, and molecular mimicry after incorporation of viral proteins into nervous tissue or sharing of epitopes between virus and host (332,333). As HIV infects macrophages, there is an upregulation of cytokines leading to broad-spectrum autoimmune disease. The most common sites are in the peripheral nervous system, i.e. nerves (182,334), nerve roots (335), muscle (336) and the neuromuscular junction (337,338). Mononeuritis multiplex, or involvement of isolated nerves, can be autoimmune or due to vasculitis producing infarcts of the nerve (339). Stroke can occur secondary to vascular or hematologic problems, such as thrombocytopenia (340), anticardiolipin antibodies (341) or vasculitis (47, 342–348). Speculation also exists that autoimmune disease contributes to dementia (349) or a multiple sclerosis-like syndrome found in early HIV infection (350,351). However, radiologists may overdiagnose multiple sclerosis due to the misinterpretation of ill-defined periventricular white matter abnormalities seen in up to 73% of cases of HIV dementia (350–353). Although the pathogenesis of these relapsing syndromes is unclear, an increase in τ/δ T cells has been described at the time of seroconversion, and these cells have been found in the demyelinating lesions and CSF of patients with multiple sclerosis (MS) (354,355). Also, monocyte infiltration through the blood vessels into the white matter near the ventricles may represent a more aggressive form of the usual HIV invasion and not a form of multiple sclerosis. As the number of τ/δ T cells decline along with other T cell depletion in late AIDS, MS becomes even more rare (356). Antibodies to myelin basic protein, although nonspecific, may contribute to dementia or MS-like syndromes (333). The contribution of antibrain antibodies to brain pathology and dysfunction in ADC has not been fully established (349,357). A severe leukoencephalopathy was recently described in seven patients receiving HAART unsuccessfully, which was not successfully controlling HIV replication (358).

Chronic headache in the early stage of HIV infection may be due to aseptic meningitis, which has been steroid responsive in rare cases (30,359).

Acute or relapsing inflammatory demyelinating polyneuropathy (IDP) due to HIV was for a time in the 1980s and early 1990s the most common cause of Guillian- Barrž

in New York hospitals (personal observation). This syndrome presents with rapidly progressive ascending symmetric weakness and areßexia, occasionally producing facial diplegia or respiratory compromise, and is sometimes preceded by paresthesias or numbness in the feet and legs. Loss of vibratory sense is common. A related inßammatory condition, mononeuropathy multiplex, can involve cranial as well as peripheral nerves (195,360), especially the facial nerve (7,361). At times the course may be more indolent or relapsing, and is known as chronic inßammatory demyelinating polyneuropathy (CIDP). Cerebrospinal ßuid contains increased protein, up to two or three times the normal upper limit, and mild pleocytosis (generally 10Ð50 lymphocytes), which sets it apart from non-HIV related cases of IDP. The presence of anti-peripheral nerve myelin antibodies (334) and the response to plasmapheresis or infusion of intravenous immunoglobulin (IVIG) lend support to its cause being a deranged immune response. Nerve biopsies reveal segmental demyelination, variable amounts of axonal degeneration, and the presence of macrophages (335,362, 363). The degree of immunosuppression can be used to distinguish these conditions from CMV polyradiculopathy, which is clinically similar but only occurs at CD4 counts below 50 cells/mm3.

Autoimmune causes of a motor neuron syndrome have been associated with anti-asialo-GM1 antibody (364).

Myasthenia gravis in HIV is similar to that in non-HIV infection (337), but with lower titers of acetylcholine receptor antibodies (338). Because memory Tcells are the Þrst subset to be lost in HIV (365) and these are responsible for development of myasthenia syndromes, it is not surprising that these are rare, even in early HIV infection.

Polymyositis presents as proximal limb or neck muscle weakness, accompanied at times by pain, cramps, or easy fatiguability, in up to 2% of patients (232,366). Occasionally the skin rash of dermatomyositis is also present (226). Elevated CPK in the serum was found in 92% of patients with muscle weakness, not all of whom had polymyositis (235,307,336,367). However, 5% of asymptomatic patients with HIV have elevated CPKs (307). More speciÞc anti-muscle antibodies such as Jo-1 have not been studied in HIV polymyositis (367). Muscle biopsy shows inclusion bodies (231) or other inßammatory cells (232,362); some show fragments of HIV provirus (368). The presence of pain helps distinguish polymyositis from the nonspeciÞc weakness, muscle wasting and fatigue seen late in AIDS (369).

Treatment of autoimmune disease, even in HIV, consists of immunosuppression with corticosteroids. This was effective in approximately 50% of patients with myositis (233) and up to 80% of patients suffering from CIDP (363). It also has been used safely in patients with mononeuruitis multiplex with good results (339). Stronger immunosuppression with cyclosporine has been used in CIDP (370). Because plasmapheresis is cumbersone and somewhat risky (371), although efÞcacious, it is used less commonly than intravenous immunoglobulin therapy to treat CIDP and myositis (7,335,360,372,373).

Because restoration of the immune system should ideally occur with the advent of HAART, all of these syndromes should be watched for in the months following initiation of therapy. A nonspeciÞc syndrome causing arthralgias, myalgias and encephalopathy known as Immune Reconstitution Disease can occur within eight weeks of beginning therapy, most commonly in patients with CD4 counts below 100 cells/µl and co-infection with hepatitis C (374). Symptoms may require anti-inßammatory treatment, even with steroids, in order to continue HAART (57). In addition, recrudescence of various opportunistic infections, sometimes manifesting atypically (375), can be seen with reconstitution of the immune system. This is most common with mycobacterial and CMV infection but can occur with any infection.

Focal Neurologic Syndromes

General Principles (see Table 18.1)

Clinically, the causes of focal central nervous system dysfunction such as tumor, toxoplasmosis, viral and bacterial infection cannot be distinguished from one another. At times, even stroke or hemorrhage can be atypical in patients with AIDS. As antiviral treatment has improved, the incidence of opportunistic conditions has steadily decreased. For example, the incidence of toxoplasmosis went from 5.4% of patients in the multicenter AIDS Cohort Study in the U.S. in 1990Ð1992 to 2.2% in 1996Ð1998, lymphoma from 2.8% to 0.4% and progressive multifocal leukoencephalopathy (PML) from 2.0% to 1.5% in that time period (266), and the overall incidence of opportunistic infection (not just neurologic diseases) has fallen by 55% between 1992 and 1997 (4). Therefore, clinicians must be even more aware of clues to help distinguish among these conditions, and must also keep in mind non-AIDS related conditions such as malignancy (376). In addition, even with successful control of HIV infection, questions remain about the ability of a reconstituted immune system to suppress opportunistic infections. Restored T cells may no longer have memory for speciÞc infections, and therefore would not present the correct antigen to the immune system (4,5,377).

With these caveats in mind, the clinician can use the following guidelines to uncover the suspected etiology of a given patientÕs neurologic syndrome. Elements from the patientÕs history (e.g. presence of headache, encephalopathy or obtundation, focal or generalized seizures, rate of symptom progression), symptom complex to localize the site or sites of pathology, history of prior treatment, including compliance with currently recommended treatments for speciÞc infections, geographic and lifestyle

background that would have provided opportunities for acquisition of various organisms and stage of immunosuppression all help predict the infection or tumor at hand (4,378Ð381). Once a focal syndrome is suspected, even in a patient with a currently non-focal neurologic exam, radiologic evaluation using contrast enhancement is required. Although some diseases are indistinguishable on MRI or CT scan, such as toxoplasmosis vs. lymphoma, many, such as stroke, PML and herpes encephalitis, display their usual characteristics on these images. MRI cannot be performed on patients with pacemakers or ferromagnetic devices, including bullets, near critical areas, and is very loud and difÞcult to tolerate for claustrophobic patients. While increasing the sensitivity of the exam, iodinated contrast accompanying CT carries risks of renal failure or allergy. Conversely, the gadolinium-DTPA used intravenously with MR scanning, while safe, does not increase the testÕs sensitivity but does further characterise the lesions seen on unenhanced scans. These enhanced lesions appear bright on T1-weighted pulse sequences. Other special MR techniques include proton density weighting, which is similar to FLAIR in its ability to show all the lesions, and gradient echo which produces a signal void in areas containing blood products, such as hemorrhagic aspergillosis or encephalitis or hypertensive hemorrhage.

Nuclear studies such as thallium-201 SPECT scan further help distinguish malignant from nonmalignant lesions, (the former being hyper-(hot) and the latter hypometabolic (cold)). SPECT sensitivity is aided by the use of a delayed scan which allows calculation of retention index (382). Additional information can be obtained from PCR or antibody studies, although the latter is less reliable in late AIDS. For example, toxoplasma encephalitis is unlikely with negative antibodies, while lymphoma becomes more likely if evidence for Epstein-Barr virus is present in cerebrospinal ßuid (381).

When the patientÕs clinical condition allows some time (i.e. the mass is not causing herniation or respiratory compromise), the easiest and safest way to determine the cause of a mass is a clinical trial. SpeciÞc therapy, beginning with treatment for the most likely etiology based on geographic origin, is initiated. If no response to therapy is seen in two weeks, brain biopsy is indicated. The risks and beneÞts of this procedure will be discussed below. If herniation is diagnosed, decompression is mandatory (unless the patient responds quickly to treatment with corticosteroids, mannitol, glycerol or other nonspeciÞc medical therapy). The beneÞt of decompression is that culture and pathologic analysis can then be performed on the mass.

The characteristic symptoms of a mass relate to its location, while the rate of symptom progression relates to its etiology. For example, a patient with an embolic brain infarct due to endocarditis presents with abrupt onset of deÞcits, which may partially resolve or may later gradually worsen as an abscess grows in the infarcted area. The clinical picture will depend on the location of the lesion, examples of which are given below. Most commonly, ßuctuation of symptoms is due to seizures with post-ictal (ToddÕs) deÞcit for several hours or days, with subsequent recovery to baseline levels of deÞcit. Steroids produce a nonspeciÞc restoration of the blood brain barrier and improvement in the edema surrounding a mass from any cause, and therefore can invalidate a trial of speciÞc antiprotozoan or antibacterial therapy (379).

Although neurologic dysfunction deÞnitely corresponds to the area of the brain involved, the diagnosis may be difÞcult in patients with multiple lesions, simultaneous involvement of multiple areas of the neuraxis such as brain and spinal cord, or focal lesions superimposed on HIV dementia, neuropathy or metabolic encephalopathy. Additional problems interpreting neurologic signs emerge when a patientÕs mental status is depressed by drug use, increased intracranial pressure or herniation, seizure activity or metabolic dysfunction. Nevertheless, it is worth trying to localize systematically, especially when neuroradiologic support is unavailable or clinical response will be used to follow treatment efÞcacy. Cortical lesions are known to cause dysfunction of higher cognitive processing, such as aphasia with difÞculty producing speech if the lesion is anteriorly located in the frontal lobe (BrocaÕs area) or difÞculty comprehending language if the lesion is posteriorly located in the temporal lobe (WernickeÕs area). Bilateral temporal lobe involvement leads to problems storing or retrieving memory. Non-dominant hemisphere involvement causes neglect of the opposite side, anosognosia (failure to recognize the illness or disability), and difÞculty with language prosody or comprehension of the emotional meaning of speech. Contralateral sensory impairment is not usually complained of, but is noticeable when testing for stereognosis (object recognition by touch), extinction (failure to notice stimulation to the opposite side of the body or visual Þeld when both sides are simultaneously stimulated) and dysgraphia (failure to recognize letters or numbers ÒwrittenÓ on the hand with light touch). Patients may notice difÞculty Þnding their way or interpreting a map. If the occipital lobes are affected, a contralateral loss of vision in the hemiÞeld will be found. If the deeper areas of the hemisphere are involved, which is often the case from toxoplasmosis or lymphoma, contralateral weakness, tremor, rigidity or rarely hemiballismus (violent movements of the opposite limbs) may occur (383). Cerebellar and brainstem involvement cause cranial nerve abnormalities such as dizziness or vertigo, diplopia, nystagmus, dysarthria and dysphagia, with intention tremor or incoordination, and severe limb weakness or numbness opposite to the facial weakness.

Once the presence of a lesion or lesions is documented, a decision must be made as to the most likely cause, and treatment begun. Fever and headache are nonspeciÞc and unhelpful. CD4 count provides some guidance, with PML or lymphoma occurring below 100/mm^3, fungi and

protozoan infections occurring below 200/mm^3 and bacterial, mycobacterial, syphilitic and non AIDS-related malignancy occurring at any CD4 count. The radiologic picture is very useful in determining probable etiology. Ring-enhancement when gadolinium or iodinated contrast injection is used is present in cases of lymphoma or other malignancy, toxoplasma or the more rarely seen mycobacterial, bacterial or fungal abscesses. The latter infections may appear in the cortex or deeper, while lymphoma and toxoplasma encephalitis occur singly or in multiple locations often in the basal ganglia, gray-white matter junction or deep white matter. Usually pronounced edema is seen, and the degree of enhancement is not different in tumor or infection. The scan often looks much worse than the patient. Viral infections cause enhancement only in the case of CMV, which is located periventricularly; CMV has become uncommon in the era of HAART. PML, despite its name, appears as a single lesion in 10% of patients (384), often in the posterior fossa (cerebellum or brainstem), and rarely enhances.

Although SPECT Thallium-201 scans have been studied extensively in lymphoma, the sensitivity, even in experienced centers, is only 86–96% and the speciþcity is 76% to 83% (382,385), which limits its usefulness. Although sensitivity may be improved (reaching 100%) by the addition of gallium scintography to SPECT scans, speciþcity remained at only 80% of a series of 21 patients reported by Lee et al. (386). In one series, technetium-99m sestamibi scans showed higher lesion-to-normal tissue uptake ratios than thallium-201, suggesting that this scanning technique may be more sensitive (387). Positron emission tomography is less widely available and no more successful than thallium SPECT (385,388).

Cerebrospinal ßuid analysis is similarly frustrating in diagnostic yield, other than the correlation between lymphoma and a positive PCR for EBV. Cytology is usually negative even in patients with lymphoma, as there may not be communication between a deep lesion and the ventricles or subarachnoid space (385). CSF serology for toxoplasma can be helpful in establishing diagnosis of toxoplasma encephalitis (TE), with no patients in Skiest's series of 24 patients having a postivie CSF titer when TE was not diagnosed, with only one false negative for TE. The serum results, however, were similar. It is prudent to obtain serum toxoplasma titers at the time of initial diagnosis of HIV infection to learn who is at risk for this infection. Miller et al., in a series of 32 patients, however, found toxoplasma serologic status unhelpful in distinguishing TE from lymphoma, preferring to rely on the fact that solitary lesions were more likely to be lymphoma, and high uptake ratios on thallium SPECT scans were also suggestive of lymphoma (384).

At times biopsy is required to make a deþnitive diagnosis, such as when lesions fail to respond to treatment, or in cases of conßicting results of supportive tests. If biopsy is performed stereotactically there is reduced morbidity and mortality, but complication rates still range from 12–23%, with a mortality rate within two weeks of 2–12%, a rate that is higher than non-HIV infected patients undergoing stereotactic brain biopsy (378). This is due to bleeding in most cases (389), iatrogenic bacterial infection in a few and seizures or postoperative neurologic deþcit in others (378,390–392). The diagnostic yield was excellent in Antinori's review of 160 biopsies (381), and in Zunt's (378) review of several studies of contrast-enhancing lesions (88–98%) and fair in non-enhancing lesions (67%), but it must be remembered that there may be problems in interpreting biopsy results. For instance, in patients with multiple lesions, not every lesion may be due to the same etiology found in the one selected for biopsy, some lesions have multiple etiologies, some biopsies are nondiagnostic, and not every biopsy yields a treatable cause of the lesion. Life expectancy after biopsy remained less than two months in patients suffering from lymphoma in Antinori's series (381), and even some infections fail to respond to appropriate therapy. Nevertheless, patients generally beneþt from knowing the cause of the intracranial pathology, when it is safe to discover it, even when the answer does not change their treatment.

Malignancies (Brain/Spine/Cord Compression/Meningeal)

Lymphoma

Recently, primary lymphoma has overtaken toxoplasmosis as the most common cause of mass lesions in the CNS in some series of patients (380,390). Lymphoma (Hodgkin's and non-Hodgkin's) occurs with advanced immunosuppression (<50 CD4 cells/mm^3). Since the brain contains no native lymph tissue, it is not known why there is a propensity for lymphoma to form there. Some theories include the central nervous system's isolation from the body's ability to suppress malignant clones, which then develop within the brain in the absence of immune surveillance; the transformation of lymphocytes after they have passed through the blood-brain-barrier; and the presence of cell surface markers on transformed lymphocytes that cause them to be attracted to the CNS. Most cases are associated with infection by the Epstein Barr virus, which presumably stimulates production of B-cells; these grow out of control when the T-cells that normally balance them are lost.

Lymphoma can present with focal signs, seizures in up to 15%, and at times, global impairment of consciousness. Obtundation is especially common with increased intracranial pressure, multiple lesions or much edema around lesions. Lymphomatous meningitis produces cranial nerve or spinal root abnormalities, without headache. Single lesions are found on MRI, which is more sensitive than CT, in up to 50% of patients and multiple lesions in 50–70% (393) (Fig. 18.8). These enhance in a ring or patchy pattern in $>90\%$ of cases (379,394), although 27%

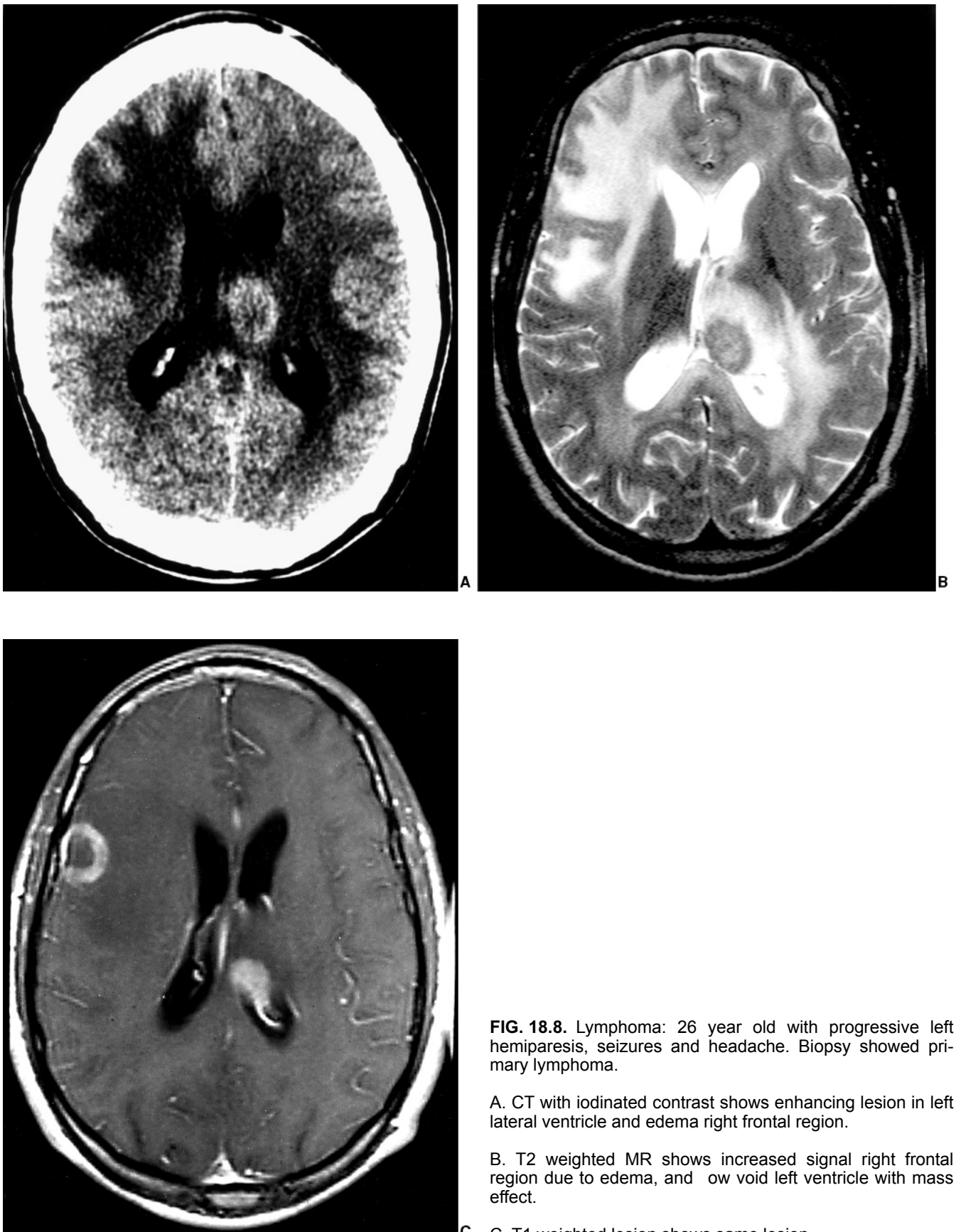


FIG. 18.8. Lymphoma: 26 year old with progressive left hemiparesis, seizures and headache. Biopsy showed primary lymphoma.

A. CT with iodinated contrast shows enhancing lesion in left lateral ventricle and edema right frontal region.

B. T2 weighted MR shows increased signal right frontal region due to edema, and ow void left ventricle with mass effect.

C. T1 weighted lesion shows same lesion.

of Thurnher's series of 60 lesions failed to enhance (395). These lesions are often found in the basal ganglia near the ependyma of the ventricles or near the meninges, and often involve the white matter (especially the corpus callosum) (396,397). There is usually marked edema and mass effect. Radiographic discrimination between tumor and infection is not possible, except in measuring the response during therapeutic trials using steroids for lymphoma and antibacterial drugs for infection. Metabolic studies are helpful, as tumor is hypermetabolic on PET, gallium and SPECT scans (385–387,398).

CSF analysis is usually nonspecific but may be required to rule out infection. Cytology for malignancy is less commonly abnormal in AIDS than non-AIDS lymphoma, where it is still only positive in less than 30% of cases (399). Occasionally lymphomatous meningitis is visible as enhanced meninges on neuroimaging, while remaining undetectable by cytologic analysis of up to 15 cc of CSF. This is attributed to the adherence of tumor cells to meningeal surfaces, preventing them from appearing in CSF (400). Using molecular techniques such as PCR-amplified nested CDR III to measure monoclonal markers of B cells, leptomeningeal involvement by tumor is more easily diagnosed (401). CSF examination is also useful in detecting PCR evidence of EB virus infection: EB viral DNA detection is both sensitive (50–100% of the time) and specific, with rare false negatives (379,381). In fact when EBV was detected in CSF, even without brain lesions, two of nine patients subsequently developed lymphoma (402). EB virus may not be the only oncogenic virus, as an isolated case of PML combined with lymphoma has suggested a possible role for JC virus (403). The same is not true of all viruses, as Kaposi's sarcoma-associated herpesvirus in CSF had no relationship to tumors in Brink's series (402). The presence of EB virus is so specific for lymphoma that a confirmatory brain biopsy is only mandated when nuclear imaging suggests malignancy, but EB virus is not detectable (392).

Once lymphoma is diagnosed, in order to direct treatment, systemic staging should be carried out and ocular and meningeal lymphoma ruled out by slit lamp exam and CSF examination, respectively. Non-Hodgkin's lymphoma occurs more often in the meninges than brain or cord parenchyma (172), while one case of angiotropic large cell lymphoma has been described in the brain (404). Survival for all patients with lymphoma used to average four to eight weeks, but in recent series this has been extended to six to twelve months, or longer with immune reconstitution.

Treatment with radiation, preceded by steroids to avoid herniation and minimize radiation induced headache, nausea and vomiting, has been successful. In cases where biopsy is not easily obtained, radiation has even been used empirically to diagnose lymphoma (4005). Radiation is given as 3–4,000 cGy in 10 fractionated sessions to the whole brain, with prolonged survival (mean 122 days) (406) in some patients, in whom death came from unrelated causes. Chemotherapy with methotrexate delivered systemically or intrathecally may achieve survivals of up to two years, but may be difficult to tolerate (407,408). Some HIV-infected patients will be able to receive chemotherapeutic regimens employed in immunocompetent patients, such as temozolomide (409) or cytarabine combined with methotrexate (410). Antiviral therapy directed against EBV (zidovudine, ganciclovir and interleukin 2) has also shown success in four of five patients treated by Raez and colleagues (411). Success can be measured by response of lesion size on follow-up imaging with MR, CT, or nuclear studies or by measurement of levels of EBV DNA in CSF (412). If HAART is successful in reconstituting the immune system, lymphoma may be contained (413–415).

Spinal cord involvement occurs with metastatic or primary lymphoma in up to 25% of cases (416), and is seen in other tumors. Presumed spread from leptomeningeal involvement is responsible, leading to patchy involvement as well as unilevel cord compression. Metastatic lymphoma is usually B-cell, immunoblastic, or occasionally small non-cleaved cell in origin (172). Radiation and chemotherapy including intrathecal methotrexate only help in 50% of patients, and survival is usually less than six months (417). HAART does not seem to improve outcome.

Lymphomatous or carcinomatous meningitis causes isolated cranial and spinal nerve root symptoms, usually without headache or meningeal signs. CSF shows an elevated protein and pleocytosis, with a low glucose present in some cases. Cytology, performed on at least 3 ccs of formalin-fixed CSF, is positive in less than 30% of patients but new molecular techniques should help improve sensitivity (400). The presence of tumor cells in CSF determines the need for intrathecal chemotherapy (407).

Kaposi's Sarcoma

Kaposi's sarcoma remains extremely rare in the nervous system (2,418,419), for unclear reasons.

Primary Brain Tumors (Glioma, Astrocytoma)

Other malignancies, including primary brain tumors, are appearing in increasing numbers of younger AIDS patients (420). In an early series, a prevalence of glioma of 0.5% among all tumors was found (421) and a total of 19 cases of glioma were reported in the AIDS literature (including postmortem and surgical pathology) up to 1994 (422). In 250 biopsies (and two craniotomies), Gildenberg diagnosed eight non-lymphomatous neoplasms (423). Up to 6% of mass lesions in two small series of AIDS patients were found to be glial cell tumors (422,424,425). Since most patients died within three months, it is unlikely that

this high frequency is simply an artifact of early discovery of tumors due to the frequent scans that AIDS patients undergo. A failure of the immune system to perform normal tumor surveillance in severely immunosuppressed patients (422) has been postulated, but not all patients have extremely low CD4 counts. In fact there is no relationship of degree of immunosuppression or CD4 count to grade of malignancy (426). Instead, HIV may play an oncogenic role for glial cells. The same cytokines involved in dementia, such as transforming GF-β, also stimulate astrocytosis and tumor growth. For example, we cared for a 35 year old woman with recurrent schwannomas in the spinal cord (427) for over ten years who finally succumbed to a highly malignant peripheral nerve sarcoma after contracting HIV, despite a relatively high CD4 count. This suggested a role for HIV in turning off an already dysfunctional tumor suppressor gene.

Metastatic Lesions

In patients who are not severely immunosuppressed, metastatic tumors must be kept in mind, especially as the risk of stereotactic biopsy in this group is low (Figs. 18.9 and 18.10) (381,392). If nuclear scans implicate malignancy and patients have negative EB virus and toxoplasma serologies, surgery is recommended. Complete removal of the lesion is especially recommended if the location is favorable, the patient medically stable and there is only one lesion. Early biopsy at least should be offered to avoid neurologic deterioration during empiric trials of antibacterial agents. If surgery is not an option, whole brain or focused beam radiation (gamma knife) may be given (428). As patients with HIV infection survive longer, disease unrelated to their infection must be kept in mind. In addition, surgery, especially biopsy, is safer and should be performed according to the standard of care.

CNS INFECTIONS (BRAIN/SPINE/MENINGEAL)

Protozoan and Helminthic Infection

Toxoplasma gondii

Until recently, toxoplasmosis was the most common cause of mass lesions in the brain of AIDS patients (429–431). In the era of HAART (surveyed in 1998), the proportion of deaths from toxoplasmosis decreased from 3.5 to 1.9% of patients in the U.S. (266,432) with a corresponding four-fold decrease in incidence in France, where up to 80% of the population is seropositive (378,433). The presentation is typical of a structural brain lesion: symptoms and signs referable to the area(s) of the brain affected, often accompanied by headache (60%), fever (70%), seizures (25%) and confusion (40%) that progress slowly over several weeks. Abrupt onset of focal deficit is described in patients with seizures or hemorrhage into a lesion. At times focal signs are superimposed on diffuse signs of HIV related dementia (379,429,430). There is a predilection for involvement of the gray-white matter junction, thalamus and basal ganglia, resulting in frequent hemiparesis or parietal signs such as neglect, denial of illness, visual field cut and hemisensory loss (379). Despite the frequency of basal ganglia lesions, movement disorders are rare (112,434). The brainstem is rarely involved (435), but sixth nerve palsies due to increased intracranial pressure are occasionally seen. Diffuse encephalitis is more rapidly progressive and often fatal (436). It was responsible for seven of 55 cases of TE in Khuong's series (437). Even before effective antiretroviral therapy was available, the widespread use of trimethoprim-sulfamethoxazole for prophylaxis of *Pneumocystis carinii* infection, which offers protection against toxoplasma as well, likely contributed to a lower incidence of toxoplasmosis (5–15%) than might have been anticipated based on seroprevalence rates (30%) in the United States (429,438–440). In addition, even in areas with much higher seroprevalence rates, such as France and Africa (presumably due to increased exposure to wild cat feces or undercooked meat), there is a variable rate of toxoplasmosis among patients with similar levels of immune depression. This suggests a role for genetic aspects of host susceptibility to infection (440), with those with histocompatibility types of HLA-DQ3 being more susceptible and those with HLA-DQ1 more resistant to encephalitis (441,442). Although the reactivation of infection, caused by the breakdown of latent cysts, followed by tachyzoite proliferation, is clearly related to failure of cell-mediated immunity due to drop in T cells, a collaboration with the humoral system, mediated through cytokines that stimulate antibody production by microglia and dendritic brain cells, has been established in animal models (441). Some variability in the severity of inflammation triggered by the tachyzoite also hinges on the parasite, as some strains use different TNF-dependent pathways to activate iNOS (441) or the transcription factor NFκB, which regulate the production of the chemokines involved in the inflammatory cascade (443). The parasite also varies in the recruitment of neutrophils across the blood-brain-barrier to suppress parenchymal infection (444).

Radiologic evaluation of toxoplasma encephalitis includes CT or MRI with contrast enhancement (Fig. 18.11), which typically reveals ring-enhancing or nodular lesions with much edema. Most patients have multiple lesions, although up to 40% may be solitary (379). On MR the lesions have decreased signal on T1-weighted and increased signal on T2-weighted images, and are typically found in the basal ganglia, thalamus or corticomedullary junction. Metabolic imaging with PET (388) or SPECT using technetium-99m sestamibi or thallium-201 (386,387) may help to differentiate TE from lymphoma or malignancy, which are found in the same locations (385).

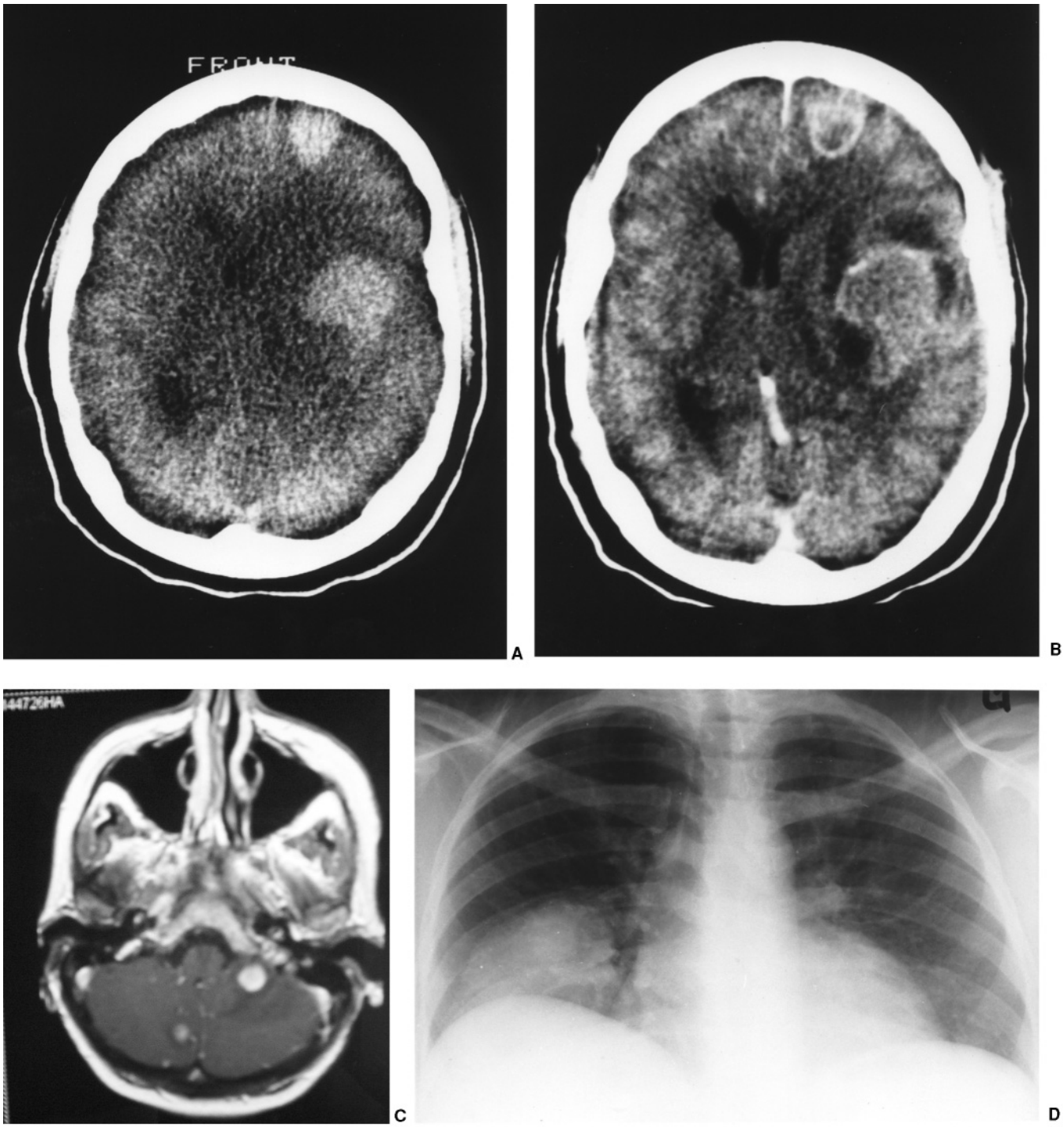


FIG. 18.9. Metastases from adenocarcinoma of lung:

A. Noncontrast CT of a 34 year old pregnant woman being treated unsuccessfully for toxoplasmosis. Hyperdense lesions are present in both frontal regions.

B. CT with iodinated contrast showing enhancement and surrounding edema of same lesions.

C. Brain MRI (T2, with gadopentate) shows two enhancing lesions in the posterior fossa. Others were seen in the cortical gray-white matter junction.

D. Chest radiograph shows large lesion in the right lower lobe, which proved to be an undifferentiated bronchogenic adenocarcinoma on bronchoscopic biopsy.

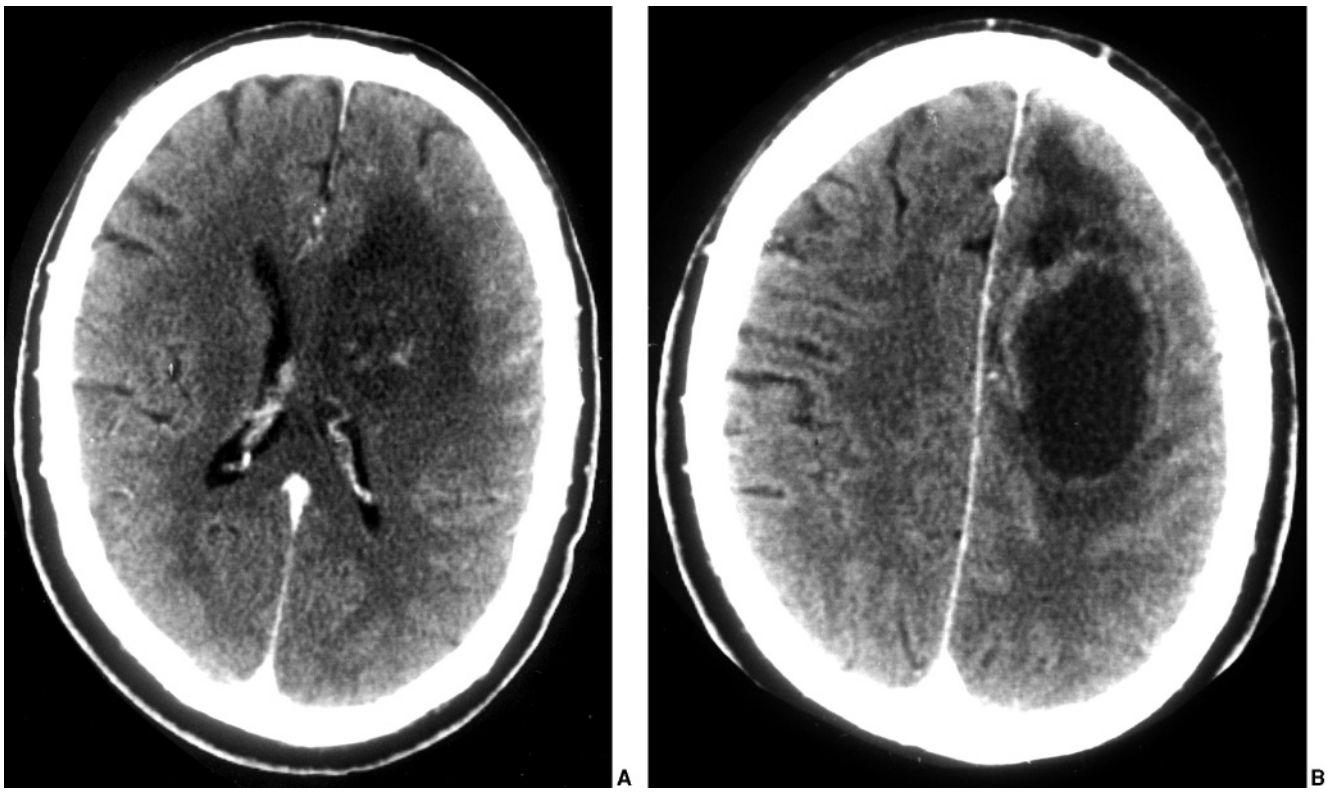


FIG. 18.10. Metastatic lesion from mucinous lung carcinoma:

A. Minimally enhancing large cystic lesion in the left basal ganglia and frontal lobe in a 54 year old heavy smoker with 900 CD4 cells.

B. Higher axial cut of same patient showing rim enhancement of lesion. Biopsy and drainage temporarily improved symptoms, which recurred two months after surgical resection. Pathology revealed mucinous adenocarcinoma.

Malignancy is hypermetabolic and infection hypometabolic. Gallium scintography also distinguishes malignancy from infection (398), but is not speciÞc for infection with *Toxoplasma gondii* as it can be positive in cases of progressive multifocal leukoencephalopathy (PML) (386) and others.

Serologic studies supplement imaging, but are not 100% sensitive. Cerebrospinal ßuid analysis using PCR plays a minor role in diagnosis (379,445) as the sensitivity, though better than that in the plasma, is only 33%. (However, a negative PCR for Epstein-Barr virus can be useful to rule out lymphoma (382,385).) Serology for IgG antibody to *T. gondii* should be performed when a patient is diagnosed with HIV, both to predict future illness (379,385,438,446) and to counsel those patients who have not been previously exposed to the parasite in avoidance techniques (379,385,438,446Ð449). Fluctuations in titer do not reßect disease activity and should not be used to measure response to treatment (447).

Treatment and prevention of TE are covered in Chapter 00 (433,450Ð452). Clinical and radiographic response is rapid, allowing a one or two week trial for diagnostic purposes. In most cases the lesions disappear completely and may calcify (435).

Spinal cord involvement (Fig. 18.12) at any level causes myelopathy or conus medullaris syndrome (171,455,456). Meningitis is rare in toxoplasma infection.

Trypanosomiasis

This protozoan, responsible for ChagasÕ disease, is endemic in Latin America and may be reactivated as patients become immunosuppressed. Two cases of enhancing mass lesions have been reported in patients with AIDS, with amastigotes seen within chronic inßammation on biopsy (457,458). One additional patient died from diffuse meningoencephalitis due to trypanosomiasis (459). An excellent review of current diagnosis and treatment strategies is presented elsewhere (460).

Schistosomiasis

A widespread infection, schistosomiasis mainly involves the liver or genitourinary system, but may produce seizures or spinal cord symptoms due to aberrant migration of worms or embolization of eggs (461). Additional neurologic symptoms due to hepatic encephalopathy may occur, as a consequence of cirrhosis and liver

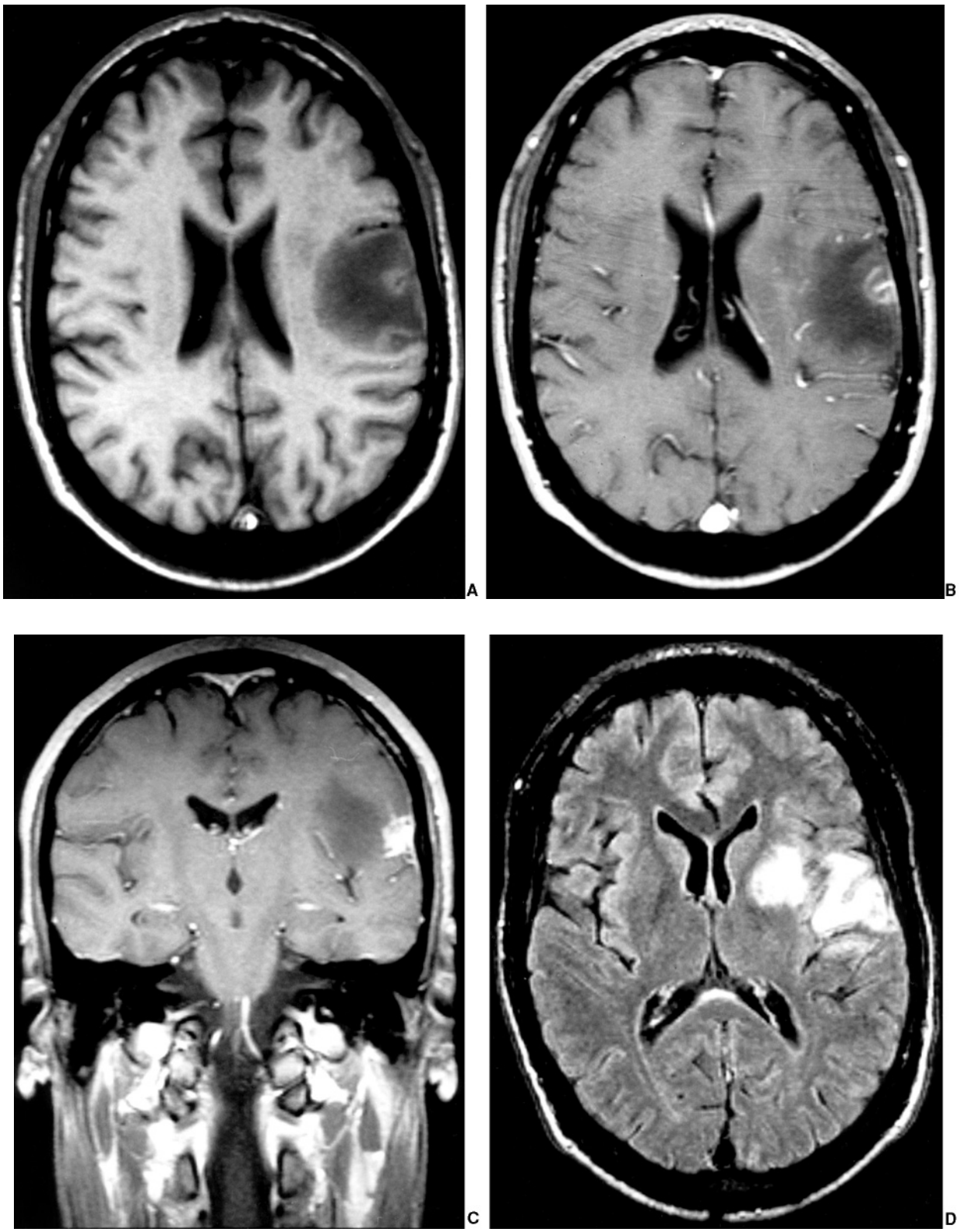


FIG. 18.11. Toxoplasmosis: 32 year old man with seizures and mild post ictal aphasia. Symptoms resolved with treatment with sulfadiazine and pyrimethamine but recurred when folic acid accidentally substituted for folinic acid.

A. Brain T1-weighted MR showing hypointense lesion in central gyrus.
B. T1-weighted MR with gadolinium showing peripheral enhancement of mass.
C. Coronal T1-weighted MR showing enhancing lesion in left inferior frontal region (same lesion).
D. T2 FLAIR image showing increased signal in left opercular and lentiform nuclei. (Same lesions 7 weeks post above treatment error).

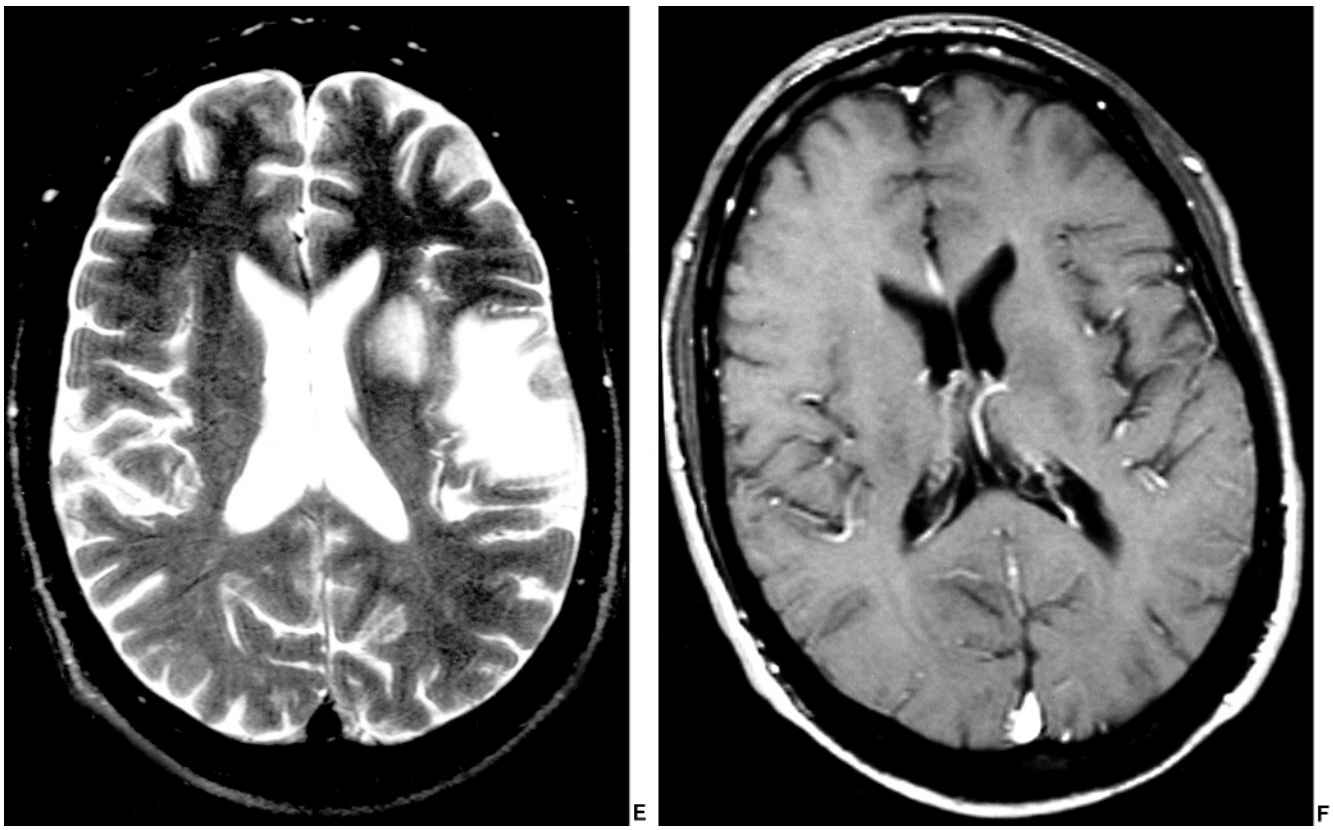


FIG. 18.11. Continued.

E. T2 weighted image showing increased signal in left corona radiata and same lesion in left frontoparietal regions, pre and post central gyrus.
F. T1 with gadolinium showing resolution of above lesions after restoration of folinic acid with antitoxoplasma treatment.

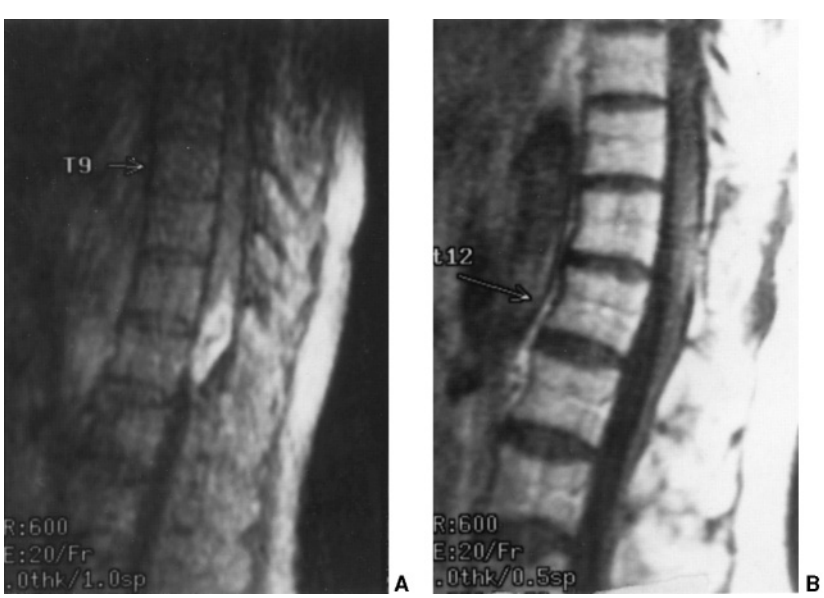


FIG. 18.12. Myelitis from toxoplasmosis:

A. Sagittal T1-weighted MRI with gadolinium of the thoracic spine of a 29 year old man with urinary retention and minimal leg weakness showing enhancing mass at T11.

B. Four weeks of treatment with sulfadiazine and pyrimethamine show resolution of the mass on gadolinium-enhanced MR.

failure. Treatment with praziqauntel works even in patients with HIV, and steroids are often given as well, to control cerebral edema. Alternative drugs include metrifonante and oxamniquine.

Cysticercosis

Although not an opportunistic infection and not more virulent in immunosuppressed hosts, *Taenium solium* infection is extremely common in many areas of the world and can be found on CT or MR of patients with AIDS (396). The most common presentation is seizure activity, with rare cases of focal neurologic deÞcit due to a mass lesion (462). Hydrocephalus occurs when there is blockage of CSF ßow due to intraventricular infection with the racemose form of the parasite, or incomplete absorption of CSF due to meningeal scarring. CalciÞcation on imaging means the parasite has been dead for some time. Eosinophilic meningitis may be found in immunocompetent patients.

Bacterial Infection

Although they are not considered opportunistic infections, bacteria must be kept in mind in the setting of HIV infection, as uncommon pathogens can be responsible for symptoms. This is especially true of children who have had limited exposure to opportunistic pathogens, of patients who have used medications that suppress the bone marrow, leading to neutropenia, and of injection drug users who suffer from skin ulcers or who improperly clean or share needles, resulting in injection of microorganisms (463). In addition to strokes and abscess formation from infected emboli, patients with bacterial endocarditis are at risk for mycotic aneurysms and subarachnoid hemorrhage (464). Nosocomial bacterial infections, including postoperative complications, are also seen more often in patients with AIDS.

In early series (pre-HAART), bacterial infections caused neurologic complications in 2% of patients with AIDS in Miami and 2Ð4% of those in New York, most of whom were intravenous drug users (Fig. 18.13). These bacterial infections can appear at any CD4 count; without a highly increased incidence in the HIV-infected population (465). Campylobacter infection may trigger some cases of Guillian-BarrŽ (466).

Bacterial infection of the brain or meninges tends to be due to encapsulated organisms such as *Streptococcus pneumoniae*. Because some bacteria, such as nocardia and listeria, are suppressed by prophylactic regimens for pneumocystis, the incidence of infection is extremely low (465). *Nocardia asteroides*, an aerobic gram-positive, weakly acid-fast bacillus, has rarely been reported to cause brain abscess (467,468); *Listeria monocytogenes,* an intracellular gram-positive bacterium, has caused abscess and meningitis (469Ð472). *Salmonella* (468), *Staphylococcus epidermidis* (473), *S. mitis* (474) and *S. pneumoniae* (475,476), have all caused cerebral abscesses (Fig. 18.14). *Streptococcal* and *Staphylococcal* organisms have also been cultured from spinal epidural abscesses

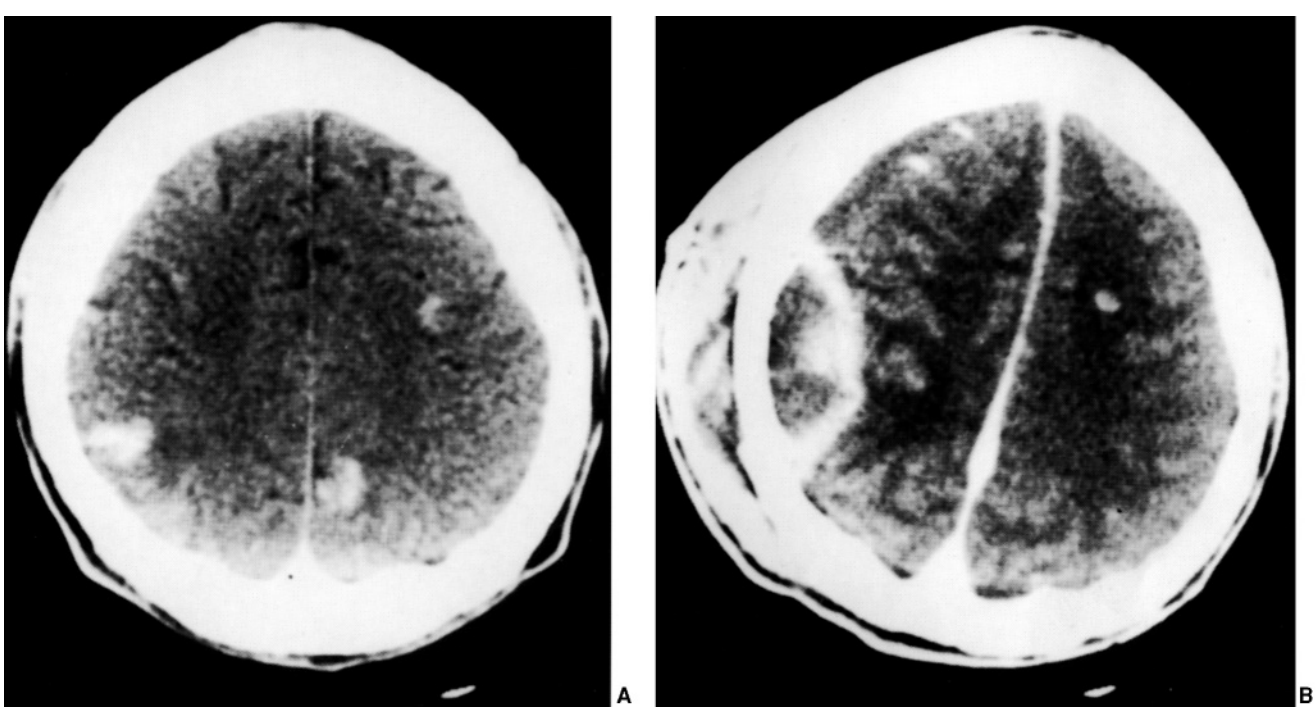


FIG. 18.13. Bacterial infection (brain abscess): CT with contrast showing enhancing lesion near craniotomy defect after a biopsy for suspected toxoplasmosis.

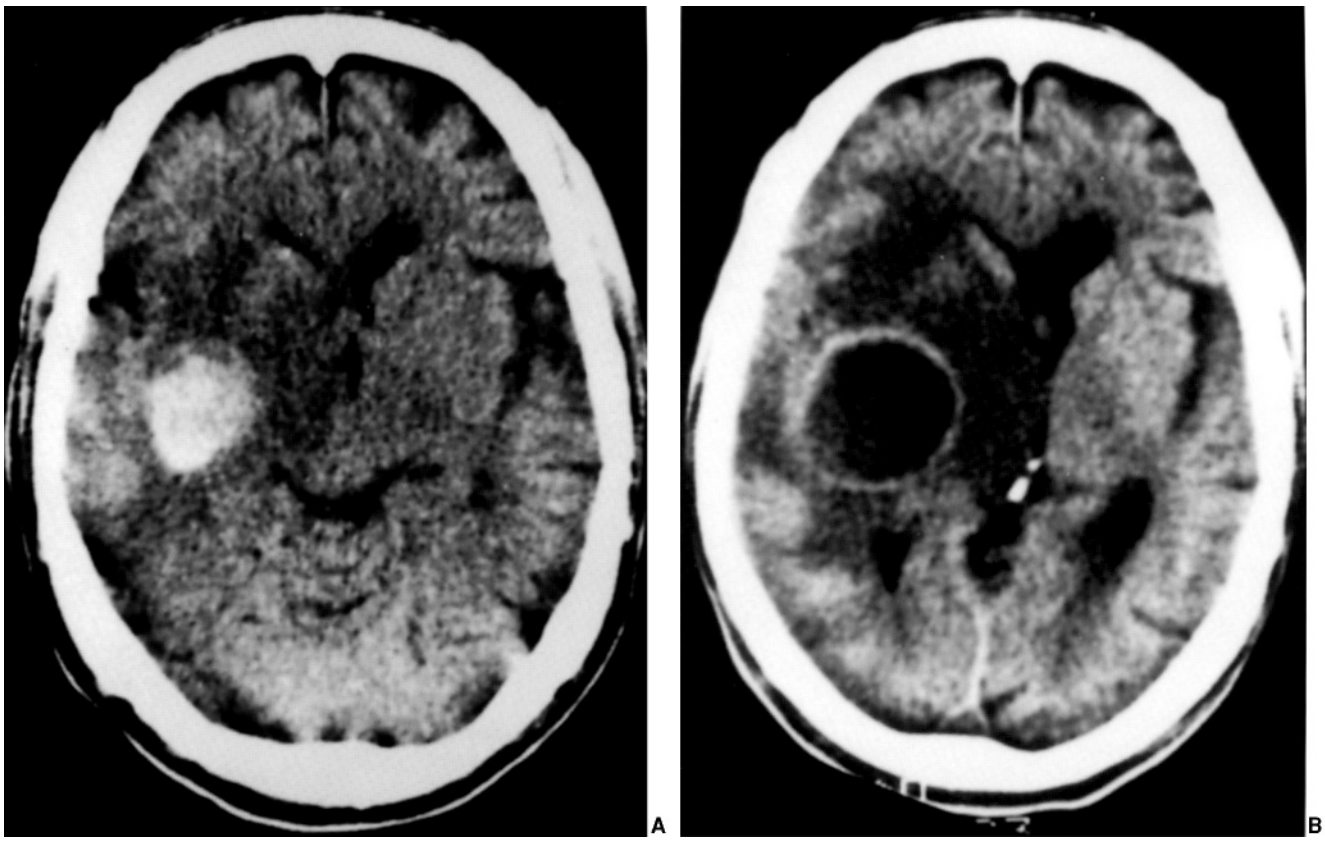


FIG. 18.14. Hemorrhage with ensuing brain abscess:

A. Noncontrast brain CT of a 40 year old hypertensive man presenting with acute left hemiplegia while septic from Streptococcus, showing hyperdense right basal ganglia mass consistent with hematoma.
B. Contrast enhanced CT of patient six weeks later when he presented with obtundation, left hemiplegia and fever. Enhancing cystic appearing mass with marked edema and mass effect appears in region of prior hematoma.

(170,477). *Escherichis coli* (478), *S. pneumoniae* and *H. inßuenzae* have produced meningitis (479). Lesions in the dural space may extend from bone involvement due to *Rochalimaea* species, which is responsible for bacillary angiomatosis and cat scratch fever (480); antibodies to this bacterium have been found in the CSF of patients with meningitis (481). Whipple's disease due to *Tropheyma whippelii*, presenting with diarrhea and a brainstem syndrome of jaw clenching, rhythmic palatal and facial myoclonus followed by supranuclear eye palsy, has been described in AIDS (112), but treatment with the appropriate antibiotics (chloramphenicol, trimethoprim-sulfamethoxazole) did not reverse the brainstem dysfunction. Many of the above infections were only diagnosed at autopsy.

In some cases, despite the inability to identify a speciÞc pathogen, features of the clinical course allow presumptive treatment of bacterial infection without culture (482). Ideally, treatment should be culture speciÞc, after drainage or resection of mass lesions and CSF analysis of meningitis.

Although not within the CNS, sinusitis is quite common and causes headache in patients with AIDS (483,484).

Spinal cord involvement generally spreads from disc space or bone infection, leading to compression of the thecal sac and underlying cord. Symptoms of epidural abscess (Fig. 18.15) include localized pain that is worse on standing, radicular pain in the area of the abscess, weakness and spasticity below the lesion, urinary frequency and urgency or a ßaccid bladder if the lumbosacral region is involved. Progression of symptoms may be acute or subacute, over a few weeks. Fever and systemic signs may be present. Tenderness and increased warmth may be present on exam, along with a sensory level to pin and temperature, upper motor neuron signs such as proximal leg weakness, Babinski signs, hyperreßexia and increased tone, or lower motor neuron signs if nerve roots are involved. Intravenous drug use, recent surgery or trauma are risks for epidural abscess in the immunocompromised AIDS patient just as they are in the general population (477). Plain radiographs may show vertebral body infection or a collapsed disc space; computed tomography with myelography or MRI with gadopentate enhancement are much more sensitive. Distortion of the spinal cord from an enhancing mass can extend for multiple levels, and is occasionally accompanied by increased signal within the

cord as well. The abscess is iso- or hypo-intense on T1 weighted image and hyperintense on T2 (485). Gallium scan can show paravertebral extension. Intramedullary enhancement is due to infection within the spinal cord itself (383).

Treatment must be determined by culture and sensitivity of infected material, which can be obtained by needle biopsy under CT guidance, or if clinically indicated, at the time of surgical decompression of the abscess. Blood cultures may be helpful. Due to the adhesions formed by these infections, decompression is often required to obtain full improvement. Duration of antibiotic use is usually six weeks, as in nonimmunosuppressed patients.

Meningitis presents with encephalopathy, the severity of which correlates with mortality. Headache, photophobia, nuchal rigidity and altered mental state all occur. The incidence of meningitis due to bacteria is not increased in HIV infection, except in children with AIDS, and is not related to T cell count (486). CSF has pleocytosis with predominance of polymorphonuclear leukocytes, hypoglycorrachia (CSF glucose $< 2/3$ serum) and an elevated protein level. Gram stain gives clues to the nature of infection, i.e. gram positive (Staphylococcus, Streptococcus, Listeria) or gram negative (Neisseria, Pseudomonas, Enterobacteriaceae) or amoebic (Naegleria, Acanthamoeba). Antibody testing is helpful for Borrelia, Treponema, and Leptospira. Bacterial antigen testing can be used if the patient has been on antibiotics at the time the culture was obtained. Adjustment in broad-spectrum therapy should be made after the results of cultures become available (usually after two to three days) (487). The exact cause of brain damage due to meningitis is unknown, but markers of excess oxidation and reactive nitrogen and oxygen correlate with outcome (488) (Fig. 18.16).

Treponemal Infection

Syphilis has long been associated with HIV infection, as chancres may facilitate acquisition of HIV and theoretically meningitis in the secondary stage of syphilis may facilitate HIV entry into the brain. In the 1980s and early 1990s the incidence of primary and secondary syphilis increased but is now stable. Controversy exists as to the reliability of serum and CSF tests in the diagnosis of neurosyphilis (490,491), and to the most effective treatment of syphilis and neurosyphilis (491–494). In the general population, syphilis progresses to neurosyphilis in less than 10% of untreated patients, an incidence that is not increased in the presence of HIV infection. For example, even in areas with high background rates of syphilis such

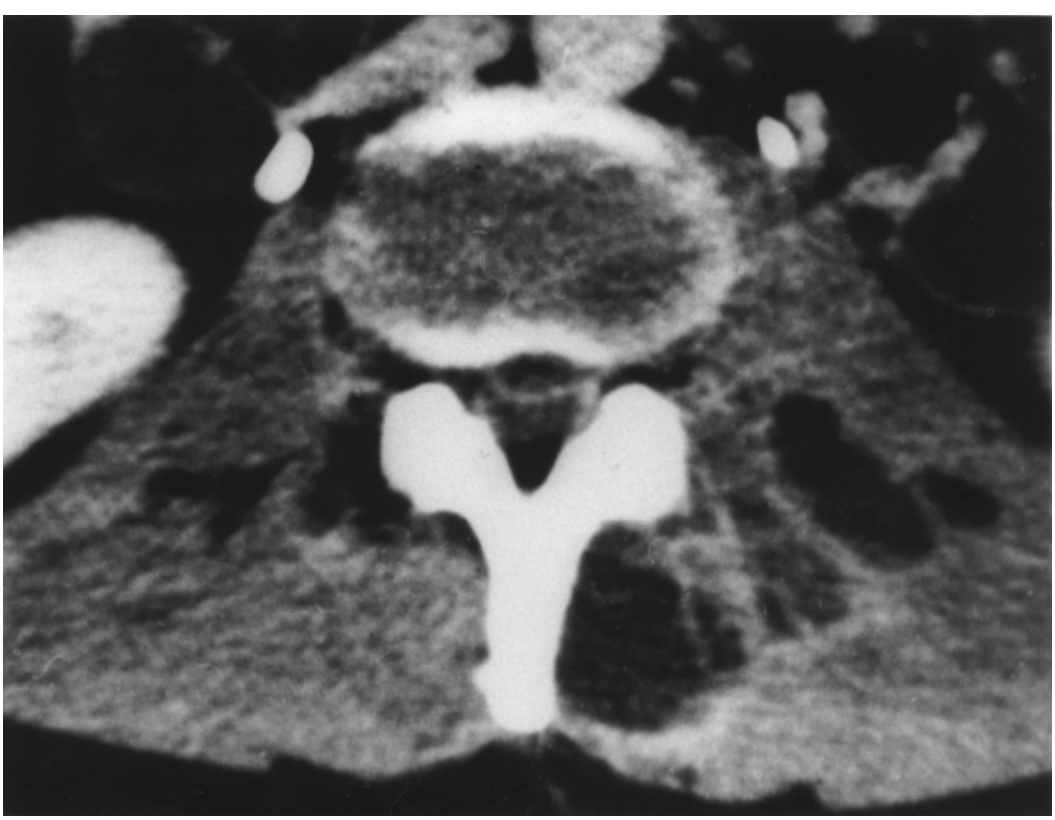


FIG. 18.15. Spinal cord epidural abscess: CT with contrast of 28 year old man with urinary hesitancy, marked back pain and mild proximal leg weakness with increased tone. Blood cultures grew *S. aureus*. Scan shows enhancing rim of dura projecting from disc space into spinal canal, and hypodense lesion with enhancing membranes in the right psoas muscle which is where abscess began.

as Miami, only 1.8% of 166 asymptomatic and 3.3% of 63 symptomatic patients in Berger's series were diagnosed with neurosyphilis based on a positive CSF FTA and VDRL (492). However, the rate of progression may be much faster, with some cases of dementia occurring within one year of onset of primary syphilis (491,495).

Symptoms of secondary or meningovascular syphilis include stroke, headache and meningeal signs. Parenchymal involvement by *T. pallidum* causes tabes dorsalis (loss of posterior column function, such as vibratory and position sense, leading to abnormal gait, lancinating pain down the back and legs, loss of bladder and bowel control and positive Romberg) and general paresis (dementia with ßorid behavior due to frontal lobe dysfunction, Argyll Robertson pupils and tremor). Gummas are very rare and act as mass lesions in the spinal cord or brain (496). CSF can have a mild mononuclear pleocytosis, mild protein elevation, increased IgG index and positive oligoclonal bands, and a reactive FTA or VDRL, although false negatives have been reported (490). Serum RPR can be negative, so a low threshold for performance of lumbar puncture is required (491,497).

High dose penicillin generally successfully treats secondary syphilis, but follow-up CSF analysis is recommended in all patients until the cell count becomes normal (498,490). Because ceftriaxone was associated with a 23% failure rate in HIV-infected patients (499), it is not recommended. Desensitization is advised for penicillin allergic patients. Maintenance therapy is not generally given, but retreatment should be given in patients whose CSF cell count fails to fall at six months, whose CSF VDRL goes up or does not decline two-fold at six months, or whose serum VDRL does not decline eight-fold at six months or rises four-fold within twelve months of treatment (500).

Neuroborreliosis

Lyme disease due to *B. burgdorferi* has been reported rarely in patients with HIV infection (501); serology is a reliable means of diagnosing extracutaneous disease. Because many people living in endemic areas are seropositive by ELISA testing, supplemental Western blot testing is necessary. The most common neurologic signs are facial palsy, meningitis, and radiculitis. Treatment is determined by the disease manifestation but most neurologic manifestations are treated with a two to four week course of ceftriaxone (502).

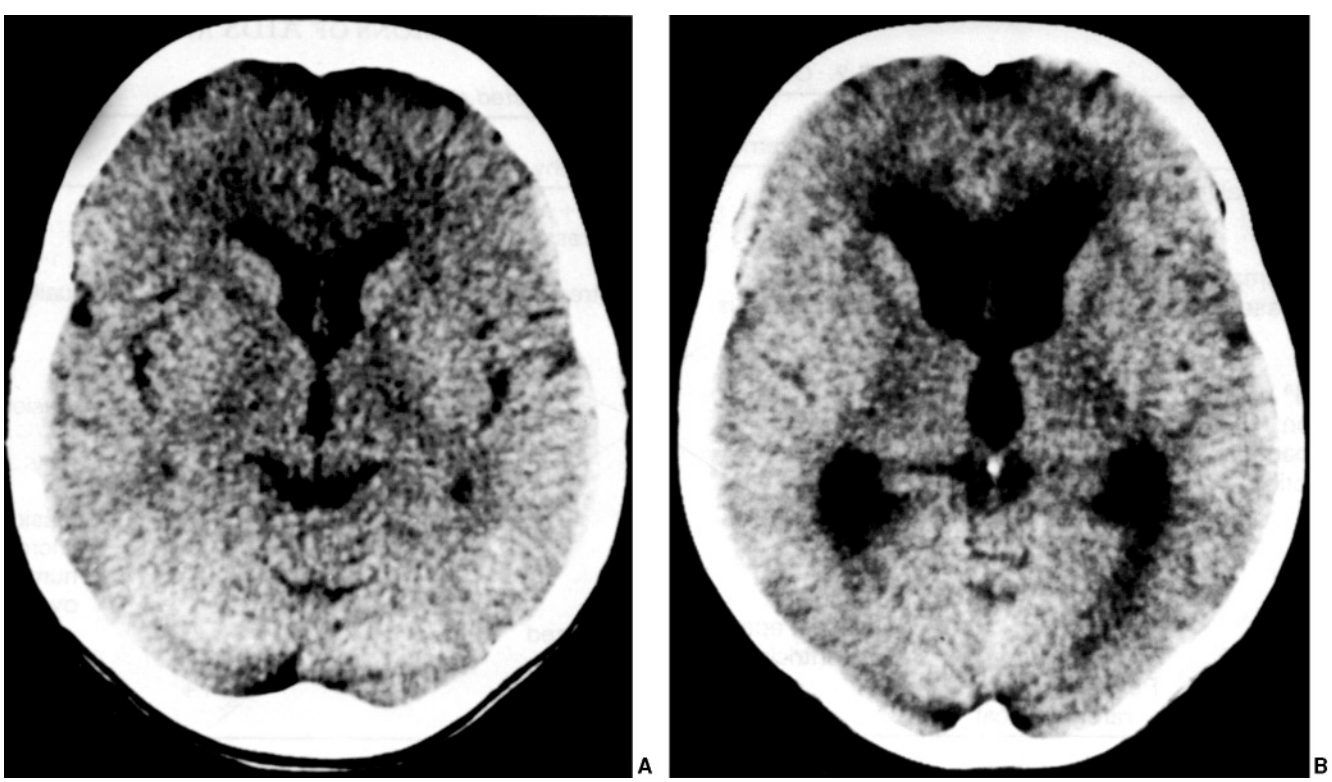


FIG. 18.16. Meningitis sequala:

A. Non contrast brain CT in a 30 year old woman with chronic otitis media due to *H. inßuenzae* showing mild atrophy and dilated sulci.
B. Same patient one month later, obtunded with increasing headache, shows communicating hydrocephalus and decreased sulci consistent with increased intracranial pressure.

Tuberculosis

Tuberculosis is still a common occurrence, especially in people immigrating to the United States (503), and occurs at increased frequency in HIV-infected patients (up to 35% overall and 6% in the CNS) (504,505). Treatment in patients with HIV infection is effective, especially if started early and drug interactions with certain antivirals are kept in mind (503,506,507). Although there are few clinical differences in TB meningitis between patients with and without HIV infection, mass lesions are somewhat more common in HIV infection, especially in intravenous drug users (508,509). Diagnosis of TB in immunosuppressed patients depends on availability of material (i.e. sputum, CSF or biopsy), for smear, culture or nucleic acid ampliÞcation techniques (510,511). Early biopsy is encouraged in the management of mass lesions, especially in patients from countries such as Haiti or India with high incidences of tuberculosis in general. CNS tuberculoma is much more common than lesions due to other members of the mycobacterium family, and can occur at any level of immunosuppression. Systemic infection due to *Mycobacterium avium* complex (MAC) does occasionally spread to the meninges or brain (478,572). Since atypical mycobacterial infection requires treatment with different agents than tuberculosis, awareness must be maintained and appropriate testing done in severely immunocompromised patients (513).

The clinical presentation of tuberculous mass lesions of the CNS shares some features with the previously described focal lesions from other opportunistic infections or tumors, such as seizures, headache and progressive neurologic dysfunction related to the region of the mass, along with fever, malaise and papilledema. What sets it apart is the accompanying presence of basilar meningitis in 10% of cases (509), especially in the basilar cisterns. This is seen in many more patients with tuberculosis than lymphoma or TE. This meningitis is particularly adhesive, leading to complications of hydrocephalous and infarction (514). Increased intracranial pressure can cause mental status changes, cranial nerve dysfunction such as abducens paresis or poor upgaze (515) and vomiting, although these are slightly less prevalent in HIV-infected patients (509).

Radiographic techniques (Figs. 18.17, 18.18A, 18.18B) useful in diagnosis of tuberculosis of the brain include MRI with contrast enhancement (514,516). Lesions are multiple and tend to be periventricular or in the corticomedullary junction. Tuberculomas are isointense on T_1 images with a hyperintense rim, and noncaseating lesions are bright on T_2, with nodular enhancement. Caseating lesions exhibit rim enhancement, and all have mass effect or edema to some degree. Tuberculous abscess results from liquiÞcation of the core of a granuloma or tuberculoma, therefore appearing larger with more mass effect or multiloculation. Computed tomography is also helpful, especially as hydrocephalous and infarction are easily detected using CT, and healed lesions may calcify (509). One study showed that SPECT is more often abnormal than CT in patients with tuberculous meningitis, but the cortical, midbrain and basal ganglia hypoperfusion that is seen does not correlate with stage of meningitis or outcome (517).

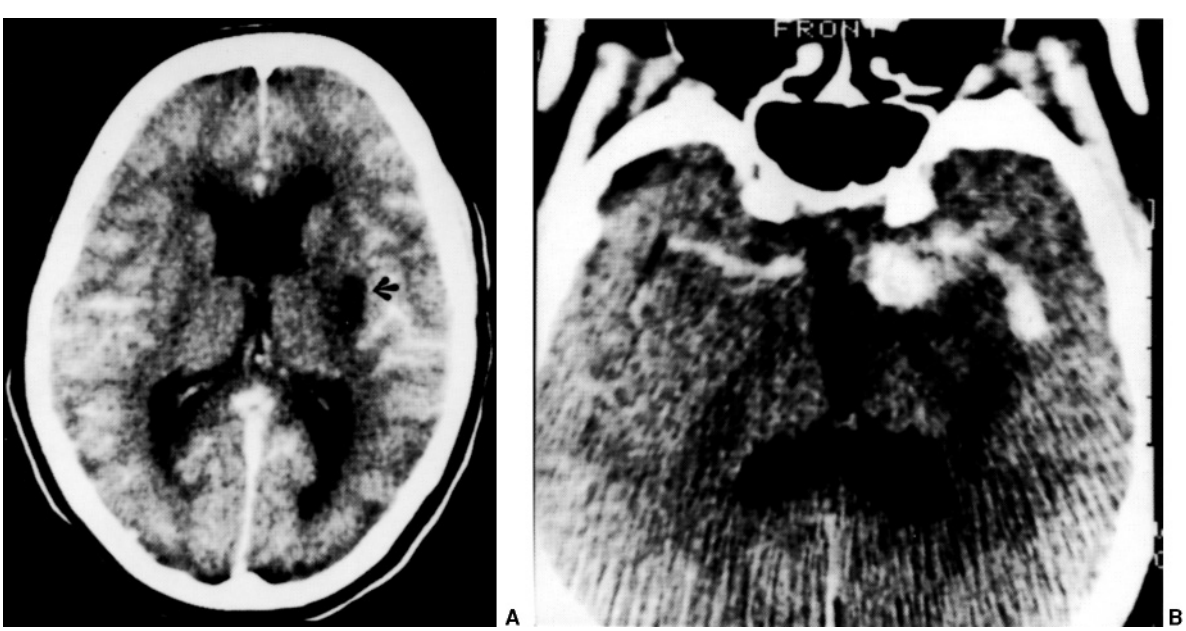
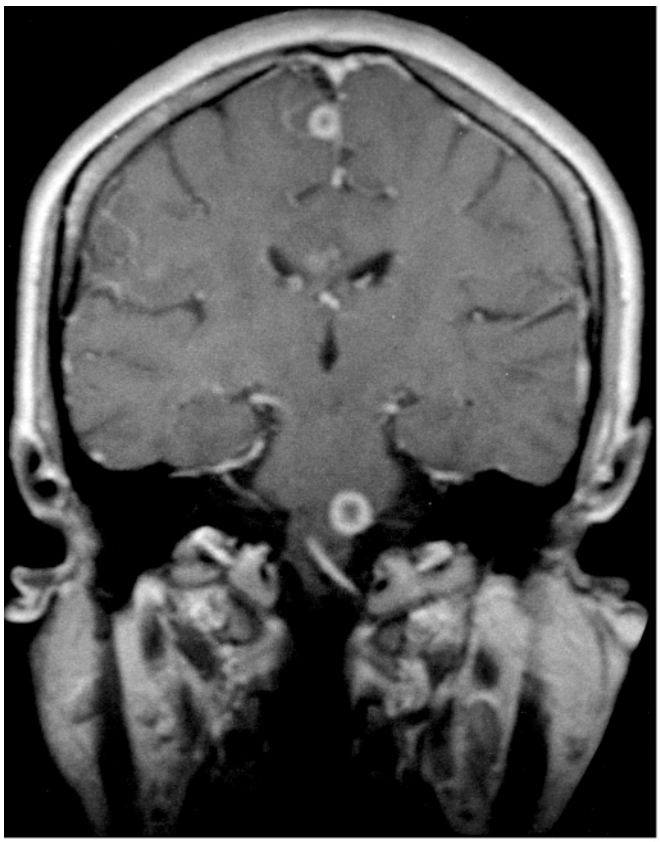


FIG. 18.17. Tuberculosis meningitis:

A. CT showing mild ventricular dilatation in a 22 year old with meningeal signs and confusion. CSF revealed low glucose, high protein, lymphocytosis and grew *M. tuberculum*.
B. CT with contrast of same patient showing meningeal enhancement.



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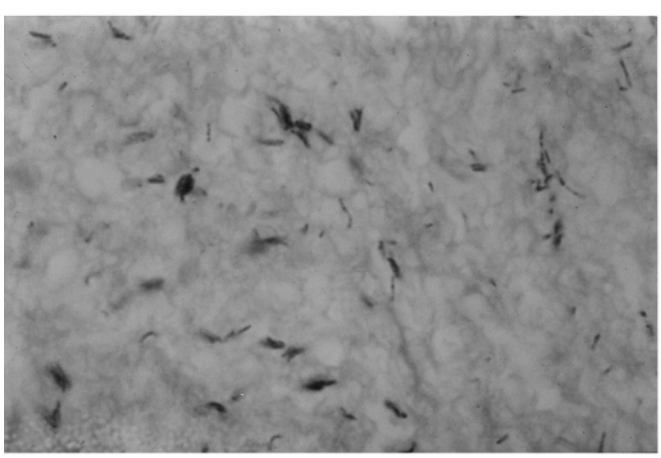

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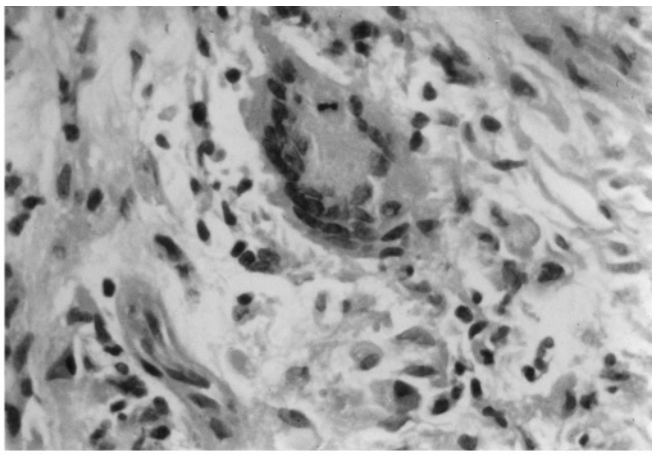

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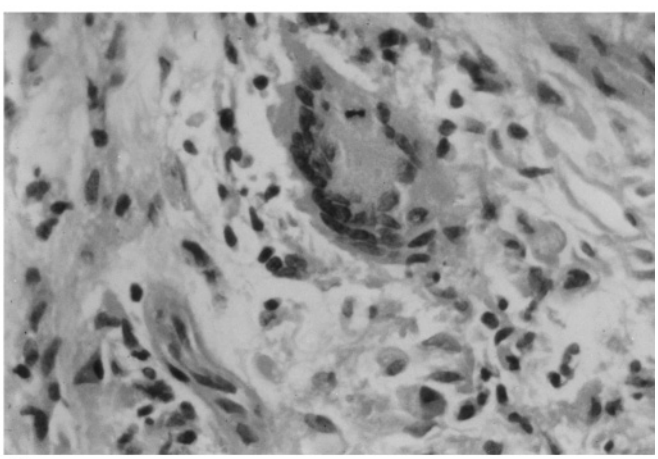


D

FIG. 18.18. Abscess from *Mycobacterium avium* and *tuberculum*:

A. MRI with contrast of a 35 year old Mexican immigrant with acute onset of left sixth nerve palsy, followed by prolonged course of other de cits. Second biopsy revealed both *M. avium intracellulare* and *M. tuberculum*.
B. Organisms staining with Acid Fast (1000 ×).
C. Necrosis in wall of abscess (Acid Fast stain, 1000 ×).
D. Multinucleated giant cells, histiocytes and abscess wall. (H&E 325 ×).

Biopsy may be required to make a deÞnitive diagnosis, and simultaneous drainage with interruption of the thick capsule wall facilitates tissue penetration of antimycobacterial drugs (518).

Treatment of tuberculosis in extrapulmonary locations is not hindered by difÞculty with tissue penetration, especially as the blood-brain-barrier is disrupted by meningitis or but is generally prolonged beyond the standard six months for most other types of tuberculosis. Although a rifamycin-based regimen is recommended, interactions with antiretroviral agents must be watched for, especially protease inhibitors (see Chapter 00) (519).

Antimycobacterial therapy should continue for six months after cultures are negative (although if cultures were originally obtained from a brain biopsy, serial scans can be substituted). Steroids are useful to control the expansion of tuberculomas, to minimize the inßammatory meningeal response and possibly to alleviate increased intracranial pressure. Steroid use is not dangerous if given for a limited time (486).

In patients with less than 50 CD4 cells, especially those not on prophylactic therapy (520), atypical mycobacteria are also rarely pathogenic, with MAC usually causing meningitis or meningoencephalitis. *M. kansasii* has been cultured in a brain abscess (521). MAC and *M. tuberculosis* may occur (522).

Spinal cord involvement is seen in patients with disseminated TB or meningitis, or extending from vertebral body osteomyelitis (Pott's disease) (Fig. 18.19). Symptoms and signs are identical to those of spinal cord compression or myelitis described earlier in bacterial infection, but more chronic and likely to reverse with treatment, even after prolonged disability. Intramedullary involvement is rare (523,524). MRI becomes abnormal at the same time that bone and gallium scans reveal enhancement of the vertebral body, but is better at demonstrating spinal cord compression (396). Myelography is substituted in patients ineligible for MRI due to metal, pacemaker, claustrophobia or inability to hold still, or where MRI is not available (396). Treatment with

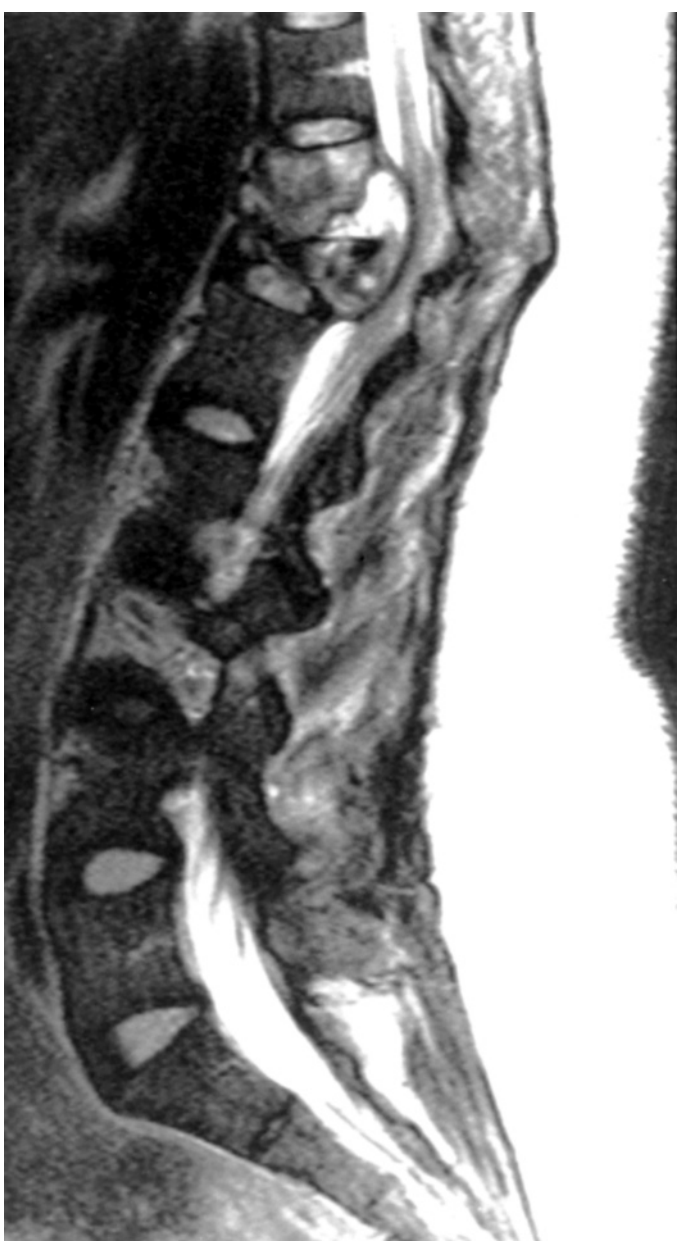


FIG. 18.19. Osteomyelitis and cord compression due to *M. tuberculum* (Pott's disease): Spine MRI with T2 weighting in a 40 year old woman with severe low back pain and urinary retention, progressing over several months. Lesion in body of T12 causing deformity of spinal column and cord compression. Other lesions present at L3 and 4, with involvement of disc space. Cultures taken at time of surgery to stabilize spine grew *M. tuberculum*.

prolonged antibiotics is required, along with spine stabilization using rods or titanium plates or cages in patients whose bone destruction renders them unstable.

Meningitis from mycobacterial infection is chronic but can become severe if unrecognized. Atypical infections are generally milder than tuberculous infection. In addition to headache, stiff neck, confusion, malaise and fever, patients can exhibit cranial nerve palsies or hemiparesis or may become comatose. Cerebrospinal ßuid is usually under increased pressure, with lymphocytic pleocytosis averaging more than 200 cells/mm^3. Neutrophils have been reported in 42% of HIV-infected patients and 27% of others (509). The average protein level is 125mg/dl in HIV-infected patients, and higher levels correlate with later stage disease. In 70% of cases the glucose level is low (525). Diagnosis of tuberculous meningitis is made by positive acid fast stain in 25% of cases (509). Cultures are positive in 60% of immunocompetent patient samples and 23% of HIV-infected patients but may take six weeks to evolve. ADA (adenosine deaminase) levels are high but non-speciÞc (509). PCR on CSF may be a more useful and rapid diagnostic test but is not 100% sensitive (526).

Treatment of meningitis is the same with or without HIV infection (See Chapter 00), but rifabutin is sometimes substituted to take advantage of its ability to treat atypical as well as tuberculous infection (509). Treatment with six months may be as effective as longer courses. Steroids diminish the inßammation and may improve cranial nerve palsies or prevent hydrocephalous. If the latter becomes symptomatic a ventricular drain or shunt may be required.

Fungal Infection

Cryptococcal infection is the most common neurologic fungal infection seen in AIDS. Other fungi also occur in severely immunosuppressed patients, and vary with the geographic exposure of the person. Most are hematogenously spread from a lung infection. Presentation and pathology can be misleading in AIDS, so various laboratory techniques are required, especially culture, which can dictate therapy. For example, a patient whose pathology and imaging suggested aspergillosis but who failed to improve on standard treatment with amphotericin, grew *Scedosporium apiospermum* from a brain abscess after two weeks, and only improved after discovering that the organism was sensitive to posaconazole (527).

Cryptococcal Infection

Cryptococcal infection has occured in up to 10% of patients with AIDS in the U.S. and 30% in Africa (528), primarily causing meningitis. However, the incidence of meningitis in the U.S. has fallen from 5.0/1,000 person-years in 1990Ð1992, to 1.5/1,000 in 1996Ð1998 (266). This decline relates to the introduction of HAART and possibly increased use of ßuconazole in this patient population (529,530). The CD4 count is <50 at presentation in most patients. Most infections are caused by reemergence of latent disease, but it is prudent to avoid exposure to bird droppings or infected soil.

Fungal meningitis symptoms are insidious, with headache, stiff neck and low-grade fever in some patients, alterations of behavior in others. The severity of symptoms, including depressed mental status, correlates with intracranial pressure elevation, as does the outcome. Seizures occur in less than 10% of patients. Meningeal signs may be absent, in which case headache is attributed to increased intracranial pressure. The latter type of headache is worse in the morning, and exacerbated by Valsalva maneuver, cough or other obstructors of venous outßow. If cryptococcal meningitis is the presenting infection of AIDS, institution of antiviral therapy may exacerbate the inßammation of meningitis, and should be delayed for several weeks. Even after initial control of cryptococcal meningitis with antifungal therapy, immune restoration with antiretroviral therapy can cause increased intracranial pressure, in the absence of meningitis. This responds well to serial lumbar punctures (531).

Lumbar puncture often reveals extremely high opening pressure, lymphocytic pleocytosis, mild protein elevation in two-thirds of patients (532) and a normal glucose level in 70% of patients (533). Yeast can appear unusual on India ink staining due to insufÞcient capsule formation. Latex agglutination is positive for cryptococcal antigen in 90% of patients with meningitis (533). Efforts to use nested and other PCR protocols may facilitate rapid diagnosis (534). The organism grows easily in culture. Poor prognostic signs found in CSF include hypoglycorrachia, white cell count $>20/mm^3$, and antigen titer $>1{,}024$ in CSF.

When the patient is very obtunded at presentation or has an exceptionally high (>500 mm/H_2O) opening pressure on lumbar puncture, it is prudent to use an effective amphotericin B preparation rather than oral ßuconazole (535Ð541), as the latter takes longer to work (536) (sterilization is accomplished by 16 days using amphotericin, instead of 30 days with ßuconazole). Although monitoring for cytopenia and treatment with growth factors is often required, the addition of 5-ßucytosine (100 to 150 mg/kg/day) for the Þrst two weeks improves outcome (533). Longer initiation treatment (six instead of four weeks of amphotericin) is recommended in patients with more than 20 white blood cells in the CSF, more than 1:32 cryptococcal antigen titer in serum or 1:8 in CSF, and a positive India ink preparation (537). Lipid preparations of amphotericin are better tolerated, with a lower incidence of chills, fever and renal failure (343,539Ð541). In those unable to tolerate amphotericin in any form, some success has been achieved with high doses of ßuconazole plus ßucytosine for the Þrst four weeks if tolerated (270,542). Response to therapy is usually based on clinical

improvement, but antigen titers in CSF can be used in uncertain cases (543).

The availability of the azole, ßuconazole, has allowed oral therapy. One obvious beneÞt of this is that indwelling venous catheters can be removed.

Attention to treatment for increased intracranial pressure also improves outcome. A CT scan is done to rule out obstructive or communicating hydrocephalous, which can be treated by ventriculostomy or shunt in the Þrst case and serial spinal taps or lumbar drain in the second. If the brain is diffusely swollen, the usual measures to lower pressure can be used, including mannitol or glycerol, steroids or possibly acetazolamide.

Once the CSF is sterile maintenance with ßuconazole, 200 mg/day (550), is recommended until there is a sustained increase in CD4 cell count (see Chapter 00). In an early study of patients followed for six months after treatment, meningitis recurred in four of 27 not receiving maintenance therapy, and none of 34 receiving ßuconazole (552).

Mass lesions, known as torulomas or cryptococcomas (Figs. 18.20A and 18.20B) are unusual and only found in chronic cases of meningitis. They are located near the brain surface or periventricular regions, probably evolving from invasion of the parenchyma from Virchow-Robin spaces of the subarachnoid space or ventricle by microcysts that are Þlled with fungi and mucus (1,468). Treatment requires prolonged antifungal therapy.

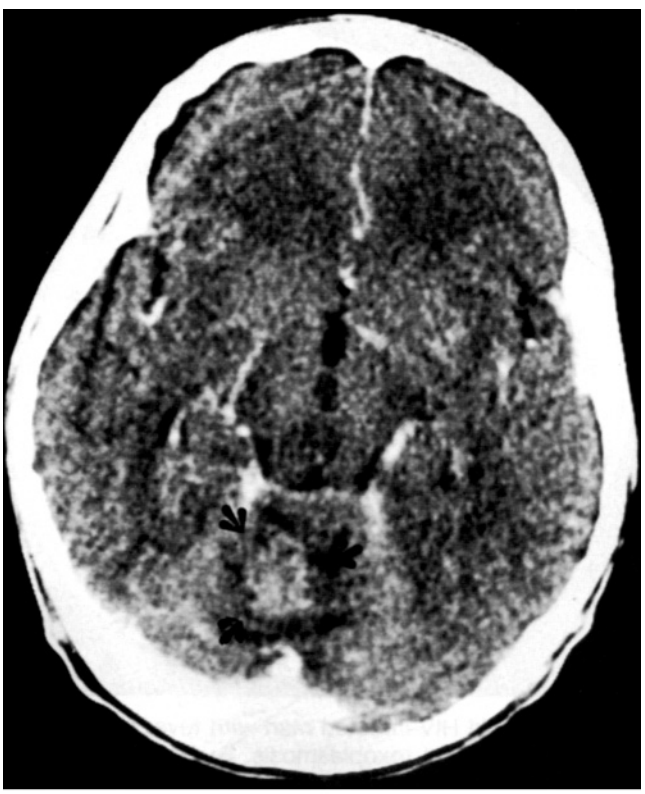
Coccidiomycosis

Opportunistic infection with this organism appears in patients who have lived in the southwest U.S., the Sonora desert of Mexico, or in Central and South America and whose CD4 count falls below 250. Serology with complement Þxation can be falsely negative in patients with very low counts or early in infection. Meningitis and other extrapulmonary infection develops in one of 200 patients whose skin test shows exposure to *C. immitis*, and is caused by miliary spread from the lungs (555). Meningitis had a mortality of 33% in Fish's study (556). Although meningitis in immunosuppressed AIDS patients presents in a similar fashion to immunocompetent hosts, i.e. chronic headache, fever and depression of mental status, in some patients extensive brain destruction due to associated endarteritis obliterans can occur and hydrocephalous may develop in those with ventriculitis. In patients with CD4 <250 living in an endemic area, the risk of developing active infection (pulmonary and meningeal) is 10% per year (557).

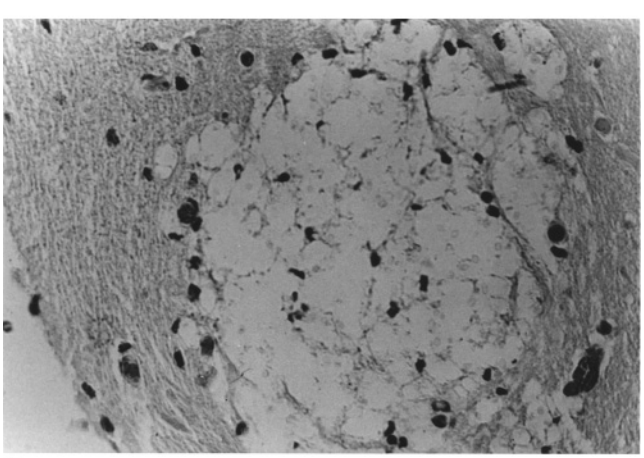
Treatment with amphotericin B infused intrathecally is traditional in meningitis, while ßuconazole at doses of 400Ð600 mg/day or itraconazole at 400Ð1200 mg/day is used in less involved patients (558). The high relapse rate mandates lifelong suppression (555).

Aspergillosis

Hematogenous spread from pulmonary or cardiac sources causes the cerebral lesions, and direct spread from the lungs is responsible for spinal cord involvement. *Aspergillus ßavus* caused cerebral lesions in two patients, and *A. fumigatus* a spinal cord mass, in a series reported by



A



B

FIG. 18.20. Cryptococcoma:

A. Contrast enhanced CT in a 32 year old with chronic cryptococcal meningitis who developed ataxic gait. Round, homogeneously enhancing lesion is seen in cerebellar vermis.
B. Biopsy of lesion shows cryptococcal yeast forms surrounding a blank space containing nonstaining capsular material (H&E 800 $\times$) (courtesy of Dr Tung Pui Poon).

Woods and Goldsmith (559). This fungal infection presents as mass lesions of the brain and spinal cord and as infarction, and is associated more with neutropenia (< 200 cells/mm^3) than with suppressed T cells, but is described in patients with CD4 < 50, or in those with neutropenia due to bone marrow toxicity of treatment, lymphoma or other bone marrow failure. In AIDS patients there are more CNS complications than in other immunosuppressed groups. Patients in the central United States are at highest risk. CT and MR findings include thick walled cavitary lesions, occasionally hemorrhagic. Precise diagnosis usually requires aspiration of tissue for culture.

A variety of treatment modalities exist but voriconazole may be the most effective (560–562). Voriconzale was recently proven effective in a trial of 277 patients, 3.8% of whom had cerebral and 5.6% sinus involvement (562).

Histoplasmosis

In endemic areas of the U.S. and Latin America, histoplasmosis becomes disseminated in up to 20% of immunosuppressed patients (563). CNS involvement occurs in 5–20% of disseminated cases and takes the form of encephalopathy, meningitis (564) and rarely abscess. Hemorrhage may occur due to associated thrombocytopenia or DIC (563). Diagnosis is made by detection of antigen or by culture of large volumes of CSF. Treatment of histoplasmosis with amphotericin has been facilitated by the introduction of lipid preparations of amphotericin B (565); abscess requires surgical debridement.

Blastomycosis, Candida, Rhizopus (Mucor) and Other Fungi

Other fungal infections causing meningitis, sinusitis or fungal CNS mass lesions are rare in AIDS, occurring in no more than 5% of patients with disseminated infection and severe immunosuppression. This is primarily attributed to lack of the main risk factor for certain fungal infections, neutropenia, in AIDS patients as compared with oncology patients. The development of resistant non-albicans candida species in those patients receiving prophylactic treatment with ßuconazole must be kept in mind (544). Special aspects of each infection include: formation of abscess more often than meningitis with blastomycosis; co-infection with candida and bacteria in brain lesions (1–3); and spread from sinus infection, with early cranial nerve dysfunction such as blindness or diplopia, in patients with the rhinocerebral form of mucor (566,567). In intravenous drug users, as well as some patients with AIDS, the cerebral form of mucor tends to involve the basal ganglia (568,569). Surgical debridement of sinus infection is often required, as endoscopic aspirations fail to diagnose the etiology of the fungus in many cases (566).

Viral Infection

Progressive Multifocal Leukoencephalopathy (PML)

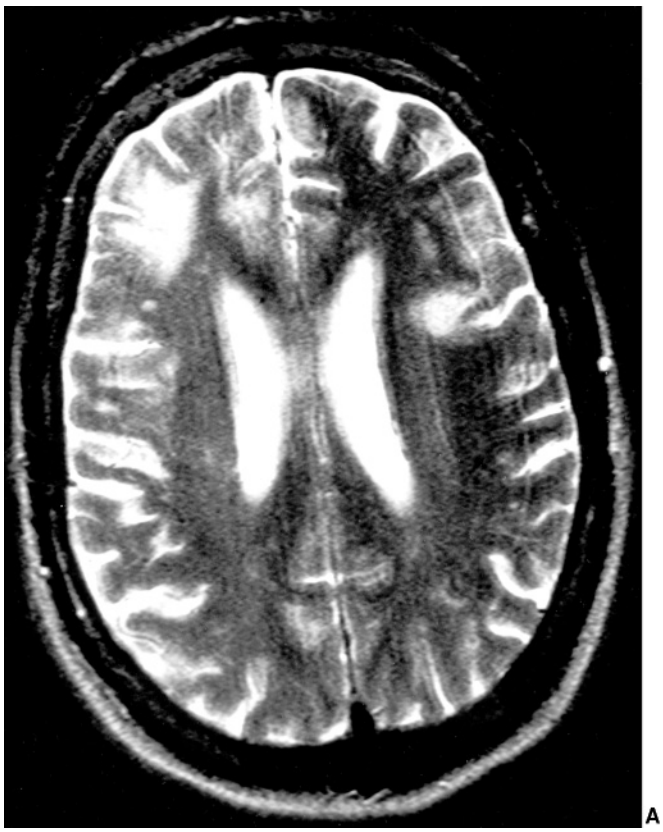
The most common viral opportunistic infection of the brain is JC polyoma virus, causing demyelinating lesions known as PML in 4% of patients with CD4 < 100. The incidence has not dropped in the era of HAART (266) but patients are surviving longer with PML (46 weeks as opposed to 11 weeks median survival) (575). PML is the AIDS defining illness in about half of patients who develop this infection (576).

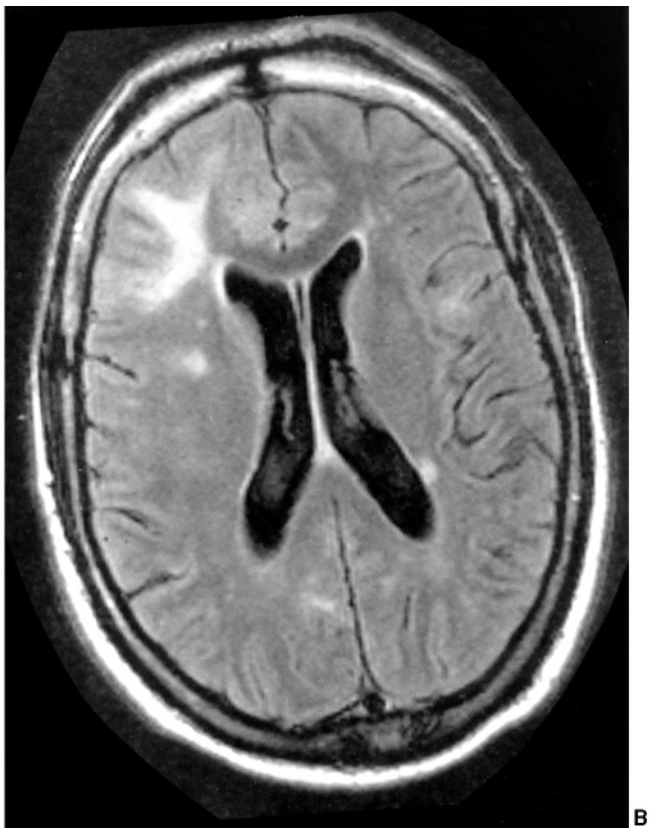
The clinical course of PML is one of insidious progression of focal signs, including hemiparesis, hemisensory loss, visual disturbance or posterior fossa signs such as diplopia, dysmetria, dysarthria, dysphagia and ataxia. Cognitive dysfunction is reported by 36% of patients and headache in 32% (576). Seizures are not seen more often than in HIV infection itself, although two patients with epilepsia partialis continua have been reported (577). A single focus is more common in AIDS patients with PML than other groups, with a predilection for the parietooccipital cortex. Involvement of white matter outside the brain, such as spinal cord or optic nerves, has not been reported in AIDS patients.

PML is suggested by the characteristic CT findings of a nonenhancing hypodense area in white matter. Hyperintense signal is seen in multiple areas on T_2 weighted images on MRI, with a single area found in about in 27% of cases (see Fig. 18.21). These generally cause no mass effect or surrounding edema and do not enhance with gadolinium, but are occasionally hemorrhagic (578). Differentiation from HIV-induced changes in the white matter include location; the demyelination of HIV is generally periventricular, not subcortical as in PML, and the demyelination is less discrete in HIV. CMV-induced white matter changes are distinguishable from PML by their location in the periventricular white matter, and by the presence of subependymal enhancement (576). Varicella virus causes demyelination by small vessel infarction, so diffusion weighted imaging can separate the MRI findings from PML (579).

Other confirmatory tests include JC virus detection in CSF (580). Amplification techniques can provide an estimate of JC viral load which correlates with the size of lesions seen on MRI, and is useful in determining prognosis (581,582). When therapy with HAART is successful in prolonging survival in PML, a lower viral load (< 4.7 log) is found at onset and the virus is cleared with therapy (583). Elevated myelin basic protein, though nonspecific, is much more common in PML than HIV or other demyelinating conditions. Similarly, MR spectroscopy reveals a homogenous pattern that is not specific for PML, but may eventually prove useful in diagnosis without biopsy (584).

The mainstay of therapy for PML is restoration of the immune system, although this transiently may exacerbate





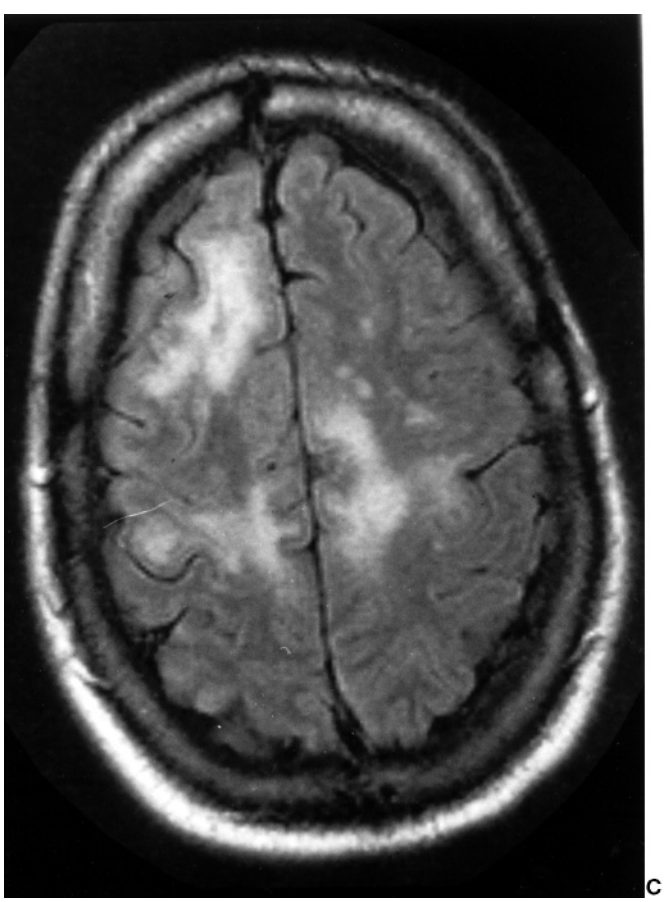


FIG. 18.21. Progressive Multifocal Leukoencephalopathy: 32 year old man with dementia and progressive left leg weakness. CT, which is not shown, revealed nonenhancing edema right frontal region.

A. T2 weighted MR shows increased signal in both frontal lobes, right larger than left.

B. FLAIR shows increased signal in right middle frontal gyrus, right corona radiata and left periventricular region.

C. Higher axial view of FLAIR shows increased signal in bilateral frontal and parietal regions.

signs of PML as inßammation occurs for the Þrst time (585). Much remains to be learned about the mechanism of HAART in PML. As HIV is often found near lesions of PML, there is evidence that the tat protein of HIV also activates JC virus, and therefore its reduction with HAART may slow the growth of JC virus (586). Evidence that improvement results from a nonspeciÞc restoration of the immune system is that agents that fail to cross the blood brain barrier, such as protease inhibitors, are more effective than CNS active antivirals such as zidovudine (587). The addition of the antiviral agent, cidofovir, promises some speciÞc efÞcacy against JC virus (583,588). Earlier trials of antivirals, including cytosine arabinoside, have all been failures (589).

Cytomegalovirus (CMV)

Although initially assumed to be the cause of dementia in AIDS, CMV has been shown to cause dysfunction of the central nervous system in only a minority of patients (590). CMV causes headache, confusion, apathy or cognitive dysfunction and rarely fever (590) and focal deÞcits that often involve the brainstem (591). Electrolyte abnormalities, speciÞcally hypo or hypernatremia, are quite common (592). Periventricular enhancement is most common (591,593), followed by enhancing mass lesions (594) or hypodense lesions similar to PML (590). CSF often has normal or mildly abnormal protein and glucose, with polymorphonuclear pleocytosis in 25% (592,593). CMV can be detected in CSF by PCR in 90% of patients (595), but false positives and negatives are common (592,593).

More commonly nervous sytem involvement of CMV is located in the spinal cord, causing myelitis with quadraplegia and a sensory level, or in the lumbar nerve roots, leading to a characteristic syndrome of polyradiculopathy (596). The latter presents in severely immunosuppressed patients (<50 CD4) with low back pain radiating in the lumbar or sacral nerve roots, followed by ßaccid paralysis of the legs and urinary retention (597). CSF reveals many polymorphonuclear cells (usually $>200/\mu l$) with low glucose (210). Although culture is not often positive (598), CMV can be detected by PCR ampliÞcation (581). MRI with contrast shows thickened nerve roots with areas of enhancement in up to one-third of patients, and should be done to exclude mass lesions (210). Electromyogram shows denervation in the paraspinal and leg muscles with normal nerve conductions (598).

Treatment of CMV infections is outline in Chapter 00 (210,598Ð602). Excellent recovery from polyradiculopathy is seen in patients whose treatment is begun early (597). HAART should be used (603) but not until speciÞc treatment for CMV has been in place for several weeks, in order to avoid the immune reconsitution disorder. The increased use of HAART has markedly reduced the incidence of CMV infection, thereby limiting accrual of patients for trials of agents (57).

Varicella and Other Herpes Viruses

Varicella zoster virus most commonly causes shingles, which is a radiculopathy syndrome with a vesicular rash in a dermatomal distribution. Shingles most frequently involves the thoracic or upper lumbar dermatomes, or the Þrst division of the trigeminal nerve. Shingles is seen in 2Ð4% of patients with HIV infection whose CD4 count falls below 200 (604), and is 17 times more common in an HIV-infected than in a non-infected homosexual population (604). Shingles presents with paresthesias (interpreted as itching), painful dysesthesias and vesicular, crusting rash in one or more neighboring dermatome distributions (Fig. 18.22) in patients with a CD4 <200, although it has been reported in HIV-infected patients with high CD4 counts as well.

Antiviral treatment limits spread from the dorsal root ganglion to the spinal cord or brain (579,605), may prevent vascular complications and decreases infectivity by clearing the virus from the vesicles (see Chapter 00) (606,607).

Adjunctive corticosteroids may be helpful in minimizing, if not preventing, postherpetic neuralgia (579). The latter chronic painful condition may be treated symptomatically with antiepileptic, antidepressant and other medications, which were discussed earlier in the section on peripheral neuropathy.

Varicella zoster virus also causes encephalitis, myelitis and stroke (especially following trigeminal neuronitis, due to granulomatous small vessel vasculopathy (579,608). Encephalitis presents with fever, ßuctuating levels of consciousness, seizures and headaches (609) but is rare, being seen in only four of 859 patients with herpes zoster radiculopathy (57).

Myelitis is seen in advanced HIV infection (610) in 1% of patients and follows the rash of radiculitis by one or two weeks. Usual symptoms such as weakness, spasticity and sensory level, relate to the level of spinal cord involved, and more rarely myoclonus is described (611). MRI may show enhancement over several levels or focally, CSF may reveal varicella DNA or antibodies (579). Overall, varicella virus is detected in 2.5Ð7% of HIV-infected patients with shingles and encephalitis, meningitis, myelitis or retinitis (612).

Herpes Simplex

Herpes simplex 2 has also caused myelitis, which is similar in presentation and treatment to zoster and CMV myelitis (174,175,613). Both *H. simplex* types 1 and 2 have caused encephalitis, usually with coinfection by CMV (614,615). A more insidious course of encephalitis in AIDS patients is not always seen (614,615), as

encephalitis can occur in patients at any level of immunosuppression. If the patient is capable of mounting an inßammatory reaction, herpes encephalitis will present typically with fever, headache, behavioral change, recurrent seizures and rapid obtundation or coma. White and red cells are present in CSF, paroxysmal lateralizing epileptiform discharges may be seen on EEG, and hemorrhagic edema is found in the temporal and frontal lobes on MRI or CT (616). CSF PCR facilitates diagnosis.

Herpes virus 6 is responsible for some cases of encephalitis (617) and myelitis (618) as well. Epstein Barr virus, though responsible for lymphoma, does not cause the ataxia, aseptic meningitis and myalgic syndromes in immunocompromised patients that it does in normal hosts.

HTLV-I or II (Human T-cell Leukemia Virus)

This chronic infection can be acquired by the same mechanisms as HIV, so coinfection with both is not unexpected (620), especially in endemic areas. Myelopathy is the most common syndrome related to HTLV-I and may be found in 73% of patients with HIV who also have HTLV-I (621). Symptoms include slowly progressive leg weakness, spasticity, urinary urgency and frequency, constipation and occasionally a concommitant neuropathy causing depressed ankle jerks and distal sensory loss. On pathologic examination the spinal cord is atrophied with loss of axons and myelin. In those whose immune function was at least partially preserved, a perivascular lymphocytic inÞltrate (622) consisting of CD4 and 8 cells surrounding HTLV-I DNA and RNA is present, with viral particles in the spinal cord as well (623).

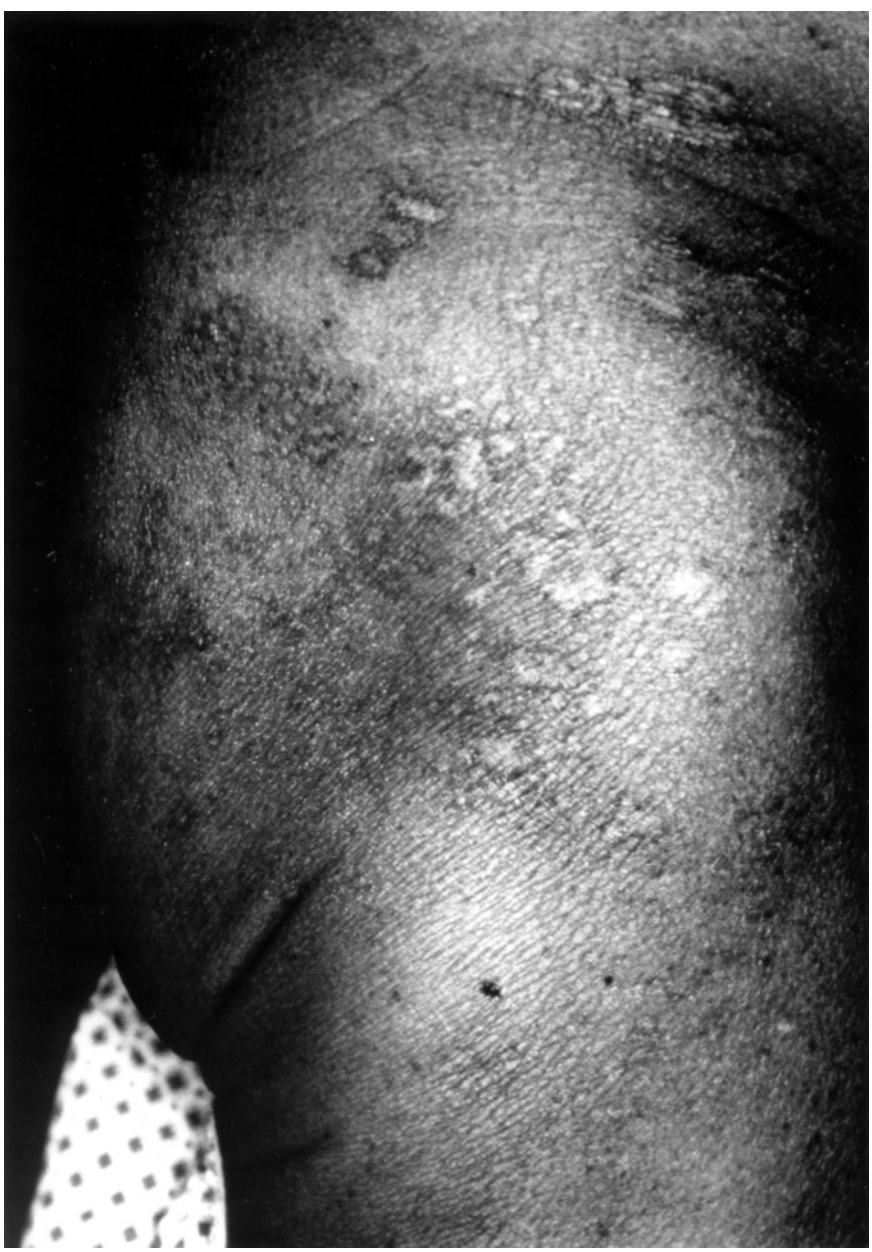


FIG. 18.22. Rash of herpes zoster: 70 year old man with right ank pain and urinary retention, with resolving rash typical of H. zoster.

Treatment is with HAART, but corticosteroids have been tried alone or in combination with HAART, especially as there is a risk of increasing inßammation in response to HAART (624). Plasmapheresis has also been used (625) but is more difÞcult to administer.

Cerebrovascular Disease

Cerebrovascular disease is discovered on autopsy in up to 34% of patients (3,26), but is often unsuspected as a cause of transient neurologic deÞcit, possibly due to the relative youth of AIDS patients. Clinical series report stroke in 1.6% of adults (626,627) and 6% of children (626). Although stroke can occur due to risk factors unrelated to HIV infection, it should be remembered that many opportunistic infections cause arteritis or thrombosis in vessels nearby. These include tuberculosis (514), varicella (especially when the trigeminal nerve is involved) (609,579,605), aspergillosis (559), cytomegalovirus (628,629), syphilis (492,495), candida, lymphoma and of course HIV itself (173,342,627,630,631). Immune complexes or cryoglobulinemia can result from hepatitis B or C infection (173). In addition, thrombosis can occur due to dehydration protein S deÞciency (632), sepsis or other stimulators of inßammatory cytokines that increase thrombosis (633). Emboli can cause stroke in patients with endocarditis (342). Anticardiolipin antibodies can be associated with thrombosis, although these are frequently Ònnocent bystandersÓnot associated with vascular events (634). New risk factors include the hyperlipidemic changes from the protease inhibitors potentially causing accelerated atherosclerosis (323).

Hemorrhage can occur in the brain as a result of a bleeding diathesis such as disseminated intravascular coogulation and thrombocytopenia, heparin used for hemodialysis, and coagulopathies from liver failure or malabsorption of vitamin K, or drug toxicities. It presents as abruptly occurring focal deÞcits, or in the case of subarachnoid or intraventricular hemorrhage, as headache, stiff neck and depressed consciousness. Headache and seizures are not uncommon accompaniments of hemorrhage. When hemorrhage occurs in a preexisting lesion there may have been a history of dysfunction in the same region, with abrupt deterioration. Blood appears hyperdense on CT and has a progressively changing appearance on MR, initially being hypointense on T2 and hyperintense on T1. If a lesion such as lymphoma (Fig. 18.23) or brain metastasis is the cause of the hemorrhage, the early scan will show mass effect or surrounding edema several hours

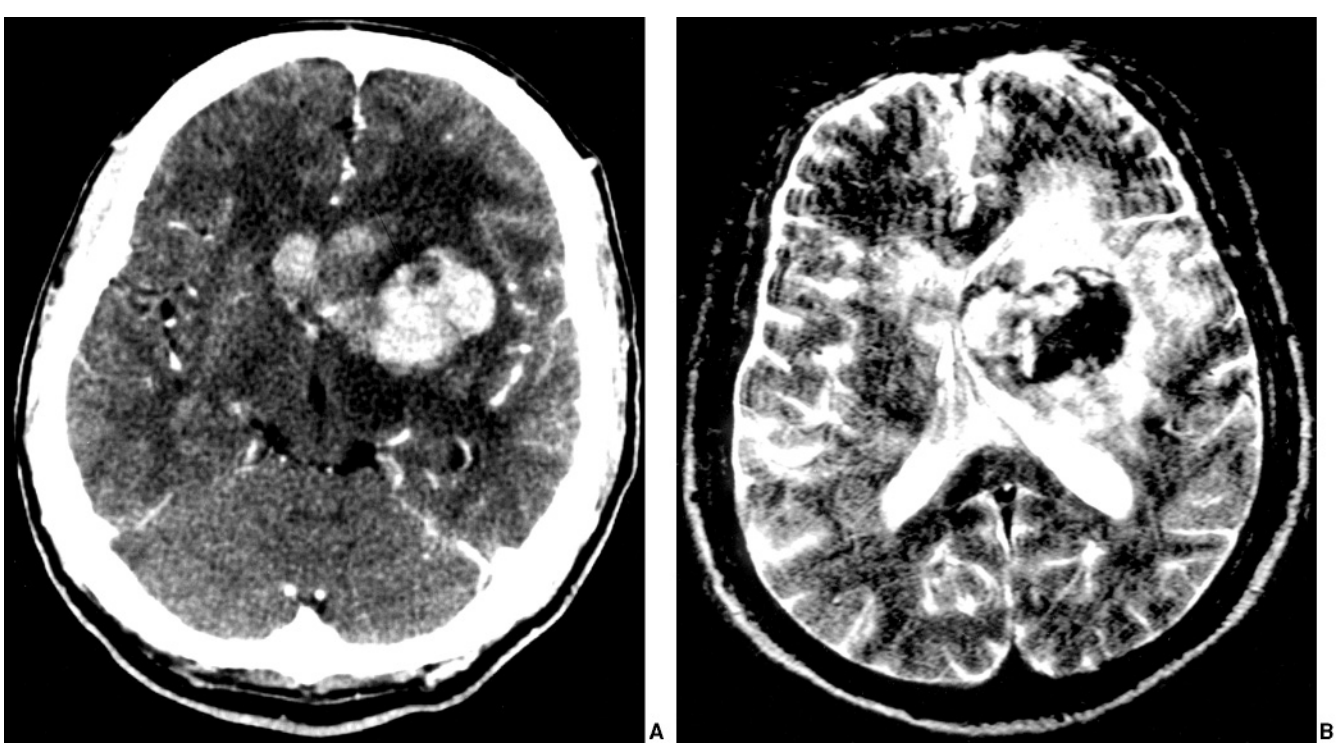


FIG. 18.23. Lymphoma with hemorrhage: 35 year old with acute onset right hemiparesis and non uent aphasia, with seizures.

A. CT with contrast shows left basal ganglia and ventricular hyperdensity consistent with blood, also in sulci. Edema surrounding lesion present on initial scan.
B. T2-weighted MR (with motion artifact) three days later shows mixed signal in lesion, consistent with resolving hemorrhage (hypointense initially, gradually lightening).

or days before it would have appeared in a spontaneous hemorrhage.

Seizures

Seizures are symptomatic of mass lesions or HIV infection itself, with peaks occurring early and late in the course of illness. Transient focal deficits may be due to seizure activity followed by Todd's paralysis. Confusion may be due to nonconvulsive status epilepticus, which is especially prevalent in patients suffering from metabolic encephalopathy, such as hepatic or hypoxic encephalopathy. Therefore, electroencephalography is mandated in the evaluation of acute confusional state in patients without witnessed seizures.

Treatment decisions are individualized based on the patient's wish to take a potentially sedating medicine, or wish to use an antiepileptic for treatment of comorbidities that can be simultaneously treated, such as pain or bipolar illness (28,29,107). Circumstances, such as need for intravenous access, will dictate the use of drugs with a parenteral form. These include phenytoin (fosphenytoin can be given intramuscularly as well as intravenously), valproic acid, phenobarbital and lorazepam or diazepam. Patients with problems with medication compliance do best with single daily dosing, which is possible with phenytoin, phenobarbital, and possibly newer agents such as leviteracetam. Patients on multiple medications may want to avoid drug interactions involving the cytochrome P450 system, which is induced by most antiepileptics, inhibited by valproic acid and not affected by gabapentin and leviteracetam. Renal failure precludes the use of the latter two drugs, and dose adjustments are required for liver failure in most others. Allergies may be increased in incidence, in HIV infection, and must be watched for.

Detoxification from epileptogenic substances, such as alcohol, cocaine or amphetamines, is also helpful where indicated.

SUMMARY

Great strides have been made in the diagnosis and management of neurologic sequelae of HIV infection, and in prevention of opportunistic infections. Unfortunately, notable exceptions remain in the areas of lymphoma, PML and other intractable infections. Even infections that have known preventive regimens, such as toxoplasmosis, present problems in terms of medication side effects, allergies, cost and the difficulties of long term maintenance of compliance. The wonderful results of efforts in new drug development are working to defeat HIV itself, thereby preserving and restoring the immune system. While these drugs have side effects and can fail due to emergence of resistance, especially within the central nervous system, they have allowed patients to reap rewards in the areas of prevention of dementia, neuropathy and other neurologic effects of the virus. The next goal will be to allow more infected people to have access to these medications, to eradicate its spread and to continue to improve diagnosis and treatment of the damaged nervous system.

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Chapter 19

AIDS Psychiatry: Psychiatric and Palliative Care, and Pain Management

Mary Ann Adler Cohen and César A. Alfonso

Psychiatric factors take on new relevance and meaning as we enter the third decade of the AIDS pandemic. Persons with AIDS are living longer and healthier lives as a result of appropriate medical care and advances in antiretroviral therapy. However, in the United States and throughout the world, some men, women, and children with AIDS are unable to beneÞt from medical progress. Inadequate access to care results from a multiplicity of barriers including economic, social, political, and psychiatric. Psychiatric disorders and distress play a signiÞcant role in the transmission of, exposure to, and infection with the human immunodeÞciency virus (HIV). They are relevant to prevention, clinical care, and adherence throughout every aspect of illness from the initial risk behavior to death. They result in considerable suffering from diagnosis to end-stage illness.

Stigma, discrimination, and fear (1Ð6) in conjunction with denial, omnipotence, and lack of awareness (7Ð9) complicate and perpetuate the HIV pandemic. The creation of a supportive, nurturing, non-judgmental healthcare environment can combat stigma and provide comprehensive and compassionate care (1,10Ð15). This chapter will provide guidelines for psychiatric and palliative care and pain management to help persons with AIDS to cope better with their illnesses, live their lives to the fullest extent, and minimize pain and suffering for them and their loved ones.

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DIAGNOSIS AND TREATMENT OF PSYCHIATRIC DISORDERS

Relevance of Psychiatric Care

There are 753,907 persons with AIDS and an estimated additional 900,000 persons currently living with HIV infection in the United States (16). An estimated 36,100,000 are living with HIV or AIDS worldwide (17). In the United States, AIDS has become the leading cause of death in men from age 25 to 44, the third leading cause of death in women age 25 to 44, and the leading cause of death in African-American women in that age group (18). It is the eighth leading cause of death overall (18). These Þgures are especially ominous because the fastest-growing HIV-infected population in the United States is that of women in the childbearing years (19) and a rapidly growing HIV-infected population is that of teenagers. The greatest risk of infection is in adolescence and young adulthood, most often through sexual contact. It is particularly relevant to public health because transmission of AIDS to men, women, and children is preventable through education, correct use of latex condoms (20), and treatment-on-demand of substance-related disorders. Non-adherence to antiretrovirals (ARVs) during pregnancy, labor, and delivery results in perinatal HIV transmission. The personal and societal costs to health, productivity, Þtness, careers, partners, spouses, parents, and children take an enormous toll in suffering. Related to this is the suffering of the loved ones and orphans (21) left behind by AIDS. The tragedy of preventable death in young and productive individuals is heightened by the multiplicity of infections, severity of illness, and the multisystem and

multiorgan involvement in this devastating illness. The illness is complicated by the psychological reactions to and psychiatric manifestations of HIV infection.

Psychiatric disorders can accelerate the spread of the virus by creating barriers to risk reduction. Risky sexual behaviors and sharing of needles in intravenous drug users account for the majority of new cases each year. In the United States, 40,000 new AIDS cases are reported each year (16) while 5,300,000 new cases are reported in the world (22).

The substance use disorders, including alcohol and other drug use, result in multidimensional obstacles to risk reduction and adherence to care. These include obvious barriers to prevention of transmission such as sharing of drug paraphenalia and more subtle barriers such as inability to adhere to perinatal antiretroviral therapy because of intoxication, withdrawal, and drug-seeking behaviors. Furthermore, intoxication and disinhibition associated with alcohol and drug use and exchange of sex for drugs result in risky sexual behaviors and difficulty with adherence to complex medical regimens. Cognitive disorders associated with alcohol and other drugs can also impair judgment and ability to adhere to care. Active substance use may interfere with adherence to prevention strategies, to medical care, to perinatal transmission, and to the care of children infected with the virus. Mortality trends indicate an increase in death from end-stage liver disease as a result of comorbid infection with HIV and hepatitis C. Substance use disorders are instrumental in hepatitis C transmission.

Other psychiatric disorders such as schizophrenia, bipolar disorder, post-traumatic stress disorder (PTSD), and cognitive disorders may be associated with HIV transmission and nonadherence to care. Risky behavior and nonadherence may result from poor judgment with regard to sexual partner choice, lack of attention to barrier contraception, and, at times hypersexuality, disinhibition, and multiple sexual partners. A further complication is the occurrence of dementia in persons with AIDS. Dementia can lead to poor judgment in sexual partner choice, unsafe sex, and disinhibition. Mood disorder with mania due either to HIV-related infections or prescribed or illicit drugs can result in hypersexuality, poor impulse control, and impaired judgment. Manic behavior includes hypersexuality and ÒsexualindiscretionsÓ as part of the diagnostic criteria for bipolar mania. When persons are psychotic, they may seek sexual contact or may become victims of sexual predators as a result of efforts to obtain love, affection, and attention or in attempting to relieve the anguish of psychosis. Post-traumatic stress disorder can lead to risky behaviors and decrease harm avoidance as a result of both dissociative phenomena and a sense of a foreshortened future. Persons with PTSD as a result of early childhood trauma may have difficulty protecting themselves from harm or may even unconsciously seek to reenact their early trauma in later life.

Delirium, dementia, depression, substance dependence, PTSD, and other psychiatric disorders complicate the course and add considerably to the pain and suffering of persons with AIDS. HIV infection and AIDS also are risk factors for suicide (1,3,10), and the rate of suicide has been shown to be higher in persons with AIDS (23–30). Psychiatric care can help prevent HIV transmission through recognition and treatment of substance-related disorders, dementia, and mood disorders such as mania. Comprehensive, coordinated care by a multidisciplinary AIDS team, including AIDS psychiatrists, can provide a biopsychosocial approach that is supportive to patients, families, and clinicians. Psychiatric interventions are valuable in every phase of infection, from identification of risk behaviors to anticipation about HIV testing; from exposure and initial infection to confirmation with a positive HIV antibody test; from entry into systems of care to managing complex antiretroviral regimen; from healthy seropositive to onset of first AIDS-related illness; from late stage AIDS to end-stage AIDS and death.

Prevalence of Psychiatric Disorders

The high prevalence of mental disorders in persons with HIV infection has resulted in closer clinical collaboration among primary care physicians, infectious disease specialists, and psychiatrists. While early studies in the 1980s showed a prevalence rate of 38% (31), more recent estimates show that 74% to 98% of persons with HIV infection who seek medical treatment have a psychiatric disorder (32–34). The prevalence of psychiatric disorders in persons with HIV infection varies according to the population studied. For example, there is a higher prevalence of delirium (acute confusional states) in acute general care units and a lower prevalence in nursing home and outpatient settings. Conversely, dementia is more prevalent in nursing home populations than in clinic or hospital populations. Table 19.1 summarizes the most recent research on prevalence of psychiatric disorders in persons with HIV infection in nursing home, inpatient, and outpatient inner city populations in the United States.

Psychiatric Disorders

While there are psychiatric disorders linked directly and indirectly to risk behaviors, HIV infection, or AIDS, people with HIV may have no psychiatric disorder or any disorder described in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) (35). We will describe the psychiatric disorders most prevalent in persons with AIDS and most relevant for primary physicians, infectious disease specialists, and other caregivers because of their impact on health, adherence, behavior, and quality of life.

TABLE 19.1. *Prevalence of psychiatric disorders in persons with HIV infection*

Diagnosis	AIDS nursing home[a] (1995-1996) N = 423	General hospital unit[b] (1989-1994) N = 433	Out patient clinic[c] (1999-2001) N = 558
Dementia	83%	22%	36%
Delirium	14%	29%	7%
Substance use disorder	83%	36%	68%
Mood disorder, depression	N/A	14%	55%
Mood disorder, mania	N/A	N/A	4%
PTSD	N/A	N/A	25%

[a] Data collected at Rivington House Health Care Facility, New York City (33).
[b] Data collected at Beth Israel Hospital, New York City. Bialer, P.A., Wallack, J.J., Prenzlauer, S.L., Bogdonoff, L., and Wilets, T. (1996). Psychiatric comorbidity among hospitalized AIDS patients vs. non-AIDS patients referred for psychiatric consultation. *Psychosomatics* 37, 469-475.
[c] Unpublished data, collected at the Jack Martin Fund Clinic, Mount Sinai Hospital, New York City.

TABLE 19.2. *Psychiatric disorders associated with HIV infection*[a]

Cognitive Disorders
Dementia
Delirium
Substance-related disorders
Mood disorders
Major depressive disorder
Due to medical condition, with depressive features
Due to medical condition, with manic features
Mixed
Dysthymic disorder
Adjustment disorders
Adjustment disorder with depressed mood
Adjustment disorder with anxious mood
Posttraumatic stress disorder
Bereavement

[a] This is only a partial list of the most prevalent psychiatric diagnoses in persons with HIV infection.

Psychiatric disorders associated with HIV infection are summarized in Table 19.2. Definitions and terminology are based on those in DSM-IV (35). Psychiatric disorders may be the first and, at times, the only manifestation of HIV infection (36–38). Early diagnosis of central nervous system (CNS) HIV-related abnormalities can lead to timely introduction of treatment with antiretroviral agents (39). Early neuropsychiatric disorders have been described (36–63), in addition to later manifestations of general psychopathology (39,41–46), dementia (47–63), psychosis (64–67), depression (68,69), and mania (70–73). These disorders can be a reaction to awareness of a diagnosis of HIV infection. Alternatively, psychopathology can be related to intrinsic involvement of the brain with HIV or opportunistic infections or cancers, such as toxoplasmosis, cryptococcosis, or lymphoma. In addition, antiretroviral therapies, treatments for opportunistic infections and cancers, and treatment for comorbid hepatitis C virus infection may have central nervous system side effects, including psychiatric symptoms.

Substance Use Disorders

In the United States and in some other countries, the AIDS epidemic is inextricably linked with the epidemic of substance related disorders. As of June 1999, 26% of all reported United States AIDS cases were associated with injecting-drug use (74). The United States is in the midst of a heroin epidemic (75). A 33-year longitudinal study of 581 men addicted to heroin revealed that nearly half of them died prematurely, primarily from drug overdoses or accidental poisonings (76). The use of heroin by injection is associated with a high mortality rate from drug overdose (76,77), and the use of drugs is associated with HIV transmission through needle-sharing, sexual transmission, exchange of sex for drugs, and through perinatal transmission. Furthermore, the use of multiple substances such as combinations of alcohol, heroin, cocaine, and benzodiazepines is associated with intoxication, disinhibition, and unsafe sexual behaviors. Persons with intoxication, withdrawal, or drug-induced persisting dementia, may make inappropriate partner choice, be victims of sexual violence, become sexually and physically violent, and exchange sex for drugs. Drug and alcohol cravings may override good judgment and result in unsafe sex.

Comprehensive psychiatric care for persons with substance dependence and HIV infection may include crisis intervention, individual psychodynamic psychotherapy, supportive psychotherapy, couple therapy, family therapy, group therapy, and specialized treatment approaches to substance-related disorders (1,2,33,78–80) including Alcoholics Anonymous, Narcotics Anonymous, and other twelve-step programs. Unsafe sex may result in not only HIV transmission, but also unwanted pregnancy, use of substances during pregnancy, and inadequate attention to prenatal care, perinatal transmission, and ultimately to early childhood abuse or neglect. With comprehensive programs (81) of support, treatment, and care, pregnant and drug addicted women with AIDS can prevent perinatal transmission and become caring mothers. It is also important to recognize that persons with substance

dependence are likely to have comorbid psychiatric disorders such as bipolar disorder, post-traumatic stress disorder, or any other psychiatric diagnosis. Conversely, persons with other psychiatric disorders such as mood disorders and schizophrenia may have substance dependence as well. Additionally, persons with AIDS and substance dependence may develop dementia and delirium from either drug-related or HIV-related causes. They can beneÞt from comprehensive psychiatric care, psychopharmacologic interventions, and complementary therapies such as relaxation response, meditation, use of writing (82) and art as creative outlets (80), as well as adequate nutrition and exercise. They can also beneÞt from incorporating and maintaining spiritual and religious connections (79,83Ð87). We will present an integrated psychopharmacologic approach to some of the commonly encountered psychiatric manifestations and symptoms that cause distress and suffering to patients and present challenges to their caregivers.

Drug Craving and Withdrawal

A person with AIDS who is actively using or craving drugs or alcohol will Þnd it difÞcult to focus on obtaining medical care, keeping appointments or taking a complex drug regimen. Intoxication, dependence, withdrawal, substance-induced mood disorders as well as comorbid psychiatric disorders interfere with adherence to care. Although rare, threats of violence and attempted assaults can be frightening to caregivers and can impede development of ongoing therapeutic relationships. A comprehensive approach to drug and alcohol treatment includes medical and psychiatric care and referral for drug detoxiÞcation, outpatient drug and alcohol treatment, and 12-step programs.

Psychopharmacologic Treatment of Drug and Alcohol Craving and Withdrawal

Drug treatment approaches to drug and alcohol cravings are currently under investigation. Several studies have demonstrated the efÞcacy of naltrexone in the treatment of alcohol cravings (88Ð92). However, we do not recommend the use of naltrexone in HIV-infected alcohol dependent individuals for three reasons. The Þrst reason is the concern about hepatotoxicity and drug side effects of nausea, vomiting, and abdominal cramping. The second reason is that naltrexone is an opiate receptor antagonist and will cause persons who are opioid dependent or on methadone as agonist therapy to go into withdrawal. The third reason is that naltrexone also blocks the analgesic effects of opioid analgesics in persons who are being treated for pain. Some studies have found several other drug treatments useful to alleviate cravings, but efforts to replicate Þndings of these studies have not been successful. The tricyclic antidepressant, desipramine, was found to be useful to decrease cocaine cravings (93,94), but placebo-controlled trials have not adequately conÞrmed these Þndings. A meta-analysis of placebo-controlled studies (95) showed that desipramine was helpful with cocaine cravings but did not improve treatment adherence. Imipramine has also been shown to decrease cravings for cocaine (96) but further review of these studies indicates that depressed cocaine users have a better response than nondepressed cocaine users. Other medications that can reduce drug cravings include anticonvulsant mood stabilizers. In a 12-week randomized, double-blind, placebo-controlled study, Halikas and colleagues (97) demonstrated that carbamazepine produced a decrease in cocaine craving. Brady and colleagues (98) reported that valproate was helpful in an open label, non-blinded study of nine patients. Isolated case reports have suggested that gabapentin might be useful for cocaine (99) and alcohol cravings (100,101). The antiparkinsonian medication pramipexole has been reported to be useful for cocaine craving (102). This was a single case report and we have not used pramipexole in any of our patients with AIDS and cocaine dependence. Placebo-controlled trials of serotonin reuptake inhibitors have not indicated efÞcacy in treatment of cocaine cravings (103).

Because gabapentin is well tolerated and does not undergo cytochrome P450 metabolism, we have used it in our patients. We have used desipramine in low doses. We have also used bupropion in low doses for both cocaine and nicotine cravings. Although there are conßicting reports (104,105) about the efÞcacy of bupropion for cocaine cravings, we have found it helpful for some of our patients. Benzodiazepines are recommended for alcohol and benzodiazepine withdrawal. A summary of our recommendations for treatment of drug craving and withdrawal in persons with AIDS is presented in Table 19.3. A protocol for persons with heroin withdrawal is presented in Table 19.4. We recommend that individuals who are actively using heroin obtain methadone maintenance as agonist therapy to prevent withdrawal and relapse. Methadone is widely used as agonist therapy for heroin dependence; well-controlled clinical trials (106Ð109) have demonstrated signiÞcant symptom improvement and treatment efÞcacy. Unfortunately, many medications prescribed for AIDS patients can alter methadone blood levels and may result in opiate withdrawal in individuals who are in full sustained remission on methadone agonist therapy.

Drug-Drug Interactions in the Treatment of Drug and Alcohol Dependence

Awareness of the drug-drug interactions of antiretroviral, antituberculous, and antifungal medications can help to prevent problems of altered blood levels of methadone and other medicines by understanding which medicines inhibit or induce cytochrome P450 enzymes.

TABLE 19.3. *Use of psychotropic medication and treatment of withdrawal and craving in persons with AIDS and substance dependence*

Indication	Medication	Dose Range
Cocaine withdrawal	Desipramine (Norpramin)	50–125 mg. hs.
	Nortriptyline (Pamelor)	50–125 mg. hs.
Drug or alcohol craving	Gabapentin (Neurontin)	100–800 mg. t.i.d.
Alcohol or benzodiazepine withdrawal	Chlordiazepoxide (Librium)	25–100 mg. t.i.d.
	Lorazepam (Ativan)	0.5–10 mg. t.i.d.

TABLE 19.4. *Methadone detoxiÞcation protocol for persons with heroin withdrawal who are not on opioid agonist therapy*

1. When heroin withdrawal is clinically evident, give methadone 20 mg po immediately.
2. Re-evaluate patient in 1–2 hours. If evidence of withdrawal persists, give an additional dose of methadone 10 mg po.
3. Repeat step 2 if withdrawal persists, for a total maximum rst-day dose of 40 mg.
4. Detoxify by 5 mg decrements every other day.

Methadone is metabolized primarily by the cytochrome P450 3A4 isoenzyme (110,111). All protease inhibitors and nonnucleoside reverse transcriptase inhibitors are metabolized by the cytochrome P450 system and may have inhibiting or inducing properties (112). Some combinations of antiretrovirals can alter methadone levels by enzyme induction or inhibition. Although ritonavir has been described as the most potent enzyme inhibitor and saquinavir as the least potent inhibitor (112,113), it is difÞcult to predict the impact of antiretrovirals on methadone levels since combination therapies with protease inhibitors, nonnucleoside reverse transcriptase inhibitors, antibiotics, and antifungals result in varied effects on induction and inhibition (112,114). Ritonavir, nelÞnavir, and efavirenz are all cytochrome P450 substrates, inhibitors, and inducers. Although ritonavir is a potent cytochrome P450 3A4 inhibitor, it decreases methadone levels by both enzyme induction of cytochrome P450 2B6 and displacement of methadone from plasma protein-binding sites (115Ð117). Although nelÞnavir has been reported to have signiÞcant inhibitory effects on cytochrome P450 3A4 *in vitro* (118), a case report indicated enzyme induction with resultant methadone withdrawal six weeks after nelÞnavir was started (119). There are also individual differences in patient responses to the combination therapies with the net effects varying considerably among individual patients. A summary of antiretroviral pharmacokinetics is presented in Table 19.5.

Drugs that have an inhibitory effect on cytochrome P450 3A4 can cause an increase in methadone to unacceptably high and even dangerous levels. On the other hand, drugs that are inducers of cytochrome P450 3A4 can

TABLE 19.5. *Antiretroviral substrates, inhibitors, and inducers of cytochrome P450 isoenzyme metabolism*

Isoenzyme	1A2	2B6	2C19	2D6	3A4
Substrates					
Protease Inhibitors (PIs)			amprenavir nel navir ritonavir	nel navir ritonavir	amprenavir nel navir ritonavir saquinavir
Nonnucleoside reverse transcriptase inhibitors (NNRTIs)			delavirdine efavirenz		delavirdine efavirenz nevirapine
Inhibitors					
PIs		ritonavir	amprenavir ritonavir	ritonavir	amprenavir indinavir nel navir ritonavir saquinavir
NNRTIs			delavirdine		delavirdine efavirenz
Inducers					
NNRTIs					nevirapine efavirenz

TABLE 19.6. *Medications known to induce enzymes, decrease methadone levels, and precipitate opioid withdrawal*

Carbamazepine
Efavirenz
Nel navir
Nevirapine
Pentazocine
Phenobarbitol
Phenytoin
Rifabutin
Rifampin
Rifapentine
Ritonavir

result in the discomfort of narcotic withdrawal. Rifampin, an antituberculous medication, is a potent inducer of cytochrome P450 3A4 and results in narcotic withdrawal symptoms in persons on methadone. When a known potent enzyme inducer is prescribed, methadone may need to be increased at a rate of up to 10 mg per day until withdrawal symptoms are alleviated. The antifungal medication, ßuconazole, is a relatively weak inhibitor of cytochrome P450 3A4, but has been shown to increase methadone by 30% (120). It is important to ascertain whether there are interactions between antiretroviral, antituberculous, antifungal, and other medications in order to prevent accidental overdose or withdrawal (121,122). A summary of these medications is presented in Table 19.6.

Drug-Illness Interactions in the Treatment of Drug and Alcohol Dependence

Issues to be considered in the psychopharmacologic approach to the treatment of drug and alcohol dependence in persons with AIDS include an awareness of the interactions of psychotropic medications and the symptoms and illnesses that are commonly encountered. Persons with AIDS may have underlying liver disease from prior drug or alcohol dependence. Some have coinfection with hepatitis C virus. Coinfection with HIV and hepatitis C virus complicates care. A treatment currently available for hepatitis C virus infection is with the combination of interferon alfa-2b and ribavirin. Interferon alfa-2b is known to cause depression, suicidal ideation, and suicide in some individuals (123,124). Musselman and colleagues (125) found that pretreatment with paroxetine helped to prevent severe depression from developing in a double-blind study of forty patients with malignant melanoma who were treated with interferon. Although we have not seen evidence of severe depression, we have found that several of our patients developed psychosis with paranoid components about two to three weeks after beginning combination treatment with interferon alfa-ribavirin. Pretreatment with an antidepressant such as paroxetine might have complicated the picture by actually precipitating or worsening psychotic symptoms. We have used gabapentin as a mood stabilizer for pretreatment and antipsychotic medication for psychotic symptoms, adding an antidepressant only if symptoms of depression ensue. Our patients are also on a multiplicity of antibiotics, antifungals, antiretrovirals, and other medications, all of which undergo hepatic metabolism. Efforts to maximize treatment efÞcacy and minimize hepatotoxicity are meaningful considerations in choice of psychopharmacologic agent.

The vulnerability of persons with AIDS to anticholinergic, extrapyramidal, hepatotoxic, and cognitive side effects of medications makes them similar to the geriatric patient population. Since persons with AIDS are also vulnerable to HIV-insomnia and HIV-neuropathy as well as mood swings, we have found that gabapentin is useful for drug and alcohol craving and have prescribed it for cravings alone or for multiple indications. Gabapentin has the advantage of not being metabolized in the body and also can alleviate mood swings, insomnia, and neuropathic pain (126Ð138). We have also prescribed desipramine or nortriptyline alone or in combination with gabapentin for patients with both HIV neuropathy and drug cravings. Valproate-induced extrapyramidal side effects and hepatotoxicity make it difÞcult to tolerate in this vulnerable population. Carbamazepine-induced anticholinergic side effects as well as hepatic metabolism also make it harder to tolerate.

Cognitive Disorders: Delirium and Dementia

Cognitive disorders may be reversible in persons with AIDS. The cognitive disorders are comprised of delirium and dementia. Delirium has been described as a syndrome of cerebral insufÞciency or acute confusional state and is 100% reversible if an underlying cause can be identiÞed and treated. In persons with HIV infection, causes may include sepsis-induced delirium, hypoxia-induced delirium with *Pneumocystis carinii* pneumonia (PCP), or hepatic encephalopathic-induced delirium due to hepatitis C cirrhosis and end-stage liver disease.

HIV dementia, unlike dementia of the AlzheimerÕs type, does not result from a direct effect on neurons themselves and can be reversed with HAART (139Ð141). With adherence to antiretroviral therapy and decrease in viral load to undetectable, dramatic improvement in cognitive function can occur with reversal of dementia. However, unless a person with dementia receives assistance, is in a structured setting, or is under direct observation, it may be very difÞcult for a person with memory impairment to adhere to a complex regimen. Cohen and Jacobson (79) described dramatic improvement in a small population of AIDS nursing home patients. Four patients with HIV encephalopathy and HIV dementia presented with moderate to severe cognitive impairment documented on mental status examination including Bender and clock drawings.

Some were disoriented, unable to perform activities of daily living, and incontinent of urine and feces. All four made dramatic improvement on antiretroviral therapy. After only one year of combination therapy including protease inhibitors, three patients had almost no detectable cognitive impairment and were able to live independently. The fourth patient is presented in the following vignette:

> Case 1: The patient was a 37-year-old disabled investment banker who was admitted to a nursing home when he was no longer able to care for himself in the community. He was confused, wandered, and was incontinent of urine and feces. He had evidence of slowing and diminished intellectual functioning. He did not understand that he had AIDS (Ò I don't even have HIV, there must be a mistake.Ó). He had evidence of cognitive impairment including constructional apraxia on clock and Bender drawings. After two years of HAART, dementia could not be detected on psychiatric examination and he no longer had constructional apraxia. He knew his CD4 counts, viral loads, full medical history, and names and doses of his medications. He was fully able to perform activities of daily living, was no longer incontinent, and was able to move out of the nursing home and to live independently. Within three years, he went from confused nursing home patient in diapers to dapper investment banker. He resumed his career and began to resume his social life. He retained an awareness of his prior cognitive deficits and was appreciative of his recovery.

Despite their frequent occurrence in general hospital populations, cognitive disorders frequently go undiagnosed (142). Even delirium, which is associated with a high mortality rate, often is overlooked. Consultation requests for persons with delirium are most often called for isolated psychiatric symptoms, management problems, or assessment of capacity to give informed consent or to refuse treatment. The cognitive disorders associated with HIV infection are even more elusive, making it difficult for inexperienced clinicians to associate aberrant behavior, mood swings or treatment refusal with the dementias and delirium associated with HIV infection, especially because they occur in young adults. Cognitive disorders occur frequently in persons with AIDS and they may or may not be associated with neurological findings. The presence of CNS involvement with *Toxoplasma gondii*, *Cryptococcus neoformans*, HIV, or cerebral lymphoma may, or may not, be indicative of the presence of cognitive dysfunction. Although cerebral atrophy is associated with HIV dementia, the degree of atrophy may not correlate with the severity of the dementia.

Delirium

Delirium is a cognitive disorder which is frequently unrecognized or misdiagnosed in persons with AIDS. Lipowski (143,144) describes delirium as a disorder of cognition including global cognitive impairment with concurrent deficits in memory, thinking, orientation, perception, disturbances of the sleep-wake cycle, and a characteristic course marked by rapid onset, relatively brief duration, and fluctuations in the severity of the disturbance. There is impaired ability to process, retain, retrieve, and apply information about the environment, body, and self. The patient's level of awareness is reduced and there can be fluctuations in level of consciousness. Thinking, perceiving, and remembering are all impaired in delirium. Delirium is also characterized by rapid fluctuations in mood, behavior, and level of consciousness. These may occur over the course of minutes, hours, or days, and may be typified by an alert patient falling asleep midsentence. ÒNodding outÓ is a sign of delirium. Delirium may be hypoactive or hyperactive and the patient may be quietly confused or agitated. Confusion is so frequently observed, that delirium has also been described as an acute confusional state and as a state of cerebral insufficiency.

Assessment of Delirium in AIDS

Delirium is 100% reversible when an underlying cause can be identified. Factors predisposing to delirium in AIDS include addiction to alcohol or drugs, brain damage, and chronic illness. Facilitating factors are psychological stress, sleep deprivation, and sensory deprivation during intensive care unit admission. Causes of delirium (see Table 19.7) include: (a) hypoxia secondary to, for example, PCP; (b) infections such as HIV encephalopathy, bacterial or fungal meningitis, or septicemia; (c) space-occupying lesions of the brain such as toxoplasmosis and CNS lymphoma; and (d) drugs, including opiates, as well as antibiotics, chemotherapeutic agents, and antiretroviral agents; (e) end-stage liver disease and end-stage renal disease.

It is not always easy to identify the cause of delirium in AIDS as is illustrated in the following vignette:

> Case 2: The patient was a 42-year-old disabled receptionist who was admitted to a psychiatric unit with symptoms of withdrawal and mutism. The patient was confused, disoriented to place and time, and was incontinent of urine and feces. She had frequent mood swings, was emotionally labile, and cried easily. She also had fluctuations in behavior. At times she was able to speak and answer questions, although not spontaneously. Most of the time she was mute and withdrawn. Ultimately after three months during which she was transferred from an acute psychiatric unit to an acute medical unit (to evaluate for underlying medical or neurological causes for her behavior) and back to psychiatry, she recovered sufficiently to return to the community. No underlying cause was identified and

TABLE 19.7. *Causes of delirium in AIDS*

Intoxication
Drugs: antibiotics, anticonvulsants, sedative-hypnotics, opiates, phencyclidine, ketamine, antineoplastic drugs, lithium, anticholinergic agents, cocaine, alcohol
Drug withdrawal
Alcohol
Opiates
Sedative-hypnotics
Metabolic encephalopathy
Hypoxia
Hepatic, renal, pulmonary, pancreatic insuf ciency
Hypoglycemia
Disorders of uid, electrolyte and acid-base balance: water intoxication, dehydration, hypernatremia, hypokalemia, hypocalcemia, hypercalcemia, alkalosis, acidosis
Endocrine disorders
Infections
Systemic: bacteremia, septicemia, infective endocarditis, bacterial pneumonia, *Pneumocystis carinii* pneumonia, cryptococcal pneumonia, herpes zoster, disseminated *Mycobacterium avium-intracellulare* complex, disseminated candidiasis
Intracranial: cryptococcal meningitis, HIV encephalitis, tuberculous meningitis, toxoplasmosis
Neurologic
Seizures: ictal, interictal, postictal states
Head trauma
Space-occupying lesions of brain: CNS lymphomas, toxoplasmosis. cytomegalovirus infection, abscesses, cryptococcoma
Hematologic
Anemia

lumbar puncture, MRI, CT, neurologic, hematologic, and metabolic evaluations were all within normal limits. No toxicology screening had been performed. On her return to the outpatient setting, she was able to recall that she had used large amounts of cocaine prior to her admission to the psychiatric unit and attributed her behavior changes to both intoxication and withdrawal. On her return visit, she was talkative, alert, calm, euthymic, and had no evidence of cognitive impairment. Delirium was most likely due to cocaine intoxication and withdrawal.

Romano and Engel (145), Pro and Wells (146), and Obrecht, et al. (147) have stressed the usefulness of the electroencephalogram (EEG) in helping to support a diagnosis of delirium. A decrease in frequency of EEG background activity is indicative of delirium, possibly as a result of a reduction of brain metabolism. EEG changes virtually always accompany delirium and make the electroencephalogram a useful diagnostic tool in persons with AIDS who manifest a change in mental status (148,149). EEG changes can aid in identifying the speciÞc etiology of delirium. Triphasic waves are characteristic of hepatic encephalopathy and generalized fast wave activity may be indicative of delirium tremens. EEG testing may also be helpful in evaluating and monitoring the course of HIV dementia (150) or diagnosing seizure-related behavior changes. A controlled study (151) of men with asymptomatic HIV infection showed that EEG and other electrophysiologic tests were the most sensitive indicators of subclinical neurologic impairment.

Treatment of Delirium in AIDS

Treatment of delirium consists of Þrst identifying and treating the underlying cause and then of maintaining hydration, electrolyte balance, and nutrition. In addition, it is important to provide an optimal environment for the patient: a quiet, well-lit room with a dim light at night, radio or television, a large calendar, easy-to-read clock, photographs and familiar objects, and, if possible, visits from familiar people. Medical and nursing support should be directed toward orientation and companionship, as well as to adequate sleep and sedation. Olanzapine 2.5 mg may be recommended as a standing order at bedtime until identiÞcation and treatment of the underlying cause have been accomplished. Olanzapine 2.5 mg is recommended as a starting dose; this can be increased gradually if necessary. Although olanzapine usually is effective in a single dose of 2.5 to 5 mg, a dose range to 10 mg may be required in some instances. For extreme agitation olanzapine can be given in divided doses of 2.5 mg every one to six hours (up to a total dose of no more than 10 mg) with or without the addition of lorazepam 0.5 mg every four to six hours.

Dementia

Dementia is a cognitive disorder characterized by loss of intellectual abilities that is sufÞciently severe as to interfere with the individualÕs social and/or occupational

functioning (35). Dementia (152) may be regarded as a global disorder of cognition, in the sense that several cognitive functions are impaired concurrently. These include memory, attention, judgment, and abstract thinking, which are decreased relative to the individual's premorbid level of performance. In HIV dementia, apraxia, agnosia, anomia, and constructional difficulty are accompanied by dropping things and poor concentration. Denial, memory impairment, and impairment of abstraction make it difficult for some patients to comprehend a diagnosis such as AIDS. Personality changes include alteration of the characteristic personality or accentuation of personality traits. Loss of cortical inhibitions may lead to promiscuity, assaultive behavior, or lack of awareness of social amenities. There may be use of obscenities by individuals who were not known to use them before. A patient may be unaware that he or she is not adequately clothed or that breasts or genitalia are exposed. These changes have a profound impact on family members, loved ones, and caregivers who might be distressed to see such changes in the elderly, but are devastated to see them in young adults.

Dementia may be associated with specific personality or behavioral manifestations if there is invasion of certain brain areas by the infectious agents or tumors associated with AIDS. If the frontal lobes are involved, the characteristics may include reduced drive, diminished self-concern, inability to delay gratification, impulsivity, lack of judgment, shallow blunted affect, perseveration, concrete thinking, and inability to change mental set (153). Other features include depression, psychosis, and anxiety. Delusions of persecution or jealousy can occur. When a patient faces a difficult series of tasks, such as during careful psychiatric examination or neuropsychologic testing, a catastrophic reaction with regression, irritability, rage, and crying may result (154). It can be averted by an examiner who is aware of this reaction and who employs a supportive approach during evaluation of cognitive capacities. The signs and symptoms of dementia are summarized in Table 19.8. The most frequent cause of dementia in AIDS is HIV encephalopathy; the causes are cited in Table 19.9. In general, differentiation of delirium from dementia may be based on criteria that include normal state of consciousness in dementia and a rapidly fluctuating course in delirium. Although in AIDS delirium may be superimposed on dementia, it is important to understand how to distinguish the two entities (Table 19.10).

Several standardized tests have been developed to screen for dementia, including the Short Portable Mental Status Questionnaire (155), the Mini-Mental State Examination (156), and the Cognitive Capacity Screening Examination (157). Unfortunately, these tests screen for moderate-to-severe cognitive dysfunction, and were developed as evaluations for cortical dementias such as dementia of the Alzheimer type. These tests may not detect subtle signs or mild impairment in persons with mild dementia or the subcortical dementia associated with HIV. HIV dementia requires careful psychiatric examination and neuropsychologic assessment. Two screening tests have been developed for HIV dementia. The first, the Mental Alternation Test (158), is analogous to Trailmaking Part B on neuropsychological testing and is a brief standardized test for very early HIV dementia. The HIV dementia scale is another standardized brief assessment (159). These tests are meant for screening and cannot serve as substitutes for a complete comprehensive psychiatric examination but can be used in addition along with general dementia screening.

TABLE 19.8. *Symptoms and signs of HIV dementia*

Symptoms	Signs
Early	
Word- nding dif culty	Cognitive Impairment
Forgetfulness	Apathy
Poor concentration	Regression
Confusion	Psychosis
Slowed thinking	Psychomotor retardation
Dif culty performing complex learned tasks	Dif culty with abstract thinking
Loss of balance	Ataxia
Poor handwriting	Tremor
Leg weakness	Paresis
Dropping things	
Late	
Disorientation	Mutism
Severe confusion	Incontinence
	Seizures
	Perseveration
	Severe regression
	Carphologia (picking)

TABLE 19.9. *Causes of dementia in AIDS*

Brain involvement
Cancers
Primary cerebral lymphoma
Metastatic cerebral lesions
Infections
Viral
HIV encephalopathy
herpes virus encephalopathy
cytomegalovirus encephalopathy
papovavirus progressive multifocal leukoencephalopathy
Fungal
cryptococcal meningitis
cryptococcoma
aspergillosis
coccidioidomycosis
Protozoal
central nervous system toxoplasmosis
Systemic involvement
Metabolic
Hypoxic or anoxic encephalopathy
Toxic substances
Chronic abuse of drugs or alcohol
Nutritional de ciency in wasting
Thiamine
Folate
B_{12}

Firesetting: An Unexpected Manifestation of HIV Dementia

Firesetting is a dangerous concomitant of HIV dementia (160). We deÞne Þresetting as an accidental or deliberate ignition of a Þre during the process of smoking or lighting a match, resulting in burn-related injuries or destruction of property. Firesetting is an extremely tragic manifestation of HIV dementia in persons with nicotine dependence.

TABLE 19.10. *Differentiation of delirium and dementia in AIDS*

	Delirium	Dementia
Fluctuation of symptoms	+	–
Drowsiness	+	–
Illusions	+	–
Hallucinations	+	–
Confusion	+	–
Carphologia (picking)	+	+
EEG background slowing	+	+
Insomnia	+	+
Impaired attention	+	+
Impaired concentration	+	+
Slow speech	–	+
Slow motor responses	–	+
Delusions	–	+
Ataxia	–	+
Leg weakness	–	+

Please note that the differentiation is *not* absolute. There is occasional overlap of symptoms and frequent appearance of delirium superimposed on pre-existing dementia in AIDS.

Fires can be set through carelessness with cigarettes even without cognitive impairment, and persons with HIV dementia and AIDS are even more vulnerable. Their vulnerability to Þresetting is multifactorial. Some individuals have HIV neuropathy and may not perceive the heat of a lit cigarette or spark, while others with visual impairment from cytomegalovirus (CMV) retinitis may not see a dangerous situation until it is too late. In persons with even mild HIV dementia, dropping things is a common manifestation of early minor cognitive/motor disorder and can result in accidental burns. Additionally, alcohol and drug intoxication can be superimposed on HIV dementia increasing vulnerability to accidental Þresetting. It is important to be aware of this dangerous concomitant of HIV dementia and to provide ßame-retardant clothing, prevent smoking in bed or in unsupervised areas, and if at all possible, to offer smoking cessation training and nicotine inhaler or transdermal system therapy to prevent Þresetting in this vulnerable population (160).

Violence

The violent patient with HIV dementia can be frightening to family members, to staff, and to other patients. Although rare, violence is extremely disruptive in the home and in the healthcare setting. Caring for the violent patient is complex and evokes strong feelings in caregivers. Education can reduce risks, prevent violence, and help to deal with trauma and its aftermath. Recognition of early warning signs and predictors of violence can help caregivers learn to defuse and deescalate potentially explosive interactions. Early warning signs include: verbal outbursts; physical aggression against objects (slamming doors, breaking objects); physical aggression toward the self; and physical aggression or threats of aggression toward others. Risk factors include prior history of violence, history of early childhood trauma, history of severe adult trauma or mortal combat, history of substance abuse, and paranoid schizophrenia. The psychiatric disorders associated with violence are summarized in Table 19.11.

Each HIV dementia patient with a history or potential for violence should be educated to become aware of precipitating factors and ways to obtain help in the face of imminent violence. The patient can be taught to reach out to staff, to use relaxation and biofeedback, and to request comforting foods such as cookies or ice cream to interrupt an escalating cycle of violence. The patient can also learn to request as needed medication or to leave an upsetting or overstimulating environment.

Caregivers should not corner a potentially violent person. Instead, they should keep at a distance of four to ten feet while speaking in a soft, reassuring, and gentle voice. The goal of the interchange is to assist the patient in regaining control and maintaining dignity. The patient

TABLE 19.11. *Psychiatric disorders associated with violent behavior*

Delirium
Dementia
Substance-related disorders
Alcohol intoxication and withdrawal
Cocaine intoxication
Ketamine intoxication
Phencyclidine intoxication
Amphetamine intoxication
Hallucinogen intoxication
Sedative and anxiolytic intoxication and withdrawal
Paranoid schizophrenia
Mood disorder with mania
Borderline personality disorder
Antisocial personality disorder
Attention-de cit disorder
Posttraumatic stress disorder

should be offered medication if it is not self-requested. Security guards, psychiatrists, and other personnel should be called promptly to minimize fear and maximize safety.

Use of creative outlets for sublimation of aggressive drives into creative activities include writing of poetry or prose, artwork, pottery, music, movement therapy, and exercise. Use of a multidimensional approach to violence includes early recognition, crisis intervention, alternative outlets, and psychotherapy. The medicines for violent behavior include combinations of antipsychotics, anticonvulsants, benzodiazepines, and β-adrenergic blockers. A summary of these recommendations is presented in Table 19.12.

Treatment of Dementia in AIDS

Gendelman and colleagues have demonstrated that HIV dementia can be reversed with HAART (141). However,

TABLE 19.12. *Guidelines for combination pharmacologic approach to violence in HIV dementia*

	Indications:	Contraindications in patients with:	Total daily dose range for standing orders oral route[a]	As needed oral Route[a]
Antipsychotics				
Haloperidol (Haldol)	Extreme agitation	Rigidity, immobility, risk for falls	0.5–20 mg	0.5–5 mg q2–4 hours
Perphenazine (Trilafon)	Little need for sedation	Rigidity, immobility, risk for falls, seizures	2–20 mg	2 mg q 6 hours
Chlorpromazine (Thorazine)	Need for sedation	Rigidity, immobility, risk for falls, seizures, endstage liver disease, cardiac disease, orthostatic hypotension	25–200 mg	10 mg q 2–4 hours
Thioridazine (Mellaril)	Need for sedation	Rigidity, immobility, risk for falls, endstage liver disease, cardiac disease, orthostatic hypotension	25–200 mg	10 mg q 2–4 hours
Olanzapine (Zyprexa)	Underlying paranoid schizophrenia with delusions and psychosis	End-stage liver disease, cardiac disease, orthostatic hypotension	10–20 mg	Not recommended
Anticonvulsants				
Gabapentin (Neurontin)	Recurrent violent and dangerous behavior	Severe renal disease	300–240 mg	Not recommended
Benzodiazepines[b]				
Clonazepam (Klonopin)	Severe agitation	Respiratory depression, liver disease, confusion, risk for falls	0.5–2 mg	Not recommended
Lorazepam (Ativan)	Severe agitation	Respiratory depression, confusion, risk for falls	1–8 mg	1 mg q 4 hours
Oxazepam (Serax)	Severe agitation	Respiratory depression, confusion, risk for falls	15–90 mg	Not recommended
Beta-Adrenergic Blockers				
Propranolol (Inderal)	Severe agitation	Asthma, emphysema, insulin-dependent diabetes mellitus, cardiac disease	10–40 mg	Not recommended

[a] Oral route is recommended to preserve dignity and autonomy.
[b] Benzodiazepines can cause paradoxical agitation in persons with delirium or dementia.

the process takes time and careful adherence to the HAART regimen. A special home care program, direct observation, or a structured setting such as a nursing home may be required in order to enable the patient to beneÞt from care if dementia impairs ability to adhere. With mild to moderate dementia, assistance from family members and reminder devices such as programmable watches or beepers and prepackaged medications may sufÞce. It is important to provide adequate care and support until cognitive function is regained. Support, psychotherapy, and family therapy must be provided for patients with AIDS-associated dementia. Ongoing therapy is beneÞcial and may lead to a less precipitous course. Antipsychotic medications help to alleviate anxiety, agitation, and psychotic symptomatology. The family should be included and caregivers should be given enough information about dementia so that they will be able to understand the patientÕs behavior and can help to orient and educate the patient as much as possible. Home care with adequate support services, nursing services, and a medical and psychiatric home care team can enable persons with dementia to function optimally and can prevent the disruption, separation, and confusion of a major environmental change.

If it is at all possible, the environment should be familiar to the patient. If the patient cannot be cared for in his or her own home, the space in a hospital or long-term care facility should be made as familiar and home-like as possible, with photographs of family members, familiar objects, and music provided. We recommend use of large clocks, calendars, televisions, and radios to ensure that the environment provides cues for orientation. Calendars should be one day per page, with only the day of the week, date, month, and year and indication of any holidays. Floors should be carpeted to prevent slips and falls. Family members should be encouraged to stay with the patient as much as possible in the Þrst few days and nights of a hospital stay. Telephones should be installed immediately upon admission. Telephones help persons with HIV dementia to maintain contact and can be programmed if telephone numbers cannot be recalled. When behavioral manifestations of dementia interfere with comfort and function and nonpharmacological interventions have been exhausted, use of antipsychotic medications is recommended. These may be helpful for psychotic manifestations of dementia, catastrophic reactions, and Òsundowning.ÓPsychotropic medication may be indicated for violence, extreme agitation, depression, anxiety, impulsivity, behavioral disinhibition, or psychotic symptomatology. Awareness of vulnerability to the extrapyramidal side effects of antidepressant, antipsychotic, and mood stabilizing medications is especially important in persons with HIV dementia.

Dementia in persons with AIDS is associated with a high suicide risk (161) and is also associated with accidental Þresetting (160). The tragedies of suicide, violence, and accidental Þresetting are compounded by the problems of loss of dignity and ability to function independently. Persons with AIDS with or without dementia can also develop depression or mania and may require treatment for mood disorders.

Mood Disorders

When depression or mania occur during the course of HIV infection, they are described as mood disorders due to medical condition if they are etiologically related to HIV, opportunistic infections, or to side effects of treatments. When an individual has had a longstanding history of recurrent or episodic periods of depression or mania that meet the DSM-IV criteria, and these have occurred prior to the onset of infection with HIV, then a recurrence would be classiÞed as either major depressive disorder or bipolar disorder, manic episode.

Major Depressive Disorder

Major depressive disorder is a mood disorder characterized by depressed mood, guilt, loss of interest in most activities (anhedonia), and hopelessness. A recent meta-analysis of ten published studies assessing the association between HIV infection and risk for major depressive disorder provided strong evidence that the frequency of major depressive disorder was twice as high in persons with HIV infection than in those who are not infected (162). There can be insomnia or hypersomnia and psychomotor retardation or agitation, diminution of self-esteem, and feelings of worthlessness and hopelessness. There may also be evidence of diminished ability to think or concentrate and suicidal ideation. Lyketsos and Treisman have described the difÞculty detecting depression especially in HIV primary care clinics where depression is often associated with substance use disorders and unemployment (163). Lyketsos and colleagues have also documented the high prevalence of depression in persons with injection drug use prior to HIV infection, upon entry to HIV primary care (164), and before progression to AIDS (165). Although research studies have not demonstrated a clear association between major depression and HIV disease progression (166), we feel that it is nonetheless important to recognize and treat depression to alleviate suffering and prevent suicide. We also have observed that depression has a negative impact on adherence which has been documented by Gordillo and colleagues (167), and ultimately this nonadherence as well as the distress of depression may contribute to illness progression as well.

Treatment for depression includes individual and family therapy and psychotropic medications. The use of antidepressants in persons with AIDS will be considered, in sections on Mood Disorder Due to Medical Condition (Section II. C. 4.).

TABLE 19.13. *Causes of AIDS-associated mania*

Illnesses	Medications
Cryptococcal meningitis	Zidovudine
HIV-encephalopathy	Efavirenz
	Steroids

Mania

Mania is a mood disorder characterized by a period of abnormally and persistently elevated, expansive, or irritable mood with grandiosity, pressure of speech, flight of ideas, decreased sleep, distractibility, buying sprees, and/or sexual indiscretions. Mania can occur early or later during the course of HIV illness, and while it is similar to the mania associated with bipolar disorder, "AIDS mania" is characterized with irritability rather than euphoria (168). The prevalence of mania is 1–2% in early HIV infection and 48% in later stages (169). The disruptive, dangerous nature of mania makes it an important diagnosis in the care of persons with AIDS. Additionally, persons with AIDS are prone to mania because of both opportunistic infections, such as cryptococcal meningitis (72,170), and medicines, such as zidovudine (73,171) and efavirenz (172). The illnesses and medicines associated with mania and AIDS are listed in Table 19.13. It is important to note that when mania is induced by an illness such as HIV encephalopathy or cryptococcal meningitis, the treatment should be directed both toward symptoms and underlying cause. It is also possible that once viral load is below detectable levels and cryptococcal meningitis has been treated, mania with mood disorders, unlike mania with bipolar disorder, is less likely to recur. Since persons with AIDS can also have bipolar disorder with manic features, this was also included.

Pharmacologic management of mania in persons with AIDS includes a combination of an anticonvulsant mood stabilizer, along with antipsychotic and antidepressant medications where indicated. Gabapentin has been investigated for use as a mood stabilizer in mania (173). Gabapentin can be started in doses of 100 mg three times a day and gradually increased to 300 mg three times a day and 900 mg at bedtime. Some individuals may require larger doses of gabapentin, up to 2,700 mg daily or more in divided doses. Although gabapentin cannot be monitored with blood levels, it is preferable to valproic acid because it has fewer side effects. Valproic acid, although highly efficacious for mania, violence, and seizures, has extrapyramidal side effects such as rigidity and tardive dyskinesia (174–176). Carbamazepine has anticholinergic side effects. Valproic acid and carbamazepine should be used with caution in persons with AIDS because of side effects and drug-drug interactions. Gabapentin can be substituted for either medication by simultaneously introducing gabapentin while tapering the other drug. Gabapentin can be started at 300–600 mg at bedtime and

TABLE 19.14. *Pharmacologic approach to mania and AIDS*

Anticonvulsant mood stabilizer[a]	Total daily dose range
Gabapentin (Neurontin)	900–2700 mg. In divided doses
Antipsychotic	
Haloperidol (Haldol)	0.5 mg.–5 mg. h.s.
Olanzapine (Zyprexa)	2.5 mg.–20 mg. h.s.
Antidepressant	
Celexa	10–40 mg. h.s.
Nortriptyline	25–125 mg. h.s.

[a] Valproic acid (Depakote) can be hepatotoxic and can cause extrapyramidal symptoms.
Carbamazepine (Tegretol) can cause central anticholinergic delirium.

TABLE 19.15. *Dangers of lithium treatment of mania and AIDS*

1. Need for electrolyte balance to prevent lithium resorption and poisoning
   - Sweating
   - Diarrhea
   - Metabolic alkalosis
   - Vomiting
2. Need for adequate renal function to permit lithium excretion
   - HIV-nephropathy
   - Heroin nephropathy
3. Similarity of symptoms of lithium poisoning to symptoms of AIDS
   - Nausea
   - Vomiting
   - Diarrhea
   - Confusion
   - Irritability

valproic acid or carbamazepine can be decreased gradually until it is discontinued, as gabapentin is titrated to therapeutic levels. The treatment of mania is summarized in Table 19.14. Lithium should be used with extreme caution in persons with AIDS because of the problems with maintaining electrolyte balance and the similarity of AIDS symptoms and symptoms of lithium poisoning. These are summarized in Table 19.15.

Mood Disorder Due to Medical Condition

In the mood disorders due to medical condition, there is a disturbance of mood with symptoms of a major depressive or manic episode. A mood disorder that occurs either after or at the onset of HIV infection is categorized as a Mood Disorder due to Medical Condition. Symptoms are identical to those described in Major Depressive Disorder or Bipolar Disorder, Manic Episode, except that they are caused by the medical condition. Mood disorder

with depression can occur with HIV infection, opportunistic infections or cancers, and many other AIDS-related illnesses. Mood disorder with mania can be caused by other AIDS-related conditions, but is more frequently associated with cryptococcal meningitis or with other cryptococcal infections. In addition, medications for AIDS-related conditions can cause both depression and mania. Some of the medications that can cause a mood disorder in persons with HIV infection are summarized in Table 19.16.

TABLE 19.16. *Medications that can cause mood disorders in persons with HIV infection*

Drug	Depression	Mania
Interferon alfa	+	–
Clonidine	+	–
Beta-blockers	+	–
NSAIDs	+	–
Amphotericin B	+	–
Cimetidine	+	–
Efavirenz	+	+
Zidovudine	–	+
Acyclovir	–	+
Captopril	–	+
Yohimbine	–	+
Isoniazid	–	+
Benzodiazepines	+	+
Steroids	+	+
Amphetamines	+	+
Penicillin	+	+
Cephalosporins	+	+

Pharmacologic Treatment of Depression

We select antidepressants to minimize side effects and to maximize efficacy while avoiding drug-drug interactions. We make an effort to choose antidepressants that are less likely to produce side effects such as agitation, insomnia, and seizures because persons with AIDS are especially vulnerable to these. As in the elderly, persons with AIDS are also vulnerable to anticholinergic and extrapyramidal side effects as well as the potentially fatal serotonin syndrome (177). All antidepressants are metabolized by cytochrome P450 enzymes. A summary of pharmacokinetics is presented in Table 19.17. The tricyclic antidepressants are metabolized by cytochrome P1A2, 2C, 3A4, and 2D6. The selective serotonin reuptake inhibitors (SSRIs) are metabolized by cytochrome P450 1A2, 2C, 2D6, 3A4 (178). Fluoxetine, the oldest of the SSRIs, is a

TABLE 19.17. *Psychotropic substrates, inhibitors, and inducers of cytochrome P450 isoenzyme metabolism*

Isoenzyme	1A2	2C19	2D6	3A4
Substrates				
Psychotropic Antidepressants				
Tricyclics	Amitriptyline Clomipramine Imipramine	Amitriptyline Clomipramine Imipramine	Amitriptyline Clomipramine Desipramine Imipramine Nortriptyline	Amitriptyline Clomipramine
SSRIs	Citalopram	Citalopram	Citalopram Fluoxetine Paroxetine	Citalopram Sertraline
Others			Maprotiline Venlafaxine	Nefazodone
Antipsychotics	Clozapine Haloperidol Olanzapine	Haloperidol Perphenazine Risperidone Thioridazine		
Anxiolytics	Diazepam			Alprazolam Midazolam Triazolam
Inhibitors				
Antidepressants	Citalopram Fluoxetine Sertaline	Citalopram Fluoxetine Sertaline	Citalopram Fluoxetine Paroxetine Sertaline	Fluoxetine Fluvoxamine Nefazodone
Antipsychotics			Haloperidol Perphenazine Thioridazine	Nefazodone
Inducers				
Mood Stabilizers				Carbamazepine

potent inhibitor of cytochrome P450 2D6 and has an extended elimination half-life with a potent active metabolite, norßuoxetine. It has important drug interactions with phenytoin (increases phenytoin), carbamazepine, and antiarrhythmics (177,178). Sertraline and citalopram have fewer side effects and drug-drug interactions than ßuoxetine (177Ð181). All SSRIs can have gastrointestinal side effects of nausea, vomiting, abdominal pain, and diarrhea. Some have psychiatric side effects of agitation, anxiety, insomnia, mania, and nervousness. Most SSRIs have some effect on sleep, primarily insomnia, although some patients experience drowsiness. Other SSRI-associated side effects, include fatigue, weight loss or sometimes weight gain, sexual dysfunction, and cognitive impairment. Neurological side effects include tremor and extrapyramidal side effects (177). The serotonin syndrome can result from the direct effect of SSRIs on serotonin reuptake and their interaction with other agents that also affect serotonergic neurotransmitters (177). The syndrome is characterized by confusion, agitation, autonomic dysfunction, myoclonus, hypperreßexia, and tremor (177) and is potentially fatal. The serotonin syndrome and the agents that can precipitate it (177) are summarized in Table 19.18. Even combinations of more than one SSRI while switching from one agent to another may precipitate serotonin syndrome so that a two-week washout period is recommended for agents known to produce features of this syndrome when used in combination (177). The most severe reactions have been described from coadministration of monoamine oxidase inhibitors, but serotonin syndromes have been described from coadministration of tricyclic antidepressants and SSRIs (177). SSRIs have been prescribed and have shown to be efÞcacious in persons with AIDS (179), but awareness of side effects and drug-drug interactions suggests cautious use and assessment on a case by case basis.

Drug-Illness Interactions with Depression

The tricyclic antidepressants include the oldest ones, amitriptyline, imipramine, doxepin, and newer agents, nortriptyline and desipramine. All tricyclic antidepressants have anticholinergic side effects and can cause urinary retention, constipation, paralytic ileus, dry mouth, and confusion from central anticholinergic delirium. Side effects of tachycardia and orthostatic hypotension make patients vulnerable to falls. Amitriptyline and imipramine should be avoided if possible because they have the most potent side effects and potential for cardiac arrhythmogenicity and falls. All tricyclic antidepressants should be used with caution and in low doses. All are efÞcacious for depression and have been used for HIV neuropathy as well. Further discussion of antidepressants for neuropathy will be presented in the section on pain. A summary of our recommendations for use of psychotropic medication for depression is presented in Table 19.19.

TABLE 19.18. *Clinical charactersitics of the serotonin syndrome*[a]

Psychiatric	Neurologic	Autonomic
Confusion	Myoclonus	Diaphoresis
Mania	Hyper exia	Shivering
Agitation	Tremor	Fever
	Ataxia	Nausea
	Siezures	Vomiting
	Coma	Diarrhea
		Tachycardia

[a] Serotonin syndrome can be caused by high-dose SSRIs or by combining SSRIs with the following agents: monoamine oxidase inhibitors, tricyclic antidepressents, other SSRIs, dextromethorplan, and L-tryptophan.

In addition to depression associated with AIDS, persons with AIDS may also be vulnerable to depression associated with their concomitant illnesses such as end-stage renal disease and end-stage liver disease. These illnesses considerably complicate treatment of depression. Their treatments themselves can also induce depression such as interferon-induced depression. It is important to monitor individuals with complicated courses of illness with both AIDS and hepatitis C-associated cirrhosis who may require interferon-ribavarin treatment (182,183). Symptoms such as pruritus associated with severe liver or kidney disease can also cause distress as well as depression that can respond extremely well to the tricyclic, doxepin, in low doses of 25 mg to 75 mg at bedtime.

Suffering at the end of life may be related to the expected bereavement, a natural response to the intense awareness of anticipatory loss of life and separation from loved ones (184). Almost all persons who are terminally ill experience these feelings, but some develop major depressive disorder (185,186). Psychostimulants can be helpful to some AIDS patients who develop end-of-life depression and have only weeks to live. Psychostimulants include dextroamphetamine, methylphenidate, and pemoline. They are especially helpful in treating depression close to death because they do not take two to four weeks to develop therapeutic levels. In severe illness they can reduce distress in patients and consequently in their families (184,187Ð189). Severely cachectic and weak individuals may experience improvement in mood and energy in only 24 hours after the Þrst dose. They have been described and used in persons with AIDS (190,191) as well as cancer. Because pemoline can cause hepatocellular damage and extrapyramidal side effects and all psychostimulants can cause confusion, we would recommend the use of low dose methylphenidate only. Methylphenidate can be started at a dose of 2.5 mg and given in the morning to prevent insomnia. It can be titrated slowly up to 5 to 10 mg in divided doses to be given in the morning and at noon. Side effects include restlessness, dizziness, palpitations, insomnia, arrhythmia, tremor, and rarely, psychosis. It is generally well tolerated in low doses.

TABLE 19.19. *Pharmacologic treatment of mood disorder with depressive features and major depressive disorder in persons with AIDS*

Psychotropic medication Category	Name	Dose range	Primary indication: Depression and secondary indications
Antidepressants			
Selective serotonin reuptake inhibitors	Citalopram (Celexa)	10–40 mg	post-traumatic stress disorder borderline personality disorder
	Sertraline (Zoloft)	50–150 mg	post-traumatic stress disorder social phobia
Tricyclics	Nortriptyline (Pamelor)	25–125 mg	neuropathy cocaine withdrawal insomnia
	Desipramine (Norpramin)	25–150 mg	neuropathy cocaine withdrawal insomnia
	Doxepin (Sinequan)	25–100 mg	pruritus insomnia
Mood stabilizers	Gabapentin (Neurontin)	100–2,700 mg	mood stabilization neuropathy drug cravings
Antipsychotics	Olanzapine (Zyprexa)	2.5–20 mg	psychotic features

Adjustment Disorder with Depressed Mood

Adjustment disorder with depressed mood is deÞned in DSM-IV as a psychological response to an identiÞable stressor that results in the development of clinically signiÞcant symptoms of depressed mood, tearfulness, or feelings of hopelessness that develop within three months after the onset of the stressor. The response is either characterized by distress that is in excess of what would be expected or by signiÞcant impairment in social or occupational functions. Although, by deÞnition, adjustment disorders must resolve within six months of the termination of the stressor or its consequences, they may persist longer if they occur in response to a chronic stressor such as a chronic illness. It is of some note that the majority of persons with AIDS do not meet criteria for adjustment disorder.

Psychosis

Psychotic symptoms such as delusions or hallucinations can occur in persons with AIDS for many reasons. They can occur because of the concomitant psychiatric disorders such as substance dependence, dementia, schizophrenia, or bipolar disorder with mania and psychotic features. They can also occur with major depressive disorder with psychotic features. Psychotic symptoms such as delusions and hallucinations are also associated with some of the prescribed medications as well as drugs of abuse and dependence in persons with AIDS. Hoffman and Cohen (personal communication) have observed new-onset of treatment-emergent psychosis in four individuals with comorbid HIV and hepatitus C virus infection when they were treated with a combination of interferon-alfa and ribavirin for hepatitis C. Psychotic symptoms are frightening to patients, their families, and caregivers. Psychotic symptoms can also be concomitants of delirium or dementia. In persons with AIDS, psychotic symptoms of new onset can be manifestations of central nervous system opportunistic infections (such as toxoplasmosis) or cancers (such as CNS lymphoma) or a manifestation of new onset cytomegalovirus retinitis with visual impairment and visual hallucinations. Whatever the etiology, new onset psychotic symptoms should be evaluated from both a medical and psychiatric standpoint. Psychotic symptoms associated with longstanding psychiatric disorders such as paranoid schizophrenia should be treated with typical or atypical antipsychotic medications and ongoing psychotherapy.

Psychopharmacologic Treatment of Psychosis

The treatment of psychotic symptoms needs to be tailored to the underlying cause. Psychotic symptoms related to cognitive impairment respond well to low dose antipsychotic medications. We have found three medications particularly useful for persons with psychosis: olanzapine, haloperidol, and perphenazine in low doses. The treatment of psychosis can alleviate frightening symptoms and improve quality of life at any stage of AIDS illness.

Drug-Illness Interactions with Psychosis

Because of the vulnerability to extrapyramidal side effects as well as hepatotoxicity, we do not recommend the use of high-dose, low-potency phenothiazine or typical antipsychotics such as chlorpromazine or thioridazine. Haloperidol, a butyrophenone, can be useful in low doses but may cause severe extrapyramidal side effects. We

sometimes recommend haloperidol in doses of 0.5 mg to 5 mg for treatment of psychosis. We prefer the atypical antipsychotic, olanzapine, over risperidone because risperidone may cause extrapyramidal reactions in persons with AIDS even in low doses. Olanzapine, one of the newest atypical antipsychotics, is the least likely to cause extrapyramidal side effects but can cause treatment-emergent diabetes mellitus (192). In persons with diabetes mellitus or a strong family history of diabetes, it may be preferable to use trißuoperazine in doses from 2 mg to 10 mg Perphenazine is a low-dose, high potency phenothiazone that is well tolerated in geriatric populations, especially in low doses. Olanzapine is highly efÞcacious in psychosis in doses from 2.5 mg to a maximum of 20 mg. Although haloperidol has been most effective in delirium-induced psychosis, olanzapine may be preferable because of its side effect proÞle. Some of the side effects of olanzapine may be desirable in persons with AIDS. These include increase in appetite, weight gain, and sedation. The possibility of treatment-emergent diabetes mellitus is of concern when olanzapine is used in combination with selective serotonin reuptake inhibitors. All selective serotonin reuptake inhibitors can induce hyperglycemia or hypoglycemia and should be used with caution in the AIDS population and with frequent monitoring of blood glucose levels.

Persons with AIDS and especially persons with HIV dementia are particularly vulnerable to the extrapyramidal side effects of psychotropic medications. These include rigidity, Parkinson-like symptoms, and tardive dyskinesia. Rigidity is dangerous because it can lead to falls. Tardive dyskinesia is dangerous because it can be irreversible and can lead to potentially serious and even lethal complications. It is an abnormal involuntary mouth and body movement disorder. Complications can develop from tardive dyskinesia because of problems in swallowing and aspiration pneumonia in severe instances. All antipsychotics and antidepressants, including selective serotonin reuptake inhibitors (193) can have extrapyramidal side effects, including tardive dyskinesia, although these are relatively rare. They are more frequently observed when antipsychotic medications are prescribed. Some persons with depression may have psychotic features requiring the use of both antidepressant and antipsychotic medication and further increasing vulnerability to extrapyramidal side effects.

Post-traumatic Stress Disorder

Post-traumatic stress disorder (PTSD) is deÞned in DSMIV as a disorder resulting from the exposure to threats of death or serious injury with a response of fear, helplessness, or horror. Although not everyone exposed to trauma develops PTSD, 14% of the United States population develops PTSD at some point during their lives. Hutton and colleagues (194) described a 33% lifetime prevalence of PTSD in 59 of 177 women prisoners with HIV risk behaviors. They found that exposure to trauma alone was not found to be associated with HIV risk behavior although other studies have linked trauma and HIV risk behavior (195Ð198). In persons referred for psychiatric consultations at the Mount Sinai AIDS Center in New York City, the prevalence of PTSD was found to be high in our HIV-infected population of both men and women. Thirty-eight percent of the 558 persons referred had histories of early childhood trauma and 25% had evidence of PTSD. One patient of all those referred had combat-related PTSD but had had early childhood trauma as well. The prevalence of PTSD in our population was high in both men and women, all of whom had early childhood physical and sexual abuse and some also had adulthood trauma such as rape, gang rape, or domestic violence. The following case will serve to illustrate:

> Case 3: The patient was a 26-year-old woman who had worked as a commercial sex worker and had a history of witnessing the murder of her sister and mother when she was four years old. Her father set both his wife and two children on Þre before he self-immolated. Only the patient survived the Þres. She survived with physical and psychological scars. She was also raped by an uncle who brutally and repeatedly assaulted her from the age of seven to thirteen years. As an adult she had a series of abusive relationships. She had symptoms of easy startle, hypervigilance, dissociation, ßashbacks, nightmares, insomnia, psychic numbing, and a sense of a foreshortened future. She was initially nonadherent with both medical and psychiatric care. She gradually became more adherent with medical care after beginning individual and group psychotherapy.

The implications and meanings of PTSD for HIV are complex. If a child is traumatized severely and chronically, the child may perceive that he or she is not valued adequately and may be unable to learn to avoid harmful situations or protect himself or herself from risky ones. Victims of severe trauma may resort to use of alcohol or drugs to escape from painful situations or memories, and the use of substances may lead to more risks because of intoxication, need to obtain substances (such as exchange of sex for drugs), and disinhibition. Commercial sex work has been shown to be associated with a high prevalence of early childhood trauma.

Since persons with PTSD have a sense of foreshortened future, a tendency to take risks, and to use drugs or alcohol, they are also likely to have problems adhering to risk reduction and to medical care. The associations among childhood trauma, PTSD, HIV risk behaviors and HIV and AIDS have implications for public health that warrant further study. There is a tremendous need for more data on the prevalence of early childhood trauma and PTSD in general AIDS populations and the feasibility of prevention of childhood trauma as a cofactor in AIDS.

TABLE 19.20. *Differentiation of major depression and bereavement*

	Uncomplicated bereavement	Major depression
General	Exhaustion, lack of strength, restlessness, inability to sit still, aimless moving, decreased capacity for organized living, "going through the motions," insomnia	Insomnia or hypersomnia, psychomotor retardation or agitation, diminished ability to function, decreased libido, headache, backache
Gastrointestinal	Empty feelings in abdomen, anorexia, tightness in throat, dysphagia, constipation	Somatic delusions ("my insides are rotting away"), anorexia, weight loss, constipation
Respiratory	Sighing respirations, dyspnea	
Emotional	Sorrow, sadness, guilt over survival, loss of warmth in other relations, hostility, preoccupation with image of deceased	Sadness, depressed mood, worthlessness, hopelessness, helplessness, decreased self-esteem, suicidal thoughts or acts
Course/Treatment	Self-limiting Working through of ambivalent feelings to achieve freedom from bondage to the deceased, readjustment to environment without the deceased, formation of new relationships, no medication	Requires intervention: Therapy, antidepressants

Uncomplicated Bereavement

Although uncomplicated bereavement is deÞned in DSM-IV in relationship to the loss of a loved one, in AIDS, as in other severe illnesses, the losses of health, functioning, body integrity, and anticipatory loss of life, may have similar consequences. Additionally, many persons with AIDS have also suffered the losses of their loved ones or of friends who have died of AIDS. A full depressive syndrome can be an expected reaction to such a loss, with feelings of depression and associated symptoms such as poor appetite, weight loss, and insomnia. However, morbid preoccupation with worthlessness, prolonged and marked functional impairment, and psychomotor retardation are uncommon and their occurrence suggests that the bereavement is complicated by the development of a major depressive disorder (Table 19.20). In uncomplicated bereavement, guilt, if present, is chießy about things done or not done at the time of the death by the survivor. Thoughts of death are usually limited to the individualÕs thinking that he or she would be better off dead or that he or she should have died with the person who died. The individual with uncomplicated bereavement generally regards the feeling or depressed mood as ÒnormalÓ although he or she may seek professional help for relief of such associated symptoms as insomnia and anorexia. The duration of ÒnormalÓ bereavement varies considerably among different individuals and cultures.

Psychodynamic and Psychotherapeutic Aspects of Care

Psychiatric care should be incorporated into HIV care in every setting and at every stage of illness. Psychiatric care has been shown to be effective in a prospective study of psychiatric treatment for persons with HIV and depression (199). Psychotherapy can provide a supportive and nurturing approach to individuals with HIV and AIDS. This can augment the support provided by friends and family or can be a welcome antidote to the poison of AIDSism, discrimination, and rejection experienced by some individuals. Psychotherapy can smooth the transitions experienced by all persons with severe illness. Examples include the transition from HIV negative to positive, from healthy seropositive to symptomatic, from symptoms to diagnosis of Þrst HIV-related illness, and from late-stage illness to death.

Psychotherapeutic interventions can also enhance HIV-related communications. Persons with AIDS or HIV infection may have difÞculty communicating about their illness to partners, spouses, children, parents, or siblings. A sensitive AIDS psychiatrist can not only smooth transitions but help with communication of information. Psychotherapy can also enable persons with HIV to adapt to their illness, work through loss of health, and cope with conßicts related or unrelated to the illness. Additionally, psychotherapy is valuable as part of the comprehensive treatment of psychiatric disorders associated with AIDS such as substance use disorders, cognitive disorders, mood disorders, PTSD, and psychotic disorders.

Psychotherapeutic modalities include crisis intervention, individual psychodynamic and psychoanalytic psychotherapy, couple therapy, family therapy, and group therapy. In addition, psychiatric care should be integrated into the comprehensive treatment of every patient through collaborative work with primary physicians, infectious disease specialists, nurses, social workers, and pastoral caregivers. An integrated multidisciplinary biopsychosocial approach includes AIDS psychiatry as part of the AIDS team. Psychiatric intervention can be of help not only to patients and families, but also to caregivers. Psychiatrists can provide bereavement therapy to families left behind by AIDS and can provide support to caregivers mourning the loss of patients. Ruiz (200) has described his work with a patient who was diagnosed with HIV during

the course of his psychotherapy whom he cared for throughout the illness, from diagnosis to death. He demonstrated the value of psychotherapy from before diagnosis to bereavement therapy for the family after the patientÕs death. Psychiatric care can alleviate suffering, improve adherence, maximize life potentials, and decrease morbidity and mortality for persons with HIV and AIDS.

DISTRESS

Persons with HIV infection and AIDS have high levels of distress from multiple sources including symptoms, medical and psychiatric illness, discrimination and stigma, as well as social, occupational, and Þnancial stresses. Persons with AIDS are also subject to the same losses, stresses, and life changes as the rest of the population and because they are living longer, to middle and old age, are also subject to other non-HIV related illnesses such as heart disease, hypertension, diabetes mellitus, osteoarthritis, cancer, and chronic obstructive pulmonary disease. The symptoms of fatigue, insomnia, pruritus may occur even in the absence of speciÞc medical or psychiatric pathology. Persons with AIDS may also have symptoms related to their treatments with HAART or with interferon alfa treatment for concomitant hepatitis C. Distress, depression, and anxiety can be measured rapidly and easily by means of the Distress Thermometer (DT) and the Hospital Anxiety and Depression Scale (HADS). Roth and colleagues (201) and Cohen and colleagues (202) have demonstrated the feasibility of using these scales in waiting room convenience samples of persons with cancer and AIDS respectively. Cohen et al found a 72% prevalence of distress, 70% prevalence of anxiety, and a 55% prevalence of depression in a waiting room sample of persons registered at an HIV clinic. We have also found the DT and HADS valuable for screening persons with HIV and HCV for interferon-ribavirin treatment and for following patients during the course of the treatment to determine the need for antidepressant or antipsychotic medications. The rapidity (Þve minutes in total for both screening evaluations) and feasibility of the DT and HADS make the screening for distress a very efÞcient way to determine appropriate interventions.

RISK BEHAVIORS

Two behaviors involved with HIV transmission, unprotected sexual intercourse and sharing of needles by injecting drug users, can be changed. Public health educational efforts, outreach programs, and education at all levels, beginning in elementary school, can help prevent HIV transmission (1). Heightening awareness about HIV transmission in medical school can help create generations of physicians who are AIDS educators (203Ð211). It is known that although up to 50% of patients are hospitalized in general care as a result of substance-related disorders (212), these are not routinely recognized and, in fact, are often left out of discharge attestations, resulting in lower reimbursement rates (213). Just asking about sexual behavior and substance use in a supportive, nonjudgmental way can help an individual become aware that he or she is involved in risky behavior, and this can lead to change. The stages of change are gradual and are described by Prochaska (214) particularly in reference to substance-related disorders.

Although needle exchange programs have been shown to decrease the rate of HIV infection in injecting drug users (215Ð226), controversies have hindered widespread access to sterile needles and syringes. Psychiatric intervention can help to diminish denial and increase referrals to drug treatment programs. Intervention in general care inpatient or ambulatory settings can be most productive, because the patient may be in a crisis and at a point where he or she is ready to make a decision to get into treatment. Similarly, ofÞces and clinics for sexually transmitted diseases, infectious disease clinics, medical clinics, gynecology clinics, urology clinics, and pediatric and adolescent clinics are ideal settings to help decrease denial and heighten awareness about avoiding HIV transmission by consistent and correct condom use (20).

> Case 4: A 17-year-old high school student who was HIV seronegative admitted to having had more than 50 unprotected sexual encounters and was currently sexually active with a 14-year-old girl at the time of referral. Although he reluctantly admitted that it might be safer to use a condom, his girlfriend refused, because they were both HIV seronegative and she had had unprotected sexual encounters with only one other person. Meeting with the teenagers, individually and as a couple, and ultimately with them and their parents, led to a visit to Planned Parenthood and to consideration of both abstinence and condom use.

> Case 5: A 43-year-old physician who was HIV seronegative refused to use condoms in a relationship with a widow who was also HIV seronegative, but whose husband of 12 years died of AIDS one year before they met. He could not be convinced to consider condom use.

Age, intelligence and even educational level (227Ð230) do not necessarily correlate with safe sexual behavior. This was documented in the study (227) of Brown University women who used birth control pills as contraceptives and averaged the same numbers of sexual partners during the AIDS epidemic as they had in the 1970s. In our own case vignettes and clinical observations, we saw that some teenagers and some well-educated professionals continued to have unprotected sexual encounters, despite their intellectual awareness of how AIDS is transmitted and despite their knowledge that an HIV seronegative antibody test does not necessarily indicate absence of the virus.

Obstacles to the education needed to create an environment conducive to changing behavior (1) include epidemics of denial, ignorance, and therapeutic nihilism in addition to AIDSism (1,6,231–233). AIDSism is a new form of discrimination, built on a foundation of homophobia, addictophobia, misogyny, and fear of contagion and death. A supportive, multidisciplinary approach can help combat the epidemics of fear and discrimination that perpetuate the AIDS epidemic and prevent educational efforts from being fully effective. This approach is also helpful in suicide prevention.

SUICIDE

In individuals with HIV infection, suicidal ideation can occur at any time, from realization of being at risk to end-stage illness (23,27,234–243). From licensing of home HIV testing kits to end-stage AIDS, issues of suicide prevention are of major concern. Because it has been shown that persons become suicidal before, during, and after HIV testing (23–26,239,240), home HIV testing kits put individuals at unnecessary risk for suicide. In an AIDS orientation course given by one of the authors (MAAC) to third-year medical students from 1985 to 1995, one or two medical students each year stated that if they tested positive, they would commit suicide. The statements were entirely spontaneous and unsolicited. They were made during discussions of strong feelings aroused while caring for patients with AIDS. In a survey of 344 university students in Kenya, most students stated that they would commit suicide if they tested HIV seropositive (240).

Some persons with AIDS have risk behaviors such as drug use that have led to alienation from families or communities. They felt isolated, lonely, alienated, and expendable, even before the diagnosis of AIDS. Suicide risk has been found to be higher in persons with chronic medical illness than in the general population (241–247). Most (23–28,237–243,248–266), but not all, studies (236,267) indicate that persons with AIDS or HIV infection are also at an increased risk for suicide. Marzuk et al. (260) studied the rate of suicide in New York City during 1985. He found that the suicide rate for men with AIDS from 20 to 59 years old was 36 times that of men from 20 to 59 without a diagnosis of AIDS. CotŽ et al. (263) studied all death certiÞcates indicating both AIDS and suicide in the United States from 1987 through 1989. He found a 7.4-fold higher rate of suicide in persons with AIDS. Drug overdose accounted for 39% of suicides, followed by Þrearms (25%) and suffocation (13%). Rajs et al. (264) reviewed 21 completed suicides in Stockholm over a period of Þve years. Medicinal drug overdose was also found to be the most prevalent suicide method. Chandra et al. (238) found in a cohort in India that 14% of patients showed serious suicidal intent six weeks after learning of their HIV-seropositive status. Older individuals (237,243) and women (242) are particularly at a higher risk for suicide. In a sample of HIV-infected persons from Milwaukee, Wisconsin and New York City, Kalichman et al. (237) found that 27% of respondents reported suicidal ideation within one week prior to the survey. In a more recent New York City autopsy study, Marzuk et al. (268) found HIV positive men of African-American and Latino-American ethnicity, aged 35 to 54, to be at the highest risk for suicide.

Erfurth et al. (28) in Munich documented that the two most common reasons for psychiatric consultation in patients with AIDS were for evaluation of suicidal behavior and for treatment of depression. Suicidal behavior was present in one out of every Þve persons with HIV seropositivity or AIDS in a general hospital population (23). Woller et al. (29) studied a cohort of HIV-infected gay men and found that suicidal behavior is related more to rejection by key persons or signiÞcant others than to disease stage or immune function.

The suicidal person with AIDS is in the midst of a crisis of expendability. The concept of expendability (269,270) is conveyed to the suicidal individual in both verbal and nonverbal ways, and hopelessness develops. Once the diagnosis of AIDS is conÞrmed, alienation and expendability compound the sense of hopelessness. Risk factors for suicide in the general population include the following (271–277): hopelessness, impulsivity, substance abuse disorder, recent illness, recent hospitalization, depression, living alone, and inexpressible grief. Persons with AIDS who are nonadherent to medical care are frequently ill and hospitalized. They feel alienated and isolated because of the discrimination against them. Hopelessness is heightened by the high mortality rate associated with AIDS and its devastating downhill course. Further complicating the picture are the issues of pain, disÞgurement, blindness, weakness, and depression associated with some of the infections and cancers comprising AIDS. Suicidal behavior can be seen in end-stage AIDS:

> Case 6: A 28-year-old aspiring artist was transferred to an AIDS nursing home with progressive multifocal leukoencephalopathy, right hemiplegia, and major depression. She stated ÒI feel that I have lost all my dreams and this is my last stop. I want to die.ÓShe made an attempt at suicide, and although medically she recovered fully, she gradually deteriorated psychologically and died despite psychotherapy and antidepressants.
>
> The multiple losses of health, Þtness, vision, mobility, hope, dreams, home, job, career, friends, and loved ones can lead to depression and suicidal behavior. Some individuals can cope better than others. The patient who made her statement about her lost dreams in a support group elicited a very sensitive response from another group member, who stated, ÒYou havenÕt lost your dreams; you can convert them to small hopes.Ó

Converting lost dreams to small hopes became a major theme of the support group and a focus of dynamic

psychotherapy. With support, networking, and family and individual psychotherapy, most individuals realize that suicide is not their only option. Some persons with AIDS have lost connections to family and beneÞt from conßict resolution and reunions following long separations resulting from distance, alienation, or behavioral complication of substance-related disorders. Psychiatric diagnoses associated with increased suicide risk include mood disorders (275), alcohol and drug dependence (273,276), schizophrenia (277), and antisocial and borderline personality disorders (241,278). Posttraumatic stress disorder frequently is misdiagnosed as borderline personality disorder. Individuals with posttraumatic stress disorder, with a history of severe childhood physical and sexual abuse and with co-morbid depression, are at a high risk for suicide. In addition to these psychiatric disorders, dementia in persons with AIDS is associated with a higher suicide risk (161). HIV dementia is associated with impulsivity, impaired judgment, affective lability, and behavioral disinhibition. Other neurocognitive illnesses, such as HuntingtonÕs disease, have an increased rate of suicide (247,279). Subcortical dementia is a corollary of both HuntingtonÕs and AIDS and may make individuals more vulnerable to suicide (161). The association between delirium and suicide has not been studied in persons with AIDS. However, patients in delirium tremens who are not adequately sedated are at a high risk for suicide (280).

Management of the suicidal person with HIV infection is a sensitive and complex issue. It is crucial to remember that all suicidal individuals have mixed feelings about suicide and can vacillate from being preoccupied with hopelessness and suicide to thoughts of and plans for the future. No one is suicidal all the time. Suicide may be an effort to gain control or to alleviate pain or alienation. Suicide is also a symptom of major depression and mood disorder, as well as a reaction to a growing realization of loss of health, strength, and cognitive capacities.

The caregiver needs to feel comfortable with taking a suicide history and to discussing suicide in depth with the person with HIV infection. The steps include the following: (a) establishment of a trusting relationship, (b) discussion of suicide and death in relation to AIDS and the patientÕs philosophies and religious beliefs, and (c) realization of the value of continuity of care and reassurance that the person with HIV infection will not be abandoned.

Suicide history-taking includes the following questions:

1. Have you ever thought about killing yourself?
2. What is it speciÞcally that made you think of suicide?
3. Have you made any plans?
4. What are they?
5. Have you ever tried to kill yourself?
6. Do you feel like killing yourself now?
7. What would you accomplish?
8. Do you plan to rejoin someone who has died?
9. Do you know anyone who committed suicide?

Far from harming the patient, being able to speak about suicidal thoughts and feelings is highly relieving. Persons with HIV infection may feel isolated and alienated. Thoughts of suicide, while on the one hand providing some measure of consolation and control, may be frightening and painful on the other. Sharing suicidal feelings with an empathic listener not only is relieving but may help the person achieve a different perspective.

In order to prevent suicide in persons with AIDS, it is important to recognize dementia, delirium, depression, and posttraumatic stress disorder, as well as the vulnerability of persons with AIDS to losses and alienation. Persons with AIDS are especially vulnerable to pain, depression, and pruritus, all of which increase suicide risk. When these symptoms can be alleviated, suicidal ideation diminishes. Persons with AIDS respond well to therapeutic modalities aimed at providing support and bolstering defenses and coping strategies. These include individual and group therapy, crisis intervention, treatment of depression, and use of relaxation techniques. Psychosocial factors are relevant to both suicide prevention and immune system function.

PSYCHOSOCIAL VARIABLES AND IMMUNE FUNCTION

Separation, loss, stress, and depression have an impact on onset of illness, progression of illness, deterioration, and mortality. The effects of separation, loss, depression, and stress on the immune system have been explored by several groups (281Ð301). In the setting of HIV infection, Silberstein (294) suggested that the association of stress and depression with alterations of cell-mediated immunity might be a cofactor in AIDS. BlumenÞeld (295) proposed that psychosocial factors play a role in the onset of opportunistic infections in persons who are HIV-seropositive. Conversely, Fawzy and colleagues demonstrated less psychological distress (296) and improved immune system function (297) when individuals with recently diagnosed malignant melanoma had group therapy for six weeks. Spiegel and coworkers have shown (299Ð301) that group therapy doubled survival time in a prospective study of women with metastatic breast cancer. Group psychotherapy for persons with HIV and AIDS has been described by Alfonso and Cohen (302) as an effective means to provide support, an outlet for feelings, a common bond, and a relief from societal stigma. The groups have been gratifying for patients and caregivers alike. Group psychotherapy for persons with AIDS has also been shown to nearly double survival time (303). Additional data on the negative impact of alcohol and drugs on the immune system (304Ð321) indicate that psychiatric intervention should be beneÞcial in HIV-related illness. Psychiatric interventions should be incorporated early (2,10,199,200,322,323) into the care of persons with HIV infection, to improve the patientÕs ability to cope and

TABLE 19.21. *AIDS palliative care: a biopsychosocial approach*

Bio	Psycho	Social
Pain	Depression	Alienation
Dyspnea	Anxiety	Social isolation
Insomnia	Confusion	Stigma
Fatigue	Psychosis	Spirituality
Nausea	Mania	Financial loss
Vomiting	Withdrawal	Job loss
Diarrhea	Intoxication	Loss of key roles
Blindness	Substance dependence	Loss of independence
Paralysis	Existential anxiety	
Weakness	Bereavement	
Cachexia	Suicidality	
Incontinence		
Pruritus		
Hiccups		

enable the clinician to recognize and treat psychiatric disorders such as depression and substance dependence.

AIDS PALLIATIVE CARE

AIDS palliative care can be deÞned as comprehensive multidisciplinary care that focuses on alleviating suffering and maximizing life potentials independent of severity, stage, or prognosis (personal communication, Daniel Fischberg). Although most clinicians emphasize the need for palliation during the terminal stage of illness, palliative care can be more than end-of-life comfort care. SpeciÞcally, palliative care relates to providing comfort at every point from initial diagnosis to end-of-life. Comfort takes on a greater role as curative care becomes less feasible and cure, care, and palliation become a smooth continuum. This approach eliminates the ÒtransitionitisÓ that may result from an abrupt change from curative to palliative at the very end of life. A biopsychosocial approach can help to delineate the complex issues in AIDS palliative care. A brief summary of the most problematic issues is presented in Table 19.21. The use of pastoral care, hypnosis, music, relaxation response, meditation, writing, and art cannot be overemphasized. The importance of networking and providing a system of support for persons with HIV from diagnosis to end of life is signiÞcant in the comprehensive care of each individual.

AIDS psychiatric care and AIDS palliative care need to be integrated and offered to persons throughout the course of illness. There is no reason for persons with a severe illness such as AIDS to have to wait until the end of their lives to obtain relief from suffering and distress. We believe that persons with severe illness need a biopsychosocial approach to care. This approach includes alleviating distress and providing a nurturing care environment. We need to address depression, anxiety, mania, confusion, psychosis, and posttraumatic stress disorder with both psychotherapy and psychopharmacotherapy. Treatment may include crisis intervention, individual, couple, family, and group psychotherapy and the seamless integration of psychiatry into primary AIDS care. Throughout the course of illness, pain and suffering need to be alleviated. Persons with AIDS may experience pain throughout the course of the illness. Pain needs to be treated as adequately during acute illness such as esophageal candidiasis or herpes zoster as it is at the end of life.

No comprehensive approach to AIDS palliative care would be complete without addressing the spiritual aspects of care throughout the course of illness and at the end of life. Spiritual care has been shown to provide comfort and solace and to alleviate suffering in persons with cancer (325Ð336) and needs to be integrated into the care of persons with AIDS.

It is important to be aware that some of the most difÞcult and agonizing symptoms can be addressed or assuaged at any stage of illness. These include not only pain, but also dyspnea, pruritus, fatigue, hiccups, and nausea and vomiting.

Symptom management strategies need to be comprehensive and integrate adequate relief of concomitant anxiety and depression. Undertreated pain and pruritus are associated with catastrophic psychological symptoms such as depression, hopelessness, and suicidal behavior. Patients with dyspnea describe feelings of intense anxiety, panic, and fear of death by asphyxiation. Intractable hiccups can lead to overwhelming exhaustion and feelings of helplessness. Anxiety, depression, panic, fear of death, and helplessness, in turn, can exacerbate the subjective distress caused by undertreated pain, dyspnea, pruritus, nausea, vomiting, and intractable hiccups. Psychiatric interventions aimed at reducing these negative emotional states can complement palliative symptom management strategies (see Table 19.22). At the end of life, one of the most important interventions is spending time with the patient, talking if possible, holding hands, and surrounding the patient with loved ones if they are available. Being with a dying patient can alleviate the terror of abandonment and the fear of dying alone. For the clinician who can

TABLE 19.22. *Palliative careÑ symptom management*

Symptom	Treatment
Intractable hiccups	Chlopromazine, olanzapine
Dyspnea	Oxygen, morphine, fan, relaxation
Nausea	Ondansetron, olanzapine
Pruritus	Doxepin
Pain (nociceptive)	Strong opioid analgesics (e.g. fentanyl, morphine sulfate)
(neuropathic)	Adjuvant analgesics (e.g. antidepressants, anticonvulsants, stimulants, antihistamines)

cope with the poignancy, intimacy, and strong feelings evoked, the experience can be rewarding.

PAIN MANAGEMENT IN PERSONS WITH AIDS

Pain is an incapacitating symptom in persons with AIDS. Undertreated pain results in an increase in psychological distress and a reduction of the quality of life. There is evidence that even in persons with metastatic cancer and multiple complications, pain can be adequately alleviated 90% of the time (331). Acute and chronic pain syndromes are associated with increased risk of suicide in persons with HIV-seropositivity (23,332,333). Pain that is undertreated leads patients to request physician-assisted suicide (334). Pain is frequently undertreated in persons with AIDS (335), causing needless suffering and leading to the dangerous concomitant of suicide. Undertreatment of pain can be a result of inadequate assessment, unfamiliarity with management strategies, unconscious resistance and fears, discrimination and stigma, and myths about analgesic use.

Prevalence Studies

The prevalence of pain in persons with AIDS ranges from 28% to 97% in various studies (Table 19.23) (336Ð343). Newshan and Winapel (344) studied a cohort of 100 hospitalized persons with AIDS who were referred to a pain consultation service. Abdominal pain and neuropathic pain were the most common complaints, accounting for 26% and 24% of patients respectively. Odynophagia, dysphagia, headache, cutaneous Kaposiõ sarcoma pain, musculoskeletal pain, and postherpetic pain were also frequently reported in this study.

Etiology of Pain in AIDS

Pain can be mediated by nociceptive, neuropathic, or idiopathic mechanisms (345). Nociceptive pain is the physiological reaction triggered by chemical, thermal, or mechanical stimuli. Injured tissue and pain receptors release substances that modulate an inßammatory response and initiate neuronal activity leading to the perception of pain (346). Nociceptive pain modulators include: bradykinin, serotonin, histamine, potassium ions, acetylcholine, adenosine triphosphate (ATP), substance P, and prostaglandins (347). Nociceptive causes of pain in persons with AIDS are summarized in Table 19.24.

Neuropathic pain results from insult to the peripheral or central nervous system (345) and 30% to 40% of persons with AIDS have peripheral neuropathy (342). Sensory neuropathy is the most common neuropathy in persons with AIDS (348Ð351). The multicenter AIDS Cohort study (352) showed that between 1985 and 1992 the annual rate of reported cases of sensory neuropathy increased by 50%. Explanations for this upward trend include longer survival with severe immunosuppression (352,353) and the increasing use of treatment regimes with antiretroviral agents (353) that produce dose-dependent neurotoxicity (354,357).

When the cause of pain is neither nociceptive nor neuropathic, it is described as idiopathic. Idiopathic pain is not to be confused with malingering or with psychogenic pain. Idiopathic pain refers to the perception of pain in the absence of identiÞable pathology. Examples of idiopathic

TABLE 19.23. *Prevalence studies of pain in persons with HIV infection*

Investigators	Study population	N	Reported pain
Schoefferman (1988)[a]	Hospitalized patients	100	53%
Lebovits et al. (1989)[b]	Hospitalized patients	96	54%
Lefkowitz et al. (1992)[c]	Hospitalized patients	130	64%
Singh et al. (1992)[d]	Hospitalized patients	72	97%
Zarowny et al. (1992)[e]	Ambulatory patients	140	55%
Reiter and Kudler (1996)[f]	Home hospice patients	200	75%

[a] Reference 336.
[b] Reference 337.
[c] Reference 338.
[d] Reference 339.
[e] Reference 340.
[f] Reference 342.

TABLE 19.24. *Nociceptive causes of pain in persons with HIV infection*

Dermatological:	Kaposi's sarcoma lesions
	Condylomata acuminata
	Folliculitis
	Impetigo
	Molluscum contagiosum
	Norweigian scabies
	Cytomegalovirus ulcerations
	Verruca vulgaris
	Psoriasis
	Abscesses
	Bacillary angiomatosis
	Seborrheic dermatitis
Oral:	Caries
	Gingivitis
	Periodontitis
	Stomatitis
	Hairy leukoplakia
	Aphthous ulcers
	Parotitis
	Angular cheilitis
	Pseudomembraneous candidiasis
	Erythematous candidiasis
Gastrointestinal:	Esophageal candidiasis
	Cytomegalovirus ulcerations
	Helicobacter pylori gastritis
	Clostridium difficile colitis
	Campylobacter colitis
	Salmonella enteritis
	Shigella enteritis
	Cryptosporidium enteritis
	Mycobacterium avium complex infection
	M. tuberculosis infection
	Giardiasis
	Entamoeba histolytica colitis
	Kaposi's sarcoma ulcerations
	Non-Hodgkin's lymphoma
	Squamous cell cancer of the anus
	CMV gall bladder infection
	CMV pancreatitis
	CMV appendicitis
	Drug-induced pancreatitis
Musculoskeletal:	Psoriatic arthritis
	Septic arthritis
	Reiter's syndrome
	Spinal epidural abscess
	Pyomyositis
	Drug-induced myopathy
Headaches:	Migraine headache
	Tension headache
	Cluster headache
	Drug-induced headache
	Cryptococcal meningitis
	Cortical toxoplasmosis
	Cortical lymphoma
	Cortical abscesses
	Sinusitis
	Aseptic meningitis
	Carcinomatous meningitis
	Bacterial meningitis
	Tuberculous meningitis
	Neurosyphilis

pain include polyarthralgias, polymyositis, and non-inßammatory myopathy.

Pain Assessment

Inadequate pain assessment is a major factor in the undermedication of pain. In a study of cancer pain management strategies (358), 76% of physicians reported having problems with pain assessment. The use of standardized pain assessment measures and sensitive, systematic clinical interviews, lead to improvement in both pain assessment and treatment. Awareness of the myths about pain and analgesia can be helpful in overcoming barriers that hinder the assessment process. Common myths that interfere with pain assessment include:

1. Patients often exaggerate their complaints of pain.
2. Latino-Americans and African-Americans exaggerate their complaints of pain and therefore need less analgesia.
3. Patients with present or past history of addiction lie about pain as part of their drug-seeking behavior.
4. The etiology of pain is often psychogenic.
5. The etiology of pain most often remains obscure.

One common misconception is that patients tend to exaggerate their complaints of pain. Research studies of patient-controlled analgesia (PCA) (359,360) show that patients tend to avoid the prospect of the complete disappearance of pain. PatientsÕ need to Òpreserve a modicum of painÓ(361) can be understood psychodynamically as a way to maintain a sense of the self that is separate from the environment and to protect the self against the prospects of existential disintegration. Patient stoicism or need to maintain the same level of pain, at a more conscious level, could be a way to avoid excessive sedation or the other side effects of analgesia or the fear of becoming addicted to narcotics. Since patients are generally reluctant to volunteer their complaints of pain (358), physicians need to assess for pain on a routine basis. A clinical interview can be helpful in determining the cause of pain. Patients with intact cognitive function can translate pain perceptions into verbal communication. The vocabulary used to describe nociceptive pain differs from that for neuropathic pain. Table 19.25 highlights this distinction.

Just as the quality of pain is helpful in determining its etiology, so is the location of the pain. Pain can be focal or referred. Referred pain can follow a dermatomal distribution, as does postherpetic pain. Many physicians rely on non-verbal expressions of pain more than what the patient communicates verbally. Non-verbal cues include crying, moaning, yelling, grimacing, splinting, rubbing, insomnia, anergia, anorexia, apathy, anxious mood, dysphoric mood, hypertension, tachycardia, and diaphoresis. While non-verbal signs may be useful in persons with dementia, delirium, depression, or aphasia, they should not be

TABLE 19.25. *Description of pain and etiology*

Nociceptive pain	Neuropathic pain
Soreness	Unfamiliar sensations (dysesthesias)
Aching	Numbness
Stabbing	Tingling
Sharp	Electrical
Dull	Pins and needles (paresthesias)
Throbbing	Extreme burning pain on light touch (allodynia)
Crushing	Burning
Pressure-like	Itching
Crampy	

necessary to validate or supersede verbal complaints of pain. Cancer pain research indicates (331,362) that nonverbal cues are no more accurate than and are no substitute for a patientÕs verbal report of pain.

Pain-assessment scales can be used both for research purposes and to enhance the clinical pain interview. They are particularly useful in persons with HIV dementia, who may have difÞculty describing the intensity, location, or chronology of acute or chronic pain (363). The Wisconsin Brief Pain Inventory (BPI) has been used in clinical research for persons with cancer and AIDS (340,364,365). It helps to determine localization and intensity of pain. It also measures adequacy of analgesia and the impact of pain on quality-of-life issues such as activity, mood, ambulation, work, relationship, sleep, and enjoyment.

Treatment Strategies

Practice Guidelines for Pharmacologic Treatment of Pain

Pain is dramatically undertreated in persons with HIV infection (333,334,366). Iatrogenic addiction to narcotics is rare (365,367). Nevertheless, the most common misconception that interferes with the use of adequate analgesia in persons with AIDS is the myth that persons with severe illness and acute or chronic pain syndromes will become addicted to narcotics. Pain treatment is complicated by myths and misconceptions:

1. Patients with pain will become addicted to narcotics.
2. Opioid analgesics at recommended doses frequently cause respiratory depression.
3. Neuropathic pain does not respond to treatment.
4. Opioids should be prescribed on an Òas neededÓ basis, rather than as standing orders.
5. Patients on methadone maintenance need lower doses of analgesics.
6. Methadone and meperidine are potent analgesics.
7. There is a ceiling dose for opioids.
8. Duration of analgesia is the same as the analgesicÕs half-life.

Acute and chronic pain is best treated on an Òaround-the-clockÓ (ATC) schedule, rather than on an Òas neededÓ or Òp.r.n.Ó basis (344,368), regardless of its etiology. ÒAs neededÓ orders for analgesia result in inadequate pain control when the length of time between doses is longer than the duration of the analgesic action of the medication ordered. The importance of routine around-the-clock orders for pain management in AIDS has been described (1). It is stressful and depressing to be in pain, but it is even more upsetting to be in pain and repeatedly be faced with the humiliation of having to ask or beg for pain medication (1). As-needed orders should be reserved for the treatment of breakthrough pain in individuals who are on an around-the-clock regimen of analgesia.

The World Health Organization (WHO) recommends a stepwise approach to analgesia for patients with cancer (369). The ÒWHO analgesic ladderÓ has been accepted by oncologists (331) and other clinicians caring for persons with AIDS (342,344). The WHO guidelines recommend a three-step process to achieve freedom from pain. Step 1 includes prescription of a nonopioid, with or without an adjuvant. If pain persists, Step 2 follows with the prescription of a weak opioid, with or without an adjuvant. If pain continues after this, then Step 3 suggests prescription of a strong opioid, with or without a non-opioid with or without an adjuvant (369). Table 19.26 summarizes the weak opioid analgesics, Table 19.27 summarizes the strong opioid analgesics, and Table 19.28 lists adjuvant agents.

Common mistakes that interfere with adequate analgesia include the clinicianÕs hesitancy to move up the analgesic ladder when the patientÕs complaints of pain persist. When moderate to severe pain is evident upon initial pain assessment, Step 1 of the analgesic ladder can be bypassed and the patient can be started on a weak or strong opioid. The WHO analgesic ladder is more effective

TABLE 19.26. *Weak opioid analgesics*

	Dose[a]	Frequency[b]	Route of administration
Codeine	30–90 mg	Q4–6 h	Oral
Oxycodone	10 mg	Q4 h	Oral
Hydrocodone	10 mg	Q4 h	Oral
Methadone	30 mg	Q4–6 h	Oral

[a] Recommended starting doses; doses in this table are equianalgesic and equivalent to 10 mg of morphine.
[b] Frequency of administration is determined based on duration of analgesia and not on half-life.

TABLE 19.27. *Strong opioid analgesics*

	Dose[a]	Frequency[b]	Route of administration
Fentanyl	100 mcg/h	Q48–72 h	Transdermal patch
Morphine sulfate	30–60 mg	Q12 h	PO, sustained release capsules
Morphine sulfate	30 mg	Q3–4 h	PO, elixir
Morphine sulfate	10 mg	Q4 h	IV
Hydromorphone	3 mg	Q3–4 h	PO

[a] Recommended starting doses; doses in this table are equianalgesic.
[b] Frequency of administration is determined based on duration of analgesia and not on half-life.
PO, orally; IV, intravenously.

TABLE 19.28. *Adjuvant analgesics*[a]

Antidepressants	Steroids	Other
Amitriptyline	Dexamethasone	Colchicine
Nortriptyline	Prednisone	Levamisole
Imipramine		Thalidomide
Desipramine	*Antiarrhythmics*	Proglumide
Doxepin	Mexiletine	Clonidine
	Tocainide	Baclofen
Anticonvulsants		Methocarbamol
Clonazepam	*Topical Anesthetics*	
Gabapentin	EMLA cream	
Carbamazepine	Capsaicin cream	
Antihistamines	*Neuroleptics*	
Hydroxyzine	Phenothiazines	
	Butyrophenones	
Stimulants		
Methylphenidate	*Calcium blockers*	
Pemoline	Nifedipine	

[a] Adjuvant analgesics may be used as rst-line drugs for the treatment of peripheral neuropathy.

for treatment of nociceptive pain than for treatment of neuropathic pain. Neuropathic pain responds better to adjuvant or coanalgesic agents such as tricyclic antidepressants and anticonvulsants. These agents are appropriate as Þrst-line drugs for the treatment of HIV sensory neuropathy or postherpetic lancinating pain (342,344,351).

Special Vulnerabilities to Analgesics

Nonsteroidal anti-inßammatory agents are relatively contraindicated in persons with AIDS who have speciÞc complications. These include: thrombocytopenia, bleeding diatheses, renal insufÞciency, renal failure, hepatic failure, congestive heart failure, peripheral edema, ascites, and abdominal pain. Although acetaminophen is widely prescribed for pain in persons with AIDS, it is hepatotoxic in persons with wasting, hepatic insufÞciency, and those who are on multiple medications metabolized by cytochrome p450 (phase 1 reaction) and glucuronidation or sulfation (phase 2 reaction) (370).

When antidepressants are prescribed for pain in persons with AIDS, the higher doses needed for analgesia can result in anticholinergic toxicity. Although amitriptyline is often used for neuropathic pain, nortriptyline is just as effective, has fewer anticholinergic side effects, and can be tolerated in higher doses in persons with AIDS. We do not recommend routine use of amitriptyline for neuropathic pain and have found that any tricyclic antidepressant is as efÞcacious as amitriptyline and is associated with fewer side effects in comparable or higher dose ranges. Although the sedating side effect of amitriptyline is often cited as a beneÞcial effect, all of the tricyclics have some sedative properties and, in adequate doses, can lead to freedom from pain, enabling persons with AIDS to sleep more soundly. Carbamazepine, hydroxyzine, and phenothiazines also have signiÞcant anticholinergic effects and can worsen confusion in persons with dementia and delirium. Stimulants lower the seizure threshold and can produce agitation, psychosis, anorexia, and insomnia in persons with AIDS. Steroids can cause mood elevation, mania, psychosis, and depression.

Meperidine is contraindicated as an analgesic for persons with AIDS. Its metabolite, normeperidine, is neurotoxic and can lead to confusion, tremors, and seizures. Although fentanyl transdermal system provides excellent analgesia in persons with AIDS, it has been

associated with delirium, although rarely. The absorption of transdermal fentanyl can be erratic in the febrile patient.

Non-Pharmacologic Treatments for Pain

Although most pain syndromes associated with AIDS respond to pharmacotherapy, other analgesic modalities should be considered as adjuvant therapies or in specific clinical situations. Surgical anesthetic procedures have been used extensively for the treatment of severe or intractable nociceptive and neuropathic malignant pain (331,345). Examples include nerve blocks and epidural anesthetic infusions of opioids. Jonsson and colleagues (371) described the successful treatment of intractable dysuria and vesical pain in a person with AIDS and interstitial cystitis. Analgesia was achieved with intrathecal subarachnoid morphine infusion, rather than with the more conventional epidural route of administration.

Radiation therapy aimed locally at painful cutaneous Kaposi's sarcoma (KS) lesions can provide adequate analgesia (372). Neurological stimulatory approaches can be useful in the treatment of neuropathy in some persons with AIDS. Among the techniques studied, acupuncture (373) and transcutaneous electrical nerve stimulation (344,374) alleviate pain in persons with HIV sensory neuropathy. Other nonpharmacological analgesic treatments studied in noncontrolled populations of persons with AIDS with reported relief, include massage therapy (375), use of support stockings for neuropathy (344), and aromatherapy (376).

Psychotherapeutic modalities can be useful in treating the anxiety and depression that often accompany incapacitating pain. Group therapy has been shown to reduce the perception of pain in persons with metastatic breast cancer (299–301), malignant melanoma (296–297), and AIDS (377–379). Supportive psychotherapy, cognitive psychotherapy, behavioral therapy, relaxation therapy, and hypnosis are effective as adjunctive interventions to reduce pain.

Treatment of Pain in Drug-Dependent Persons with AIDS

Drug addiction can be defined as compulsive drug-seeking behavior that interferes with judgment and social functioning, coupled with the development of tolerance due to repeated drug use and withdrawal upon the discontinuation of drug use. Drug-seeking behavior is primarily a result of drug withdrawal and secondarily a function of the development of tolerance. When drug-dependent persons with AIDS complain of pain, the clinician needs to take into account these variables. Manipulative and sociopathic behaviors diminish when pain is adequately treated in persons with AIDS and drug dependence. Clinicians often feel that drug addicted patients manipulate doctors and the healthcare system in order to obtain narcotics. Persons with AIDS who also complain of pain rarely lie or exaggerate their need for analgesics. Persons with HIV infection and opioid dependence have a higher tolerance for narcotics and need higher doses of potent analgesics to adequately treat their nociceptive pain (380). The prevalence of pain in persons with AIDS does not differ when populations of injecting drug users are compared with non-drug users (335,344, 365). However, the authors have found that pain is greatly undermedicated in drug-dependent persons with AIDS.

Pain control in persons with either a history of drug dependence or who are active drug users can be adequately achieved by setting aside unnecessary concerns or hesitations. With previously addicted patients who are now currently drug free, clinicians should follow the same guidelines for administration of analgesics that are recommended for persons who have never been addicted. For those patients who express concerns about use of opioids for pain relief, it is helpful to reassure them that adequate pain relief will not lead to relapse. Clinical research has shown that former drug users who have HIV infection do not have increased complaints of pain or greater need for opioids than those without addiction history (381). Injecting drug users with HIV infection and pain do not exaggerate their complaints and need dual treatment for addiction and pain. Agitation and behavioral dyscontrol result from both drug withdrawal states and severe pain. Agitation is not to be dismissed as manipulative behavior but be understood as an extreme manifestation of physiological distress. Injecting drug users and persons on methadone maintenance as agonist therapy for drug dependence have a high tolerance for opioids. For patients not on methadone maintenance but in heroin withdrawal, as is the case in many acute care settings in inner city hospitals, a methadone detoxification protocol is effective in treating heroin withdrawal. Guidelines for treatment of heroin withdrawal are summarized in Table 19.4.

When a person with AIDS and pain is maintained on a standing dose of methadone or treated with methadone for heroin withdrawal, the pain should be treated as a separate problem (345). The patient's methadone dose should not be thought of as analgesia, but rather as agonist therapy for relapse and withdrawal prevention. Adequate doses of weak or strong opioids should be prescribed as if the individual were not already on an opioid, even if the methadone dose is high. Methadone for relapse prevention will target opioid tolerance needs and prevent withdrawal but will not provide analgesia for pain.

CREATION OF A SPECIAL HEALTHCARE ENVIRONMENT FOR PERSONS WITH AIDS

Anyone with a severe or potentially fatal illness needs a non-judgmental, nurturing, loving environment throughout

the course of the illness. The families, loved ones, and caregivers are also in need of a special kind of understanding, gentle support, and availability. Persons with AIDS are more vulnerable because of the epidemics of fear, discrimination, ignorance, and therapeutic nihilism. They need an unconditionally loving ego-supportive environment. They need psychological, medical, spiritual, and social support. Creation of a supportive environment leads to better education, behavior change, and improved care.

Healthcare environments are not necessarily supportive even if they are designed to be. An elegant example is a controlled study (382) of the effect of operating room temperature on rate of infection, duration of healing, and length of post-operative hospital stay. The study indicated that the chilly temperatures (65¡F.) maintained to prevent heavily gowned and gloved surgeons and surgical support staff from perspiring under hot operating room lights resulted in lowering of surgical patientsÕ body temperatures while under general anesthesia. By warming intravenous ßuids administered during surgery and providing blankets, Kurz and colleagues found that the patients with the warmer environment maintained their normal body temperatures and had lower rates of wound infection, shorter lengths of postoperative stay, and faster rates of wound healing. The warming of the operating room environment was efÞcacious in decreasing both morbidity and cost of care.

Warming of the healthcare environment for persons with any illness is important, but it is crucial when the illness is associated with sex, drugs, infection, and death and has led to epidemics of fear and discrimination (1). One way to warm the environment is to create a multidisciplinary team to provide comprehensive compassionate care. The multidisciplinary team approach has been accepted in the medical community in speciÞc clinical settings. In general care and ambulatory settings, the team approach is familiar in the care of persons with cancer, end-stage renal disease, addiction, chronic pain, dementia, and eating disorders. The multidisciplinary team is particularly relevant for the HIV epidemic. Persons with AIDS are vulnerable not only to immunosuppression and opportunistic infections, but also to cancer, end-stage multiorgan disease, addiction, chronic pain syndromes, dementia, and even anorexia and malnutrition. The establishment of multidisciplinary teams to provide comprehensive compassionate care to persons with AIDS has been described as a way to combat the epidemics of AIDS and AIDSism (1). To provide a warmer environment for persons in the HIV epidemic is a major challenge; we would like to provide guidelines for creating this environment and overcoming obstacles to care.

Medical Guidelines

The care of persons with AIDS can be rewarding for physicians. When orientation, education, resources, and ongoing support are available, institutions, physicians, and persons with AIDS beneÞt. When a physician can get to know a patient with AIDS as a person, doctor and patient can develop a supportive relationship that diminishes the barriers of discrimination and AIDSism. The hallmark of this relationship is a nonjudgmental and loving connection between the doctor, patient, and the family. It is through this relationship and the use of participatory decision-making (**38?**,384) that the foundation for trust and climate for risk reduction can be established. A basic understanding that the physician will be there to provide competent care, respect the patientÕs wishes, enable participatory decision-making, and provide relief from pain and suffering, is therapeutic and diminishes requests for physician-assisted suicide.

Psychologic Guidelines

Recognition and treatment of depression, delirium, dementia, and substance-related disorders provide comfort for patients, family, and staff in the AIDS epidemic. Although some persons with AIDS have no psychiatric disorders, everyone with a risk behavior has anxiety, and nearly everyone with AIDS or other manifestations of HIV infection is under stress, anxious, sad, fearful, and even suicidal at times. It is helpful to incorporate consultation-liaison psychiatric services into both the function and leadership of comprehensive multidisciplinary programs, to provide a biopsychosocial approach and support for staff. It is especially important for psychiatric staff to take the lead in providing a loving, compassionate, and nurturing approach to care.

Spiritual Guidelines

The importance of the spiritual dimension in the comforting and healing process cannot be overemphasized. For patients who have strong religious support networks, the process of living and dying with AIDS becomes easier. For those who do not, introducing a spiritual support system may make a difference in providing solace and comfort care. Chaplains should be available in institutional settings and spirituality should be a part of an ongoing dialogue in the ambulatory setting. It is important to explore each individualÕs spiritual and philosophical belief system and to address these issues at his or her level of understanding and frame of reference (385).

The spiritual aspect of care must be entirely nonjudgmental, providing only solace and comfort, without introduction of rigid or dogmatic attitudes. It is important to integrate chaplaincy into the multidisciplinary team for a reciprocal teaching and learning process to ensue.

Ethical Guidelines

A supportive healthcare environment must ensure access to care, freedom from discrimination, and right to self-determination. Each personÕs right to autonomy and dignity must be respected throughout the course of HIV-related illness, from diagnosis to death. Ethical decision-making centers around the soundness of medical decisions by balancing beneÞcence against nonmaleÞcence. Autonomy requires that patients with capacity have the right to choose among medically indicated procedures or treatments and to refuse unwanted care.

ConÞdentiality

Our patients have taught us, all too poignantly, how important conÞdentiality is. An individual who is struggling with as devastating an illness as AIDS deserves not only humane care but also conÞdentiality. This is especially important in a society in which individuals may be discriminated against because they have AIDS. It is crucial that a patientÕs rights to privacy and conÞdentiality remain protected in the hospital setting, as well as in the community. There are several difÞculties with this because of the nature of the illness. Universal precautions should be used for all patients and in all healthcare settings. Education about universal precautions and conÞdentiality can combat discrimination. These concepts should be part of ongoing education as well as initial orientation for all hospital and healthcare employees.

Paradoxically, however, the issue of conÞdentiality has yet another aspect. Sexual partners of persons with HIV infection are at risk for AIDS. A person with HIV infection should be encouraged to inform a partner or potential partners and should be taught methods of risk reduction. Physicians should obtain consent from a patient to inform partners or trace contacts. This becomes further complicated when infected persons are illegal aliens or fugitives. In general, most people with AIDS are eager to inform their partners and to take whatever precautions necessary to prevent the spread of the illness. Unfortunately, not all infected persons have the courage, wisdom, or cognitive capacities to enable them to cooperate in this way. Issues of conÞdentiality can be exquisitely painful and may not always have simple answers.

Employees or Students with HIV Infection

Employers need to develop policies in order to prevent discrimination against employees with HIV infection. When the employers are hospitals and medical schools, AIDS policies need to emphasize conÞdentiality. The rights of employees to conÞdentiality must be protected. Employees or students with AIDS should be treated in the same way as all other employees. Persons with AIDS should be allowed to attend school or work until severe illness, disability, incapacitation, or death intervenes. The burdens and losses associated with AIDS are painful enough. There is no reason to add more losses. Employees with AIDS within our hospital systems have worked for as long as they were able to and left work only because they chose to do so. This policy was reinforced by the support of both the medical staff and administration. The preservation of a job enables a person with AIDS to continue to support himself or herself Þnancially, to maintain dignity and self-respect, and to have a sense of purpose. If the patient is also supporting a family, they too will be protected.

Ethics of HIV Testing

The HIV test is a test for antibodies to HIV. Although most individuals develop antibodies and become HIV seropositive within six weeks to six months of initial exposure, in a small fraction of cases, the time period may be longer. This latency period can present a public health hazard by falsely reassuring an individual who is infected that he or she is not. On the other hand, there are also rare false positives that may lead to unnecessary anxiety in an individual who is, in fact, uninfected.

HIV testing should be voluntary and anonymous, and it should not be used to screen prospective employees, insurance applicants, marriage license applicants (386), or students. HIV testing should not be done without both pre- and post-test counseling and written informed consent (387). ConÞdentiality of HIV test results should be carefully maintained and should not be disclosed without a speciÞc written release of information.

Early detection of HIV seropositivity can lead to early prophylactic medical intervention, prolong life, and prevent transmission of HIV (388). Voluntary anonymous testing of individuals with risk behaviors should be encouraged.

The availability of home AIDS test kits provides greater access to conÞdential testing. The beneÞts are complete conÞdentiality and easy accessibility. Ready access to testing is important because of the availability of antiretrovirals to decrease HIV transmission in pregnancy (389). It is also important because persons who are diagnosed early may beneÞt from HIV medical care and antiretroviral therapy. However, the beneÞts of home testing must be balanced against the vulnerability of individuals to anxiety, depression, and suicide. Testing is a time of crisis and turmoil and anonymous telephone counseling is far from adequate, even if it includes medical referrals. The availability of home AIDS testing kits should signal physicians and other clinicians the importance of providing HIV counseling and testing to patients with whom an ongoing relationship can help provide the necessary support and mobilize a family or social support network.

Ethical Issues and Advance Directives

The person with HIV infection needs to be able to have a way of maintaining dignity and humanity from the day of diagnosis until the day of death. Caregivers need to begin discussions early, while an HIV seropositive person is healthy and can plan for his or her future. A healthy seropositive pregnant woman needs to be able to discuss her choice of a surrogate parent for her child, should she become ill, disabled, or die. All HIV seropositive individuals should be encouraged to designate someone whom they trust to act on their behalf and to make medical decisions for them, should they no longer have the capacity to make them for themselves. The advance directives can be documented in the form of a living will or medical durable power of attorney. The designation of someone trusted can be codiÞed in a healthcare proxy, which is a document designating a healthcare agent. The process of making choices about advance directives should be part of a three-way discussion among the HIV seropositive individual or person with AIDS, his or her primary physician, and the healthcare agent. The discussion may involve the patientÕs philosophy of life and death, belief systems, and resolution of conßicts about some of these difÞcult issues. The discussion should include all forms of decision-making, including medical care and end-of-life decisions such as do-not-resuscitate (DNR) orders and foregoing life-sustaining treatment.

Ethical Issues Involved with Foregoing Life-sustaining Treatment

The evaluation of whether life-sustaining treatment should he initiated, maintained, foregone, withheld, or withdrawn depends on the values and preferences of the patient. Life-sustaining treatment is deÞned as mechanical ventilation, renal dialysis, chemotherapy, antibiotics, and artiÞcial nutrition and hydration. Patients need to understand both the risks and beneÞts of artiÞcial nutrition and hydration (390). They need to understand that tubes can be uncomfortable, that central lines can become infected, that intravenous lines can inÞltrate. Even though we advocate use of no mechanical restraints, the lines, tubes, and intravenous ßuid poles serve as tethers and anchors and tend to immobilize patients, leading to a Òcascade to dependencyÓ described by Creditor (391). No distinction is made between withholding, withdrawing, or foregoing life-sustaining treatment. There are also some indications that when artiÞcial hydration and nutrition are withheld, individuals may not be uncomfortable because of endogenous opioid secretion, as well as administration of analgesics for pain. Physicians have an obligation to relieve pain and suffering and promote the dignity and autonomy of dying patients in their care (390). This includes providing effective palliative treatment even if it may foreseeably hasten death (390). Providing a model for comfort, care, and a nurturing supportive environment includes changing many traditional healthcare practices and ensuring participatory decision-making styles.

Ethical Issues Involved with Determination of Capacity for Decision-making

Every individual should be presumed to have the capacity for decision-making and the right to self-determination. Every patient should be able to decide whether to stay in a healthcare facility, whether to accept medical care, refuse procedures, or sign out against medical advice (AMA). When it appears to the physician that the patient is unable to understand or reason about his or her medical care, the physician can make a determination of decisional capacity. This determination is an assessment of a personÕs capabilities for understanding, communicating, and reasoning about speciÞc issues. Mental illness per se, including dementia, schizophrenia, or depression, does not necessarily preclude decision-making. Any attending physician can determine decisional capacity. If an attending physician Þnds it difÞcult to make a determination, he or she can call upon a psychiatrist to assist. Guidelines for determination of decision-making capacity are summarized in Table 19.29.

One of the most frequent concerns in the healthcare setting is whether or not an individual has the capacity to decide about DNR. The patient with HIV dementia may still be able to decide about DNR. In order to be able to give informed consent for DNR or no emergency cardiopulmonary resuscitation (CPR), an individual must be able to understand the following:

1. The general nature of the illness, its severity, and implications.
2. The meaning and implication of respiratory or cardiac arrest (Òif my lungs or heart stop, I will dieÓ).
3. The distinction between CPR in an otherwise healthy person who has sudden trauma or arrhythmia and CPR in end-stage illness with severe compromise of health, immunity, organ function, and cognition.
4. The nature of CPR and the need for mechanical ventilation, intubation and external cardiac massage.
5. The consequence of a DNR order.
6. The fact that an individual can change his or her mind about DNR.
7. A DNR order does not imply withholding or withdrawing care. Those are entirely separate issues.

An individual with late-stage AIDS and HIV dementia demonstrated his capacity to understand the implication of a DNR order, in the face of cardiopulmonary arrest, by stating ÒItÕs goodbye, Charlie.Ó

Understanding issues of decisional capacity in individuals with HIV dementia can help preserve the delicate balance between safety and autonomy in the care of persons with AIDS.

TABLE 19.29. *Guidelines for determination of decisional capacity*

Who can determine decisional capacity?
- Any physician can determine decisional capacity.
- If a physician is unable to make the determination, it is appropriate to consult with a psychiatrist for assistance in the assessment.
- No psychiatric assessment is necessary if the primary physician can make the determination.
- This concept is in accord with both patient's right to self-determination and generally accepted bioethic principles.

Competency vs. Capacity
- Although the terms are often used interchangeably and have similar meanings, they are differentiated in ethics terminology.
- Capacity is a clinical determination while competency is a legal determination.

Myths about Capacity for Decision-Making
- Mental illness precludes decision-making.
- History of mental illness precludes decision-making.

Lack of decisional capacity in one area precludes decision making in another area.

Facts about Capacity for Decision-Making
- Psychopathology per se does not preclude decisionmaking.
- A person with dementia, schizophrenia or depression can make decisions unless the psychopathology interferes with the process, as in the following examples:
  - Severe depression preventing an individual from deciding to consent to care because of a clear wish to commit suicide in response to command hallucinations.
  - Severe dementia preventing an individual from understanding the illness or its treatment.
  - Schizophrenia incorporating treatment into a delusional system preventing an individual from being able to understand the illness.
- Determination of capacity for decision-making is highly speci c.

Types of Decisional Capacity
- Decisional capacity for treatment planning
- To give informed consent for a procedure
- To refuse procedures
- Decisional capacity for discharge planning
- To sign AMA
- To participate in discharge planning
- Decisional capacity for advance directives
  - To decide about DNR
  - To designate a Health Care Agent

Decisional Capacity for Other Issues
- To care for a newborn
- To handle nances
- To testify in court-testimonial capacity
- To write a will-testamentary capacity

Examples of Decisional Capacity

To give informed consent for lumbar puncture an individual must understand:
- The nature of the illnesses suspected-meningitis or infection of the brain lining.
- The nature of the procedure-a needle introduced into a space between the bones in the spine to get some uid out to test for what is wrong.
- The risks of the procedure.
- The bene ts of the procedure.
- The consequences of not having a lumbar puncture when meningitis is suspected.

To participate in discharge planning the individual must be able to understand:
- The nature of the illness or illnesses.
- The extent of his or her disability.
- The need for treatment and types of assistance required.
- The need for shelter, clothing, and speci c dietary requirements.
- How to obtain medical care.
- How to direct home help.
- How to negotiate activities of daily living.

To handle nancial affairs the individual must be able to understand:
- The nature of his or her assets.
- Where to keep assets.
- How to access assets.
- The nature of liabilities.
- Whom he or she would trust with information about resources.
- Whom not to trust and how not to become a victim of designing persons.

Each determination is speci c for the type of decisional capacity being evaluated and no blanket determination of decisional capacity is valid.

CONCLUSIONS

Persons with AIDS are living longer, healthier, lives because of good medical care, antiretroviral therapies, and prophylaxis for some of the previously fatal complications such as PCP (392). In order for persons living longer to live more comfortable lives, with preservation of independence and dignity, it is important to establish special nurturing, supportive, and loving healthcare environments. The ÒwarmingÓ of the internal and external environment for persons with AIDS can be accomplished through programs of education about psychiatric care, pain management, and decision making capacity. These special healthcare environments can enable persons with AIDS, their loved ones, and caregivers to meet the challenges of AIDS with optimism and dignity.

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Chapter 20

The Gastrointestinal and Hepatobiliary Systems in HIV Infection

Donald P. Kotler and Pierre M Gholam

Gastrointestinal (GI) dysfunction is common in HIV-infected individuals, with or without AIDS, as it is in other immune deÞciency disorders (1). The specialized functions of mucous membranes, such as nutrient absorption by enterocytes or gas exchange by pulmonary epithelium, require intimate contact with the external environment but render them vulnerable to pathogens. These conßicting needs, i.e. having intimate contact while remaining a barrier to the external environment, promoted the evolution of complex immunological and non-immunological defenses.

Gastrointestinal involvement in HIV infection and AIDS has serious consequences. Chronic enteropathy is associated with increased mortality (2). Progressive malnutrition, which may be associated with intestinal disease, is an independent predictor of death in patients with AIDS (3Ð5). Chronic diarrhea has been associated with diminished quality of life (6). Despite impressive advances in the treatment of the underlying viral infection, chronic diarrhea remains a source of morbidity in HIV-infected patients. In addition, new therapies have been associated with drug-induced toxicities that target the gut and liver.

The aim of this chapter is to describe the effects of HIV and AIDS upon the GI tract and the liver. Diagnosis and management are organized into a series of clinical syndromes.

MUCOSAL HIV INFECTION

The immune defenses of the mucous membranes are linked as a common mucosal immune system. The gut-associated lymphoid tissue is composed of lymphoid aggregates in the tonsils, PeyerÕs patches, and mucosal lymphoid follicles, plus diverse immunologically active cells in the lamina propria. Different subpopulations of mononuclear cells are found in the lamina propria and in the epithelium. The mucosa contains elements of both humoral (secretory) and cell-mediated immunity. The non-immunological defense system includes gastric acid, salivary, pancreatic and biliary secretions, secreted mucus, and intestinal motility. A stable lumenal ßora also contributes to homeostasis (7).

Mucosal immunity in HIV infection has received relatively little study. Studies have demonstrated decreases in CD4+ lymphocytes in intestinal mucosa, plus an increase in the population of suppressor T cells (CD8+) (8). Depletion occurs early in the disease course (9,10). Lamina propria lymphocytes, as opposed to macrophages, may be the cellular target for HIV-1 infection in intestinal mucosa. Lamina propria lymphocytes express CD4, plus chemokine receptors that are secondary receptors for HIV, including CCR5 and CXCR4 (11). In contrast, lamina propria macrophages express CD4 but not CCR5 or CXCR4. The reduced permissiveness of macrophages to HIV-1 appears to be due to the near absence of surface CCR5 on resident intestinal macrophages. Impaired permissiveness of intestinal macrophages to HIV-1 may play an important role in the low prevalence of HIV-1 mRNA-expressing macrophages in the lamina propria during HIV-1 infection *in vivo*.

Very few studies have examined speciÞc immune functions in the GI tract of HIV-infected individuals. Eriksson demonstrated normal responses to oral vaccination with cholera toxin B subunit (12) in HIV-infected subjects, which contrasts with studies demonstrating poor systemic antibody responses to tetanus vaccination (13). Evidence of speciÞc anti-HIV activity of lamina propria lymphocytes has been reported (14).

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Many investigators have detected cellular reservoirs for HIV in the GI tract by various methods (15–20). Primary intestinal cell lines and colonic tumor epithelial cell lines have been productively infected with HIV (21). The possible role of HIV in intestinal disease is discussed below. Viral burden can be quantitated in intestinal mucosa. Rectal HIV RNA content was shown to fall to a similar extent as in peripheral blood in response to highly active antiretroviral therapy (HAART) (22).

CLINICAL SYNDROMES

General Principles of Evaluation and Treatment

Proper clinical management of the HIV-infected patient requires an appreciation of differences in disease presentation. Multiple enteric complications may coexist in AIDS patients, reaching almost one third of patients in one study (23). One organism may produce several different clinical syndromes, while many organisms can produce identical clinical syndromes. Disease complications of AIDS are notable for their chronicity, susceptibility to suppression, and resistance to cure, so that treatments must be given chronically. The speciPc pathogens producing disease in AIDS patients are different from those that usually affect immunocompetent individuals. However, HIV-infected individuals also are subject to usual illnesses. In either case, the pathologic features usually match the clinical symptoms and physical Pndings.

Pathologic processes may affect the GI tract with either a focal or diffuse pattern. Focal ulcers may be found anywhere in the GI tract. They may be infectious, noninfectious or neoplastic. Certain viral infections, such as cytomegalovirus (CMV) infection, and fungal infections, such as histoplasmosis, produce multifocal disease. The GI tract also can be involved diffusely. The most common diffuse lesion is candidiasis of the oral cavity and/or the esophagus. The epithelial cell layer of the small intestine is a target for protozoal infestations including *Cryptosporidium parvum*, *Isospora belli*, and the microsporidia, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*. The lamina propria and submucosa may be involved by chronic infections such as *Mycobacterium avium* complex (MAC). Bacteria such as salmonella, shigella and campylobacter may cause a diffuse colitis or enterocolitis. The pathologic processes described above may lead to several types of clinical syndromes: disorders of food intake, dyspepsia, diarrhea, anorectal diseases, tumors, hepatobiliary diseases, pancreatic diseases, gastrointestinal bleeding, and the acute abdomen.

Advances in the treatment of HIV infection with highly active antiretroviral therapy (HAART) has allowed for effective suppression of viral replication, often to undetectable levels, and immune reconstitution. Mortality rates and the incidence rates for many opportunistic infections have fallen dramatically in recent years (24). However, GI problems in HIV-infected patients have not disappeared and continue to provide clinical challenges. There are several reasons. A substantial proportion of HIV-infected subjects are unaware of their infection status, so that underlying HIV infection often is part of the differential diagnosis of diarrhea in a patient not previously known to be HIV-infected. Thus, HIV infection must now be included as part of the differential diagnosis of the causes of chronic diarrhea. In addition, some people are aware of HIV infection but are not currently under medical care, or do not take HAART in spite of immune dePciency. In other patients, viral infection is resistant to available therapies and patients may suffer immune deterioration despite attempts at virologic control. In addition, HAART therapy is available to only a minor percentage of HIV-infected subjects worldwide, and the disease complications of AIDS, including diarrheal illnesses, continue unabated in most developing countries, so that HIV infection must be considered as an underlying disease in immigrants.

Which patients require extensive evaluation is an important question. Many patients develop GI symptoms at some point during the course of the illness, but a large proportion of the episodes are self-limited. Extensive evaluation in all cases is expensive, burdensome, and often unrewarding. Evaluation is mandated for patients whose symptoms are persistent, increasing in severity or associated with fever and/or weight loss. When such symptoms have been present for more than one month, it is likely that they will continue indePnitely, if untreated.

Disorders of Food Intake

Etiology and Pathogenesis

Candidiasis is a very common complication of HIV infection. Candidiasis decreases taste sensation and affects swallowing, in addition to causing oral or substernal discomfort. Most cases are due to *Candida albicans*. Hairy leukoplakia, a hyperkeratotic lesion found along the sides of the tongue or adjacent gingiva, may be mistaken for candida. As opposed to candidiasis, hairy leukoplakia is asymptomatic and does not affect food intake. Severe gingivitis or periodontitis also affect eating. Ulcerations, due to herpes simplex or CMV, and idiopathic ulcers cause pain and interfere with eating. Mass lesions, such as KaposiÕs sarcoma or lymphoma, occur in the oral cavity and can interfere with chewing or swallowing.

The esophagus is affected by the same lesions as is the oral cavity. A particularly striking lesion is the idiopathic esophageal ulcer, which may produce debilitating symptoms (25,26). As opposed to herpetic ulcers, no etiologic agent is found on biopsy of the ulcer. Studies have documented the presence of HIV RNA in these ulcers, though this Pnding is non-speciPc. HIV also has been detected in transient ulcers associated with seroconversion (27).

Patients also may experience diminished food intake in the absence of oral or esophageal disease. Focal or diffuse neurologic lesions can affect food intake, as can speciÞc medications. AIDS patients with malabsorption do not increase food intake sufÞciently to account for calories lost in feces (28). Anorexia also is a feature of systemic infections, and appears to be an effect of altered cytokine release (29).

Diagnosis

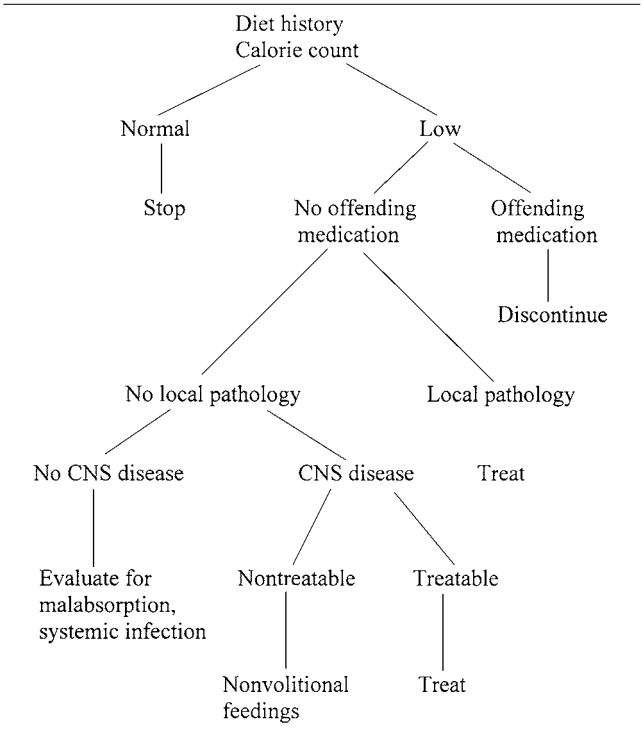
The etiologic diagnosis of disorders of food intake can be approached using a diagnostic algorithm (Table 20.1). Local disease can be discerned from a careful history and physical examination, assisted by ENT or dental consultation. The presence of malabsorption usually is suggested by history (see below) and can be conÞrmed with a small intestinal biopsy or an absorption study. Systemic disease is suggested by fever or other localizing signs and symptoms. The clinician should not conclude that anorexia is due to a medication until other possibilities are ruled out, or the patient responds positively to a supervised trial of medication withdrawal.

Candidiasis is a diffuse lesion in the esophagus and may occur independently of thrush, though the lesion is most ßorid in the soft palate and posterior pharynx. Various clinical forms are recognized including the typical plaque-like thrush, erosive candidiasis, atrophic candidiasis, and angular stomatitis. The diagnosis can be suggested by barium studies (Figure 20.1) and conÞrmed by biopsy or brushings. The lesion is quite distinct from oral hairy leukoplakia, which is found along the sides of the tongue, and, occasionally on the adjacent buccal mucosa, and has a Þssured or serrated border and cannot be scraped from the underlying mucosa. In an AIDS patient with suspected esophageal candidiasis, it is advisable to treat empirically and only evaluate patients with persisting symptoms (30).

All esophageal ulcerations should be investigated by direct examination and biopsy. Herpetic esophagitis presents as groups of small, shallow ulcers containing herpetic inclusions in the epithelial layer. CMV may produce esophageal ulcers with typical CMV inclusions in the ulcer base. No etiologic diagnosis can be reached in a signiÞcant percentage of patients with esophageal ulcers. These ulcers may be large and deep, with undermined edges and a clean base. Bleeding and perforation have occurred.

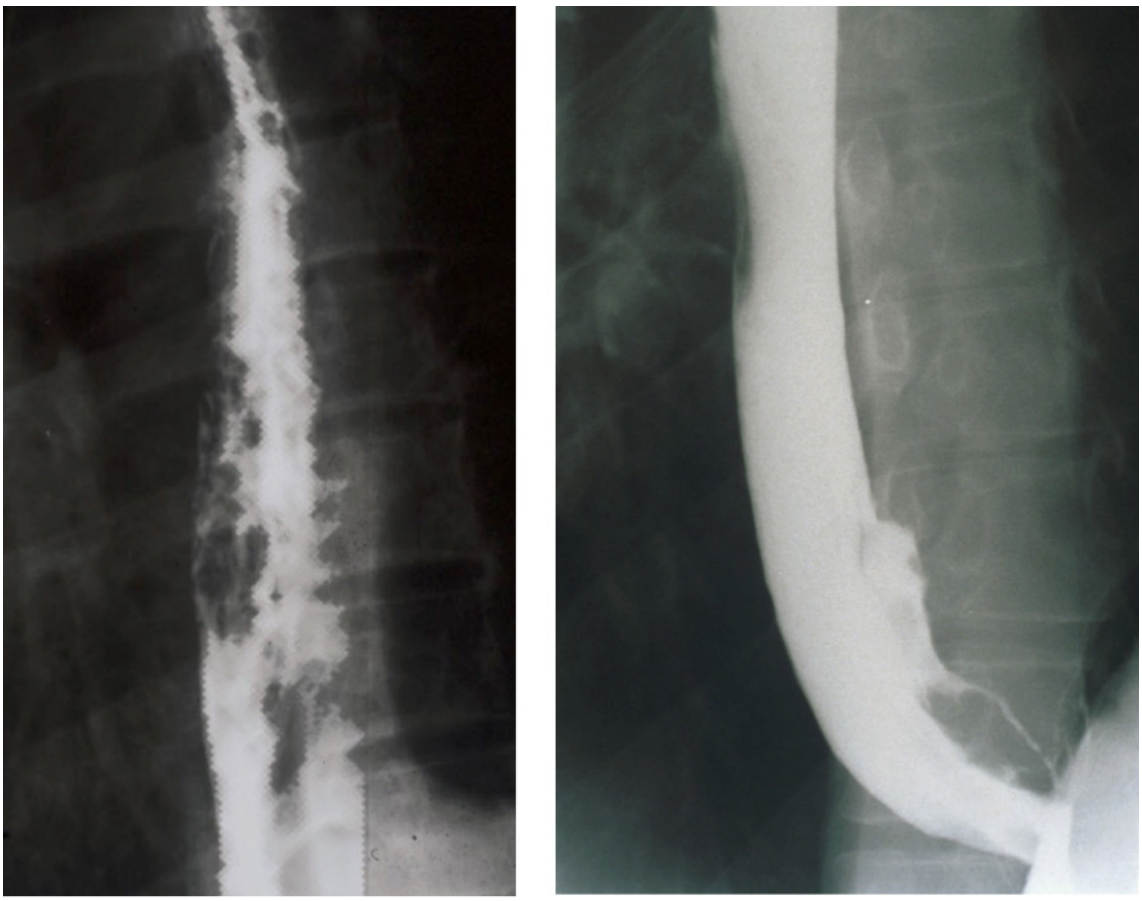
Overall, studies have demonstrated a decline in the incidence of both oral and esophageal disease in subjects on HAART, with the possible exception of HPV-induced lesions.

TABLE 20.1. *Diagnotic algorithm for disorders of food intake*



Treatment

The speciÞc management of eating disorders is based upon the precise cause of the problem. Oral candidiasis responds to a variety of antifungal therapies including the topical therapies, nystatin and clotrimazole, and the systemically active azole drugs. Esophageal candidiasis is best treated using systemically active compounds, since the organism is invasive. The infection may become resistant to azole therapy in some cases, or be due to other yeasts, such as *Candida glabrata* (31), for which intravenous amphotericin B therapy may be required. Treatment options for hairy leukoplakia include acyclovir 800 mg po 5 × /day for two to three weeks (directed to Epstein-Barr virus, the etcologic agent), then 1.2Ð2 g/day, or tretinoin (Retin A) 0.025% or 0.05% solution applied 2 to 3 × /day. Herpes simplex virus infection responds to oral treatment with acyclovir. Discontinuation of therapy after induction therapy and use of maintenance therapy only if there are frequent relapses is recommended. Ganciclovir or foscarnet are effective treatments for CMV esophageal disease (32). Deep, painful aphthous ulcers not due to an identiÞable pathogen may respond promptly to locally injected or systemic corticosteriods (25,26), though the danger of worsening immune suppression in patients with AIDS should be kept in mind. Corticosteroid therapy was associated with an increased risk of developing CMV infection in one study (33). The ulcer also may recur after steroid therapy is discontinued. Studies have shown the effectiveness of thalidomide for the treatment of idiopathic esophageal ulcers. The signiÞcant neurologic and sedating side effects of thalidomide as well as teratogenicity in women have restricted its use to registered physicians and pharmacies.



(a) (b)

FIG. 20.1. (a) Barium esophagram demonstrating diffuse mucosal involvement as a result of candida esophagitis. (b) Barium esphagram demonstrating an idiopathic ulcer plus a stula tract.

Salivary gland enlargement frequently complicates AIDS. The concerning manifestations of this entity are cosmetic appearance, pain from distension and xerostomia. For painful or cosmetically disÞguring cystic lesions, needle aspiration and CT scan will distinguish cystic and solid lesions (34). Biopsy is needed if malignancy is suspected, although most are benign cystic lesions. Fine needle aspirate permits microbiologic analysis and decompression. For xerostomia, sugarless gum and artiÞcial saliva may offer some relief. Pilocarpine may be necessary for refractory cases.

A major complication of oral and esophageal diseases is decreased food intake. Dietary consultation with creative diet planning and choice may be quite beneÞcial in milder cases. Caloric supplementation with formula diets also may be helpful. In patients with local lesions that cannot be treated successfully or in refractory cases of anorexia, some form of non-volitional feeding is required. Nutritional repletion has been reported in response to total parenteral nutrition (TPN) (35) and to enteral feeding regimens (36,37). Nasoenteric tubes can be used, though there are problems with cooperation in long-term use, plus the possibility of precipitating or exacerbating sinus disease. Percutaneous endoscopic gastrostomy feedings are efÞcacious and well tolerated by AIDS patients, and can be continued indeÞnitely.

DYSPEPSIA

Etiology and Pathogenesis

Nausea and dyspepsia are very common symptoms in AIDS but rarely dominate the clinical picture. The stomach may be involved by disseminated infections such as CMV, MAC or fungi, or by tumors. Gastritis due to *Helicobacter pylori* has been found, but its incidence may be lower in HIV-infected than in non-infected patients (38). Some medications, such as nonsteroidal antiinßammatory agents, promote gastric ulceration and produce dyspepsia. Early studies have shown a high incidence of

achlorhydria in AIDS patients (39,40). Achlorhydria was not related to diarrhea or to small bowel bacterial overgrowth in a more recent study (41).

Clinical symptoms are nonspecific. The presence of weight loss or fever implies a serious complication such as a systemic infection or ulcerating tumor. Dyspepsia is due to a low-grade pancreatitis in some patients and may precede the diagnosis of biliary tract disease in others (see below). As in the non HIV-infected person, gastric pathology may be detected even in the absence of gastric symptoms.

Treatment

Symptomatic *Helicobacter pylori* in an AIDS patient is treated in a similar manner to other patients. The diagnosis of CMV or MAC infection is an indication for systemic anti infective therapy. Widespread or ulcerating Kaposi's sarcoma is an indication for systemic chemotherapy, as is the presence of lymphoma. Associated eating disorders should be evaluated and sufficient caloric intake assured.

DIARRHEA AND WASTING

Etiology and Pathogenesis

Alterations in bowel habits are common in HIV-infected patients. Most of the available information relates to the pre-HAART era. Unexplained intestinal dysfunction and injury was an early observation (42), and the term "AIDS enteropathy" had been applied to this group of patients.

Prevalence

The prevalence of enteric pathogens varies greatly in reported series (23,43–48) and has decreased dramatically since the advent of HAART therapy (49,50). The prevalence rates and specific types of enteric pathogens differ in patients seen by primary care physicians and GI consultants due to selection of the most severe and protracted cases for referral, and elimination of those that improve or resolve after routine workup and non-specific or symptomatic treatment. We prospectively evaluated 250 HIV-infected individuals referred for GI symptoms in the pre-HAART era and identified an enteric pathogen in 83% of 141 AIDS patients with diarrhea (23). Two or more coexisting infections were found in 28% of AIDS patients with diarrhea. Thus, AIDS patients with chronic diarrhea have a very high chance of harboring one or more enteric pathogens.

A few studies in the current era have evaluated different diagnostic algorithms including type of endoscopic procedure, specific diagnostic samples, and cost-effectiveness, with no general consensus (51–53).

Small Intestinal Pathogens

Cryptosporidiosis

Cryptosporidium parvum, a coccidian related to *Toxoplasma gondi*, is a well recognized etiologic agent for chronic diarrhea (54). The illness is self-limited in immune competent patients but may be protracted in AIDS patients. The clinical course in AIDS is variable based upon immune function (55) and ranges from spontaneous remission to persistent, debilitating disease. A histopathologic study demonstrated variation in the intestinal location of cryptosporidiosis in AIDS (56). Most patients (65%) had diffuse small intestinal involvement while a smaller percentage of patients have cryptosporidial ileocolitis, without evidence of jejunal disease. Patients with small intestinal localization had more severe histopathology, malabsorption, more often received parenteral hydration or nutritional support, and had significantly shorter average survival. The factors underlying the variation in disease location are unknown.

Microsporidiosis

Microsporidia are primitive protozoa only recently recognized to infect man, though they have long been known as pathogens in animals. Two species have been identified in intestinal biopsies from AIDS patients, *Enterocytozoan bieneusi* (57) and *Encephalitozoon intestinalis* (58). While *E. bieneusi* infection is limited to enterocytes, *E. intestinalis* has been shown to disseminate. Prevalence rates in different population groups range from 2–50% (23,45,46,59–61). *E. bieneusi* has been detected in immunocompetent individuals with a self-limited diarrheal illness (62). This observation implies that human infection may be widespread and could be a significant cause of community-acquired or traveler's diarrhea.

Little is known about immunity to *E. bieneusi* infection. Resistance to Encephalitozoon infection is related to the presence of interferon-secreting lymphocytes (63).

Other Protozoa

Isospora belli has been reported frequently in AIDS patients from Haiti and West Africa, though fewer cases have been seen in the continental United States (54). The symptoms are similar to those of cryptosporidiosis. Organisms may be rare in stool specimens and in biopsies, so the diagnosis may be missed.

Giardia lamblia is not a common cause of acute diarrhea in AIDS. Drug therapy with quinacrine or metronidazole is indicated if cysts or trophozoites are found, though suspicion of other etiologies should be high. Like giardiasis, amebiasis is not a common cause of severe illness in HIV-infected patients. In the majority of cases,

Entamoeba histolytica appears to act as a commensal organism. While therapy should be given, the possibility of coexisting pathogens should be considered. Other parasites have been reported in AIDS patients, including *Strongyloides stercoralis*, which may produce a hyperinfection syndrome. *Blastocystis hominis*, which is felt by some authors to be an enteric pathogen, has been found in HIV-seropositive people with diarrhea.

Enteropathogenic Bacterial Infection

A syndrome of chronic diarrhea and malabsorption associated with bacterial-epithelial cell adherence and damage has been recognized (64–65). The infection is localized to the terminal ileum, cecum and colon. It is unclear if the same mechanism applies in AIDS patients, or if the immune deficiency renders the mucosa vulnerable to "non-pathogens." Alternatively, the problem might result from community-acquired, low-grade intestinal infection that cannot be cleared due to the severity of the immune deficit.

Enterocolitis

Cytomegalovirus

Disseminated CMV infection is a frequent and serious complication in AIDS and can present as a variety of gastrointestinal syndromes including oral and esophageal ulcers, esophagitis, gastritis, isolated intestinal ulcers, terminal ileitis, spontaneous intestinal perforation and focal or diffuse colitis. Colitis is the most common GI problem produced by CMV in AIDS patients (66). The major cell affected is the vascular endothelial cell. Clinical disease occurs as a result of vasculitis and presents as focal or multifocal ulcerations that become progressively diffuse. Colonic perforation, "toxic megacolon" or progressive wasting with refractory diarrhea are possible clinical outcomes in untreated cases.

Mycobacterium Avium Complex

Infection with *Mycobacterium avium* complex (MAC) is a common cause of intestinal disease in AIDS patients with severe immune suppression. The infection probably is acquired through the ingestion of contaminated water. Clinical disease results from infiltration of infected macrophages into tissue compartments, similar to Whipple's disease (67), and from the effects of cytokines released by the macrophages. The luminal gastrointestinal tract is involved by MAC infection. Massive thickening of the proximal intestine may occur. Small intestinal biopsy reveals infiltration of the lamina propria by macrophages containing large numbers of acid-fast bacilli.

Bacterial Enteritides

Bacterial enteritides in AIDS have distinctive features. Infections with species of salmonella, shigella or campylobacter occur in HIV-infected patients, with or without AIDS (68). In one study, shigella infections tended to occur early in the disease course, while salmonella and campylobacter infections were more common in AIDS patients. It is unclear if the incidence is increased over the surrounding population, though enhanced susceptibility could be related to decreased gastric acid secretion, as noted above. The clinical presentation of salmonella infection may be reminiscent of the classical descriptions of typhoid fever (69).

Antibotic-Associated Colitis

Antibotic-associated colitis, related to elaboration of *C. difficile* toxin, has been reported in AIDS patients (70). AIDS patients may be particularly vulnerable to this complication, as they often receive broad-spectrum antibiotics. Clindamycin use and prolonged hospitalization were found to be risk factors for its development. The clinical syndrome is similar in AIDS and non-AIDS patients.

Adenovirus

Adenoviruses are occasionally isolated from rectal swabs or biopsies from AIDS patients with diarrhea and a stable or deteriorating course. Electron microscopic studies have demonstrated cytoplasmic inclusion bodies containing adenovirus in superficial epithelial cells, including goblet cells (71).

Role of HIV in Intestinal Disease

Several authors have suggested that HIV may play a role in intestinal disease. Most AIDS patients with diarrhea but very few HIV-infected individuals without AIDS have identifiable enteric pathogens (23,72,73). HIV RNA and protein antigen expression may correlate with clinical symptoms as well as histopathologic alterations, including lymphoid cellularity and cytokine expression (72,74).

Clinical Symptoms

The ability to localize the pathologic process presumptively to the small intestine or colon is valuable in directing and streamlining a diagnostic workup. Important

information often can be obtained from the clinical history. Symptoms related to small intestinal infection are typical of malabsorption. Patients complain of three to ten nonbloody bowel movements per day, with urgency but no tenesmus. When severe, the diarrhea is associated with dehydration and electrolyte abnormalities, typically hypokalemia and hypomagnesemia. A mild hyperchloremic acidosis may be seen. Stool volume is variable but often large. Patients may have little diarrhea or even formed stools at times, yet suffer from episodes of profuse diarrhea at other times. The diarrhea does not occur consistently thoughout the day and is often worst at night or early in the morning. There may be no *speciPc* food intolerances, as diarrhea is worsened by any signiPcant food intake. However, stool volumes are decreased by fasting. In some patients, stool volumes are very high (up to 10 liters/day) and not affected by food intake, implying a secretory process. The infections producing malabsorption usually are not associated with fever or anorexia, though food intake may be decreased voluntarily to avoid diarrhea. A notable exception to this rule is MAC. Weight loss typically is slow and progressive, or patients may stabilize around a lower weight.

Enterocolitic diseases produce typical symptoms of colitis. There are numerous (up to 30) small volume bowel movements that occur at regular intervals throughout the day and night. Cramping and tenesmus may occur but usually are not severe. The clinical course often is associated with fever, anorexia, rapid and progressive weight loss and extreme debilitation.

Diagnosis

Stool examinations are an integral part of the diagnostic workup of an HIV-infected individual with diarrhea. Many organisms can be detected in the stool. Special diagnostic techniques are available for cryptosporidia and microsporidia, though there is relatively little clinical experience with the latter techniques. While molecular biological techniques are available experimentally, they have not been used in clinical situations (75). However, a substantial proportion of patients have enteric pathogens that can be detected only by intestinal biopsy (Figs. 20.2–20.6), so that negative stool examinations are incomplete as a workup. An algorithmic approach may be used to evaluate AIDS patients with diarrheal illnesses (Tables 20.2, 20.3).

The ability to diagnose *E. bieneusi* on tissue biopsy has improved greatly (Fig. 20.3). Initially, there was complete reliance upon transmission electron microscopy. The recognition of characteristic patterns of cytopathology by light microscopy, coupled with conPrmatory special stains (76) coincided with a marked increase in the number of diagnoses made. Microsporidial infection is associated with variable degrees of villus atrophy and crypt hyperplasia. There is an increased parasite burden in the proximal jejunum and distal duodenum, compared to the proximal duodenum (77). In some cases, villus heights are almost normal and there is marked crypt hyperplasia. In other cases, there is signiPcant villus atrophy, usually with less marked crypt hyperplasia. *E. bieneusi* causes a typical pattern of cellular injury, which is concentrated in the upper third of the villus. The histopathology and ultrastructural characteristics have been reviewed in detail (57,78,79).

The spectrum of endoscopic lesions in CMV colitis varies from essentially normal appearing mucosa, to scattered groups of vesicles or erosions, to broad shallow ulcerations that may coalesce. CMV usually causes a pancolitis, though the cecum and right colon may be affected earlier than elsewhere. Histopathologic examination demonstrates characteristic intracellular inclusions

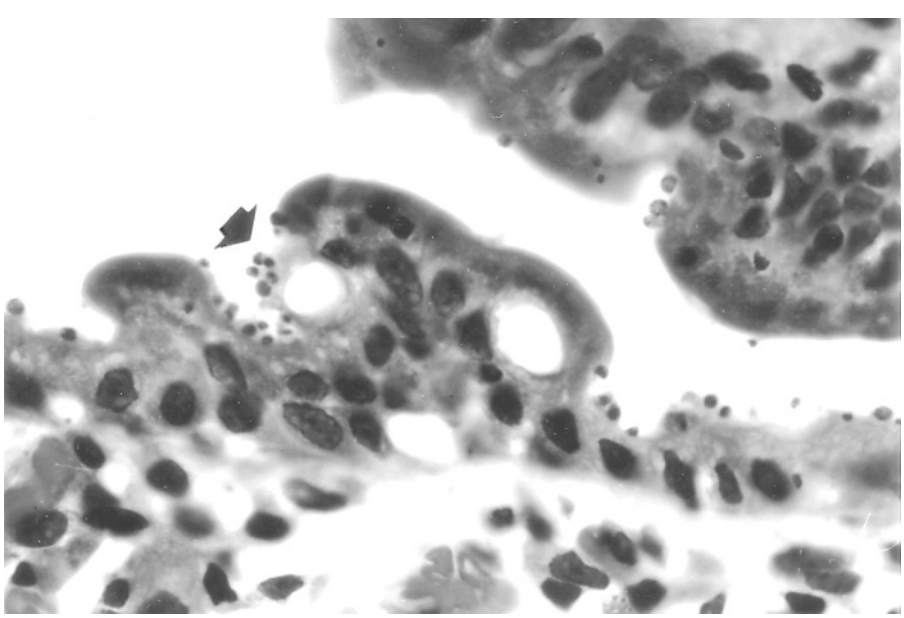


FIG. 20.2. Cryptosporidial infection of the intestine. Organisms are seen at the level of the epithelial cell brush border (arrow) (H&E 400 ×)

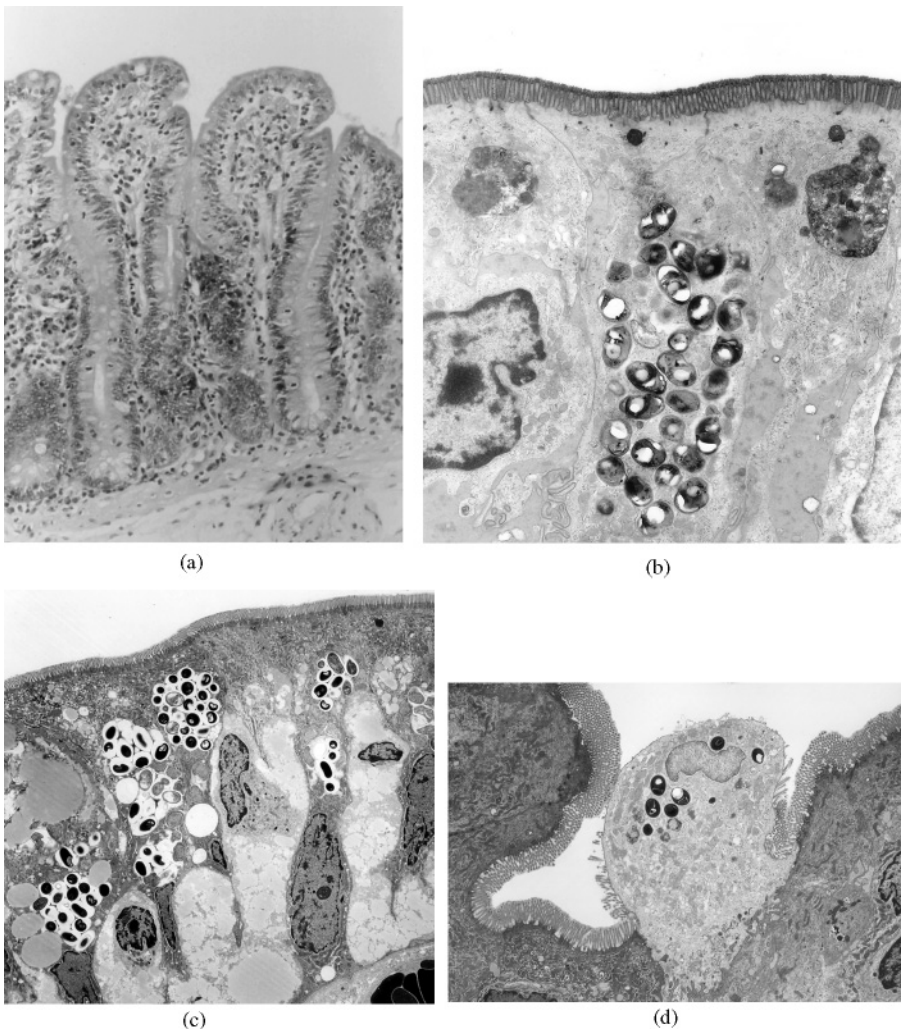


FIG. 20.3. Microsporidial infection of the small intestine. (a) Partial villus atrophy and crypt hyperlasia (H&E 125 ×). (b) Transmission electron micrograph of *E. bieneusi* in epithelial cells (2,000 ×). (c) Transmission electron micrograph of E. intestinalis in epithelial cells (2,500 ×). (d) Transmission electron micrograph of *E. bieneusi* spores in a sloughing epithelial cell (5,000 ×).

(Fig. 20.5). Specialized immunohistochemical or in situ hybridization techniques are available and may increase the sensitivity of diagnosis.

The diagnosis of mycobacterial infection is made by culture or histology. Stool smears may demonstrate acid-fast bacilli, but the Þnding is not speciÞc for tissue invasion. Mucosal thickening on barium X-rays and thickening of the intestinal wall plus enlargement of mesenteric and retroperitoneal nodes on CT scan are characteristic of MAC, though lymphoma or fungal infections have similar appearances. Histologic demonstration of acid-fast bacilli in intestinal tissue is straightforward (Fig. 20.6). Diagnosis by biopsy often can be made in one day, as compared to cultures, which may take as long as six weeks to become positive. Molecular hybridization techniques are being developed to allow species identiÞcation on tissue sections.

The diagnosis of antibiotic-associated colitis is the same in AIDS and non-AIDS patients. The diagnosis of bacterial enterocolitis should be straightforward with routine evaluation. Blood cultures should be part of the workup of suspected infectious diarrhea with fever in an HIV-infected patient, as salmonella may be cultured from blood but not from stool. An unusual feature of salmonella infections in AIDS patients is the tendency for clinical and/or microbiological relapse after antibiotics are discontinued.

Diarrhea Induced by Antiretroviral Drugs

Diarrhea is a commonly reported complaint in subjects on protease inhibitors and nucleoside analogs. Studies done three years into the HAART era have suggested that the incidence of chronic diarrhea in AIDS patients did not change, however the etiologies of diarrhea did change signiÞcantly, with an increased incidence of noninfectious causes and a decreased incidence of opportunistic infectious causes. This shift in etiologies coincides with the introduction and increased use of HAART in the populations studied. The exact mechanisms underlying the development of drug-induced diarrhea is uncertain although fat malabsorption has been associated with HAART therapy (80). The HIV protease inhibitors saquinavir, ritonavir, and nelÞnavir but not indinavir have

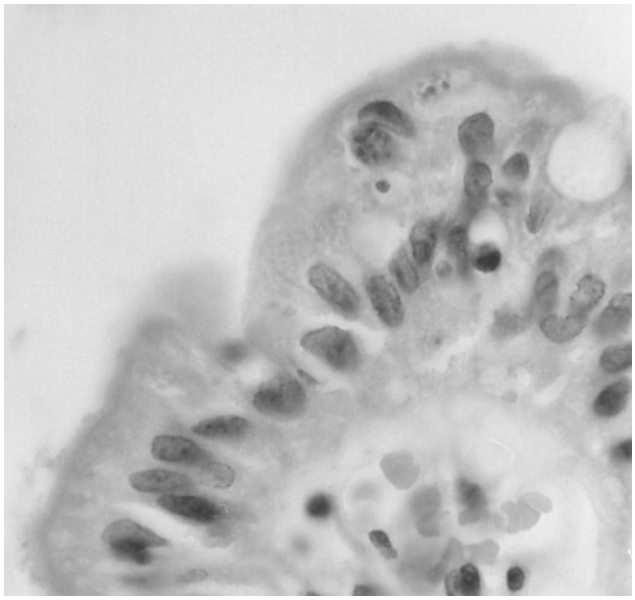


FIG. 20.4. Isoporiasis of the small intestine. A developing form is located in the epithelium (H&E 440 ×)

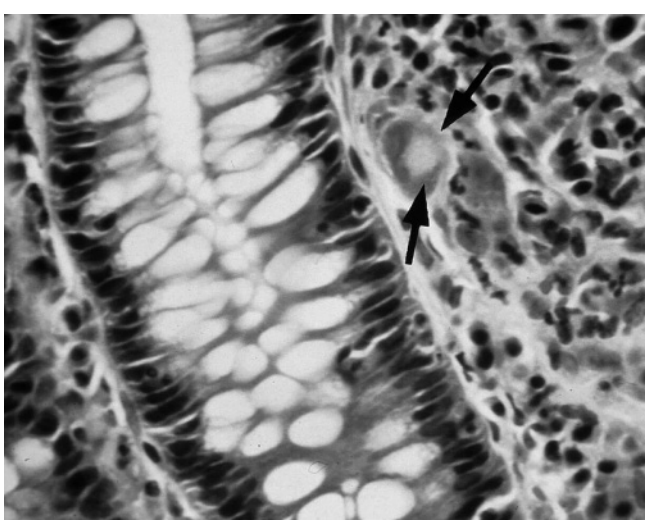


FIG. 20.5. Cytomegalovirus colitis. Intranuclear inclusion in an endothelial cell (H&E 250 ×)

been shown to impair the epithelial barrier in human intestinal cell lines (81).

Treatment

Treatments for the patients with diarrheal diseases can be divided into antimicrobial therapies, and treatments for the associated diarrhea, dehydration and malnutrition. There is no known effective therapy for cryptosporidiosis. Many agents have been tried, and the results have been disappointing. Some patients improved during treatment with paromomycin (Humatin, Parke Davis) while controlled trials showed only modest improvement and no

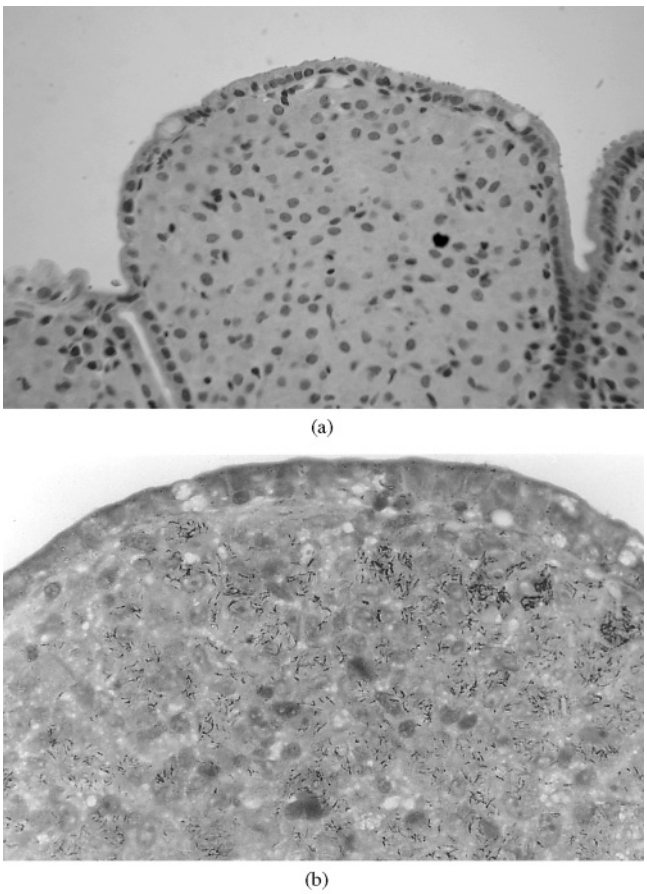
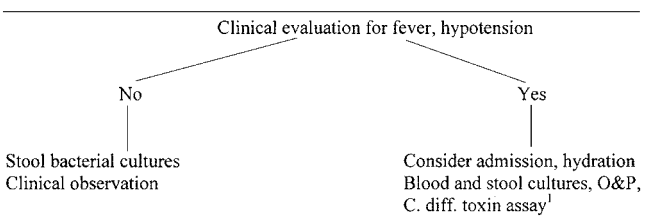


FIG. 20.6. (a) *Mycobacterium avium complex* infection of the small intestine. Foamy macrophages located in the super cial lamina propria (H&E 200 ×). (b) *Mycobacterium avian complex* infection of the small intestine. Acid fast organisms by Ziehl Neilson (H&E 200 ×).

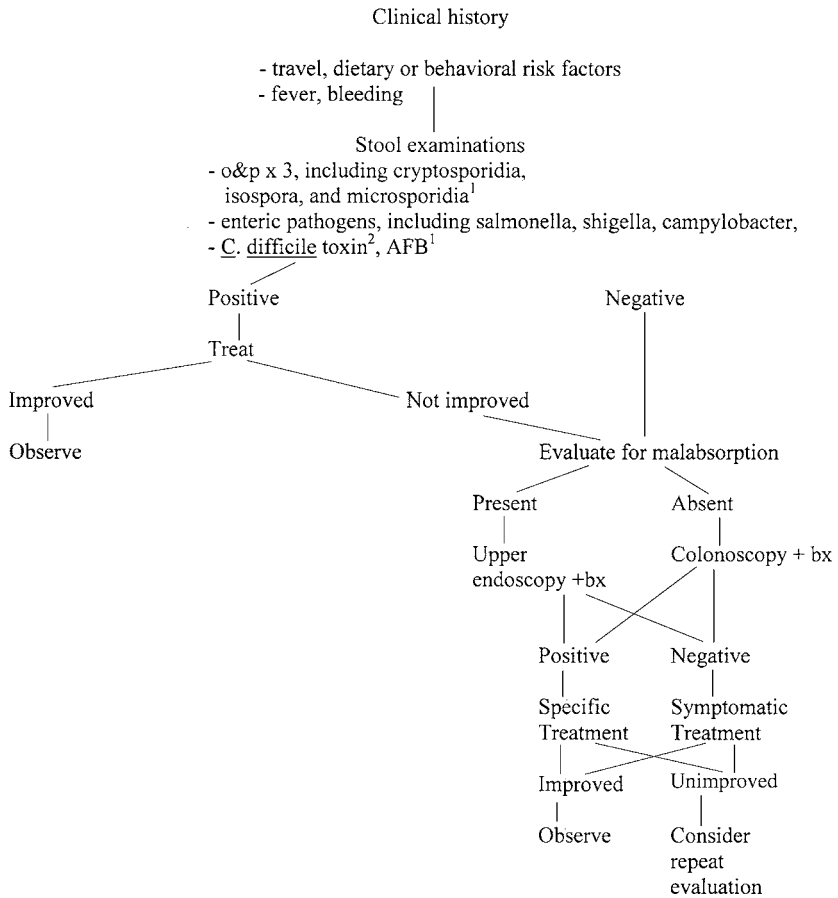
cures (82,83). An uncontrolled trial of paromomycin plus azithromycin showed good response in terms of clinical symptoms and oocyst excretion (84). Azithromycin alone is ineffective. Clarithromycin or rifabutin prophylaxis for MAC prophylaxis may reduce risk of cryptosporidiosis (85). Nitazoxanide (Unimed Pharmaceuticals, Buffalo Grove, IL) has been reported as effective in the treatment of cryptosporidiosis but is not approved by the FDA and clinical trial data, including ACTG 192, shows minimal or no beneÞt. Overall, HAART with immune reconstitution is

TABLE 20.2. *Evaluation of acute diarrhea (less than seven days)*



[1] Recent or concurrent antibiotic use or hospitalization.

TABLE 20.3. *Evaluation of chronic diarrhea (greater than 14 days).*



[1] If CD4 + lymphocyte count < 100/MM[3].
[2] Concurrent or recent antibiotic use.

the only consistently effective treatment for cryptosporidiosis (86Ð88).

Few studies of drug therapy of *E. bieneusi* infection have been published (89). Albendazole (Glaxo Smith Kline) is ineffective as treatment, but is effective therapy for *E. intestinalis* infection (90). Anecdotal success in the treatment of *E. intestinalis* has been reported with itraconazole, ßuconazole, atovaquone, and metronidazole (91). Experimental drugs include Fumagillin, TNP-470, and Ovalicin. HAART with immune reconstitution is best therapy, especially for the 80Ð90% of cases involving *E. bieneusi* (92Ð94).

Isosporiasis may be treated with trimethoprim sulfa. Due to a high rate of recurrence, repeated courses or chronic maintenance with this drug may be needed. Some have advocated treatment indeÞnitely unless there is immune recovery. The optimal duration of high-dose therapy is not well deÞned. There has been one case report of refractory infection that responded to pyrimethamine plus sulfadiazine (95).

CMV colitis appears to occur less often in subjects on PIÕs and also has a lower rate of recurrence in subjects on HAART. Survival outcomes are also signiÞcantly better in patients with CMV colitis who are on HAART. Two agents are clinically effective in the treatment of CMV colitis. The most widely used is ganciclovir. A double-blind, placebo-controlled trial demonstrated clinical and antiviral beneÞts of ganciclovir therapy (96). Ganciclovir therapy also has been associated with nutritional repletion and with prolonged survival. Oral ganciclovir is available for maintenance therapy. Other analogues with better bioavailability have been developed. Foscarnet also has activity against CMV as well as HIV.

Several drugs have *in vitro* efÞcacy against MAC including the macrolide clarithromycin (Biaxin), as well as ethambutal (Myambutal), rifabutin (Mycobutin) and clofazimine (Lamprene). Other drugs with *in vivo* or *in vitro* efÞcacy include amikacin, ciproßoxacin, cycloserine, and ethionamide. Three drugs: azythromycin, rifabutin and clarithromycin, have been shown to be effective prophylactic agents. Combination of azithromycin plus rifabutin was associated with an even higher reduction in the expected number of cases of disseminated MAC at the expense of reduced tolerability (97). In a double-blind, placebo-controlled trial, clarithromycin prevented 69% of expected cases of *M. avium* complex disease. In another

placebo-controlled study of prophylaxis, azithromycin prevented 59% of expected cases. Either drug was more effective than rifabutin, although rifabutin prevented 55% of expected cases (98). Prophylaxis failures with rifabutin usually involve clarithromycin sensitive strains; failures with clarithromycin or azithromycin often involve clarithromycin-resistant strains. In the setting of immune reconstitution, primary prophylaxis may be discontinued when the CD4 count increases to $>100/mm^3$ for >3 months (99,100,101). It appears that secondary prophylaxis (prior disseminated MAC) may also be discontinued when the CD4 count is >100 plus HAART $\times$ 6Ð12 months.

The treatment of enterocolitis due to *Salmonella*, *Shigella*, or *Campylopbacter* sp. is modiÞed in that intravenous therapy with antibotics is used commonly, due to the frequent occurrence of bacteremia. In addition, repeated or chronic courses of antibotics such as trimethoprim sulfa or ciproßoxacin often are needed because of disease recurrence. Since many enteric bacterial pathogens are intracellular pathogens, it is possible that disease recurrence is a function of impaired intracellular macrophage killing due to deÞcient T cell help. Furthermore, the intracellular reservoir may protect the organisms from lysis by antibiotics. Further studies are needed to determine if complete eradication of the enteric bacterial pathogens in AIDS is possible, or if chronic suppressive therapy will be needed.

Treatment of chronic bacterial enteropathy with broad spectrum antibiotics has brought clinical improvement in several patients. The difÞculty in choosing antibiotics is the inability to determine which bacteria in stool are the offending organisms. In addition, the widespread use of antibiotics could lead to the development of multi drug resistance strains (102).

Other Therapies

Maintenance of adequate ßuid balance and nutritional status are important clinical tasks, especially in patients with malabsorption syndromes. Oral rehydration solutions may help maintain hydration status, but are hypocaloric and may promote wasting if used excessively. The goal of hydration therapy is to maximize ßuid intake while minimizing diarrheal losses. A low fat, lactose-free diet may be beneÞcial and medium chain triglycerides are useful adjuncts in the treatment of patients with signiÞcant malabsorption. On the other hand, polymeric formula diets generally are tolerated poorly and lead to substantial diarrhea. A variety of anti-diarrheal therapies may be used. Some patients with non-speciÞc diarrhea or ileal dysfunction respond well to the bile salt binding resin cholestyramine. The most commonly used antidiarrheal agents are loperamide and opiates, though escalating doses often are required. Octreotide has been used in the treatment of diarrhea of several etiologies, with mixed results. However, excess ßuid losses from the small intestine due to malabsorption or abnormal secretion will overcome any pharmacologically produced inhibition of motility and lead to diarrhea.

Nutritional therapy of AIDS patients with diarrhea depends entirely upon the pathogenic mechanism underlying the diarrhea. Different approaches are required in patients with and without malabsorption. Nutritional maintenance may be impossible by the enteral route in patients with severe small intestinal disease, and parenteral nutrition may be required (36). However, the use of elemental diets, which contains simple sugars, amino acids and medium chain triglycerides, may give similar results to TPN in some cases (103).

Nutritional support is less effective in patients with enterocolitis associated with systemic infections. The metabolic rate is elevated, and metabolic derangements promote protein wasting irrespective of intake. Alterations in lipid metabolism often result in the development of fatty liver when nutritional support is attempted. In one study, total parenteral nutrition resulted in weight gain, but the increase was due entirely to an increase in body fat content, while body cell mass did not change (36). While nutritional support might help prevent progressive protein depletion, the key to successful therapy is proper diagnosis and treatment of the speciÞc disease complication.

Treatment of PI-associated diarrhea is largely nonspeciÞc; most of the available literature is published only in abstract form and is based primarily on retrospective and survey data. Agents for which some efÞcacy has been shown for treatment of PI-associated diarrhea include oat bran, psyllium, loperamide, calcium carbonate, and pancrealipase.

ANORECTAL DISEASE

Etiology and Pathogenesis

Anorectal diseases seen in AIDS patients include both infections and tumors. Herpes simplex virus is the most common infectious agent found. The primary lesion occurs at the pectinate line. Vesicles in the anal canal may be missed as they rupture during defecation or examination. Herpes infection in AIDS patients most often presents as a painful, shallow spreading perineal ulcer. A smaller group of patients present with idiopathic ulcers, originating at the anorectal junction. Perianal and intraanal condylomata occur in AIDS patients as well as non-AIDS patients and are related to infection with human papilloma virus. The lesions may be enlarged and ßat (leukoplakia) and may show dysplasia on histologic examination. The lesions are likely precursors to squamous cell cancers. Tumors in the anorectal region include Kaposi's sarcoma, lymphoma, and squamous cell carcinoma or its variants.

Hemorrhoidal disease also is seen frequently. Factors predisposing to hemorrhoids may have predated the HIV

infection. Severe diarrhea or proctitis may promote local thrombosis, ulceration and secondary infection. Fleshy skin tags, resembling those seen in Crohn's disease, also are seen. Thrombosed hemorrhoids occur frequently, but it is unclear if the incidence is higher in AIDS patients than in a comparable population.

A variety of classic venereal diseases can produce anorectal ulcerations. Diagnosis and therapy of *Neisseria gonorrhoese* proctitis is similar in AIDS and non-AIDS patients. Syphilis may have an atypical presentation in HIV-infected subjects, and serologic diagnosis is affected by the presence of immune deficiency. Chlamydia are prevalent in sexually active groups. The frequency of chancroid, caused by *Hemophilus ducrei*, in HIV-infected patients is unknown. Rectal spirochetosis has been recognized in homosexual men with or without HIV infections (104). The infection usually is asymptomatic and an incidental finding on evaluation.

Diagnosis

In many cases, the correct diagnosis can be made by inspection. If necessary, the diagnosis can be confirmed by culture or biopsy. Specialized techniques, such as in situ hybridization, are available for the diagnosis of papilloma virus infection but are rarely needed clinically.

Treatment

Resolution of herpetic lesions occurs after treatment with oral or intravenous acyclovir. Herpes simplex virus resistant to acyclovir has been demonstrated in patients with refractory ulcerations. The use of foscarnet or ganciclovir may bring resolution. Anorectal ulcers containing CMV respond to antiviral therapy. Symptomatic idiopathic ulcers may respond promptly to intralesional corticosteroid therapy and healing may occur after repeated treatments. Areas of leukoplakia can be followed clinically while large or enlarging lesions should be excised. Some caution should be placed on surgical therapy. Poor wound healing may occur, especially in severely malnourished patients, patients with serious, untreated diseases such as CMV, and patients with continued diarrhea due to nutrient malabsorption.

Epidermoid cancers, including squamous cell and cloacagenic cancer, occur in anal skin and rectal glands, respectively. While these cancers rarely metastasize in immunocompetent persons, they may do so in patients with AIDS. For these lesions, management after diagnostic biopsy includes excision, chemotherapy, or laser photocoagulation. Laser therapy of rectal Kaposi's sarcoma also is effective.

TUMORS

The incidence of Kaposi's sarcoma in AIDS has declined in the HAART era. Kaposi's sarcoma in AIDS is indistinguishable, histopathologically, from classic Kaposi's sarcoma, endemic forms of Kaposi's sarcoma found in Africa, or the form that occurs during immunosuppressive therapy. Visceral involvement in AIDS patients with Kaposi's sarcoma is more common than in non HIV-infected individuals (105). Visceral involvement may be asymptomatic. The diagnosis is made by visual inspection and confirmed by biopsy, though endoscopic biopsy may be falsely negative if the tumor is in the submucosa. No treatment is needed in most cases. Kaposi's sarcoma is responsive to chemotherapy, which can be used in symptomatic patients or in the event of rapidly progressive disease. Pegylated liposomal doxorubicin (PLD, Caelyx) in the treatment of AIDS-related KS is more effective and less toxic than bleomycin plus vincristine (BV) or adriamycin plus bleomycin plus vincristine (ABV). Cost-effectiveness analysis suggests that PLD is preferable over liposomal daunorubicin, and other regimens and seems to offer a better quality of life.

A high prevalence of extranodal, high-grade non-Hodgkins B cell lymphomas has been noted in AIDS patients. Gastrointestinal lymphomas in AIDS are biologically aggressive, especially the Burkitt's subtype. The lesions may respond to chemotherapy, using combination therapies. Sporadic reports of AIDS patients with carcinomas in the gastrointestinal tract have been published but a heightened incidence has not been documented convincingly.

HEPATOBILIARY DISEASES

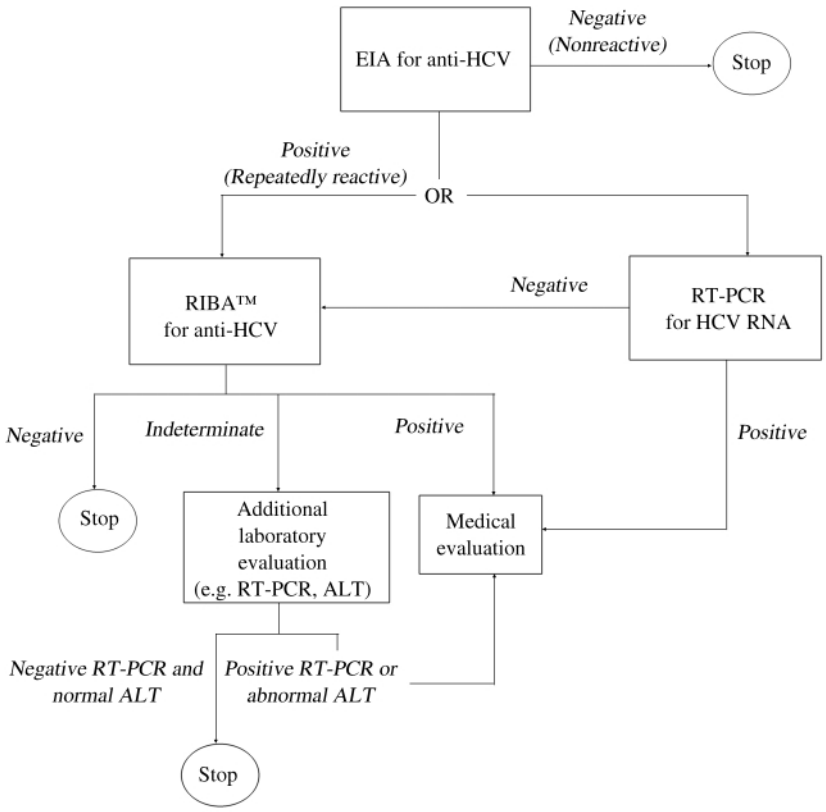
Etiology and Pathogenesis

Liver dysfunction is common in AIDS patients. Paradoxically, the perceived and documented morbidity and mortality from liver disease in HIV-infected individuals has grown in magnitude and importance during the era of HAART as the incidence and severity of other opportunistic infections has decreased. In general, HAART does not influence the long term outcomes of hepatitis B or C.

There are three distinct clinical syndromes of AIDS-related hepatobiliary disease: diffuse hepatocellular injury, granulomatous hepatitis and sclerosing cholangitis. Drug-induced liver injury also may occur, and is most commonly reported with nevirapine (Viramune, Boerhinger Ingelheim, Ridgefield, CT) and ritonavir (Norvir, Abbott Laboratories, Chicago, IL).

Many HIV-infected patients have had prior exposure to hepatitis B. A relatively high percentage of HIV-infected individuals have circulating hepatitis B surface antigen and e antigen. There is no evidence that immune deficiency will cause a reactivation of prior hepatitis B. On the other hand, hepatocyte injury as a direct result of viral or other cytopathy is unaffected by the immune deficiency so that delta hepatitis and drug-induced hepatitis are as severe in HIV-seropositive as in HIV-seronegative people.

TABLE 20.4. *Hepatitis C virus (HCV)-infection-testing algorithm for asymptomtic persons*



Granulomatous hepatitis in AIDS occurs in the setting of disseminated systemic infection. The causative organisms include mycobacteria and fungi, such as *Histoplasma capsulatum*, *Coccidiodes imitis*, and *Cryptococcus neoformans*. Viral diseases such as CMV also may affect the liver. Rare cases of disseminated protozoal infection has been reported. Disseminated pneumocystosis also may involve the liver.

A syndrome of sclerosing cholangitis has been recognized to occur in AIDS patients (106). The disease in AIDS bears a striking resemblance to the non-AIDS variety, though the disease in AIDS may be more rapidly progressive. The etiology and pathogenesis of sclerosing cholangitis are as obscure in AIDS patients as they are in non-AIDS patients. Some patients have been shown to have biliary involvement with protozoa, namely cryptosporidia and microsporidia. Other patients have been shown to have CMV infection of the liver and biliary tree. In other patients, no etiologic agent is identified.

Chronic hepatitis is believed to be one of the leading causes of morbidity and mortality in HIV infected subjects in the HAART era. An unexpected development of the application of HAART therapy was the observation that liver disease has become one of the most common underlying cause for death in HIV-infected individuals. The improvement in survival in patients on HAART has led to the development of chronic liver disease in many of the estimated 60% of HIV patients who are co-infected with chronic hepatitis C. Factors that promote HCV progression are HIV-co-infection and alcohol ingestion of $\geq$50 gm ETOH/day (107–109). HCV has a disputed effect on HIV progression.

USPHS/IDSA guidelines recommend that all HIV-infected persons should be tested for HCV infection using the EIA screening assay for anti-HCV antibodies; positive tests should be confirmed by either the recombinant immunoblot assay (RIBA) or by reverse transcriptase PCR (RT-PCR) for HCV RNA (110). The CDC-recommended algorithm for detecting HCV is summarized in Table 20.4. HCV RNA assays (quantitative and qualitative) are required to confirm chronic hepatitis C infection. Serologic tests for anti-HCV may be negative with acute HCV infection and with severe immunosuppression (CD4 $<100/mm^3$); HCV RNA may be useful in these settings. Quantitative HCV RNA assays are widely available using RT-PCR or b DNA technology. These tests show a threshold of detection of 100–1,000 copies/mL and they are positive in 75–85% of persons with anti-HCV and $>95\%$ of persons with acute or chronic hepatitis C. Rigorous quality control is necessary for these assays in terms of specimen preparation for laboratory submission. Genotype assay identifies six genotypes and >90 subtypes. Genotype 1 accounts for 70% of HCV infected

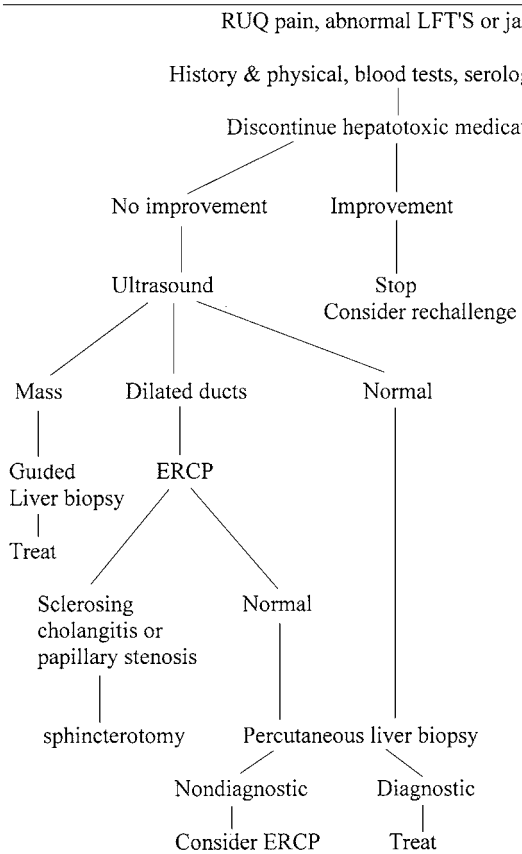
persons in the United States and is associated with a poor response to therapy compared to genotypes 2 and 3.

Patients co-infected with HCV and HIV should be advised not to drink excessive amounts of alcohol, be evaluated for HCV treatment with interferon plus ribavirin, and be vaccinated for hepatitis A, if susceptible.

Indications to treat in HCV/HIV co-infection include biopsy evidence of bridging Þbrosis or moderate inßammation and necrosis. HCV RNA levels and ALT levels do not adequately predict prognosis. Ribavirin appears to work as well in HIV-infected patients as in non-HIV patients, but causes more anemia in the presence of HIV (111). Peggylated interferons have a longer half-life than interferon, permitting once weekly adminstration. Comparative trials with peginterferon (180 μg SC q wk) vs. interferon alfa 2-a (3Ð6 million units SC $3\times$/wk) $\times$ 48 wks in patients with chronic HCV, in the absence of HIV infection, showed peginterferon to be superior in terms of virologic response and liver function tests (ALT) at 72 weeks (112,113). The results of peg interferon monotherapy are inferior to those of interferon and ribavirin.

Other patients with abnormal liver chemistries have macrovesicular or microvesicular fatty inÞltration, or other nonspeciÞc changes. Peliosis hepatis has been described in AIDS patients and is associated with infection by a rickettsia-like organism (114).

TABLE 20.5. *Algorithm for hepatobiliary disease in AIDS*



Clinical

There are few speciÞc clinical signs to indicate liver involvement except in cases of diffuse hepatocellular injury or obstruction with jaundice. The suggestion of liver disease usually is based upon the Þndings of abnormal liver function tests in serum samples. Mild hepatomegaly and abnormalities in liver function tests are seen commonly and often are of no clinical importance. When part of an obvious systemic process with chronic fevers and wasting, the presence of progressively rising serum alkaline phosphatase activity and an enlarged liver provide the opportunity for performing diagnostic procedures with a high yield of diagnoses expected. Patients with sclerosing cholangitis present with non-speciÞc abdominal complaints, sometimes associated with pruritis. Severe liver dysfunction with liver failure is rare but can occur in patients with hepatitis C or delta hepatitis infections, and has been seen in a few patients with sclerosing cholangitis.

Diagnosis

Evaluation for liver disease can be approached with the assistance of an algorithm (Table 26.5). The indication for biopsy is a progressive rise in liver function tests, not the absolute level of these enzymes. The techniques used to diagnose liver and other visceral diseases are the same in AIDS and non-AIDS patients. Focal lesions may be detected with ultrasound of CT scans and approached by directed biopsy. Percutaneous liver biopsy has been used to diagnosis MAC, tuberculosis, and fungal infections. Liver biopsy reveals focal collections of histiocytes, which may be organized into poorly formed granulomas, scattered throughout the parenchyma. Giant cells are seen only rarely. The responsible organisms often can be identiÞed by special stain on the biopsy. Mycobacteria are the most common organisms detected in clinical series (115). Acid-fast stains usually disclose large numbers of organisms, especially in cases of MAC. This Þnding differs from the usual paucity of acid-fast bacilli in non-AIDS related tuberculosis (116). Since tuberculosis cannot be differentiated from MAC by biopsy alone, culture of the liver also should be done and therapy based upon clinical judgement until results are known. Histoplasmosis and coccidiodomycosis can be diagnosed with great certainty on biopsy using speciÞc histologic stains. However, an aliquot also should be cultured for fungi. In addition to percutaneous liver biopsy, percutaneous needle aspiration of retroperitoneal nodes using sonographic or CT guidance is an effective means of making a diagnosis of infection or tumor.

The diagnosis of sclerosing cholangitis (AIDS cholangiopathy) can be made by ERCP (Fig. 20.7), though

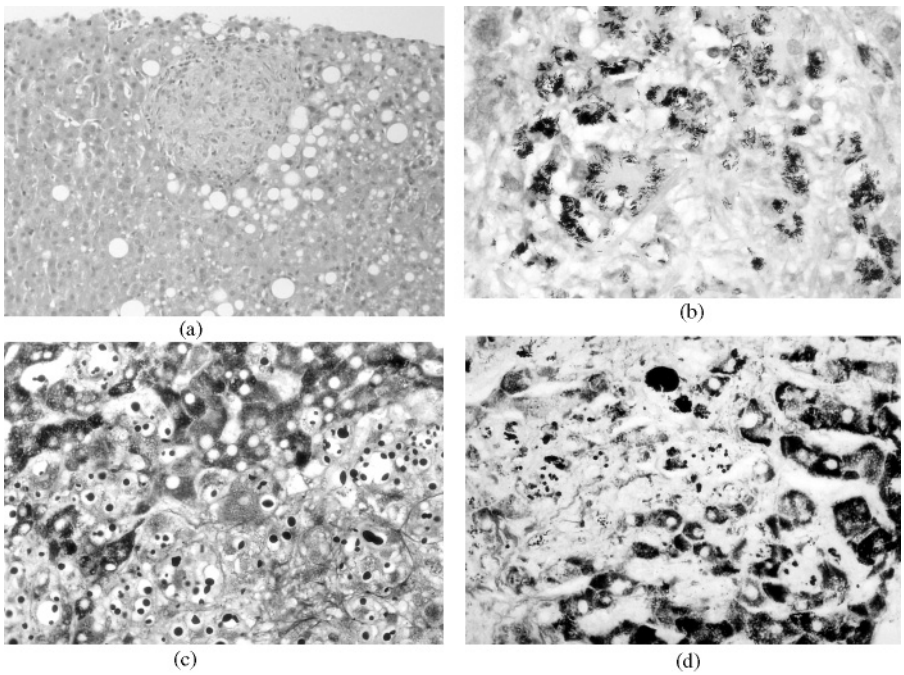


FIG. 20.7. Granulomatous hepatitis. (a) Granuloma located in the hepatic parenchyma (H&E 250×). (b) Acid fast bacilli located in macrophages (Zhiehl Nielson 480×). (c) Cryptococcal hepatitis (GMS 250×). (d) Histoplasma hepatitis (GMS 250×).

sonographic or CT studies also may support the diagnosis. Endoscopic retrograde cholangiography demonstrates multiple areas of narrowing and dilatation of the intrahepatic and/or extrahepatic ducts, sometimes with ulceration and plaque formation. Examination of bile and pancreatic juice may reveal bacterial overgrowth, viruses, and/or cryptosporidia. The absence of ductal dilatation on imaging studies does not rule out sclerosing cholangitis.

Treatment

The diagnosis of mycobacterial or fungal infection is indication for antimicrobial therapy. Likewise, the diagnosis of lymphoma or widespread Kaposi's sarcoma is indication for systemic antineoplastic therapy. Ongoing studies will determine the efficacy of treating hepatitis C in co-infected patients. Treatment of cholangiopathy is limited to sphincterotomy for pain relief, which does not affect disease progression. The question of liver transplantation has arisen, because patients with progressive liver disease, but stable HIV infection, are being seen in increasing numbers, and clinical experience in organ transplantation and its postoperative management is increasing.

PANCREATIC DISEASES

Pancreatic disease in AIDS has received little attention and may not be recognized pre mortem. The pancreas may be involved in systemic diseases such as CMV, MAC, fungi, Kaposi's sarcoma, or lymphoma. Drug-induced pancreatitis is the most commonly recognized form. Hyperlipidemic pancreatitis has been observed. There are no reports of chronic pancreatitis occuring as a specific complication of HIV infection. Pancreatic insufficiency is an uncommon cause of fat malabsorption in AIDS patients.

GASTROINTESTINAL HEMORRHAGE

Etiology and Pathogenesis

Gastrointestinal hemorrhage is not a common consequence of AIDS, but serious or life-threatening bleeding does occur (117,118). Bleeding may result from the same conditions occurring in the non-HIV infected patient as well as the tumors and ulcers seen in AIDS. Many causes of GI bleeding associated with complications of AIDS have been reported, including localized solitary ulcers in the esophagus or bowel, bleeding duodenal or gastric ulcers with or without associated use of nonsteroidal antiinflammatory agents, and extensive mucosal KS.

The clinical presentation of gastrointestinal hemorrhage in an AIDS patient is the same as in a non-AIDS patient and the basic concepts of resusitation also are the same.

Diagnosis

The endoscopic and radiologic techniques available for the evaluation of GI hemorrhage should be used. Bleeding lesions often can be visualized by endoscopy and controlled locally, while diagnostic material is obtained.

Treatment

The basic approach to GI hemorrhage is the same in AIDS and non-AIDS patients. The proper manangement

of GI bleeding depends upon the precise cause and undoubtedly is related to local expertise. An attempt to determine the cause should be made in every case of hemodynamically signiÞcant bleeding. In addition to possible local control, the nature of any underlying lesions must be determined. If related to a solitary ulcer, surgical excision should be considered. Proper management of bleeding neoplasms involves effective local control followed by systemic chemotherapy. Techniques that have been utilized successfully include injection sclerotherapy of bleeding lesions of KS, and angiographic embolization of a bleeding ulcer. Thus, the proper approach to GI bleeding in an AIDS patient probably is not different from an immunocompetent patient.

THE ACUTE ABDOMEN

Etiology and Pathogenesis

Abdominal pain is an important symptom in AIDS patients. The major enteric pathogens such as cryptosporidium, CMV, salmonella, and shigella cause abdominal *cramps* and not *pain*, though widespread MAC infection may present with chronic abdominal pain. Severe acute abdominal pain often is a sign of a signiÞcant pathologic process, such as a perforated viscus. The clinical signs of abdominal tenderness, guarding and rebound have the same signiÞcance in AIDS patients as in immunocompetent patients. It is important to remember that AIDS patients may develop peritonitis for the same reasons as patients without AIDS, plus reasons speciÞc for AIDS itself. Cholecystitis may occur but usually is acalculous. Perforated viscus occurs in AIDS, but the cause may be a solitary intestinal ulcer rather than peptic ulcer disease or diverticulitis. Malignant intestinal obstruction usually is due to Kaposi's sarcoma or lymphoma rather than adenocarcinoma. Kaposi's sarcoma and lymphoma also may be responsible for perforation (119) or be the leading edge in an intussusseption.

Clinical

The clinical presentations of appendicitis, cholecystitis or generalized peritonitis are the same in AIDS and non-AIDS patients and the correct diagnosis can be made using similar criteria. It is important to recognize that extensive MAC infection in the retroperitoneum may lead to liquefaction necrosis of retroperitoneal nodes and the production of peritonitis similar to that seen in tuberculosis peritonitis. In this circumstance, surgery is not helpful and should be avoided.

Diagnosis

While the physical Þndings in a patient with an acute abdomen may be unaffected by the presence of AIDS, the laboratory Þndings may differ. This is particularly true of the blood count. Elevations of the leucocyte count with a left shift count may be absent, especially if there is preexisting leukopenia or prior treatment with myelosuppressive drugs such as zidovudine. Imaging studies such as CT scan may be valuable in detecting extraluminal collections of pus or ßuid. Isotopic imaging studies such as an indium-labeled white blood cell study or gallium scan may be falsely negative in the presence of severe leukopenia.

Treatment

While the indications for surgery are the same in the AIDS and the non HIV-infected patient, the expected results may differ. The possibility of unusual pathogens is higher in AIDS patients, and the possibility of impaired wound healing is increased in debilitated patients, irrespective of HIV infection. The reported incidence of postoperative complications and mortality was high in the pre-HAART era (119), but this is due, at least in part, to the seriousness of the underlying illness, and other complications. However, recovery after surgery is possible and may be followed by prolonged survival.

Some centers approached the question of laparotomy in AIDS patients with caution (120,121), while other centers have shown that speciÞc subgroups of patients, such as patients undergoing splenectomy for refractory thrombocytopenia, had an acceptable postoperative mortality (122Ð125). Further experience has shown that, with the appropriate indication and clinical status, laparotomy can be carried out with expectation of clinical beneÞt. Laparoscopic surgery also can be performed and lead to signiÞcant clinical beneÞt.

Operating on a patient with AIDS entails extra personal risk to members of the surgical team, both from needle sticks and from contact with blood or body secretions by mucous membranes. The risk of nosocomial infection by hepatitis, tuberculosis or other illnesses during surgery has long been recognized. The potential risks to the surgical team can be minimized by wearing extra layers of gloves, using face masks or other devices to shield the mucous membranes from splattering of ßuids, separating needles from suture material and removing them and other sharp instruments from the immediate surgical Þeld, and using laparoscopic techniques when possible.

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Chapter 21

Neoplastic Complications of HIV Infection

Paula O'Connor and David T. Scadden

The hallmark of human immunodeÞciency virus (HIV) infection is decreased cellular immunity resulting from the preferential infection and destruction of CD-4 T-cells. This impairment of the immune system decreases the ability of HIV-infected individuals to contain certain viral infections, to respond to tumor antigens or to maintain normal cytokine homeostasis. The neoplasia seen in the setting of HIV infection is a consequence of these abnormalities. KaposiÕs sarcoma (KS), primary effusion lymphoma (PEL), plasmablastic/multicentric CastlemanÕs disease (MCD), non-HodgkinÕs lymphoma (NHL), HodgkinÕs disease (HD), and anogenital carcinomas are all seen with increased frequency in the setting of HIV infection. All but a subset of NHLs are preceded by viral co-infection with KaposiÕs sarcoma herpes virus (KSHV), Epstein-Barr virus (EBV), or human papilloma virus.

In this chapter the pathogenesis, clinical presentation, and treatment of the neoplasias known to arise more frequently in the setting of HIV infection will be reviewed. In addition, we will brießy discuss the complications commonly encountered while treating HIV-infected individuals with chemotherapy and comment upon the concurrent use of HAART during treatment for HIV-associated neoplasia.

KSHV RELATED DISEASE

KaposiÕs Sarcoma

Epidemiology

Initially described in 1872, KaposiÕs sarcoma (KS) was a rare tumor seen in individuals of Mediterranean, eastern European, and sub-Saharan extraction. It was called classic KS when diagnosed in elderly individuals from the Mediterranean and eastern Europe, and endemic KS when diagnosed in individuals from sub-Saharan Africa.

In the 1980s increased rates of KS in young gay men heralded the onset of the AIDS epidemic. At its peak incidence, approximately 20% of patients with AIDS had KS. This epidemic form of KS represented a 73,000 fold increased incidence of KS above that seen in the general population (1,2). KS was seen most frequently in men having sex with men (2). People infected with HIV via blood product contamination or intravenous drug use comprised less than 5% of those with HIV and KS. Promiscuity and oral fecal contact were subsequently identiÞed as risk factors (3), raising the suspicion of an infectious cofactor. In 1995, Moore and Chang detected KSHV, also known as Human Herpes Virus 8 (HHV8), in the lesions of patients with KS using a comparative analysis of the genetic differences between KS lesions and normal skin (2a).

The role played by race and gender in the development of KS is not well understood. KS has consistently been shown to have decreased incidence in women, but early reports of decreased incidence in African American men have not been consistently reproduced (2b).

KS was the most prevalent neoplasm arising in the setting of HIV infection at the peak of the AIDS epidemic. With the advent of highly active anti-retroviral therapies (HAART), KS incidence has declined sharply. This has been conÞrmed by investigators in Australia, Europe, and the U.S. (4Ð6). In some cases the declining incidence of KS antedated the widespread use of HAART. However, the rate of decline accelerated signiÞcantly with its introduction.

Reasons for the decreased incidence of KS prior to the initiation of HAART include earlier detection of HIV infection and a declining incidence of AIDS (7). Public health initiatives to prevent the spread of HIV may perhaps have contributed to the decline by decreasing transmission rates of KSHV. Improved opportunistic infection prophylaxis and treatment may have further contributed to the

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decline in KS by reducing levels of inßammatory cytokines known to synergize with HIV-1 Tat to promote angiogenesis and KS (8). Other effects of HAART which may be relevant include decreasing the stimulation of KSHV replication by HIV-1 Tat and preserving or restoring immune function necessary to control viral infection (9). In addition, recent animal model experiments have suggested that protease inhibitor anti-HIV medications may directly impair angiogenesis and progression of KS (10).

KS Pathology and Pathogenesis

KS is a multifocal angioproliferative disease. KS lesions consist of endothelial cells lining ectatic vascular spaces surrounded by spindle cells. These spindle cells are considered to be the neoplastic component of the KS lesion. The spindle cells are admixed with an inßammatory inÞltrate and extravasated red blood cells giving the lesions their characteristic red-purple hue.

KSHV has been detected in the endothelial cells and spindle cells of the KS lesion (11). The virus is a complex gamma herpesvirus with genes encoding many cellular protein homologs including bcl-2, interleukin 6 (IL-6), macrophage inßammatory proteins (MIP-I, MIP-II), and cyclin D-1. Like other well known gamma herpesviruses such as Epstein-Barr virus (EBV) and herpesvirus saimiri (HSV), KSHV encodes for gene products that are capable of inducing malignant transformation (12,13) when transfected into cells. Among these is the latent nuclear antigen LANA. LANA has been shown to transform rat Þbroblasts through its interaction with the retinoblastoma susceptibility protein (pRB) (13). The pRB protein plays a signiÞcant role in cellular proliferation, differentiation, and senescence. Other transforming proteins encoded by KSHV include K-1, K-12 (14,15), but it should be remembered that each of these genes has been tested in artiÞcial *in vitro* settings. It is unclear if these gene products contribute to tumor formation *in vivo* or whether chemokine or cytokine homologues affect local microenvironments or whether the constitutively active chemokine receptor encoded by KSHV is able to alter cellular physiology sufÞciently to facilitate oncogenesis.

The ability of KSHV to encode gene products that transform cells, the virusÕ ubiquitous presence in KS lesions of classic, endemic and epidemic type and evidence that KSHV infection precedes tumor transformation, all strongly support a causative role for the virus in KS (16Ð18). The issue of whether KS is a clonal malignancy is more complex. Judde et al demonstrated monoclonal terminal repeats in KS lesions suggesting outgrowth of the tumor from a single clone of KSHV infected cells (19). Others have analyzed the patterns of X-chromosome inactivation similarly demonstrating the clonality of KS cells (20Ð22). However, some studies have suggested polyclonal activation (22) rendering the clonal issue still unresolved.

Immunosuppresion appears to play a central role in the development of KS despite the fact that KS has been described in patients with variable CD-4 cell counts. It generally occurs with advanced immunosuppression, however, and has been described in patients immunosuppressed for reasons other than HIV infection such as organ transplantation (23Ð26). Such tumors are generally quite responsive to immune restoration with rapid regression seen when immunosuppressive drugs are reduced. Similarly, Cattelan and colleagues reported a correlation between tumor response and increased CD-4 count and decreased HIV-viral load (27). As immune restoration occurs in the months following initiation of HAART, the majority but not all patients with KS will have improved tumor control.

Clinical Presentation and Diagnosis

HIV associated KS differs from classic KS in its proclivity to present systemically, involving the skin, the GI tract, and the viscera. Although KS in AIDS patients most commonly presents as erythematous, non-tender, macular, papular lesions involving the skin, it also commonly involves mucosal surfaces of the oropharynx, upper and lower respiratory tract, and gastrointestinal tract and may involve abdominal viscera. KS frequently involves the lymphatics and lymph nodes, in which case patients present with associated edema local and distal to the site of involvement. Edema is commonly found in advanced KS due to the combined heightened permeability of KS vasculature, the release of vascular endothelial growth factor (VEGF) by tumor tissue enhancing permeability of normal vessels and tumor disruption of lymphatic drainage.

At the height of the U.S. AIDS epidemic, KS was among the most frequent presenting signs of HIV infection. While no longer as common, it is not an infrequent indicator of HIV positivity, though not the sole cause of hyperpigmented macules in HIV-infected individuals. The diagnosis of cutaneous KS requires differentiation from other non-blanching skin conditions such as bacillary angiomatosis. Bacillary angiomatatosis is seen with increased frequency in patients with HIV infection and may be differentiated from KS by obtaining a punch biopsy of the skin and staining to exclude Bartonella infection. As this entity is curable with antibiotics, it is clearly important to distinguish it from KS.

The diagnosis of pulmonary, gastrointestinal or visceral KS should be entertained when a patient with HIV infection and a known history of KS presents with unexplained cough, chest pain, hemoptysis, shortness of breath, gastrointestinal bleeding or abdominal pain. It must be noted, however, that patients with KS may present with disease limited to the skin, the viscera, or involving both the skin and the viscera. The presence of disease in one area does not rule out its presence elsewhere.

The gastrointestinal tract is the most common site of extra-cutaneous KS. It most commonly involves the stomach, the small and large bowel (28). It may be detected using endoscopy or barium upper and lower gastrointestinal series. Endoscopy reveals ßat and nodular mucosal lesions while barium studies reveal nodular KS lesions and diffuse wall thickening. CTs may also reveal luminal disease and give information regarding lymphadenopathy, masses, and organ involvement as well. Like mucosal KS, hepatic and splenic KS may occur but are most often asymptomatic and not necessarily a basis for aggressive therapy.

Pulmonary KS is the second most common site of extracutaneous disease and is the most life threatening manifestation of the disease. There are no deÞnitive radiologic criteria for the appearance of pulmonary KS on x-ray or CT scan, however pulmonary KS may appear as a coarse interstitial pattern with thickened bronchovascular bundles or multiple poorly deÞned nodules scattered throughout the lung. Pleural effusions frequently accompany interstitial disease and may be hemorrhagic. Parenchymal lung disease generally does require aggressive management with systemic chemotherapy to avoid signiÞcant respiratory compromise.

Staging

The staging system for KS devised by the AIDS Clinical Trial Group (ACTG) divides KS into good and poor risk categories based upon tumor burden, immune status, and HIV-1 viral load (29,30). Good risk patients are those with KS limited to the skin, lymph nodes, and oropharyngeal palate. Palatal lesions in good risk patients must be ßat. Good risk patients are considered those with a CD-4 count greater than 200 cells/mm^3, no B symptoms (fever, night sweats, $>10\%$ weight loss) and no previous history of opportunistic infections, including thrush. Poor risk patients are those with a large tumor burden, deÞned as bulky oropharyngeal, gastrointestinal, and pulmonary disease. KS lesions with associated edema and ulceration are also placed in the large tumor burden category. CD-4 cell counts less than 200 cells/mm^3, B symptoms, a history of opportunistic infections and other illnesses associated with advanced HIV disease are characteristic of poor risk patients.

Therapy

KS therapy may be local or systemic. The approach is guided by the impact of the tumor on the patient, risk for treatment related toxicity, and immune status. Patients should be questioned about pain, edema, bleeding, respiratory compromise, abdominal pain, difÞculty eating, isolation as a result of visible skin lesions, HIV stage and HIV therapy before deciding upon a treatment plan. Patients with good or poor risk disease who have not initiated anti-retroviral therapy should begin such therapy before embarking on speciÞc KS treatments. HAART, alone, is capable of inducing KS remission in some patients in 8Ð12 weeks (31Ð34). Patients presenting with poor risk disease and impending visceral crisis may initiate cytotoxic therapies, along with HAART, to control their disease more rapidly. These cytotoxic therapies may then be tapered or discontinued once disease control has been established.

Long-term cytotoxic therapies may be considered for those patients presenting with poor risk disease despite effective HAART therapy. When disease control has been established, the cycle length of the therapy may be tailored to maximize quality of life and maintain disease control.

The response of KS lesions to therapy is variable and depends upon the duration and extent of the lesion. Long-standing lesions are frequently large, nodular, and darkly pigmented. They are less likely to resolve completely. Lesions may become ßattened, exhibit central clearing, and decreased edema but frequently maintain some pigmentation because of extravasated red blood cells and hemosiderin staining of the skin. This hyperpigmentation can be expected to gradually fade but the pace of resolution is often measured in months to years.

Local Therapies

Intralesional vinblastine, cryotherapy, laser therapy, and radiotherapy given in single and multi-dose fractions have all been used with varying rates of success to treat localized good risk KS (35Ð37). The beneÞts of these approaches include minimal side effects and a short duration of therapy. Their complications include pain, Þbrosis, ulceration, mucositis, secondary infection and scarring.

Topical 9-cis-retinoic acid (Aliretinoin) has also been approved by the U.S. Food and Drug Administration (FDA) for KS treatment. It is effective when used topically and in some cases when given orally (38). The side effects include headache, dry skin, hyperlipidemia, and pancreatitis when it is given orally. Topical therapy needs to be judiciously applied as local reactions can be substantial.

Interferon-alpha has been used to treat KS locally as well as systemically. The known anti-HIV, anti-angiogenic, anti-proliferative, and pro-immunologic activities of interferon were thought to optimally suit it for treating KS, and it can be effective in producing KS remissions, taking approximately 10 weeks to induce maximal effect. However, ßu-like symptoms, depression, leukopenia and liver function abnormalities may be limiting. In general, interferon should be coupled with HAART and doses as low as 2 million units/tiw have been shown to be active in the setting of combined anti-retroviral and interferon therapy (39).

Systemic Therapies

Chemotherapeutic agents used to treat KS include Paclitaxel, Vinblastine, Vincristine, Etoposide, Bleomycin, Doxorubicin, and Daunorubucin (40–46). Caution must be used in interpreting the response rates of therapeutic trials using these agents. Due to the frequent residual pigmentation, bidimensional tumor measurements often underestimate meaningful response rates in more recent clinical trials. However, other parameters of benefit were sometimes used in older studies tending to inflate response rates, though perhaps these are more closely aligned with patient and clinician estimates of benefit. ABV (Adriamycin, Bleomycin, and Vincristine) combination therapy has a response rate of 50–88% but also has a substantial side effect profile. Nausea, vomiting, peripheral neuropathy, neutropenia and Raynaud's phenomenon are among those side effects that are frequently described. As a consequence investigators have more recently used single agents with good activity but fewer side effects to treat KS.

Liposomal Doxorubicin (20 mg/m2) has induced responses in 46–50% of treated subjects in phase III trials when given at 3 week intervals as compared with a 25% response rate in subjects treated with ABV (47). Liposomal Daunorubicin (40 mg/m2) given at two-week intervals has also been tested against ABV (48). The response rates were equivalent but the toxicity significantly less with the single agent.

Paclitaxel (100 mg/m2) appears to be the most effective single agent regimen for KS, producing response rates of 50–70% when given at two-week intervals (46,49). These responses are seen in naïve subjects as well as those who have been previously treated with liposomal doxorubicin (50). The side effects of paclitaxel and liposomal doxorubicin are typically mild and include mild cytopenias and fatigue. Paclitaxel may also cause peripheral neuropathy, joint aches, and alopecia.

The duration of remission induced by liposomal doxorubicin or paclitaxel is variable ranging from months to years. Patients failing one may be treated with the other with excellent response. The interval of therapy with liposomal doxorubicin or paclitaxel may be tailored to each individual patient. All patients should begin with therapy at two to three week intervals depending upon the agent used. Once disease control is established the interval between courses of therapy may be extended. Chemotherapy may be reduced in frequency based on durability of tumor response once a stable HAART regimen has been established.

Angiogenesis inhibitors such as Thalidomide, IM-862, TNP-470, Col-3, and vascular endothelial growth factor (VEGF) receptor inhibitors have been used to treat KS in phase I and II trials given the vascular nature of the tumor (51–54). They have produced variable response rates and their role in future KS treatment remains to be determined.

The incidence of KS among patients receiving antiviral therapy with ganciclovir or foscarnet is low suggesting a role for anti-viral drugs in the control of KSHV. Ganciclovir, Foscarnet and Cidofovir have been shown to control KSHV *in vitro* (55–58). Cidofovir has also been shown to decrease KSHV cytoviremia, and induce KS remission in scattered anecdotal reports (59–61). The response to antiherpesvirus drugs is generally not substantial enough to merit their routine use for tumor therapy, however.

The decreased incidence of KS in women has led to an investigation of hormonal agents in the control of KS. An associated protein found in certain preparations of human chorionic gonadotropin (HCG) has been shown to induce KS regression (62,63). Once again its utility remains to be determined.

Multicentric Castleman's Disease

KSHV is a lymphotropic virus similar to EBV. KSHV infection has been implicated in the development of multicentric Castleman's disease (MCD) and primary effusion lymphoma, lymphoproliferative disorders seen with increased frequency in patients infected with HIV. As in the case of KS, KSHV transcripts have been detected in tissue samples of MCD, however, the role of KSHV in the development of this disorder has not been well defined.

Epidemiology

Castleman's disease was first described in 1956. It is a rare disorder that is divided into two broad categories, unicentric or localized and multicentric. Unicentric Castleman's occurs in non-HIV-infected individuals, while multicentric Castleman's, the plasmablastic variant, occurs most frequently in the setting of HIV. KSHV has been almost uniformly detected in cases of plasmablastic MCD (64–67).

Pathology and Pathogenesis

Plasmablastic MCD is characterized by preservation of typical lymph node architecture despite a pronounced interfollicular plasma cell infiltrate (11–19). KSHV infected B-cells in the mantle zone express lytic and latent antigens including vIL-6 and LANA (66,68,69). vIL-6 is homologous to hIL-6, which plays a significant role in B-cell proliferation, while LANA has transforming potential.

The mechanisms by which KSHV may induce MCD are not clear, however, expression of the known B cell proliferative factor, vIL-6 produced by KSHV has been consistently noted (69a,69b). Of note, the rhesus rhadinovirus, which is closely related to KSHV and also encodes a vIL-6 homolog, induces an MCD-like syndrome when

inoculated into SIV (simian immunodeficiency) infected macaques (70–72). In addition to virus specific products, however, clinical studies have indicated that active MCD correlates with higher circulating levels of host derived B cell stimulating cytokines such as IL-6 and IL-10 (72a). The relative contribution to B cell proliferative drive of KSHV gene products versus host immune activation is not clear, but multiple stimuli appear to interact in the development of this often devastating complication of HIV.

Judde and his colleagues examined the terminal repeat segments of KS, MCD, and primary effusion lymphoma samples to assess their clonality (19). No clonality was found among the terminal repeats of the MCD samples. This syndrome may represent a polyclonal proliferation in response to KSHV infection of B cells.

Clinical Presentation and Therapy

Multicentric Castleman's disease is characterized by fever, lymphadenopathy, and organomegaly. Diagnosis requires a lymph node biopsy. The clinical course of MCD occurring in the setting of HIV is often aggressive, perhaps more aggressive than that occurring in the non-HIV infected (67,73,74). Durable clinical remissions despite therapy are rare. Up to 25% of patients go on to develop non-Hodgkin's lymphoma (75,76) and concurrent KS is common.

Therapies used to treat MCD include chemo and radiation therapy, antiviral, and anti-CD-20 monoclonal antibodies (74,77,78). Chemotherapy has been utilized most often but anti-CD 20 antibodies appear to have substantial activity in limited anecdotal reports. No reports of combination therapy are available to date.

Primary Effusion Lymphoma

Primary effusion lymphoma (PEL) accounts for $<3\%$ of the non-Hodgkin's lymphomas encountered in the setting of HIV infection (79). It is generally an agonal complication of advanced HIV disease (80). Like KS, it is most often found in HIV positive homosexual and bisexual men. PEL has been described in non-HIV infected individuals. In these cases patients are generally older and immunocompromised (81–83).

PEL cells are characterized by the uniform expression of KSHV and near uniform expression of EBV gene sequences (84–86). The cells exhibit immunoblastic cytology, indeterminate immunophenotype, and B-cell genotype, evidenced by immunoglobulin gene rearrangement. CD-45 and CD-30 are frequently expressed (84,87). MYC rearrangements are absent and BCL-6 mutations have been described (88). Latent KSHV and EBV gene products are capable of inducing transformation. Beyond this little is known about the pathogenesis of PEL. Constitutive activation of NF-kB may play a role in preventing apoptosis of PEL cells, while vIL-6 may promote cell growth (89,90).

Patients with primary effusion lymphoma typically present with pericardial and pleural effusions or new onset ascites with scant lymphadenopathy. There is no staging system. Treatment is palliative. Radiation, modified CHOP, and Liposomal Doxorubicin and Daunorubicin have been utilized with minor success (91–94).

EBV Related Disease

The gammaherpesvirus EBV has been implicated in the development of lymphoid malignancies including HIV-associated Hodgkin's disease (HD), primary CNS lymphoma, systemic AIDS related lymphoma (S-ARL) and PEL. The role of EBV in AIDS associated Burkitt-lymphoma is unclear, but appears to be small. EBV has been implicated in the development of nasopharyngeal carcinoma. This does not occur with increased frequency in the setting of HIV infection.

EBV is a lymphotropic virus that avoids immune detection by establishing latent infection. Differential gene expression during latent infection is associated with the development of different tumors. Latency pattern I is associated with the development of BL and PEL; latency pattern II with the development of HD and nasopharyngeal carcinoma; and latency pattern III with CNS, S-ARL, and post transplant lymphoproliferative disease (PTLD). EBV latent membrane protein 1 (LMP-1) is expressed in latency patterns II and III. The LMP-1 interacts with tumor necrosis factor receptor (TNFR) signaling pathways that activate NF-kB effecting apoptosis and cell growth. LMP-1 has demonstrated transforming potential (95).

The mechanism by which HIV infection promotes the development of EBV lymphoproliferative disease is poorly understood. However, it is clear that virally induced immune activation in the setting of decreased cellular immunity, and viral immune evasion play pivotal roles.

Hodgkin's Disease

Epidemiology and Clinical Presentation

The excess risk for HD in the setting of HIV has been measured at 3–18 times the risk for HIV negative individuals. 80–100% of these are EBV associated (96,97). The predominant histologies are mixed cellularity and lymphocyte depleted. Cases of HIV-associated HD in patients with CD-4 counts <10 and as high as 1300 cells/mm^3 with a median between 120 and 250 cells/mm^3 have been reported (98–101). The risk for HD appears to rise with advancing immunodeficiency. Gender, ethnicity, and intravenous drug use do not appear to alter the risk for HD development (102–104).

HIV-associated HD is typiÞed by advanced stage and the presence of B-symptoms: fever, night sweats, and >10% weight loss. Non-HIV associated HD is characterized by the spread of disease via contiguous lymph-node groups. HIV-associated HD frequently spreads in a non-contiguous pattern and frequently involves extranodal sites (98,101,105,106). Among the sites reported are the tongue, rectum, skin, and lung. The bone marrow is the most frequently observed site of extranodal involvement, occurring in 40Ð50% of patients at presentation.

The advanced stage and extra-nodal presentation of HIV-associated HD may be a reßection of altered HD biology in the setting of immunocompromise but may also be a reßection of late diagnosis. The diagnosis of HD requires differentiation from the persistent generalized lymphadenopathy seen in HIV infection, chronic infection with mycobacteria and fungi, CMV, and non-Hodgkin's lymphoma. Enlarged lymph nodes should be biopsied if they are rapidly growing or unchanged over weeks. It should be remembered that needle aspirates are often non-diagnostic in HD and that excisional biopsy is often required.

The Ann-Arbor staging system is used to stage HIV-associated and non HIV-associated cases of HD.

Therapy

The treatment approach for HIV-infected patients with HD is the same as that for HIV-negative patients. Complete response rates of 45Ð65% have been reported by groups using Adriamycin, Bleomycin, Vinblastine, and Dacarbazine (ABVD), without growth factor support or HAART (100,107). The median survival was 15Ð18 months. No data on response rates with the ABVD regimen since the advent of HAART have been reported. An Italian group using Epirubicin, Bleomycin,Vinblastine ± Prednisone (EBV ± P) with concomitant pneumocystis prophylaxis, AZT or DDI, and growth factor support reported CR rates of 66% with a three year survival rate of 53% (108). This same group has reported the preliminary results of a 20 patient study utilizing the Stanford V regimen in which the CR rate was 80% and the PR rate 15% (109).

Primary CNS Lymphoma

The development of primary CNS lymphoma is an increasingly uncommon and generally lethal complication of advanced AIDS. These lymphomas accounted for up to 15% of all ARL prior to HAART and their incidence was 1,000-fold greater than that seen in the general population. These lymphomas occur in the setting of profound immunosuppression, in patients with CD-4 counts generally less than 50. With the advent of HAART the incidence of primary CNS lymphoma has plummeted (110,111).

These tumors are aggressive, B-cell in origin and EBV related. They express the type III EBV latent gene proÞle of lymphoproliferative disease and are very comparable to post-transplant lymphomas in that regard. Their clinical presentation may be focal or non-focal. Typical symptoms include confusion, headache, seizures, and in some cases cranial neuropathies.

CT and MRI imaging usually reveal multiple ring-enhancing peri-ventricular lesions, however, single lesions have also been described. Attempts to differentiate toxoplasmosis, the most common CNS mass lesion in patients with AIDS, from CNS lymphoma indicate some differences based upon the size and location of the lesion; larger than 2 cm and peri-ventricular favors NHL, extending across the midline occurs only with malignancy. Brain biopsy represents the gold standard for making the diagnosis of primary CNS lymphoma (112). However, lumbar puncture with evidence of EBV DNA using PCR is 100% sensitive and 98% speciÞc for the diagnosis in some studies and is signiÞcantly less invasive (113). Single photon emission computed tomography (SPECT) thallium 201 and positron emission tomography (PET) scanning may also help in making the diagnosis (114,115). Increased uptake is seen with primary CNS lymphoma.

Therapies for primary CNS lymphoma are limited. They include radiation and HAART. Whole brain radiotherapy (30Ð60 Gy) combined with steroids results in response rates of approximately 75%, with a median survival of approximately three months (116). Combination chemotherapy and radiation appears to add only toxicity while high dose methotrexate may offer a reasonable alternative to radiation therapy (117Ð120). HAART has been associated with spontaneous remissions without other systemic or local therapies but is generally used in addition to other therapies (121). Given the universal involvement of EBV in primary CNS lymphoma, a regimen of ganciclovir, azidothymidine (AZT), and IL-2 was developed (122). This regimen appears to have some anti-tumor effect, but the results are preliminary.

Systemic AIDS-Related Lymphoma

The most common systemic ARLs are aggressive B-cell tumors. Immunoblastic, diffuse large cell (DLCL), Burkitt, and Burkitt-like lymphomas occur with relative risks of 650, 110, and 260, respectively (1,2,123). Indolent NHLs may also occur with increased frequency in the setting of HIV, but the magnitude of increase is modest. A relative risk 14-fold greater than that seen in the general population has been reported (1). Risk factors for the development of S-ARL are not completely deÞned. Immunosuppression is among the risk factors as is viral co-infection but their precise roles are not completely clear. 35Ð65% of S-ARLs are EBV related and less than

3% are KSHV related. Median CD-4 counts at diagnosis range from 50–191 cells/mm^3 for immunoblastic and DLCL variants and higher for the Burkitt and Burkitt-like variants (3,124–126).

The impact of HAART on the development of S-ARL is still in the process of being deÞned. S-ARL incidence has been reported to be decreasing as well as to be remaining static (126a,126b). Similarly, the degree of immunosuppression at which S-ARL develops has been reported to be increased, as well as decreased (127–129). These differences likely reßect patterns of access to HAART and other medical care. Overall, the most recent and longest studies have indicated that the overall decline in S-ARL is approximately 50% (129a).

The majority of patients presenting with S-ARL are Caucasian and male. However, the numbers of Black, Latino, and female patients with S-ARL appear to be increasing, likely reßecting changes in the pattern of HIV infection. Differences in the rates of NHL among Caucasians and other ethnic groups may also be a reßection of polymorphisms of the CXCR-4 chemokine receptor, which confers increased risk for NHL and is most often found in Caucasians (130,131).

Pathogenesis and Pathology

HIV promotes the development of NHL, EBV related or unrelated, by impairing immune surveillance, producing chronic antigen stimulation, and dysregulating cytokine expression. This allows for oligoclonal B-cell proliferation during which genetic lesions capable of producing malignant transformation accumulate, resulting in the development of a malignant clone. Among the genetic rearrangements noted in S-ARLs are BCL-6, and c-myc rearrangements. These are found in approximately 33 and 40% of DLCLs, respectively (132–137). C-myc rearrangements have been found in 70–100% of HIV associated Burkitt and Burkitt-like lymphomas (138–140). P53 and Ras mutations have also been reported (135,141).

The histologic features of S-ARL are similar to those of NHL in HIV-uninfected patients. S-ARLS, however, tend to display a greater number of mitotic features consistent with a higher growth fraction and more aggressive natural history (142,143).

Plasmablastic NHL is a rare S-ARL (144). The tumor cells are plasmablasts expressing CD-138. There is weak or no expression of CD-20 and CD-45 (145). Plasmablastic lymphomas typically present in the oral cavity and jaw (146,147).

The majority of S-ARLs are of B-cell origin. However, T-cell ARLs have been described. Demonstration of HIV sequences upstream of the c-fes proto-oncogene suggests a direct role for HIV in the development of a subset of these lymphomas.

Clinical Presentation and Staging

Extranodal disease is a characteristic of S-ARL with 50–60% of patients presenting with disease limited to extra-nodal sites. The most common sites of extranodal disease include the bone marrow, CNS, and GI tract. The small bowel, ileum and rectum are the areas of the GI tract most commonly involved. The presenting symptoms of S-ARL are highly variable given these unusual sites of involvement. B symptoms, fever, night sweats, and weight loss are common, often reßecting a large burden of disease.

Diagnosis of ARL requires documentation of a clonal process utilizing ßow cytometry and/or pathology with immunophenotyping stains and cytogenetic analysis. The initial evaluation of patients suspected to have ARL should include CD-4 count, HIV viral load, notation of previous anti-retroviral therapy and opportunistic infections, uric acid, and LDH, as these factors may inßuence treatment options, reßect prognosis, and risk for complications during therapy. Microbiologic evaluations for *Pneumocystis carinii*, cytomegalovirus, *Toxoplasma gondii*, Mycobacterial disease, and Cryptococcus should be undertaken in patients presenting with B symptoms and CD-4 counts less than 200 cells/mm^3.

Staging for S-ARL is similar to that employed for lymphoma patients without HIV infection. It should include CT imaging of the chest, abdomen and pelvis with contrast in addition to the studies noted above. Head CT or MRI, and lumbar puncture should also be included in the initial evaluation, given the higher risk for CNS involvement in patients with ARL (117).

Therapy

CD-4 count less than 100 cells/mm^3, age over 35, advanced stage (III/IV) and intravenous drug use are among the prognostic indicators identiÞed for poor prognosis prior to the advent HAART (148,149). Subsequent analyses have identiÞed LDH, Karnofsky status, prior AIDS deÞning illness, extranodal disease with extensive bone marrow involvement, and high grade histology as predictors of risk (127). The International Prognostic Index has been validated for use in the setting of HIV (150), though the magnitude of the studies has been limited to date.

For those patients successfully using anti-retroviral therapy, treatment options for ARL are increasing and include dose intensive therapies. HAART has made aggressive therapies previously considered untenable in AIDS, generally more tolerable and the rationale for intensive therapy more reasonable. Prior efforts to minimize the toxicity of anti-tumor therapy due to the generally poor functional status of the patients have largely been supplanted by efforts to maximize the chance for cure of these aggressive, but chemosensitive tumors.

Half-standard dose therapies such as m-BACOD were tested prior to the advent of HAART. They were shown to be active with equivalent tumor outcomes, but have reduced toxicity compared with full dose chemotherapy (151). However, results from other studies using half dose CHOP have raised concern about inferior tumor control (152,153) and current practice is to restrict such dose compromised regimens to those patients with advanced AIDS who are considered HAART failures or unable to tolerate full dose therapy due to concurrent complications.

Outside the setting of end-stage AIDS, full dose regimens such as CHOP have become standard practice with complete response rates documented in the 48–63% range (152–154). Further, recent experience with infusional regimens has suggested that dose delivery may offer promising alternatives. Infusional cyclophosphamide, doxorubicin and etoposide (CDE) over a 96-hour interval was tested under the direction of Sparano and colleagues and was shown to be highly active with a complete response rate of 58%, confirmed at 46% in a follow-up multi-institutional trial (155,156). The U.S. National Cancer Institute (NCI) has tested dose adjusted EPOCH (157,158) and demonstrated durable complete responses in over 75% of patients. The recent improvement in the treatment of aggressive lymphomas by adding rituxan to CHOP has prompted testing of this regimen in ARL in a multi-center phase III trial by the AIDS Malignancy Consortium (AMC), which is nearing completion. Combining rituxan with infusional CDE was well tolerated and resulted in a highly encouraging 86% complete response rate in a preliminary report by Tirelli and Sparano (156).

Second line therapy has always been problematic in S-ARL due to poor tolerability and response rates. However, with the impact of HAART, there is considerably better tolerance and efforts to use more aggressive regimens are again underway. The use of high dose regimens requiring autologous stem cell rescue has been reported to be reasonably tolerated with no difficulty in engraftment (159,160). Non-myeloablative allogeneic transplants are also of considerable interest, but should only be undertaken in the context of clinical trials.

It remains an issue of debate as to whether anti-HIV medication should be continued during the course of cytotoxic chemotherapy. Concurrent anti-retroviral (stavudine, lamivudine and indinavir) in combination with CHOP chemotherapy was tested by the U.S. AMC assessing pharmacokinetic as well as clinical parameters. While no untoward or unexpected toxicities were observed, the clearance of cyclophosphamide was reduced by approximately 50%. In contrast, no alteration in the clearance of doxorubicin or indinavir was noted (160a). A report in which multiple regimens were used documented a higher incidence of hematologic and neurologic toxicity in those receiving HAART along with chemotherapy, which was offset by an improvement in infection and survival rates (154). An alternative approach was adopted by the U.S. NCI where all anti-retrovirals were held during EPOCH chemotherapy. A rise in HIV viral load and decline in CD-4 counts was observed as expected, but both parameters normalized following re-introduction of medications at the end of tumor therapy (161). Weighing the relative tolerance of the anti-virals with the potential for interactions needs to be considered in deciding whether to continue antiretroviral agents; but the anti-HIV medications need to be either stopped or a combination continued, half-measures are to be avoided to prevent the emergence of HIV resistance.

Another therapeutic consideration in the care of patients with S-ARL is whether to use prophylactic CNS therapy to prevent the high incidence of CNS relapse (up to 20%) (117) noted early in the HIV epidemic. Recent data would suggest that in addition to clinical features which have been associated with CNS involvement outside the setting of HIV infection, such as paranasal sinus, bone marrow or testicular involvement, EBV status of the tumor may by important in S-ARL. Cingolani and colleagues documented increased risk for CNS relapse when EBV was present in the primary tumor ($p = 0.003$) (162). These data suggest that CNS prophylaxis with intrathecal chemotherapy may be more selectively applied though no treatment trials have tested this hypothesis.

HPV Related Disease

An increased incidence in genital squamous neoplasia has been documented in HIV-infected individuals since the 1980s (163). Cervical and anal intraepithelial neoplasia (CIN, AIN), presumed to be precursor lesions for invasive squamous cell carcinoma, are the most common manifestations of this disease. However, only invasive cervical carcinoma (ICC) is classified as an AIDS-defining illness, and it is not clear if invasive oral or cervical cancer is increased in the HIV-infected population. Risk factors for the development of squamous neoplasia include immunosuppression (CD-4 < 200), concurrent infection with HPV subtypes 16 and 18, early sexual activity, promiscuity, history of sexually transmitted disease and anal receptive intercourse (164–167).

The magnitude of the problem posed by genital squamous cell neoplasia in the setting of HIV infection is difficult to ascertain. This is well demonstrated by reviewing the rates of CIN and ICC in women dually infected with HIV and HPV. While dually infected women have been shown repeatedly to have a higher incidence of CIN (168,169), whether there is an increased incidence of ICC has been debated. Italian and American linkage analyses have demonstrated increased rates of ICC among HIV-infected women, while studies from Africa and Australia have not (166,170). AIN lesions are reported to occur in 25–45% of HIV-infected homosexual and bisexual men engaging in anal receptive intercourse (171). Progression from low to high-grade dysplasia was documented in 15% of patients observed over 21 months (172).

Despite this an increased incidence of invasive squamous cell carcinoma in men having sex with men has not been convincingly demonstrated.

Expansion of screening programs for genital squamous neoplasia and improved cancer registry documentation of HIV status may help clarify the true extent of this problem. However, whether to institute therapeutic intervention for dysplastic disease, and whether these are truly pre-malignant lesions, remain open areas of debate.

Pathology and Pathogenesis

Cellular disorder, atypia, and the appearance of atypical mitoses are the deÞning characteristics of squamous intra-epithelial lesions (173). Progression of this pattern of cell growth below the basement membrane is described as invasive.

The role of HIV in the development of squamous intraepithelial neoplasia is not well understood. HPV gene products E6 and E7 have demonstrated transforming potential. HIV induced cytokines, growth factors, and proteins like HIV-Tat may alter local cellular immunity, or effect HPV transcription and replication (174,175)

Clinical Presentation and Therapy

Genital squamous neoplasia is typically asymptomatic. Low-grade anal intraepithelial lesions may present as anal condyloma. These may cause discomfort or anal leakage depending upon their size. Invasive squamous disease of the cervix or anus may present with pain or bleeding.

Screening for cervical neoplasia with an annual Papanicolaou (Pap) smear is recommended for HIV-infected women with CD-4 counts >200. Bi-annual exams are recommended for women with CD-4 counts <200. Guidelines for screening the anal canal in men have not been established. Anal pap smears, anoscopy, and anal colposcopy are being used to screen high-risk patients at some medical centers (176,177), though the value of such is unproven at present.

CIN may be treated with ablation or excision using cryotherapy, lasers, cone biopsies and the loop electrosurgical excision procedure. AIN may be treated with excision, imiquimod cream, and observation with biopsy depending upon the size and placement of the lesion (178Ð180). Imiquimod therapy may only be used to treat lesions on the anal verge. CIN and AIN lesions frequently recur in patients with persistent HPV infection so repeat screening and treatment is necessary.

Invasive genital squamous neoplasia should be treated with the same approach used in HIV-negative patients. Combination chemotherapy and radiotherapy may produce bone marrow suppression and radiation induced skin injury, but these may be tolerated with supportive care (181Ð183).

Chemotherapy for AIDS-Associated Malignancy in the Era of HAART

Questions regarding the safety of the co-administration of HAART and chemotherapy have been addressed by several investigators (152,184Ð186). It is clear from these studies, that HAART may be administered safely with at least some regimens of dose intensive chemotherapy. Questions remain, however, regarding the necessity of HAART therapy for maximal tumor control and the timing of HAART initiation in previously drug na•ve or resistant patients.

The magnitude of immunosuppression produced by cytotoxic therapies for HIV-associated malignancies depends upon the underlying malignancy, the pre-existing level of immunosuppression, and the regimen intensity. The greater risk for opportunistic infection in patients undergoing chemotherapy must be weighed against the need for tumor control. HAART may be a way to tip the scales. It may increase the likelihood of achieving complete remission by improving immune function directed against tumor and associated viral antigens, decreasing HIV-associated stimulation of oncogenic viruses, and decreasing the production of inßammatory cytokines, like IL-6, that promote tumor growth and lymphoid stimulation.

HAART is not without risk, however. Zidovudine may cause signiÞcant bone marrow suppression limiting chemotherapeutic options and increasing the risk for opportunistic infection. Protease inhibitors may block the cytochrome P-450 pathway leading to altered metabolism of chemotherapeutic drugs such as cyclophosphamide. Didanosine may potentiate the peripheral neuropathy associated with chemotherapies such as paclitaxel and vincristine. The non-nucleoside reverse transcriptase inhibitor delavirdrine has been associated with severe potentiation of paclitaxol toxicity. In general, the p450 effects of NNRTIs raises concern about particular, unpredictable interactions.

In our institution patients who are taking an effective HAART regimen are encouraged to continue it as long as it is tolerated with the exception of NNRTIs or AZT. Patients who are unable to take their drugs as scheduled because of nausea and vomiting are encouraged to stop their regimen completely rather than compromising dose or schedule of anti-retrovirals to prevent the development of HIV drug resistance. Patients who need to discontinue their HAART regimen have been able to re-establish viral control once their regimen was restarted and this practice is now commonly applied in some centers. HAART na•ve patients are encouraged to begin anti-retroviral therapy after their ability to tolerate chemotherapy has been deÞned to permit clearer discrimination of the source of potential toxic reactions.

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AIDS and Other Manifestations of HIV Infection
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Chapter 22

Hematologic Manifestations of HIV Infection

John P. Doweiko

The Þrst human retroviruses were discovered in the late 1970s (1). Type one human immunodeÞciency virus (HIV-1), previously known as HTLV-III or lymphadenopathy-associated virus, is a member of the Retroviridae family and the Lentivirus subfamily (2,3). Other members of this subfamily are known to infect other mammalian species. Phylogenetic studies suggest that HIV-1 is a result of cross-transmission from simian viruses, although the exact ancestral point is still unknown (4). HIV-1 has now diversiÞed into at least seven different subspecies (clades). These clades differ in ease and mode of transmission, virulence, and global distribution (4).

HIV-1 infection may have had its origins in the 1940s; it reached epidemic proportions forty years later. Currently, it is estimated that over 1 million people in the United States and over 30 million people world-wide have been infected, making this the greatest lethal epidemic known to man (4,5).

Despite having a relatively simple structure, HIV-1 has a rather complicated mechanism of replication (6). Its genome is encoded within a single strand of RNA that is enclosed within a ÒshellÓ of P-24 protein (7)This is itself contained within a glycoprotein envelope that is studded with the transmembrane P-41 protein to which is attached the GP-120 protein (2,3). The GP-120 glycoprotein is necessary for binding to the CD4 protein of the target cells (8).

HIV-1 is genetically more complex than other members of the Retroviradae family (3). It has a genome of 9.7 Kb in total length, and contains three genes that are characteristic of replicative retroviruses (2,3). The *Gag* gene encodes for the core structural proteins of the virus, the *Pol* gene encodes for the viral enzymes (reverse transcriptase, integrase and protease), and the *Env* gene contains the genetic information for the surface glycoproteins of the virus (8). In addition, the genome of HIV-1 also includes six other genes that are not encountered in other retroviruses and are currently poorly understood (2).

Certain genes of HIV-1 are error-prone, particularly those encoding reverse transcriptase and the envelope proteins. Having this propensity to mutate *in vivo*, progression of the infection within a host is associated with evolution toward a quasi-species composed of viral variants (9Ð11). This allows for extensive genomic variation to develop from a single infecting event.

The CD4 protein is contained on T lymphocytes and cells of the monocyte/macrophage line and is a member of the immunoglobulin superfamily (12). This molecule is in high concentration on T-helper cells and monocytes, designating these cells as the major targets of the infection (13Ð16).Although the CD4 antigen may be the sole high-afÞnity cellular receptor for HIV-1 (13,14), other cell surface proteins act as co-receptors and markedly augment entry into the cell by a factor of at least a ten thousand-fold compared with cell membranes that lack these other proteins (12,17). These other molecules include the chemokine receptors (12). CXCR-4 (fusin) is a co-receptor for strains of HIV-1 that primarily invade T-cell lines. The beta-chemokine receptor CCR5 serves as a co-receptor for strains that infect cells of monocytic lineage (12,17). HIV-1 can also bind to cells by means of other molecules, albeit with much less afÞnity. These alternative receptors include the galactosylcereamide on the cells of the central nervous system, and complement receptors when the virus is part of an immune complex (12).

Other cells also express surface CD4 antigen, rendering them vulnerable to infection by HIV-1 (18). These include Þbroblasts and related cells such as glial cells, and the stromal reticular cells of the bone marrow (13,14). Cells may be infected via routes other than the CD4 antigen (13,14), including galactosylceramide on neurons and enterocytes (12). Entry into cells may also occur via Fc receptors when HIV-1 in bound to antibody within immune complexes (12).

Binding of HIV-1 to the CD4 antigen allows the virus to enter the cell. In addition, other important events that

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result from this binding are inhibition of signal transduction by the T cell CD3 receptor, and secretion of a multitude of cytokines by the infected cell that include interleukin-1B, TNF-alpha, and GM-CSF (12,19).

Some strains of HIV-1 preferentially infect monocytes, while others display selectivity for CD4 lymphocytes (13). This is important to the evolution of the intricate syndrome that results from this viral infection. The major sequellae of HIV-1 infection are degradation of cellular immunity (20). The clinical manifestations of this are the opportunistic infections and neoplasms. The other major result of HIV infection is an ultimately detrimental cytokine response that is deleterious in that it accelerates HIV replication and promotes tissue injury (21–25). During all stages of HIV infection there is dysregulation of cytokines: IL-6, TNF-alpha, Interferon-gamma, and IL-10 tend to be overexpressed, while IL-2, IL-4, and IL-12 are undersecreted (23,26,27). Many of the cytokines that are hyperexpressed result in upregulation of HIV replication (23,28).

The monocyte, as well as the CD4+ lymphocyte, plays an important role in the pathogenesis of HIV infection. Cells of monocytic lineage are central to the complex network of growth factors and cytokines that sustains and regulates the hematopoietic and immune systems (28). In the early phases of infection, monocytotropic strains of HIV-1 predominate over lymphocytotrophic strains (20,29). While HIV assembles almost exclusively on the plasma membrane of CD4 lymphocytes, it can assemble and accumulate within cytoplasmic vacuoles of monocytes and macrophages where it remains hidden from the immune system (16). Infection of cells of the monocyte/ macrophage line is not lethal, making these cells important reservoirs of HIV-1 and vectors for spread of the virus throughout the body (5,24,29). In non-lymphoid tissues, such as the central nervous system, local infection is predominately sustained by cells of monocytic lineage (29,30). Derivatives of the monocyte/ macrophage cell line such as glial cells of the central nervous system may be infected by and harbor the virus (31). Within these cells, the virus may replicate more rapidly than in other tissues (31). Progression of HIV-1 infection is associated with a shift toward more lymphocytotropic variants of the virus (29). This is important to the cellular immune dysfunction that is characteristic of AIDS (24).

One life cycle for HIV is about 1.2 days *in vivo*, with about 0.9 days of this being intracellular and the remainder of time representing the half-life of the virion within the tissues and/or blood (32,33). It was previously thought that after the initial viremia of HIV infection, the virus entered a "latent phase" during which viral replication took place at a very reduced rate. More recent studies have shown that viral replication persists throughout the course of the HIV infection (34,35). During the clinical "latent" phase those infected may be only minimally symptomatic. A large reservoir of HIV-1, however, is sequestered within lymphoid and other tissues wherein viral replication continues (34,36,37), resulting in a ceaseless destruction of the architecture and function of the lymphoid tissues.

HIV-infected persons produce and destroy about 30% of the total body viral burden daily (32,33,38), amounting to billions of viral particles. The clearance of HIV virions is relatively constant during the course of the HIV infection, regardless of the CD4+ lymphocyte counts (25,33). The decline in the CD4+ lymphocyte count that occurs with progression of HIV infection is due to the destructive capacity of HIV-1 for these cells eventually exceeding the replicative capacity of the body (32,33,36,38–40). This results in a progressive deterioration of the cellular immune system (39,40).

Unlike the viral production rate that tends to be fairly constant, there is large variation in the CD4 lymphocyte production rate from patient to patient (25,33). This may explain, in large part, the variations in disease course that occurs among patients. Some of those infected with HIV-1, however, do not succumb to the infection as rapidly as do others (41). In long-term survivors, the viral burden in the plasma and peripheral blood-mononuclear cells is less by several orders of magnitude than in less fortunate patients with similar duration of disease (42,43). These long-term survivors seem to have a vigorous viral-inhibitory CD8 lymphocyte response and a stronger neutralizing antibody response than is typically seen (42). Genetic alterations in the chemokine receptors may also confer some resistance to HIV-1 and promote survival (12).

SPECIFIC CYTOPENIAS IN PATIENTS WITH HIV INFECTION

Infection with HIV-1 is associated with suppression of hematopoiesis (43–46) (Table 22.1). The hematologic perturbations encountered with HIV-1 infection not only have morbidity of their own, but also hinder therapy directed toward the primary viral infection and those toward the secondary infectious and neoplastic complications (47–52). The need to reduce doses or interrupt therapy due to poor hematologic tolerance may cause emergence of drug-resistant organisms, and progression of infections and/or neoplasms (47,48).

TABLE 22.1. *Causes of cytopenias in HIV infection*

Ineffective hematopoiesis
Effect(s) of HIV-1 on stem cells
Effect(s) of HIV-1 on marrow stromal cells
Nutritional de ciencies
Alterations in growth factors
Alterations in cytokines
Myelosuppressive medications
Autoimmune peripheral destruction
Opportunistic infections involving the marrow
Tumor involvement of the marrow

Anemia

The most common, and well studied, cytopenia associated with HIV-1 infection is anemia. Although anemia occurs less often in the era of HAART than in the past, it remains a signiÞcant clinical problem. The degree of anemia correlates with the stage of HIV-1 infection. Prior to the introduction of HAART, 10Ð20% of people were anemic at the time of presentation, and 70Ð80% eventually become anemic with progression of the infection (49,53). In patients receiving HAART, mild anemia (deÞned as a hemoglobin level of 9Ð11 grams/dL) occurs in about 13% of patients. Severe anemia (hemoglobin levels less than 9 grams/dL) has an overall incidence of about 5% (54,55), particularly in those with advanced HIV infection. In this group, the one-year incidence of anemia was almost 37% in those with an AIDS-deÞning opportunistic infection, about 12% in those with a CD4 count less than 200 and/or a CD4 percentage less than 14, and about 3% in those without clinical or immunologic AIDS (56).

The major cause of anemia in HIV-infected patients is impaired erythropoiesis. The anemia is typically normochromic and normocytic and associated with an inappropriately low reticulocyte count (57). Macrocytosis is unusual, and tends to occur in those treated with zidovudine (57,58). Iron stores are almost always normal or elevated, and there is typically a decrease in serum iron along with a parallel decrease in the total iron binding capacity that is characteristic of the Òanemia of chronic disease.ÓSerum ferritin levels are often increased (57), and these levels tend to parallel the severity and duration of infection with HIV-1 (59Ð61). There may be a positive correlation between iron levels and the replication rate of HIV (62).

While decreases in serum B-12 levels are found in about 20% of HIV-infected patients (62,63,65,66), it is not clear to what extent these low levels contribute to the cytopenias of HIV infection (60,63,67,68). Patients usually do not have other manifestations of B-12 deÞciency, and typically do not improve markedly with parenteral repletion (57,65,66). It has been demonstrated in the non-HIV-infected population, that serum B12 levels may also have poor predictive value (69): Anemia may be absent in patients who are found to be B12 deÞcient using assays that rely on vitamin B12-dependent metabolic pathways (69,70). Macrocytosis may be absent with B-12 deÞciency (70). The low B-12 levels that occur with HIV infection seem to be due to altered serum transport of the vitamin (63,66), but there may be abnormal absorption with advanced HIV infection (63). Considering limited evidence that cobalamins may hinder binding of HIV-1 to the CD4 molecule (65), and the ease and relatively low expense of parenteral administration, this therapy should be considered in an anemic HIV-infected patient with low B-12 serum levels.

While some HIV-infected patients are Coombs positive, this is usually non-speciÞc and not a major contributing factor to the anemia. Sensitive assays show that 2Ð44% of asymptomatic HIV-infected patients are direct Coombs positive, compared with up to 85% of those with AIDS (48,57). This contrasts with about 1% of non-HIV infected blood donors who are Coombs positive (71). While these antibodies may be reactive with speciÞc minor antigens on erythrocytes, most commonly the U and i antigens (48,50,57), they are most commonly due to non-speciÞc binding of anti-phospholipid antibodies or deposition of immune complexes on erythrocytes (48,50,57). Immune hemolysis is very rare (48,51).

Paraproteinemia may occur in upwards of half of HIV-infected patients (57). The peripheral smear may demonstrate rouleau; dimerization or comigration of these proteins during electrophoresis may result in the appearance of a monoclonal protein (57). However, a polyclonal pattern on electrophoresis is more common, and the paraproteinemia does not cause or contribute to the cytopenias seen in HIV-1 infection (57).

Clinical studies have demonstrated that anemia is an independent risk factor for increased morbidity and risk of mortality in HIV-1 infection. In this capacity, anemia is not merely a marker for the degree of severity of the viral infection (72Ð76): The prognostic information provided by the hemoglobin level is independent of the CD4 count, age, history of opportunistic infections, use of prophylaxis of infection, antiretroviral therapy (72), and viral load (77,78). In a study of over 6700 patients, it was demonstrated that a 1 gram/dL decrease in the hemoglobin level was associated with an increase in the hazard ratio for death by 57Ð70% (74,77). Recovery from anemia, either spontaneously or by intervention, is associated with improved survival (54,72,74,79).

Neutropenia

Granulocytopenia tends to occur concomitantly with anemia (60). While 10Ð30% of HIV-infected patients may be neutropenic, this may increase to about three-quarters of those who meet criteria for a diagnosis of AIDS (50,52,60,62). Review of the peripheral smear reveals a variable deÞciency of neutrophils, lymphocytes, and perhaps monocytes; atypical lymphocytes may be seen. Vacuolization of the monocytes is a typical Þnding, and hypolobulation of the neutrophils may occur which may imitate a left shift (60,80).

Impaired myelopoiesis is the major cause of the leukopenia associated with HIV-1 infection (60,81Ð83). Myelotoxic medications should be considered Þrst, as drugs may be the primary cause of granulocytopenia in up to 80% of cases (84). While about a third of patients have antibodies on circulating neutrophils, their presence correlates neither with the incidence nor the severity of the neutropenia (57).

In addition to the direct and indirect effects of HIV on granulocyte numbers during the course of HIV infection,

granulocytes undergo a progressive decline in chemotaxis (85) and oxidative capacities (86–90). Furthermore, there is a progressive acceleration in the rate of apoptosis (86,87). Granulocytes from HIV-infected patients tend to be less pliable than those from controls, making them problematic with respect to microvascular disease or diapedesis into sites of infection (89,90). These qualitative defects in granulocytes tend to improve with protease inhibitor therapy and/or growth factors (G-CSF, GM-CSF) (86).

Thrombocytopenia

While anemia and granulocytopenia tend to occur concomitantly, with a severity that parallels the course of HIV-1 infection, thrombocytopenia can occur independently of other cytopenias and at all stages of HIV infection (49,52,60,91–93). Thrombocytopenia may occur in 30–60% of HIV-infected patients, regardless of the risk group (47,49–51,90,92–95). Of these patients, 16–40% of those with platelet counts below 50,000 may have clinically-significant bleeding (92,95). By itself, however, thrombocytopenia is not a prognostic factor in HIV infection (96,97).

The causes of thrombocytopenia in HIV infection include reduced bone marrow production and immune and non-immune destruction (98). While immune mechanisms and reduced platelet life span may be more important in the early stages of HIV-associated thrombocytopenia, decreased production may be relatively more important in the later stages of disease (98,99).

Mechanism of Platelet Destruction in HIV-Related I.T.P.

The majority of HIV-infected patients have antibodies coating the surface of platelets (100–102). Some of these antibodies are due to non-specific binding of immune complexes (49,103). However, molecular mimicry between the GP-160/120 antigen of HIV-1 and GPIIb/IIIa of platelets may lead to production of more specific antibodies to the platelet surface (100,101,104–107). These antibodies shorten the average platelet life-span by two-thirds (108,109). The presence of antibodies on the surface of the platelets does not, however, correlate well with the platelet count, due to defective reticuloendothelial clearance in HIV-infected patients (110–112). In addition to immune destruction, infections and fevers that occur in patients with HIV infection decrease the life-span of circulating platelets. Splenomegaly that may occur in HIV infection results in some degree of sequestration of platelets. Other causes of non-immune destruction of circulating platelets are hemolytic-uremia syndrome and thrombotic thrombocytopenia purpura that occur more commonly in HIV-infected patients (91,113,114).

Despite stimulated megakaryocytopoiesis that occurs with HIV infection, there is a progressive reduction in the productive capacity of megakaryocytes to counter ongoing peripheral destruction (108,115–118). Megakaryocyte precursors in HIV-1 infected patients demonstrate an increase in apoptosis compared with normal controls and the degree of this has been shown to correlate inversely with the circulating platelet count (93). HIV-1 may directly suppress platelet production in that megakaryocytes are potential targets of infection by the virus (103,117,118), and this may result in quantitative and morphologic abnormalities of megakaryocytes (98). HIV-1 indirectly suppresses platelet production by exposing or altering antigens on the surface of the megakaryocyte that then renders them targets of antiplatelet antibodies (91,100). Compounding this is the dysregulation in cytokines and growth factors that occur during HIV infection that alter platelet production (115,119).

Treatment Modalities for HIV-Associated Thrombocytopenia

There are no well-controlled, prospectively randomized trials of the various treatment options for HIV-associated thrombocytopenia (57) (Table 22.2). Spontaneous remissions may occur in 10–20% of patients (59,120). A sudden elevation in the platelet count, however, may indicate deterioration in the immune system and herald the onset of AIDS (91,120). In those who have a sustained decrease in platelet count, therapy is not always necessary, since the incidence of significant bleeding episodes may be low despite low platelet count (121–123). It is, however, difficult to predict the risk of bleeding based solely upon the platelet count (124).

In those with thrombocytopenia associated with HIV infection, administration of zidovudine may result in elevation of the platelet count in approximately 30% of patients within twelve weeks of initiation of therapy (57,91,92,119,120). Although some studies have shown a dose-response, this has not been demonstrated in others (58). While monotherapy with other nucleoside reverse transcriptase inhibitors has not, as yet, been shown to have this effect (118), small studies have shown that a statistically significant number of patients with HIV-associated thrombocytopenia have a sustained increase in platelet count within a few weeks of starting therapy with HAART (125,126).

Similar to the effects of zidovudine, dapsone may elevate platelet counts in a minority of patients within three weeks of initiating therapy (102). While the mechanism of the effect of zidovudine is not clear, that of dapsone may be due to a reduction in phagocyte-mediated destruction (102).

Corticosteroids will elevate the platelet count in 40–80% of patients with HIV-associated immune thrombocytopenia; long-term remissions, however, occur in only

TABLE 22.2. *Treatment modalities for HIV-associated thrombocytopenia*

Modality	Acute Response Rate (% of patients)	Durable Response Rate (% of patients)
Spontaneous remissions	10–20%	10%
Nucleoside analogs	20–30%	10%
Dapsone	20–30%	10%
Corticosteroids	40–80%	10%
Vincristine	10%	10%
Anabolic steroids	10–30%	10%
High-dose ascorbic acid	Case reports to date	
Interferons	Small and uncontrolled studies	
Anti-D (anti-Rh) antibody	70–80%	10%
Splenectomy	60–90%	60%
Low-dose splenic irradiation	40–60%	40%

10–20% of cases (91). Although chronic, low-dose steroids may be effective in maintaining an acceptable platelet count (111), side effects preclude their use. No controlled trials thus far demonstrate any adverse effects of short-term steroids on HIV infection (111).

Patients who have failed the treatment modalities noted above have been tried on other therapies. Vincristine and anabolic steroids have an overall response rate of only about 10% (127,128). High-dose ascorbate (2–4 grams per day) over several months has also been shown in small studies to increase platelet counts; the mechanism and durability of the response is not entirely clear (91). Interferons, particularly interferon alpha, have been shown in controlled trials to have some efficacy in those with zidovudine-resistant thrombocytopenia (57,129). Small studies have shown an acute response of almost 70% (130); the doses required for this, however, often preclude its use. One potential mechanism by which interferon-alpha restores platelet production may be by increasing levels of interleukin-6 (131), a cytokine with trophic effects on megakaryocytes.

Infusions of gamma globulin offer the potential for a rapid elevation in platelet count with an acute response rate of 70–90% (132,133); the median response duration is on the order of three weeks. Sustained remissions from a single course of such therapy occur in less than 10% of patients (91). A similar acute response rate of 75% is offered by anti-D (Anti-Rh) antibody (134), but sustained remissions from a single administration in less than 10%. As with gamma globulin infusions, re-administration is effective in elevating the platelet counts in over 85% of those who initially responded (91,135–137). Unlike gamma globulin, however, the response may take up to three weeks to occur and there may be some hemolysis; anti-D antibody is not effective in splenectomized patients or in those who are Rh negative (134). Both immune globulin and anti-D antibody may work by increasing production of thrombopoietic cytokines such as IL-6 from cells of the reticuloendothelial system (138). Intravenous gamma globulin and anti-D globulin may also increase the platelet count by saturating complement receptors on the cells of the reticuloendothelial system and, thus, hindering platelet destruction (139).

When other therapies fail, splenectomy needs to be considered. This procedure has an acute response rate of 60–100%, and durable responses occur in at least 40–60% (91). These short and long-term response rates are better than those encountered when splenectomy is used for therapy in classic immune thrombocytopenia (140). No studies to date have demonstrated any detrimental effects of splenectomy on HIV progression (124,141,142). This procedure can, however, result in artificial elevations of the CD4 count due to redistribution of the lymphocyte pool (141). Consequently, splenectomized patients may develop opportunistic infections at higher CD4 counts than otherwise expected (141). A compromise to splenectomy is offered by low-dose splenic irradiation (102,143). Small, uncontrolled studies have demonstrated an acute response rate of 70% with durable responses occurring in about 40% of patients (143). Total doses are about 900–1,000 cGy over a month. Some degree of splenic function is maintained by low-dose radiation (144).

MARROW ABNORMALITIES IN HIV INFECTION

The bone marrow in the majority of patients with HIV infection exhibits morphologic aberrations (145,146) (Table 22.3). The incidence increases with progression of

TABLE 22.3. *Typical features of the bone marrow in HIV infection*

Cellularity:	Increased in 50–60% Normal in 35–40% Hypocellular in 5%
Dysplasia in one or more cell lines occurs in over 70%	
Granulocyte > Erythrocyte > Megakaryocytic	
Lymphoid aggregates occur in 20%	
Fibrosis occurs in 20%	
Less commonly seen:	Eosinophilia Plasma cell infiltrates

HIV infection (62,147). Although common, none of the marrow abnormalities seen with HIV infection, however, are speciÞc for the disease (148,149).

Hypercellularity of the bone marrow is the most common morphologic Þnding encountered in HIV-1 infected patients (150Ð152). This occurs in 50Ð60% of cases and is due to absolute hyperplasia of one or more of the non-lymphoid cell lines (147). There may be mild myeloid hyperplasia, but more often, the myeloid to erythroid ratio tends to remain close to normal (149). Since much of the hypercellularity may not represent effective hematopoiesis, the marrow cellularity correlates neither with the peripheral blood counts nor with the stage of HIV infection (149,152,153). Hypocellularity of the marrow is rare, occurring in less than 5% of cases and is usually a manifestation of advanced HIV infection (47,51,62). In the end-stages of HIV infection, atrophy or necrosis of the marrow may occur (149).

Dysplasia of at least one cell line occurs in over 70% of HIV-infected patients (51,62,117). This is similar to that due to the myelodysplastic syndromes and is largely not distinguishable from the latter on morphologic criteria alone (51,154). Dysplasia of the granulocyte series is the most frequent occurrence, with vacuolization of the granulocyte precursors in the marrow and in the peripheral blood neutrophils (155). Erythrocytic dysplasia is somewhat less common, seen in 50Ð60% of HIV-infected patients, and dysplasia of the megakaryocytes is seen in about one-third of HIV-infected patients (151). In general, the degree and frequency of dysplastic changes in the marrow increase with progression of HIV-1 infection and also with concurrent opportunistic infections (50).

Evidence of defective transfer of iron from the reticuloendothelial system to the maturing erythroid cells is encountered in over 60% of cases (153). Evidence of iron overload may also be encountered.

Less common aberrations include lymphoid aggregates and increased numbers of lymphocytes. This is encountered in about 20% of patients and occurs despite the peripheral lymphopenia that is characteristic of HIV infection (50,80). A similar proportion of those with advanced HIV infection has focal or diffuse increases in reticulin deposition in the marrow (50,62). In general, marrow Þbrosis increases in incidence and severity with progression of HIV-1 infection and with marrow involvement by fungal or mycobacterial infection (57). Other non-speciÞc morphologic changes that may be seen in the marrow include increases in eosinophils and plasma cells, and histiocytic erythrophagocytosis (62,156).

CAUSES OF CYTOPENIAS IN PATIENTS WITH HIV INFECTION

Hematopoiesis is a process that is both constitutive and inducible. Constitutive hematopoiesis is largely under the direction of the colony stimulating factors (157), while inducible hematopoiesis is more within the realm of action of other cytokines and interleukins that modulate hematopoiesis during situations of altered demand (158,159). These cytokines are released from marrow Þbroblasts, endothelial cells, T cells and monocytes as a response to a multitude of stimuli (157,158). The hematologic perturbations that occur in association with HIV infection may be a direct or indirect result of HIV-1 on stem cells and the marrow stromal cells. The alterations in growth factors and cytokines that occur as a result of HIV infection contribute to hematopoietic abnormalities (160). Other factors that merit recognition are opportunistic infections and neoplasms invading the marrow, and myelosuppressive medications.

Altered Hematopoiesis Due to Tumor, Infection, Medications

HIV infection is associated with the development of lymphomas as well as Kaposi's sarcoma and squamous cell carcinomas. The incidences of neoplasms that occur with HIV infection are likely to increase as the infectious complications of AIDS are better controlled. The bone marrow is affected in about one-third of those with AIDS-related lymphomas (161), most commonly, small non-cleaved cell lymphoma (162,163). The extent of replacement of the marrow by these malignant cells does not, however, correlate well with the peripheral blood counts (161). The antineoplastic drugs needed to treat these tumors are myelosuppressive, and the dose reductions that may be needed to preserve hematopoietic function hinder therapy of the tumors.

The medications used to treat HIV infection and the opportunistic infections that occur with AIDS, cause disease-stage and dose-dependent suppression of hematopoiesis (33,57). All of the dideoxynucleoside analogues can inhibit hematopoieis at sufÞciently high doses, with zidovudine being the major offender (164,165). Other myelosuppressive drugs include pentamidine, trimethoprim, sulfonamides, ganciclovir, and pyrimethamine (164). Medications that are not typically associated with decreases in hematopoiesis may cause this effect when given to those with altered hematopoietic potential such as occurs with HIV infection. Furthermore, concurrent administration of myelo-suppressive medications are synergistic in the potential to cause bone marrow suppression.

Several opportunistic infections that result from HIV-induced immunosuppression may cause or contribute to marrow failure. Myco-bacterial infections, particularly *Mycobacterium avium complex* but also disseminated *Mycobacterium tuberculosis* and others, and fungal infections, most common of which are *Cryptococcosis* and *Histoplasmosis*, are capable of widely disseminating throughout the bone marrow and thus may reduce hematopoietic potential. The marrow may reveal a disseminated mycobacterial or fungal infection prior to other

indications of these infections in an HIV-infected host (154,166). Special stains and cultures of the marrow for these organisms may be helpful and may be positive prior to peripheral blood cultures turning positive (154,166, 167). There is, however, not a general consensus on this point in that some studies have shown no difference in the time for blood and simultaneously-obtained bone marrow cultures to become positive (168). Small studies have shown that marrow examination or cultures are positive for mycobacteria and/or fungi in 75–84% of patients who are subsequently found by other diagnostic methods to have these infections (154,166,168). However, with respect to mycobacterial disease, the marrow has histologic evidence of infection in less than 30% of cases (168).

The most common manifestation of these infections within the marrow is diffuse infiltration with loose aggregates and clusters of macrophages (59,169). The ability to detect involvement of the bone marrow by mycobacteria and fungi correlates with the number of macrophages in the marrow (59). Although these cells may organize into granulomas, the tendency to do so lessens with advancing immunosuppression (57). Pseudo-Gaucher cells may also be seen as a manifestation of such infections (170).

Opportunistic infection of the marrow by viruses other than HIV-1 is an important cause of marrow failure. In this regard, *Cytomegalovirus* and *Parvovirus* have special significance. *Hepatitis B* and *C*, however, merit recognition as causes of marrow suppression in an HIV-infected patient (171,172).

Cytomegalovirus may cause suppression of hematopoiesis and may also be associated with autoimmune destruction of blood cells (173). Neutropenia, anemia that may be hemolytic, and thrombocytopenia either alone or in combination can be seen with *Cytomegalovirus* infection (174). This virus can infect bone marrow progenitor cells, rendering them less responsive to colony stimulating factors (173–175). Furthermore, these infected cells may serve as reservoirs of latent *Cytomegalovirus* within the marrow, causing further problems with advancing immunosuppression (173). *Cytomegalovirus* can infect the bone marrow stromal cells, interfering with their hematopoietic supporting functions, largely by decreasing local growth factor production by these cells (173,176,177). Despite the hematologic problems that *Cytomegalovirus* may cause, it does not cause distinctive histologic changes of the marrow, and it is best cultured from the buffy coat of the blood and not from the marrow itself (173).

HIV-infected patients may become infected with *Parvovirus* B-19 which may result in marrow suppression. These patients often do not have the manifestations of Fifth's disease (fevers, rash and arthralgias) that are seen in immunologically normal patients (178). The major hematopoietic target of *Parvovivus* B-19 is the erythroid progenitor (179). This is the only permissive cell of the hematopoietic system for the virus. Morphologically, such an infection results in giant pronormoblasts (178) and erythroblastopenia that can persist, if those infected are unable to make antibodies to the virus (172,179). *Parvovirus* also has an inhibitory effect on myeloid and megakaryocyte progenitors that may result in varying degrees of neutropenia and/or thrombocytopenia (178–180). Immunosuppressed patients may not be able to make specific IgM antibody to *Parvovirus* and this hinders spontaneous recovery from the infection as well as interferes with the usefulness of serology in diagnosis (181). Treatment of *Parvovirus* B-19 infection in those who are unable to make antibodies to this virus infection includes intravenous gamma globulin to reduce serum viral concentrations (178,181). Simultaneous infection with *Parvovirus* B-19 and HIV-1 does not preclude a response to erythropoietin (178).

Human *Herpes Virus-6* is the etiologic agent of another viral exanthem of childhood, roseola. Infection usually occurs in the first three years of life and then establishes latency (182). The primary target of this virus is the CD4 lymphocyte, as well as cells of monocytic lineage (182–184). Immunosuppression may result in loss of latency; subsequent exposure of marrow precursor cells to this virus inhibits their ability to respond to growth factors, and infection of lymphocytes causes further suppression of T cell function (182,183). When HHV-6 suppresses hematopoiesis, it is usually leukocytes that are most affected, followed by platelets and then erythrocytes (184). This virus also enhances immunodeficiency and upregulates HIV-1 replication (184).

Colony Stimulating Factors and HIV Infection

There is a vast network of cytokines composed of over twenty growth factors (185). Important components of this array are the colony stimulating factors that include G-CSF, M-CSF, GM-CSF, IL-3, Stem Cell Factor, and erythropoietin (158,186). These glycoproteins regulate passage of hematopoietic cells into the cell cycle and into the processes of terminal maturation (187). The predominant activity of these cytokines is to suppress apoptosis (188–190).

Major sources of colony stimulating factors are T and B lymphocytes, NK cells, vascular endothelial cells, smooth muscle cells, and fibroblasts (158,159). Many of these cells are targets of infection by HIV-1, and when they are infected, their ability to make growth factors progressively diminishes (160,191). The monocyte, an important primary target for infection by HIV-1, is central to the network of cytokines, interleukins, and growth factors that support and regulate hematopoiesis (159,169,192). While infection of cells of monocytic lineage by HIV-1 enhances their secretion of inflammatory cytokines, it simultaneously diminishes their ability to secrete hematopoietic growth factors (193).

Hematopoietic Growth Factors

Production of G-CSF, GM-CSF, and M-CSF increases during the early phases of HIV infection (45). This may partially explain the hypercellularity of the bone marrow that is often seen at this stage of HIV infection (160). This is largely due to the effects of low concentrations of IL-1 and other inßammatory cytokines, such as interferon-gamma and tumor necrosis factor-alpha, on cells within the bone marrow that produce these growth factors (160,194,195).

As HIV infection advances, however, levels of these inßammatory cytokines progress beyond levels that stimulate hematopoiesis to levels that inhibit it (160,193). Furthermore, with advancing HIV infection, the increasing levels of inßammatory cytokines that occur alter receptors on target cells to make them less responsive to growth factors (194Ð198). Other cytokines that inhibit hematopoiesis, such as transforming growth factor-beta (TGF-beta) are produced in increasing amounts with advancing HIV infection (160,191).

A major stimulus to the production of these negative regulators of hematopoiesis is products of HIV itself. The *Tat* protein is released from infected monocytes and lymphocytes and is taken up by other cells and stimulates them to release proinßammatory cytokines that include TNF-alpha, IL-1-alpha, IL-1-beta, and IFN-gamma (199Ð203). HIV-1 *Nef* protein also may be released from infected cells and induces IL-6 production and release by peripheral blood lymphocytes (204).

The pharmacokinetics of growth factors that are administered to cytopenic patients depend upon the dose, amount of glycosylation, and the route of administration. Cytokines may have effects in combination that are not seen with each alone (160,185). Exogenous administration of growth factors offers the potential of ameliorating some of the adverse effects of HIV infection on hematopoiesis. Prescribing growth factors permits the administration of myelosuppressive medications without dose reduction or interruption of therapy (48). With the exception of erythropoietin, the potential uses of growth factors have been investigated in uncontrolled studies only (205).

Granulocyte Colony-Stimulating Factor (G-CSF)

G-CSF is produced by activated monocytes, stimulated endothelial cells, and Þbroblasts. Exogenous administration of G-CSF results in a sustained, dose-dependent rise in circulating neutrophil counts (81,157). In HIV-infected patients on a stable HAART regimen, G-CSF may also increase the concentration of CD4+ and CD8+ lymphocytes as well as NK cells (206). High doses may result in modest elevations of monocytes (157,207). The relative and absolute elevation in neutrophil count is due to an increase in the number of divisions of neutrophil precursors in the marrow, and a decrease in their maturation time (208). *In vitro*, G-CSF augments neutrophil functions such as chemotaxis and phagocytosis, migration of granulocytes into sites of infection, and the oxidative functions of these cells (209,210).

Granulocyte-Monocyte Colony-Stimulating Factor (GM-CSF)

GM-CSF is produced primarily by stimulated Þbroblasts and endothelial cells, but also by T cells. Administration of this growth factor, as with G-CSF, results in a dose-dependent elevation in neutrophils and monocytes. The kinetic basis of the elevation in neutrophils differs from that of G-CSF: GM-CSF prolongs the circulating half-life of these cells rather than decreasing the production time as does G-CSF (157,211). Unlike, G-CSF, however, it also results in a signiÞcant elevation in eosinophils and monocytes (212,213). GM-CSF has been shown to augment the phagocytic activity of monocytes and macrophages in those infected with HIV-1 (214).

GM-CSF and G-CSF have different effects on HIV-infected cells (81,186,215). While G-CSF does not seem to alter HIV replication in cells that are targets for the growth factor, GM-CSF can stimulate HIV replication *in vitro* in infected cells of monocytic lineage (212Ð217). Moreover, activation of monocytes by HIV infection induces them to produce GM-CSF and to stimulate T cells, endothelial cells, and Þbroblasts to produce this growth factor, and this in turn augments HIV replication in the monocytes (158,159). When GM-CSF is administered in conjunction with zidovudine, the result is enhanced antiviral effects due to an increase in the concentration of the active drug within monocytes (212,213,216,218). Thus far, this effect has been shown only for zidovudine, and because of this GM-CSF may be administered concomitantly with zidovudine in HIV-infected patients (172).

Erythropoietin

The majority of HIV-infected patients with anemia have adequate erythropoietic capacity but are unable to augment this during times of demand due in large part to relatively inadequate erythropoietin levels (57). Inappropriately low endogenous levels (< 500 mU/cc) of serum erythropoietin are seen in 75% of AIDS patients regardless of the medications they are receiving (48,160,219). Proinßammatory cytokines not only decrease erythropoietin production, but also alter the sensitivity of erythroid precursors to this growth factor (160,194,220,221). One study has demonstrated that over 20% of HIV-infected patients, particularly those with higher endogenous erythropoietin levels, have autoantibodies to erythropoietin (222).

Administration of erythropoietin may reverse the suppression of erythropoiesis that occurs in response to

proinßammatory cytokines (194,223,224). In placebo-controlled trials, exogenous administration of erythropoietin increased the hematocrit and improved the quality of life for patients with AIDS (219,223). Elevation in erythrocyte counts was dose-dependent (47,48). Initial concerns about stem-cell exhaustion or lineage diversion did not occur (48). Erythropoietin neither promoted nor prevented HIV replication (48,215).

Despite these promising results, not all patients respond to administration of this growth factor. The beneÞts are principally seen in those with baseline erythropoietin levels of less than 500 mU/cc (48). About 25% of patients on zidovudine will not have a signiÞcant elevation in hematocrit with concurrent administration of erythropoietin (48).

Administration of erythropoietin offers an alternative to transfusions, the latter having potential morbidity. Transfusions may be immunosuppressive (225,226), having been shown to cause decreases in the ratio of CD4 to CD8 lymphocytes, and reductions in natural killer cell number (227). Transfusions may enhance HIV replication (228,229), perhaps accounting for the observation that the time to progression to AIDS is shorter in those who are transfused (227,228). Furthermore, transfusions risk exposure to new blood-borne infectious agents (48), and there is a risk of alloimmunization transfusion reactions despite the immunodeÞciency induced by HIV-1 infection (48,149).

Stem Cell Factor (SCF, Kit-Ligand)

S-CSF is a multipotential growth factor that is produced by marrow stromal cells (200). It acts on cells of myeloid, lymphoid, and mast cell lineage (230). For optimal stimulatory effects, it acts in synergy with other, more lineage-restricted, colony stimulating factors (230–232).

Levels of endogenous stem cell factor decrease as HIV infection advances, and these levels directly correlate with overall survival (232). Reduction of these levels may be due to progressively defective function of the stromal cells that make stem cell factor (232). When administered to HIV-infected patients, S-CSF increases hematopoiesis in a dose-dependent fashion and does not alter HIV expression (233).

Interleukin-3

This growth factor is produced by activated T cells and has direct effects on granulocytes, monocytes, and mast cells, and indirect stimulatory activities on erythroid production and T lymphocytes (234). Administration results in a dose-dependent increase in circulating granulocytes, erythrocytes, and platelets (234).

The loss of T cells that is characteristic of advancing HIV infection results in reductions in endogenous IL-3 levels (235–237). This may contribute to the myelosuppression associated with advancing HIV infection (218). Suboptimal concentrations of IL-3 may act in synergy with other growth factors to counter the myelosuppression that occurs with HIV-1 infection. The mechanism involves preventing the apoptosis of hematopoietic progenitor cells that occurs in the presence of HIV and in the absence of growth factors (190).

While some have found IL-3 to potentiate HIV expression in monocytes *in vitro*, others have found no consistent effects on viral activity (238). Like GM-CSF, it may augment the antiviral effects of zidovudine by elevating the intracellular levels of the active form of this antiretroviral drug (14,148,218,239).

Bone Marrow Progenitor Cells in HIV Infection

Results of studies on HIV infection of bone marrow progenitor cells have been conßicting (147). While there are data indicating progenitor cells to be targets of HIV infection (60,119), others have revealed that CD34+ bone marrow progenitor cells are infrequently infected with the virus (240–246). Some investigations have shown that progenitor cell numbers in the marrow and peripheral blood (152,247,248) are decreased in HIV infection (51,66,146), while others have found no signiÞcant differences in the numbers of these cells in HIV-infected patients when compared with normal patients (242,245, 249,250).

Reconciliation of these ostensibly conßicting results comes about when consideration is given to several factors. Studies on the effects of HIV-1 on hematopoietic progenitor cells need to consider the different strains of virus that are known to occur and evolve with advancing infection. Monocytotropic strains of HIV-1 may be more prone to alter hematopoiesis than are lymphocytotropic strains (249,251). There are also differences in the cytopathic capacity of different strains of HIV-1 (243,245), with some strains known to impair hematopoiesis in a dose-dependent manner (243,252–254).

It is difÞcult to obtain highly puriÞed populations of CD34 cells from bone marrow (246). When this impediment is overcome, however, it becomes apparent that only a minority of CD34+ progenitor cells are also positive for CD4 and therefore only a relatively unimportant number of progenitor cells are major targets for HIV-1 (244,255–258). Direct infection of hematopoietic cells by HIV-1 does not seem to make a major contribution to the hematopoietic defect that occurs with HIV-1 infection (190,201,249,259,260).

The presence or absence of accessory cells within marrow cultures is also important to the myelosuppression that occurs *in vitro* in the presence of HIV-1. T cells, monocytes, adipocytes, Þbroblasts and vascular endothelial cells are important components of the bone marrow stroma and inßuence hematopoiesis by production of

growth factors (160,191). These cells, particularly the stromal cells of monocytic lineage, are targets for HIV and may serve as reservoirs of virus within the bone marrow (192,261). When infected with HIV-1, these cells are less able to make growth factors (60,67,193). As the major function of growth factors may be to prevent apoptosis, the deficiency in local production permits marrow progenitor cells to undergo this process (249). Furthermore, these cells may produce inhibitors of hematopoiesis such as TGF-beta, platelet factor 4, interferons, and tumor necrosis factor-alpha (239,262–264). *In vitro* depletion of marrow cultures of these cells increases hematopoiesis (259,261, 265).

Progenitor cells of HIV-1-infected patients may be impaired without productive infection by HIV-1 (249). Protein products of HIV-1 can directly inhibit marrow progenitor cells, and this effect does not depend upon the presence of active virus (146,266). The GP-120 and GP-160 envelope proteins of HIV-1 have been shown to cause a dose-dependent decrease in viable CD34+ cell counts by a process that stimulates apoptosis in these hematopoietic cells (190,257,267). Preincubation of HIV-1 with neutralizing anti-gp-120 antibody prevents the apoptosis that occurs in hematopoietic cells when envelope gp-120 interacts with the low levels of CD4 antigen present on these cells (190,249). However, prevention of apoptosis does not occur if HIV-1 or recombinant gp-120 treatment of hematopoietic cells is followed rather than precedes by the addition of gp-120 antibody (249). This implicates the engagement of CD4 antigen present in low levels on progenitor cells as a trigger to programmed cell death thus depleting the hematopoietic reserve (249,258). Anti-CD4 and anti-GP-120 antibodies themselves also may induce apoptosis in hematopoietic cells (265,268). The mechanism that permits anti-CD4 antibodies to do this may be via altering protein kinase C activity and intracellular calcium levels (210,266).

Indirectly, GP-120 can suppress hematopoiesis by inducing other cells to produce cytokines that inhibit hematopoiesis such as tumor necrosis factor-alpha (259,269). Protein products of the *Tat* and *Nef* genes cause suppression of hematopoiesis either directly (211), or indirectly by causing other marrow stromal cells to produce inhibitors of hematopoiesis such as TGF-beta and TNF-alpha (259,269,270).

Another product of HIV-1, the Vrp protein, also plays a role in suppression of hematopoiesis (271). This 96 amino acid protein is important to the efficiency of infection of mononuclear phagocytic cells by HIV-1. It activates these cells and in so doing increases HIV replication. Activation of these cells also promotes premature phagocytosis of hematopoietic cells (271).

Other factors need to be recognized when one looks at studies that investigate the effects of HIV-1 on bone marrow progenitor cells. At least with respect to megakyarocytes, HIV-1 infection induces alterations in surface antigens that renders them potential targets of antiplatelet and anti-HIV-1 antibodies (115,119). Anti-retroviral drugs may influence the results of experiments on the presence of HIV-1 in marrow progenitors (245,272). Protease inhibitors have been shown to decrease apoptosis in bone marrow cells by a mechanism that seems to be independent of their effects on HIV replication (273).

Taken all together, the data indicate that the effects of HIV-1 on the marrow progenitor cells are largely indirect rather than a result of direct infection of these cells. The summation of these indirect effects on hematopoietic cells is a reduction in the potential of the progenitor cells to differentiate and proliferate (253,254).

Other Cytokines and Interleukins

Interleukin-1

Interleukin-1 is one of the primary mediators of the acute phase response (274). This cytokine is produced largely by cells of monocytic lineage (274–276), but is also produced by dendritic cells, B and T lymphocytes, NK cells, fibroblasts, and vascular endothelial cells (1). Interleukin-1 causes fever and anorexia, and contributes to a catabolic state (275). With respect to hematopoiesis, it induces secretion of colony stimulating factors by accessory cells in the marrow, particularly G-CSF, M-CSF, and GM-CSF (158,277), and also IL-6 (158,274). In this way, it enhances hematopoiesis (124,223). Prolonged secretion of interleukin-1, however, induces secretion of tumor necrosis factor-alpha and other inflammatory cytokines that suppress hematopoietic activity (263,274,277). *In vivo* and *in vitro*, levels of interleukin-1 tend to increase with progression of HIV-1 infection (275,278).

Interleukin-2

Interleukin-2 is a primary growth factor for T cells (158,159) and secondarily stimulates proliferation and differentiation of B cells (186). Interleukin-1 and TNF-alpha are the major stimuli for production of IL-2 by T cells, and these cytokines also increase IL-2 receptor numbers and binding capacity on the T cell membrane (158,159,186).

Interleukin-2 can indirectly decrease hematopoiesis by its ability to induce synthesis of interferon gamma by other cells (158). More importantly, production of IL-2 during the chronic inflammatory state of HIV infection augments replication of HIV-1 in infected cells (279,280). In this way, production of this cytokine directly upregulates HIV-1 production, and indirectly contributes to the suppression of hematopoiesis that is characteristic of advancing HIV-1 infection.

Interleukin-6

HIV infection is associated with increased production of IL-6 primarily by monocytes (143). This may occur as a

result of the interaction of GP-160 antigen with the CD4 receptors of these cells (131,193,280). IL-6 may also be produced by stimulated B cells, T cells, and Þbroblasts (158,231,277). Circulating neutrophils and eosinophils as well as vascular endothelial cells have been shown to produce this cytokine in the presence of HIV-1 (281,282). HIV-1 Tat protein has been shown to directly and indirectly upregulate IL-6 expression (270).

Interleukin-6 can act in synergy with IL-3 to enhance hematopoiesis (158,283Ð285). In this regard the elevated levels of IL-6 that occur with advancing HIV-1 infection (264,278) would be expected to upregulate hematopoiesis (131,193). This cytokine, however, also induces hepatocytes to produce acute phase proteins (131), primarily IL-1, and this directly and indirectly down-regulates hematopoietic potential (231,254,286). Furthermore, IL-6 can upregulate HIV-1 production by infected cells (256,279), and may cause T cell proliferation thereby expanding the pool of HIV-infected cells (231).

Interferons

The interferons are a family of cytokines that are produced by leukocytes (interferon-alpha), Þbroblasts (interferon-beta), and cells of lymphocyte and monocytic lineage (interferon-gamma) (25). The latter may be produced by cytotoxic T lymphocytes upon contact with target cells that present HIV antigens (25).

Serum interferon levels tend to increase with progression of HIV infection (241,287,288). Interferons in general, and INF-alpha in particular, inhibit marrow progenitor cells (288Ð290). This effect may be mediated by inducing secretion of other cytokines such as TNF-alpha, and IL-1 from marrow monocytes and T cells (263,277,291).

Interferon gamma has been shown to upregulate chemokine receptors, particularly CXCR-4, on bone marrow progenitor cells (292). This molecule is the co-receptor for strains of HIV that primarily infect T cells, strains that tend to occur later in the course of HIV infection. The CXCR-4 molecule is also present on maturing megakaryocytes and platelets (292,293), thus providing a potential explanation for the decrease in platelet production that occurs late in HIV infection.

Tumor Necrosis Factor

Tumor necrosis factor-alpha (cachectin) is produced by stimulated monocytes and macrophages (148,159,277), T lymphocytes (25), stimulated B cells (294) and vascular endothelial cells (276). Although it is not produced constitutively by HIV-infected cells, it can be produced in response to diverse stimuli concurrent with HIV infection (275,276,295). The Tat protein of HIV-1 may activate TNF-alpha genes *in vitro* (196,278). Cytotoxic T lymphocytes are capable of producing TNF-alpha upon contact with target cells presenting HIV-1 antigens (25), and monocytes and macrophages are capable of producing TNF-alpha after contact with GP-120 (30,259). This does not require the presence of live virus (259).

TNF-alpha levels are elevated in the sera of HIV-infected patients (259,275,278,296), and rise progressively with the disease. *In vitro*, TNF-alpha can maintain HIV expression in chronically infected cells (277,297,298). TNF-alpha is, therefore, important to the autocrine and paracrine regulation of HIV infection (211).

TNF-alpha can act as both a positive and negative regulator of hematopoiesis (259,276,299). TNF-alpha has indirect suppressive effects on hematopoiesis by inducing the production of IL-1 by monocytes that, in turn, suppress hematopoiesis (158,263). It can alter the production of growth factors by marrow stromal cells as well as modulate the expression of cell surface receptors for growth factors on cells that are targets of HIV infection (158,259,274,276,299).

The concentration of TNF-alpha is important to the quantitative effect on hematopoiesis (299,300): Low concentrations stimulate IL-3 and GM-CSF-induced colony formation, but high levels inhibit the actions of these growth factors (158,263). TNF-alpha also has differential effects upon various hematopoietic cell lines: The same concentration of TNF-alpha that stimulates growth of committed granulocyte and monocyte progenitor cells inhibits erythroid growth (300). There are at least two receptors for TNF-alpha, a 55 kD and a 75 kD receptor, and these seem to use different signaling pathways and result in different effects on hematopoiesis. While the inhibitory effects of TNF-alpha on progenitor cells involves p55 and p75, the p55 receptor alone mediates the stimulatory effects on progenitors (299,300).

Transforming Growth Factor-Beta (TGF-beta)

Transforming growth factor-beta is a stimulatory agent for some cell lines, Þbroblasts in particular, but is a potent inhibitor of hematopoietic proliferation (262,301,302). It has a reversible, suppressive effect on marrow progenitors (262). It acts on early cells in hematopoiesis in a multipotential and non-lineage speciÞc fashion (262). Its mechanism of action may be via down regulation of cell surface C-kit expression and IL-1 receptors (301).

Levels of TGF-beta increase progressively as infection with HIV-1 advances (302). The *Tat* protein of HIV-1 upregulates production of this cytokine in a direct and indirect manner (270). Moreover, TGF-beta is capable of inducing its own synthesis (302).

In addition to its effects on hematopoiesis, TGF-beta is a potent endogenous immunosuppressive factor (302). It down-regulates activity of B cells, T cells, monocytes and macrophages (302). Its overall effect is to augment the immunosuppression and hematosuppression characteristic of HIV-1 infection (302Ð304). Moreover, TGF-beta enhances HIV-1 expression in infected cells (305).

THE HEMATOPOIETIC MICROENVIRONMENT AND HIV INFECTION

The bone marrow stroma consists of cellular and acellular components that provide a structural framework organizing hematopoiesis into a non-random distribution that is conÞned to the bone marrow (306Ð308). The cellular component of the bone marrow stroma consists of endothelial cells, Þbroblasts, macrophages, adipocytes, and reticular adventitial cells (309). Adhesion events between hematopoietic cells, the stromal cells, and the extracellular matrix are important to the regulation of hematopoiesis (310,311). In addition to providing sites of attachment for hematopoietic cells, the marrow stromal cells produce growth factors and cytokines that regulate proximately hematopoiesis (306,308,310Ð312).

The cytokines that are released in response to infection with HIV-1, particularly IL-1 and TNF-alpha, alter the adhesion molecules on the marrow stromal cells (313). Alterations in the attachment events between marrow stromal cells and hematopoietic cells (313) may result in suppression of hematopoiesis (311).

HIV-1 infection of accessory cells within the marrow adversely alters hematopoiesis (309,314,315). Monocytes and lymphocytes within the marrow are important cellular components of the bone marrow stroma, and regulate hematopoiesis by their capacity to produce locally growth factors, particularly GM-CSF and IL-3 (316). During the early phases of infection by HIV-1, lymphocytes and monocytes within the bone marrow may increase production of these growth factors; this may partially explain the marrow hypercellularity commonly seen during this stage of HIV infection (147,316).

As the viral infection progresses, however, lymphocytes and monocytes within the marrow begin to produce increasing levels of IL-1 and IL-2 (263,277,316) that not only decrease hematopoietic potential (263,274,277), but also up-regulate HIV replication within these and other cells (148,263,276,277). As noted above, these cells may also serve as a reservoir for HIV within the bone marrow (317,318).

Non-hematopoietic cells within the bone marrow are also important to the structure and function of the bone marrow microenvironment. A major component of the stroma is the reticulum cell (Þbroblast). This accounts for 60Ð70% of the volume of the marrow framework (235). The marrow reticulum cell is a target of HIV infection (261,319,320). When infected with HIV-1, these cells are less able to support hematopoiesis (317,318,320,321) due to impaired ability to secrete growth factors (322). Moreover, HIV-infected marrow stromal cells may also secrete factors that inhibit hematopoiesis (201,246). Medications used to treat HIV infection, such as zidovudine, have the potential to inhibit the growth and development of these stromal cells in addition to that of the hematopoietic cells themselves (272).

The endothelial cells that line the marrow sinusoids are also important to the structure of the marrow (309). These cells are essential to the homing of circulating hematopoietic cells and, in addition, regulate migration of marrow cells into the circulation. They also secrete growth factors. These cells are targets for HIV-1 (281,322), and tend to be infected early during the course of infection (309). When infected by HIV-1, they undergo alterations in surface receptors, and their ability to secrete growth factors is hindered, thus adversely altering their ability to support hematopoiesis (309). In addition, these cells may serve as reservoirs of HIV-1 within the bone marrow (322).

COAGULATION ABNORMALITIES IN HIV INFECTION

Clotting disorders are not uncommon issues encountered with HIV infection (323,324). The most common of these are due to antiphospholipid antibodies. This class of proteins may bind to cardiolipin, anionic phospholipids (the ÒlupusanticoagulantsÓ), and beta-2-glycoprotein I. The latter is a phospholipid binding protein that inhibits coagulation and platelet aggregation (325Ð327). All of these antibodies occur as a result of the abnormal immune responses characteristic of HIV infection. These antibodies are found in 20Ð66% of HIV-infected patients (328Ð333). Titers tend to increase with active opportunistic infections (333).

The antiphospholipid antibodies were once thought to be clinically relevant leading to thromboembolic phenomena (325) both arterial and venous (334). However, the risk of thromboembolic events associated with antiphospholipid antibodies in HIV-positive patients may be less than that encountered in the HIV-seronegative population due to a lower incidence of antibodies to beta-2-glycoprotein I in the HIV-positive population (326,327). Antibodies to this protein may be central to the pathophysiology of the antiphospholipid antibody syndrome (326,327).

Thromboembolic events may also occur as a result of reduced levels of active protein S (324,335Ð338). This peptide is the cofactor for Protein C, acting to localize active Protein C to the phospholipid surface. Mean total and free protein S levels are signiÞcantly lower in HIV-infected patients with or without thromboses than in healthy male controls (324,336,337). The levels of free and total protein S correlate with CD4 counts(339,340). Protein S binds to C4b-binding protein, and increases in C4b-binding protein with the chronic inßammatory state encountered with HIV infection results in greater binding of Protein S, so less is available to prevent aberrant thrombotic events (337). Another mechanism that may contribute to lower levels of active Protein S in HIV-infected patients is the presence of anti-Protein S antibodies in these patients (341). These antibodies bind Protein S, thus decreasing the unbound and active forms of the protein (338).

Heparin cofactor II, a thrombin inhibitor, is lower in HIV-infected individuals than in healthy controls (342). The levels of this anti-thrombotic molecule are decreased further in those with advanced HIV infection (342).

Several of the opportunistic viral infections that result from the immunosuppression induced by HIV-1 may cause or contribute to the pro-thrombotic state characteristic of HIV infection. Quiescent endothelial cells have control mechanisms that restrict expression of pro-coagulant phospholipids to areas of vascular injury (343,344). *Cytomegalovirus* and *Herpes simplex* types 1 and 2 can convert vascular endothelial cells from a non-coagulative to a pro-coagulative phenotype (343–345). The mechanism of this seems to be alterations in surface phospholipids to forms that are active in the coagulation system (343,344).

Increases in levels of antigenic von Willebrand factor, a marker of endothelial injury (109), correlate with CD4 lymphocyte counts. Decreases in plasminogen activator inhibitor levels also occur in those infected with HIV-1 (338) and may contribute toward a prothrombotic state. Other abnormalities in coagulation that may occur with HIV infection include isolated deficiencies in prothrombin levels (344), and abnormal platelet aggregation (343,344). Acquired circulating anticoagulants with anti-factor V activity also have been reported, as well as deficiencies in heparin cofactor II (333,346). The hypo-albuminemia that occurs with advancing HIV infection may cause fibrin polymerization and fibrinolytic defects (323,347).

Thrombotic thrombocytopenia purpura (TTP)-hemolytic uremia syndrome (HUS) has been reported to occur more frequently among patients with HIV infection (347,348). Although the exact etiology of this consumptive coagulopathy is unknown, therapy with plasma exchange has been shown to be effective as therapy (349).

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Chapter 23

Cardiac Manifestations in Human Immunode ciency V irus Infection

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Over 40 million people worldwide are infected with human immunodeÞciency virus (HIV). As the AIDS epidemic spreads, it is clear that cardiovascular problems associated with this infection are going to become more prominent (1,2). Cardiac disease in HIV-infected patients may be multifactorial, caused by infectious or neoplastic complications or their therapies, any of the established causes of cardiac disease in other patient populations, and perhaps HIV infection of the myocardium itself. Knowledge of the relative frequency of each form of heart disease in HIV-infected patients is currently evolving.

Appreciation of cardiovascular dysfunction in HIV infection dates back to a necropsy study in 1982 which described cardiac involvement in a patient with AIDS and Kaposiõ sarcoma (3). Subsequently, cardiac involvement in patients with HIV infection has been described in multiple autopsy (4Ð17) and echocardiographic series (18Ð25). However, the exact prevalence of cardiac involvement in HIV-infected patients is uncertain.

Important clinical syndromes described in patients with HIV infection that involve the heart include cardiac tamponade resulting from pericardial effusion or hemorrhage, dilated cardiomyopathy, other forms of myocardial failure, myocarditis, refractory ventricular tachyarrhythmias and/or sudden death, and systemic thromboembolic disease caused by infectious and non-infectious thrombotic endocarditis, left ventricular aneurysm, myocardial infarction coincidental with the use of the protease inhibitors, and sudden infant death syndrome (26Ð28) (Table 23.1). In one large autopsy series from Europe, cardiac disease was the cause of death in 9.1% of patients dying from HIV infection (29).

The prevalence of cardiac involvement in patients with HIV infection, as demonstrated by echocardiographic examination, has been shown to be much greater than that seen in autopsy series, especially in critically ill hospitalized patients (30). As demonstrated by Blanc et al. in a prospective echocardiographic study of HIV patients admitted to an intensive care unit, few patients presented with clinical evidence of cardiac disease (5/68), but echocardiographic abnormalities were identiÞed in 55 of 68 patients (81%) (22). Herskowitz et al., in their echocardiographic study, showed that patients with CD4 counts $< 100\ mm^3$ had higher rates of left ventricular dysfunction (31) than those with higher CD4 counts. According to Barbaro et al., who examined 952 asymptomatic patients with HIV infection during a mean follow-up period of 60 ± 5.3 months, an echocardiographic diagnosis of dilated cardiomyopathy was made in 8% of patients, with a higher incidence seen in those patients with CD4 counts $< 400\ mm^3$ and in those who received therapy with zidovudine (18). Often the clinical manifestations of cardiac disease may be obscured by the systemic manifestations of HIV infection. Risk factors for myocardial disease other than HIV infection (systemic hypertension, coronary artery disease, alcoholism, intravenous drug use) may also be present (25,32Ð38).

MYOCARDIAL DISEASE

Opportunistic bacterial, fungal, protozoal and viral infections, possible direct HIV infection, non-speciÞc myocarditis and neoplasia can involve the myocardium in HIV-infected patients with advanced immunodeÞciency

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TABLE 23.1. *Cardiac conditions associated with human immunodeÞciency virus infection*

MYOCARDIAL DISEASE

Opportunistic Infections
- Bacterial: Mycobacterium tuberculosis
  Mycobacterium avium complex
- Fungal: Cryptococcus neoformans
  Aspergillus fumigatus
  Candida albicans
  Histoplasma capsulatum
  Coccidioides immitis
- Protozoan: Toxoplasma gondii
  Pneumocystis carinii
- Viral: Cytomegalovirus
  Herpes simplex
  Coxsackie B virus

Direct HIV Infection
Lymphocytic Myocarditis
Non-In ammatory Myocardial Necrosis: Microvascular spasm
Catecholamine excess
Nutritional De ciency
Hypoxic Injury
Toxic Lesions: Anti-HIV drugs
Drugs used for opportunistic infections or malignancies
Arteriopathy
Cardiomegaly & Cardiomyopathy:
- Right ventricular hypertrophy or dilation with pulmonary hypertension resulting from:
  - pulmonary infections
  - pulmonary emboli
  - plexogenic pulmonary arteriopathy related to HIV infection
- Biventricular dilation (congestive cardiomyopathy)

Neoplasia: Kaposi's sarcoma
Lymphoma

ENDOCARDIAL DISEASE

Marantic endocarditis (non-bacterial thrombotic endocarditis)
Healed bacterial endocarditis
Infective endocarditis: bacterial
fungal

PERICARDIAL DISEASE
Infectious
- Bacterial: Myobacteria (M. tuberculosis, M. avium complex, M. kansasii)
  Nocardia
- Viral: Herpes simplex
  HIV
  Coxsackie
- Fungal: Histoplasma
  Cryptococcus

Non-infectious: Uremia

Neoplastic: Kaposi's sarcoma
Lymphoma

Fibrinous Pericarditis
Idiopathic

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(Table 23.1). The complete etiologic spectrum of myocarditis has not yet been delineated. In an autopsy study, Kaul and associates (39) described myocarditis in 46% of patients with HIV infection. In 80% of these patients no speciÞc microorganism was found, whereas in the remaining 20%, one or more opportunistic pathogens was present.

In the aforementioned study by Barbaro et al. (18), all patients found to have echocardiographic evidence for a dilated cardiomyopathy, underwent endomyocardial biopsy. A histologic diagnosis of myocarditis was made in 83% (63/76) of these patients. Inßammatory inÞltrates were predominantly composed of CD3 and CD8 lymphocytes. Some of the patients with evidence for HIV in cardiac tissue were also co-infected with Coxsackie virus group B, cytomegalovirus (CMV), or Epstein-Barr virus (EBV) (18).

Myocardial involvement in HIV infection has been classiÞed into the following categories: infectious or lymphocytic myocarditis; non-inßammatory myocardial necrosis; dilated cardiomyopathy; and inÞltrative myocardial disease caused by neoplasia such as KaposiÕs sarcoma or high grade lymphoma (40).

Bacterial, Fungal and Protozoan Opportunistic Infections

The number of reported opportunistic infections in HIV-infected patients involving the heart continues to grow (Table 23.1) (37). Multiple simultaneous infections may also occur (41,42). Common pathogens include *Toxoplasma gondii*, *Mycobacterium tuberculosis* and *Cryptococcus neoformans*.

Opportunistic infections, neoplasia and other pathologic conditions which occur in patients with HIV infection often have been explained in terms of deÞcient immune surveillance that stems from a reduced number of T helper (CD4) lymphocytes. However, in studies by Himelman et al. (24) and Akhras et al. (25), there was lack of a quantitative difference in the number of CD4 cells in HIV-infected patients with and without cardiomyopathy or myocarditis. In contrast, other studies have demonstrated that the degree of immunosuppression, as evidenced by a decreased CD4 lymphocyte count, correlated strongly with echocardiographic evidence of myocardial dysfunction (31,43). However, cardiomyopathy may occur before advanced immunodeÞciency develops (CD4 $< 200\ mm^3$) (18).

Reactivation of infection is the proposed explanation for many of the HIV-associated opportunistic infections (44,45) including infections caused by *P. carinii*, *T. gondii*, *H. capsulatum*, *C. neoformans*, *M. tuberculosis* and *M. avium-intracellulare*. Myocardial involvement, when present, is usually not extensive and often occurs in the absence of signiÞcant histologic damage by these organisms. Often the myocardium is an incidental site of infection. *T. gondii* and *M. tuberculosis*, however, are two pathogens with an apparent tropism for the myocardium or pericardium (45). Cardiac toxoplasmosis was diagnosed at necropsy in 12% of AIDS cases in an autopsy study by Hofman et al. (46). In this study, myocardial lesions were generally asymptomatic and were not discovered until necropsy. Myocarditis is not a common Þnding with *M. tuberculosis* (47). More common is the Þnding of pericardial involvement as part of widely disseminated tuberculosis.

Viral Myocarditis

The histologic Þnding of mononuclear inßammatory inÞltrates in myocardial tissue suggests a viral cause for myocarditis, although this could also reßect an autoimmune process. Viral agents, such as CMV, Herpes simplex, Coxsackie, as well as HIV itself, are seriously considered as etiologic agents. Intranuclear inclusions characteristic of CMV infection have been reported in the myocardium of some patients (14,48,49). Other studies have demonstrated CMV antigen and CMV immediate early gene expression within myocytes from HIV-infected patients with cardiomyopathy (50,51), suggesting non-permissive infection of myocytes without classical intranuclear inclusions. Myocyte infection may be necessary to trigger cellular and humoral-mediated cardiac injury. Because it is well known that CMV infections can cause tissue necrosis without associated inßammatory changes, it is still possible that CMV or other viruses, including HIV, are the responsible agents in patients with HIV infection in whom myocardial necrosis without inßammation is seen (48). Coxsackie B virus has also been associated with myocarditis in patients with AIDS (52). EBV is another opportunistic pathogen which can be reactivated to cause disease in immunosuppressed patients. Chronic B-cell stimulation mediated by EBV in the absence of modulation by functional T helper lymphocytes may be an etiologic factor in the development of cardiac lymphomas (53).

HIV Myocarditis

Hypothetically, HIV may damage myocytes either by direct infection with ultimate cytolysis or by Ònnocent bystander destructionÓ as proposed by Ho and coworkers (54) in their explanation for the presence of neuroglial cell damage in HIV infection-associated subacute encephalitis. Autocrine and paracrine factors, lymphokines, or other enzymes released by HIV-infected lymphoreticular cells may be involved in disease pathogenesis by mechanisms other than immunosuppression (54). HIV has been documented in cardiac tissue by culture (7), southern blot (55), *in situ* hybridization (56,57), and polymerase chain reaction (58). Because there is no direct correlation with

any histopathologic Þnding or clinical heart muscle disease (56), it is difÞcult to make a direct link between the presence of HIV in the myocardium and the induction of myocarditis. In one study (18), HIV nucleic acid sequences were detected by in situ hybridization in 76% of patients with dilated cardiomyopathy and 57% of patients with a histologic diagnosis of myocarditis, which would suggest that there is a pathogenetic relationship between HIV and myocarditis or dilated cardiomyopathy. However, in most cases, the myocytes with a positive hybridization signal were sparse and were not surrounded by inßammatory cells.

Flomenbaum et al., in an autopsy study, described large numbers of multi-lamellated membrane bodies predominantly associated with the mitochondria of myocytes (55), and it is believed that this membranopathy may be an HIV infection-speciÞc abnormality related to the development of heart muscle disease. Cotton (59) reported on the observation that large amounts of HIV proteins rather than the whole virus can be found bound to muscle Þbers in heart muscle. Other investigators, using different methods, also found HIV viral proteins in the human heart (5,55Ð58). These studies suggest that the cardiac myocyte is at least an induced target for HIV. However, a direct link between the presence of HIV in the myocardium and the induction of myocarditis has not been established.

Autoimmunity and the Heart

Various cytokines and hormones may play an important role in HIV-related cardiac dysfunction; these include endothelin (ET-1), tumor necrosis factor alpha (TNFα), atrial natriuretic factor (ANF) and nitric oxide (NO), substances which may be deleterious to cardiac myocytes (60Ð62).

Recent studies suggest that inducible NO synthases (iNOS) and TNFα can inßuence the clinical course of cardiomyopathy in patients with AIDS (63,64). Endomyocardial biopsy specimens from 82 HIV-infected patients with dilated cardiomyopathy were compared with biopsy material from 80 patients with idiopathic dilated cardiomyopathy and were processed for determination of immunostaining intensity of TNFα and iNOS and for virological examination. Staining intensity was greater in patients with AIDS cardiomyopathy than in the other group and correlated inversely with the CD4 count (64). These data suggest that cytokine-mediated cell signalling plays an important role in AIDS cardiomyopathy.

Acierno (47) suggested that myocardial damage could relate to hypersensitivity resulting from uncontrolled hypergammaglobulinemia from altered T cell function. HIV-infected patients show high concentrations of immune complexes in their sera, but the relationship of this Þnding to direct myocardial damage still needs to be determined.

Herskowitz and other investigators (65Ð69) have proposed an autoimmune mechanism for HIV-related myocardial disease, similar to that described with anti-myosin antibodies. They postulate that an HIV gene alters the cell surface of the muscle Þber, eliciting a progressive autoimmune reaction. A series of experiments have revealed the presence of circulating cardiac autoantibodies to a variety of antigens in patients with cardiomyopathy without HIV infection and in a majority of patients with cardiomyopathy and HIV infection. Patients who are HIV-infected without cardiomyopathy do not demonstrate these autoantibodies. Immunohisto-chemical, immunopathological and serological studies in humans with HIV infection and cardiomyopathy have shown that the majority of inßammatory cells are CD8 positive and express abnormally large amounts of Class 1 and 2 histocompatibility markers, interleukin-1 and other cytokines, which may contribute to the induction of heart speciÞc autoimmunity (65,69a).

Non-Inßammatory Myocardial Necrosis

It is common to Þnd areas of myocardial necrosis in patients dying of AIDS without associated inßammation. Necrosis of myocytes may be brought about by the long-term stresses that HIV- infected patients are subjected to as a result of their disease, mediated by excessive catecholamine stimulation (70). Microvascular spasm causing transient and focal or widespread ischemia and necrosis has been implicated as another pathogenic mechanism (71), especially for the development of congestive cardiomyopathy (72). Microvascular spasm may also be an aggravating factor in HIV infection-associated myocarditis (47).

Vascular Lesions (Arteriopathy)

Several types of arterial vascular lesions have been described in patients with HIV infection. These include an arteriopathy with and without aneurysmal formation, a ÞbrocalciÞc lesion, and endothelial proliferation in association with Kaposiõ sarcoma with episodes of coronary artery thrombosis (73Ð76).

Joshi and coworkers (74) reported the occurrence of arteriopathy in children with HIV infection. Two types of lesions were described: inßammatory and ÞbrocalciÞc. Inßammatory lesions consisted of a vasculitis or perivasculitis in the brain and myocardium. FibrocalciÞc lesions consisted of intimal Þbrosis with fragmentation of elastic tissues, Þbrosis and calciÞcation of the media with variable luminal narrowing. This has been observed in many organs, including the heart and has involved small and medium-sized arteries. In one case, three aneurysms of the proximal right coronary artery were seen in association with intraluminal thrombosis and myocardial infarction. The left coronary artery had similar ÞbrocalciÞc lesions but of lesser severity and without aneurysmal

formation. None of the lesions showed evidence of inflammation or necrosis of vessel walls (74). The pathogenesis of fibrocalcific arteriopathy is unclear. Based on its clinicopathologic and immunologic features, it appears to be distinctive and separate from Kawasaki's disease and idiopathic arterial calcification of infancy (73–76). Causative factors that have been suggested include: (1) HIV itself; (2) persistent and unchallenged antigenic stimulation; and, (3) endogenous or exogenous elastases (77). These observations in children suggest the possibility that an arteriopathy will be seen with increasing frequency and severity as longer survival rates of children with HIV infection are achieved (74,77).

In an autopsy series of HIV-infected adults, Paton et al. described the presence of major eccentric atherosclerotic lesions involving the coronary vessels with significant luminal obstruction (78). The mean age of subjects in this series was 27 years, and no detectable risk factors for coronary artery disease were found other than tobacco smoking at moderate levels.

Mild aortic root dilation was recently observed in children from 2 to 9 years of age with vertically transmitted HIV infection (79). The aortic root size was not significantly associated with markers for stress modulated growth, however, aortic root dilation was associated with left ventricular dilation, increased viral load, and lower CD4 counts.

Autonomic Dysfunction

Neild et al. studied heart rate variability as a marker of cardiovascular autonomic tone and found it to be reduced in HIV-infected patients (80). All components of heart rate variability were reduced in patients with AIDS ($p < 0.0001$) when compared with controls. Therefore, HIV may be associated with global autonomic dysfunction which is not related to heart disease (80).

Drug-Induced Myocarditis or Vasculitis

A number of drugs used in the treatment of HIV infection and its infectious or neoplastic complications have been associated with a variety of heart problems. Some have been shown to induce structural changes in the heart and blood vessels with resultant dysfunction. Others have direct toxic effects, manifesting as acute or chronic myocarditis.

Pentamidine has been associated with severe ventricular arrhythmias including torsades de pointes (10). When used intravenously it can also cause orthostatic hypotension, especially if infused too quickly (81). Trimethoprim-sulfamethoxazole has also been associated with torsade de pointes (82), as well as hypokalemia through its action on renal tubules. Neither of these drugs, which are used to treat *P. carinii* pneumonia, have been described to cause myocarditis or congestive heart failure in patients with or without HIV infection (83,84). Amphotericin B, used in severe fungal infections, has been reported to cause arrhythmia, hypotension, hypertension, electrolyte disturbances (notably hypokalemia and hypomagnesemia), and cardiac arrest.

Cocaine abuse has been associated with myocarditis and dilated cardiomyopathy (85–88). Whether intravenous drug use is an additional risk factor for myocarditis or myocardial dysfunction among patients with HIV infection is still uncertain (33,89). Cho et al. (33) observed that the prevalence of idiopathic myocarditis and dilated cardiomyopathy was significantly higher in a group of HIV-infected patients who had intravenous drug use as their primary risk factor, as compared to male homosexuality, a finding confirmed by our own clinical experience. Turnicky et al. (35) concluded in their study that intravenous drug use is an independent risk factor for myocardial diseases.

Chemotherapy used for treatment of Kaposi's sarcoma in patients with HIV infection includes vincristine, vinblastine, doxorubicin and α-interferon. Cardiomyopathy is a well-recognized complication of doxorubicin (90,91). Hypertension and unexpected myocardial infarction have been reported with vinblastine (47). Adverse cardiovascular effects of α-interferon are hypotension, hypertension and tachycardia (91–94). Three patients, after prolonged and high-dose therapy with α-interferon, developed dilated cardiomyopathy with profound myocardial dysfunction which improved after cessation of therapy (95).

Investigators demonstrated that zidovudine could induce myocardial and skeletal muscle mitochondrial changes (96–98) related to zidovudine-induced inhibition of mitochondrial DNA replication. Zidovudine and didanosine, two reverse transcriptase inhibitors, have been proposed as both deterrents to and inducers of cardiomyopathy (99,100). Lipschultz et al. (101), however, compared 24 HIV-infected children receiving zidovudine with untreated HIV-infected children, and did not find a decrease in myocardial contractility associated with use of the drug. Lipschutz et al. recently studied a larger group of infants born to HIV-infected women from birth to five years of age with echocardiographic studies every four to six months. They found that zidovudine was not associated with acute or chronic abnormalities in left ventricular structure or function in infants exposed to the drug in the perinatal period. No child over the age of 10 months had depressed fractional shortening (102).

Lipodystrophy and vascular complications can occur during treatment with protease inhibitors (103–109). Initial case reports have described the development of lipid abnormalities and vascular events that were closely related to the initiation of protease inhibitor therapy. It was suggested that the important risk factors for atherosclerosis and atherothrombosis be characterized in order to establish which patients might be at risk for vascular

events on protease inhibitors (106). In a recent review on hyperlipidemia associated with protease inhibitor use, it was suggested that lipid abnormalities generally consist of elevated triglycerides and total cholesterol levels, with reduced HDL cholesterol levels. The pathophysiological mechanism is unknown, but a recent hypothesis is that the catalytic region of HIV-1 protease has a 60% homology to regions within 2 proteins that regulate lipid metabolism: cytoplasmic retinoic acid-binding protein-1 and LDL receptor-related protein. Inhibition of these proteins by protease inhibitors may result in abnormalities in lipid metabolism leading to hyperlipidemia, fat redistribution and insulin resistance (104,107). The clinical signiÞcance of these abnormalities in terms of risk of coronary events, however, has not been established. Coplan et al. conducted a cross protocol analysis of randomized phase 3 clinical trials sponsored by the pharmaceutical industry that examined treatment with the protease inhibitors, indinavir, nelÞnavir, ritonavir and saquinavir (110). A total of 7668 subjects were randomized to treatment with nucleoside analogues plus a protease inhibitor and 3318 to treatment with nucleoside analogues only. Rates of myocardial infarction did not differ between the subjects receiving protease inhibitors compared with control subjects, and the rates were similar to those reported in population-based epidemiological studies of the general population. The mean age of the patients in these trials was 37 years and mean follow up time was one year (110). Observational data from Kaiser Permanente also failed to show an increased incidence of coronary events in patients receiving protease inhibitors (111).

Use of anabolic steroids has increased in patients with the wasting syndrome of AIDS. Physicians need to be alerted to the possibility of toxicity from this treatment including a risk of myocardial infarction (112).

Nutritional DeÞciency

The terminal stages of HIV infection often involve signiÞcant cachexia and nutritional problems such as selenium and vitamin B deÞciencies, which can cause myocardial dysfunction (113,114). Kavanaugh-McHugh et al. (113) documented that selenium deÞciency is common in malnourished pediatric AIDS patients. Selenium supplementation in this subgroup of AIDS patients has been shown to improve cardiac function (113). Vitamin B1 deÞciency in sick patients may be a cause of cardiomyopathy. However, Acierno (47) feels that at most, it would be a secondary factor. In an autopsy study, Lewis described concurrent cardiac atrophy in subjects who were seriously wasted (115). Umana et al. found that the left ventricular mass index was signiÞcantly higher and left ventricular fractional shortening signiÞcantly lower in HIV-infected patients with recent substantial weight loss compared to normal healthy controls (116). A recent study by Martinez-Garcia et al. demonstrated that HIV-infected patients had a reduced left ventricular mass index with diastolic abnormalities (117).

Clinical Implications of Myocarditis

Many cardiac syndromes have been described in patients with HIV infection, some of which may be progressive and disabling or fatal. Clinical manifestations of myocarditis can include chest pain, dyspnea, fatigue, peripheral edema, dizziness and syncope, although the majority of patients are asymptomatic (40).

Successful therapy for *T. gondii* myocarditis has been reported (118). Therefore, there should be a low threshold for considering this diagnosis in toxoplasma seropositive HIV-infected patients with advanced immunodeÞciency who develop heart failure suddenly (41).

Levy and colleagues (119) made an antemortem diagnosis of myocarditis by endomyocardial biopsy in two patients with known cardiac dysfunction. After treatment with azathioprine and prednisone, a repeat myocardial biopsy showed resolution of the myocardial inÞltrate in one patient who remained asymptomatic without medication during 15 months of follow up. Similar observations were made by Kaul et al. (39). However, there is a report of spontaneous regression of cardiomyopathy in a patient (120). In addition, the Myocarditis Treatment Trial did not support the routine treatment of non-HIV-infected patients with myocarditis with immunosuppressive therapy (121). In patients with heart failure and myocarditis, digoxin should be used with caution because there is an increased sensitivity to the drug. At present, there is no standard methodology for diagnosing myocarditis. Myocardial biopsy may be diagnostically helpful, but has limited sensitivity due to non-diffuse involvement. The presence of speciÞc classes of ventricular ectopic beats may represent a simple and sensitive EV6 marker of HIV-related myocarditis. These beats correlate signiÞcantly with abnormalities in systolic and diastolic echocardiographic parameters (19). Cardiac nuclear scans, such as the gallium 67 scan, the technetium 99 pyrophosphate scan, and the antimyosin antibody scan, are currently used to evaluate patients suspected to have myocarditis. A positive scan is supportive but not diagnostic of the disease. The gallium 67 scan has a limited sensitivity of 36%, with an excellent speciÞcity of 98% (122). The indium 111 antimyosin antibody scan has a sensitivity of 83% and a speciÞcity of 53% (123,124).

CARDIOMEGALY AND CARDIOMYOPATHY

Right ventricular hypertrophy and dysfunction are common Þndings in autopsy series of HIV-infected patients. The most probable cause is pulmonary hypertension related to hypoxia (125), secondary to recurrent pulmonary infections with *P. carinii* or other interstitial lung diseases (47,115). Reports have suggested a possible

association between HIV infection and primary pulmonary hypertension (37,126–130). Mette et al. (127) reported three HIV-infected homosexual men with primary pulmonary hypertension independent of drug use and pulmonary disease. The condition did not appear to be due to direct infection of the pulmonary vasculature by HIV, but to an autoimmune process. Possible etiologies of pulmonary hypertension and cor pulmonale in HIV-infected patients appear diverse, and may include recurrent viral, bacterial, parasitic or fungal pulmonary infections; HIV related interstitial pneumonitis and fibrosis; necrotizing angiotis secondary to intravenous drug use; and thromboembolic events. In a recent review of 131 HIV-infected patients with pulmonary hypertension (131), the interval between diagnosis of HIV infection and diagnosis of pulmonary hypertension was 33 months. Sixty-six patients died during a median follow up period of 8 months, and it was concluded that 82% of the cases were related solely to HIV infection. The appearance of unexplained cardiopulmonary symptoms in HIV-infected patients should suggest pulmonary hypertension (131). In a case series of 56 HIV-infected patients, 7.1% of patients had pulmonary hypertension not related to other well known associated conditions (132). Despite low HIV RNA viral loads and an efficacious antiretroviral drug regimen, pulmonary hypertension worsened. Antiretroviral therapy seems not to prevent or reduce the RV pressure gradient in pulmonary hypertension. It may be that in individuals with an immunogenetic predisposition, high levels of secretion of cytokines and endothelin-1 stimulated by a trigger other than HIV may be at work (132). Aguilar et al. (133) studied six patients with severe pulmonary hypertension associated with HIV infection and treated them with continuous intravenous epoprostenol infusions. This treatment resulted in rapid decreases in both mean pulmonary artery pressure and pulmonary vascular resistance and an increase in cardiac output with similar hemodynamic improvements when these patients were restudied at one year.

Congestive dilated biventricular cardiomyopathy is increasingly being recognized as an important manifestation of HIV infection. Both Cohen et al. (7) and Anderson and Virmani (26) reported the association between HIV infection and dilated cardiomyopathy in clinicopathologic studies. Subsequently, both Himelman et al. (24) and DeCastro et al. (21) documented dilated cardiomyopathy with echocardiography in a large series of patients with HIV infection. The most common echocardiographic findings in their series (21,24) and that of Hecht et al. (23) were impaired left ventricular fractional shortening and left ventricular dilation. It is estimated from echocardiographic studies that cardiomyopathy may be present in up to 30–40% of patients (7,9,23,24,41,43). Herskowitz et al. (31) reported on the prevalence and incidence of left ventricular dysfunction in a large cohort of patients with known HIV infection using two-dimensional echocardiography. They described a 14.5% prevalence of hypokinesis and an incidence of 18% per year, most of which occurred in patients who had low CD4 counts. A subgroup of these patients developed symptomatic heart failure. Similarly, DeCastro and associates (134), in a prospective study showed that about 10% of patients with AIDS developed acute global left ventricular dysfunction.

The pathogenesis of cardiomyopathy still remains uncertain. Shannon et al. (135) studied simian AIDS-associated dilated cardiomyopathy in Rhesus macaques, and found that left ventricular ejection fraction was significantly depressed in those chronically infected with pathogenic SIV compared with those infected with non-pathogenic SIV. Furthermore two-thirds of macaques that succumbed to simian AIDS had myocardial pathology which included lymphocytic myocarditis, coronary arteriopathy and complete vessel occlusion and associated myocardial infarction and necrosis (135). A primary culture of a subpopulation of human fetal cardiac myocytes could not be infected with wild type HIV1, suggesting that direct infection of cardiac muscle fibers is not the cause of HIV heart disease (136). The presence of other viruses, i.e. EBV, CMV or Coxsackie, in certain of the biopsies done by Barbaro et al. (18) suggests a role for these viruses in the pathogenesis of myocarditis. Bowles et al. used polymerase chain reaction to demonstrate that 42% of HIV-infected patients with myocardial inflammation had CMV and adenoviruses besides HIV in their hearts (137). However, some patients with adenovirus had congestive heart failure but no myocarditis, an observation that raises doubt regarding the possible role of adenoviruses as an etiological agent.

Myocarditis has been suggested as the cause for cardiomyopathy (5,134,138–140). However, many reports describing dilated cardiomyopathy do not describe myocarditis at autopsy (115). It has been postulated that in these patients, the active myocarditis had already resolved or that the altered immune response masked the presence of myocardial infection with opportunistic viruses or HIV.

Gross pathologic findings in patients with cardiomyopathy include increased heart weight, biventricular or four chamber dilation, and a pale-appearing myocardium (7,15). On microscopic examination, these cases have shown evidence of both hypertrophy in focal areas and focal areas of atrophy with diffuse loss of myofibrillar elements and vacuolization of the atrophic myocytes (7,15). Focal areas of inflammatory infiltrate have been seen containing lymphocytes, histiocytes, and occasional plasma cells in the myocardial interstitium (115). Electron microscopic studies have revealed loss of myofibrils, lipid deposition, and an increase in the number of mitochondria.

Clinical Implications of Cardiomyopathy

In the early years of the HIV epidemic, clinical cardiac disease was rarely described. However, more recent

studies have described patients with symptomatic cardiac dysfunction. The onset of congestive heart failure often heralds the preterminal stage of the disease (25).

The underlying cause of heart failure has prognostic value in patients with unexplained cardiomyopathy. Patients with cardiomyopathy due to HIV infection may have a worse prognosis compared with other patients with cardiomyopathy. In a recent study by Felker et al. (141), patients with HIV cardiomyopathy had a worse survival over a 4.4 year follow-up period compared to patients with idiopathic cardiomyopathy (adjusted hazards ratio for death was 5.86 with the 95% conÞdence interval, 3.92 to 8.77) (141).

In another study the median survival was 101 days in HIV-infected patients with cardiomyopathy as compared with 472 days in HIV-infected patients with normal hearts who were at a similar stage of disease (142). A longitudinal prospective study of HIV-infected infants and children found that left ventricular fractional shortening determined by echocardiography was a signiÞcant independent predictor of overall mortality, even after adjustment for age, CD4 cell count, and progressive neurologic disease, demonstrating the clinical importance of cardiac function in the outcome of HIV-infected patients (143). The degree of left ventricular dysfunction correlated with the extent of immune dysfunction at baseline but not over time, suggesting that the CD4 count may not be a useful surrogate marker of HIV associated left ventricular dysfunction. Another study in children also showed that depressed left ventricular function correlated with CD4 counts at baseline but not longitudinally (144). The same study showed that development of encephalopathy correlated well with decreased fractional shortening of myocardial muscle Þbers. Hornberger et al. found that vertically transmitted HIV infection may be associated with reduced left ventricular size but no alteration in cardiac function in the fetus in utero (145).

Dyspnea is one of the most common symptoms of HIV infection and because of the high prevalence of pulmonary disease, it is presumed to be secondary to it. However, with the increasing prevalence of cardiac dysfunction, myocardial disease has to be considered in the evaluation of patients, especially when dyspnea is out of proportion to underlying pulmonary disease. Chest x-rays are relatively insensitive for detecting cardiac dysfunction, but they may show evidence of cardiomegaly. EKG Þndings are usually non-speciÞc. Hsai and coworkers (146) found that late potentials on signal-averaged electrocardiograms performed on HIV-infected patients were both common and intermittent, but were not related to contractile abnormalities. Echocardiography shows cardiac abnormalities in excess of 30% of patients in some studies (22), often segmental abnormalities at the level of the interventricular septum (21). Barbaro and co-workers found an early impairment of systolic and diastolic function in asymptomatic HIV-infected patients (CD4 □600) (20). In their multicenter echocardiographic and echo-doppler study, 1236 asymptomatic New York Heart Association class I HIV-infected patients were compared to 1230 healthy subjects. An analysis of the echocardiographic data revealed a reduction of 19.7% in ejection fraction, an increase of 55.7% in wall motion score, a reduction of 34.6% in the E/A ratio and an increase of 19.7% in the isovolumetric relaxation time in HIV-infected subjects compared with healthy controls. Echocardiographic alterations were observed in 57.2% of HIV-infected subjects and in 13.7% of control subjects (20).

Although the etiology of cardiomyopathy is most often unclear, treatment of congestive heart failure is the same as for patients without HIV infection. Treatment for opportunistic infections and neoplasia may need to be altered. Intravenous treatment should be given in lower volumes, and cardiotoxic drugs, such as doxorubicin and daunorubicin, should be avoided (33).

NEOPLASIA OF THE HEART

Kaposiõ sarcoma and malignant lymphoma affecting the heart have been described in patients with HIV infection (16,147). Kaposiõ sarcoma is, by far, the most common malignancy affecting the heart.

Kaposiõ Sarcoma

Kaposiõ sarcoma is a neoplasm which possibly arises from endothelial cells. Heart involvement in Kaposiõ sarcoma is usually part of a widely disseminated process. In 1983, Autran et al. (3) Þrst described Kaposiõ sarcoma involving cardiac tissue in HIV-infected patients. Kaposiõ sarcoma originates in the visceral or parietal pericardium, and less frequently in the myocardium (148). Rarely are both the pericardium and myocardium involved together. Kaposiõ sarcoma may also involve the adventitia of the coronary arteries and involvement of the great vessels has also been reported (148). Various groups have reported its predilection for the epicardium and subepicardial fat (148,149). At autopsy, the prevalence of cardiac Kaposiõ sarcoma in HIV-infected patients with other evidence of Kaposiõ sarcoma was reported by Cammarosano and Lewis (148) to be 4 out of 21 (20%) and by Silver et al. (16) to be 5 out of 18 (28%). A few cases of fatal cardiac tamponade in patients with epicardial Kaposiõ sarcoma have been reported (150,151), and one patient presented with pericardial constriction (39). A common observation in all studies was the paucity of clinical cardiac dysfunction even in cases with extensive myocardial involvement.

Malignant Lymphoma

Malignant lymphoma involving the heart, although infrequent, has been described in HIV-infected patients.

Most of these non-Hodgkin lymphomas are high grade with Burkitt-like cells (small and non-cleaved), reticulum cell sarcomas, or large cell immunoblastic sarcomas (16,152). In the majority of cases they appear to originate from B cells, with most of them displaying a monoclonal pattern of immunoglobulin staining. In some cases the EBV genome was shown in the proliferating cells (152). Extranodal sites of lymphoma are common in HIV-infected patients, and involvement of the heart can occur as part of this disseminated process (16,97,147,153–155).

Despite their rarity in general (156), primary cardiac lymphoma has been reported in HIV-infected patients (157–159), and many theories have been put forward for their pathogenesis (160,161): (1) chronic antigenic stimulation; (2) impaired immune surveillance; (3) reactivation of latent oncogenic herpes group viruses; (4) impaired immunoregulation; (5) carcinogenic effects of immunosuppressive, cytotoxic or other drugs; (6) genetic susceptibility; and (7) interleukin-6 production (161).

Lymphomas involving the heart are characterized by one of two different pathologic patterns: diffuse infiltration that imparts a pale appearance to the heart; or involvement of the epicardium, myocardium and endocardium in the form of focal circumscribed nodules (156,157).

Clinical manifestations of lymphomatous involvement of the heart may include cardiomegaly, pericardial effusion, congestive heart failure, or progressive heart block (157,159). Sudden death is a rare event (159), and over one-half of the patients have no clinical evidence of cardiac dysfunction. Gallium-67 and blood pool isotope studies can aid in the diagnosis (162,163). Echocardiographic and EKG studies demonstrate non-specific findings. The outcome is usually poor, and the optimal approach to therapy is still not known (164).

ENDOCARDIAL DISEASE

Marantic Endocarditis

The most common endocardial lesion seen with HIV infection is non-bacterial thrombotic endocarditis, also known as marantic endocarditis (148,165,166). Any of the valves can be involved, but left-sided lesions are the most common. Marantic endocarditis is also associated with chronic wasting disease, malignancies and hypercoagulable states (148,165,166). In a study of HIV-infected patients with marantic endocarditis (148), vegetations were friable and systemic embolization occurred. The diagnosis of marantic endocarditis is very difficult to make before death, even with the help of echocardiography (167).

Bacterial Endocarditis

Infective endocarditis is infrequent in AIDS, despite the fact that intravenous drug use is an important risk factor for HIV infection (39). The most common organism causing bacterial endocarditis in HIV-infected intravenous drug users is *Staphylococcus aureus* (>75%) (168).

One study reported no significant difference in hospital mortality among patients with endocarditis whether or not they were HIV positive or negative. However, the investigators did note a higher frequency of left ventricular dysfunction in the HIV-infected group (169). Bacterial endocarditis may be more virulent in immunocompromised hosts as compared with normal hosts (11).

Fungal Endocarditis

Henochowicz and colleagues (170) described one patient with HIV infection who had *Aspergillus* endocarditis diagnosed at autopsy. The patient did not have a cardiac murmur, nor a positive blood culture for *Aspergillus fumigatus* or any evidence of cardiac dysfunction. At autopsy, pulmonary aspergillosis, myocardial abscesses and cerebral emboli were found. *Cryptococcus* and *Candida* fungal endocarditis have also occurred in AIDS patients, particularly among intravenous drug users. In patients with fungemia, signs of systemic or cerebral embolism may indicate the presence of endocarditis (40).

PERICARDIAL DISEASE

Pericardial disease is a frequent cardiovascular manifestation of HIV infection and is often associated with a shortened survival (171). The prevalence of a demonstrable effusion based on echocardiography varies from 10–59% (26,39,115,134,172) and the development of an effusion suggests endstage HIV disease (171). The spectrum of pericardial disease in different reports ranges from asymptomatic effusions detected by echocardiography to fatal tamponade and constrictive pericardial disease. In 15 autopsy and echocardiographic series involving 1139 patients with HIV infection, the average prevalence of pericardial disease was 21% (173). Pericarditis is usually non-specific in origin and can occur with or without pericardial effusion (174). Levy et al. found no difference in the prevalence of pericardial effusion between HIV-infected patients with and without AIDS (119). Initial symptoms and signs are frequently subtle, but may include chest discomfort and findings of tamponade and hypotension (39). Low pressure tamponade is a phenomenon that is sometimes seen in AIDS patients who are severely dehydrated and cachectic; the severe volume depletion may cause reduced right ventricular filling pressures, and even a minimal pericardial effusion may cause a hemodynamically significant tamponade (173). In this setting, jugular venous distention or pulsus paradoxus may be absent (175). Most cases of pericardial effusions are asymptomatic and without an identifiable cause. However, in those that are symptomatic, about two-thirds

are caused by an infection or neoplasm. Etiology includes Kaposi's sarcoma and various microorganisms such as *M. tuberculosis* (8), *M. avium-complex* (176), *Cryptococcus neoformans* (177), Herpes simplex (178), *Nocardia asteroides* (179) and pyogenic bacteria such as *Staphylococcus aureus* (Table 23.1). In 66 published cases of cardiac tamponade in patients with HIV infection, 26% were caused by tuberculosis, 17% were pyogenic, and 8% were caused by atypical mycobacteria. Lymphoma and Kaposi's sarcoma each accounted for 5% of these effusions. Less frequent causes of tamponade were *C. neoformans*, CMV and *M. kansasii* (173). The cause of asymptomatic effusions often remains uncertain. Culture of pericardial fluid in these cases is usually unrevealing. Some pericardial effusions are attributable to viral pericarditis, uremia or congestive heart failure in patients with HIV-associated cardiomyopathy. Echocardiographic evaluation has proven to be an excellent diagnostic tool in detecting and monitoring pericardial effusion (21,24,39,172).

Small asymptomatic pericardial effusions in patients with HIV infection do not require diagnostic evaluation, and may be followed with serial echocardiography; however, symptomatic pericardial effusions should be investigated to find a potentially treatable infection or neoplasm (173). Non-steroidal anti-inflammatory drugs may be helpful therapeutically, but corticosteroids should generally be avoided because concurrent infections may be exacerbated by steroid therapy.

The value of pericardial fluid analysis in HIV-associated pericardial effusions is controversial with diagnostic yields ranging from 0 to 100% (150,172,180–182). HIV infection is the most common underlying disease in recent retrospective and prospective reports of patients undergoing pericardiocentesis in urban settings (172,182,183). Reynolds et al. (182) have suggested that mycobacterial disease is a common etiology for large HIV infection-related pericardial effusions. HIV-infected patients from the inner city who have pulmonary infiltrates and fever are at risk for tuberculosis as the cause of pericardial effusion (184). Based on available evidence, the diagnostic use of pericardiocentesis in this population remains questionable. However, the procedure can provide unexpected diagnostic information and often demonstrates a total lack of concordance between infections in other organ systems and that in the pericardium (172).

Pericardiocentesis may not only be diagnostic, but also therapeutic in the case of tamponade. Pericardial biopsy may increase the diagnostic yield. An open drainage procedure usually done by the subxiphoid approach may be necessary for large pericardial effusions. Open drainage is required for pyogenic pericardial effusions (173).

THERAPEUTIC IMPLICATIONS

Since cardiac complications in HIV infection are often clinically non-apparent or subtle initially, periodic screening of these patients with both an EKG and echocardiogram is probably worthwhile, specifically in the subgroups of patients with a low CD4 count (< 400 mm^3) or in those patients receiving therapy with zidovudine. Because of the concern that patients receiving highly active antiretroviral therapy may develop a higher incidence of vascular complications and lipid abnormalities, risk factors for atherosclerosis should be evaluated before starting treatment with these drugs. Care should be given to proper nutrition of the HIV-infected patient because there is evidence that nutritional deficiency can initiate or aggravate a cardiomyopathic condition. Patients who have acute myocarditis should be treated when an arrhythmia develops or when there is evidence of myocardial dysfunction. Drugs used to manage congestive heart failure may include digoxin, diuretics, angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, or beta-adrenergic blockers.

Patients with treatable infections of the myocardium, heart valves, and pericardium should receive the appropriate antimicrobial regimens. The appearance of unexplained cardiopulmonary symptoms in a patient with HIV infection should stimulate prompt evaluation of left and right ventricular function and the possibility of pulmonary hypertension should be investigated. The development of pulmonary hypertension portends a poor clinical outcome. Continuous infusion of intravenous epoprostenol has shown hemodynamic benefit in a small case series (184a).

Treatment of AIDS-related neoplasms of the pericardium or myocardium should be based on providing symptomatic relief. If cancer chemotherapy is being used, those agents that can cause myocardial depression should be avoided.

CONCLUSION

HIV infection is responsible for significant morbidity and mortality worldwide (185), and cardiac disease is increasingly being recognized as a complication of HIV infection. Cardiac diseases in HIV infection include myocarditis, myocardial necrosis, cardiomyopathy, arteriopathy, endocarditis, pericarditis, pericardial effusion, and neoplasm. These conditions are often not detected by clinical examination, and current knowledge is based almost exclusively on echocardiographic and autopsy studies which have detected abnormalities in over 30% of patients. AIDS is providing a clinical venue for gaining insights into the pathogenesis of rare cardiac diseases such as lymphocytic myocarditis, Kaposi's sarcoma involving the heart and dilated cardiomyopathy. In the future, observational and clinical trials may yield more information on the impact of treatment on these conditions and will be necessary for obtaining data concerning the mechanisms of myocardial damage in AIDS.

The cause and pathogenesis of myocarditis, cardiomyopathy and pericardial disease remain unresolved in

most situations. Opportunistic infections, vasculitis, hypoxia, an autoimmune process, catecholamine excess, adverse effects of medication (e.g. zidovudine) and nutritional deficiencies have been suggested as possible causes. There is now some evidence to suggest that HIV infection itself may be an important cause of myopericardial disease. In more than 80% of patients, no specific cause for myocarditis has been found.

Once myocardial failure develops, it has a poor prognosis despite the observation that patients often respond to traditional heart failure therapies. Pericardial involvement is frequent, often asymptomatic, and usually manifested as an idiopathic pericardial effusion, although neoplasms and opportunistic infections may also be seen. The value of pericardial fluid analysis is controversial. Endocardial involvement is most likely related to marantic endocarditis, however, bacterial endocarditis may also complicate the course of patients who are intravenous drug users. Infection with HIV does not appear to make patients more susceptible to bacterial endocarditis. Physicians should be alert to the high frequency of cardiac involvement in patients with HIV infection which might impact on the treatment and supportive care that these individuals require.

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Chapter 24

Renal Manifestations of HIV Infection

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HIV-1 related diseases of the kidney encompass a spectrum of disorders and a diversity of clinical syndromes. They are often multifactoral in etiology, but a few very speciÞc diseases are known to be direct consequences of HIV-1 infection in the kidney. The others appear to be renal manifestations of systemic complications of HIV-1 infection or are related to drug therapy. A useful approach to the diagnosis of kidney diseases in general is to distinguish between the syndrome of acute renal failure (ARF) and chronic renal insufÞciency. The former is commonly encountered in the hospital setting, caused by combinations of tissue hypoperfusion, systemic infection, and/or the use of nephrotoxic drugs. Chronic renal insufÞciency is more commonly encountered in the outpatient setting, and can be due to one of many speciÞc disease entities. A clear separation of diseases into these two categories is not completely appropriate, however, because several diseases that are considered chronic in nature, such as HIV-associated nephropathy (HIVAN) and immune-complex glomerulonephritis, may present as the syndrome of acute renal failure. The physician must also consider the stage of the underlying HIV-1 infection, the presence of concomitant illnesses, current medications, the rate of fall in glomerular Þltration rate (GFR), abnormalities in urinary sediment, and urinary protein excretion.

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ACUTE RENAL FAILURE

Acute renal failure (ARF) is a syndrome deÞned as an abrupt, detectable fall in GFR in an individual with otherwise stable kidney function. The causes of ARF found in the setting of HIV-1 infection are listed in Table 24.1.

Ischemic Acute Renal Failure

Ischemic acute renal failure from sepsis or hypotension induces acute renal failure usually as a result of acute tubule necrosis (ATN). The endothelium of the glomerular microcirculation and the renal tubular epithelial cells are most susceptible to ischemic injury. The functional abnormalities that result from this insult include a reduction in glomerular Þltration rate (GFR) and defects in urinary concentrating ability.

Under physiologic conditions, the kidney responds to hypotension or renal vasoconstriction by conserving salt and water. Urea is reabsorbed more efÞciently and the increase in blood urea nitrogen that occurs is referred to as prerenal azotemia. Daily urinary volume falls to as low as 500 ml/day, urinary sodium concentration falls below 10 meq/l, and urinary osmolality approaches 800 mosm/l. After ischemic injury, however, daily urinary volume may fall, may stay the same or can increase. Thus, the volume of urine unless the patient is anuric (urine volume $<$ 500 ml/day) is a poor index of renal function.

A much better index of renal function in distinguishing ischemic vs. prerenal azotemia in the setting of oliguria is the analysis of concentrating functions of the kidney. In prerenal conditions such as hypotension or sepsis, the kidney concentrates the urine and avidly resorbs sodium. Urine speciÞc gravity increases well above the plasma level of 1.010 and urine Na concentration falls to $<$ 5 meq/l. In the setting of renal injury, however, urinary speciÞc gravity rarely exceeds 1.010, and the urinary Na

TABLE 24.1. *Common causes of acute renal failure in HIV-1 infection*

Cause of ARF	Pathophysiology	Clinical presentation and diagnostic clues
Ischemic ARF (also known as acute tubule necrosis (ATN))	Hypoperfusion induces ischemic injury in renal endothelial and tubule cells.	Often accompanies a systemic in ammatory response syndrome. Clinical picture is marked by oliguria with defective Na and H_2O reabsorption (urinary speci c gravity (SG) □1.010 and urine Na concentration (U_{NA}) > 20 meq/l). Pigmented urinary casts observed on microscopic examination of urine.
Prerenal azotemia	Hypoperfusion reduces GFR by lowering effective glomerular ltration pressure. There is no ischemic cell injury.	Develops with severe intravascular volume depletion or low cardiac output. Oliguria is present but urinary Na and H_2O reabsorption are preserved (urinary SG > 1.018 and U_{NA} < 20 meq/l.) Urine sediment is normal.
Hepatorenal syndrome	Seen with chronic liver disease, advanced liver failure and portal hypertension. Marked abnormalities in arterial hemodynamics and vasoactive hormone production.	Recognized by progressive oliguric renal failure associated with chronic and acute liver disease. Chemical composition of the urine is similar to prerenal azotemia (SG > 1.018, U_{NA} < 10 meq/l), but does not improve with intravenous uids.
Drug induced Nephrotoxicity		
Aminoglycosides	Selectively transported into proximal tubule cells where drug accumulates in high concentration.	Nephrotoxicity is dose-dependent, daily urinary volumes exceed 500 ml (non-oliguric ARF) and is reversible.
Amphotericin B	Direct tubule epithelial membrane injury associated with acute reductions in GFR due to reduced renal blood ow .	Dose dependent nephrotoxicity characterized by renal tubule dysfunction including acidosis, hypokalemia and hypomagnesemia.
Acyclovir	Drug is secreted into tubule lumen where solubility may be exceeded. ARF is due to intratubule obstruction and perhaps direct tubule toxicity.	Urinary sediment is characterized by needle-shaped crystals, hematuria and pyuria.
Foscarnet	Electrolyte disorders suggest direct tubule toxicity.	ARF often accompanied by hypercalcemia, hypomagnesemia and hypokalemia.
Cidofovir	Direct toxicity on tubule cell, drug is eliminated by the kidney and secreted by proximal tubule cells.	Incidence of ARF is decreased with co-administration of probenecid
Pentamidine	Mechanism of toxicity is unknown. Drug is concentrated in the kidney but not eliminated by the kidney.	Non-oliguric acute renal failure develops after 7–10 days of therapy. Non-speci c urinary ndings include microscopic hematuria and pyuria.
Drug-induced interstitial nephritis	Methicillin,* rifampin, trimethoprim-sulfamethoxazole and other sulfonamides, NSAIDS, phenytoin	Methicillin* associated allergic interstitial nephritis can present with eosinophilia, eosinophiluria, fever and skin rash. Other drugs may induce interstitial nephritis without other allergic components.
Crystalluria	Indinavir, sulfadiazine, acyclovir	Indinavir is associated with needle-shaped, fan or rectangular crystals. Sulfadiazine with “shocks of wheat”- appearing crystals and acyclovir with needle-shaped crystals that are birefringent under polarized light.
Myoglobin-induced (rhabdomyolysis)	Multifactorial pathophysiology in which nephrotoxic potential is accentuated in the setting of hypovolemia and/or renal vasoconstriction.	Myoglobinuria develops as a direct effect of HIV-1 infection, especially in the acute retroviral syndrome. Also associated with cocaine use and drug treatment of dyslipidemia. CPK levels usually > 10,000 U/L.
Glomerular and vascular disease.	HIVAN, glomerulonephritis secondary to hepatitis C, post-infectious glomerulonephritis, lupus nephritis, microangiopathic hemolytic anemia.	As a group, these disorders are distinguished by either heavy proteinuria (HIVAN) or hematuria with red blood cells casts (acute glomerulonephritis). Certain diseases, such as HIVAN and glomerulonephritis, more commonly present as chronic renal failure syndromes.

* Methicillin is no longer in clinical usage.

concentration is usually >20 meq/l. With ATN, cellular elements also appear in the urinary sediment. Numerous necrotic, pigmented (muddy-brown) tubule epithelial cells or casts are usually easy to observe. The chemical indices and the characteristic urinary sediment conÞrm the diagnosis of ischemic acute renal failure in the proper clinical setting. Once ischemic injury has been established, the usual course is a one to four week period of oliguria and reduced GFR. Morbidity is quite high during this period from sepsis and ßuid and electrolyte disorders and early aggressive dialysis reduces the complication rate of ATN. Treatment during this time is focused on correction of underlying hemodynamic abnormalities, on establishment of proper dietary intake of calories and protein to minimize tissue catabolism, and on management of ßuid and electrolyte disorders, particularly hyponatremia, hyperkalemia, and metabolic acidosis. Hemodialysis should be initiated as soon as the diagnosis is clear and the blood urea nitrogen concentration (BUN) approaches 80 mg/dl to prevent complications of uremia. Recent studies indicate that more frequent dialysis in critically ill patients with ARF is associated with better outcomes (1).

Prerenal Azotemia

Prerenal azotemia is a syndrome in which effective Þltration pressure in the glomerular microcirculation is reduced because of reduced renal blood ßow, but the hypoperfusion is not severe enough to induce ischemic cell injury. The hepatorenal syndrome is one of the more classic examples of this hemodynamic condition. As a consequence, GFR is reduced but the tubule response to hypoperfusion is physiologic. Patients are oliguric, but the urine speciÞc gravity exceeds 1.020 and urinary Na concentration is <5Ð10 meq/l. The fractional excretion of Na or FE_{Na} is particularly useful in this setting; it is measured as the ratio of Na to creatinine clearance.

$$\begin{aligned} \%\, FE_{Na} &= (U_{Na}V/P_{Na})/(U_{Cr}V/P_{Cr}) \times 100 \\ &= (U_{Na}/P_{Na})/(U_{Cr}/P_{Cr}) \times 100 \\ &= (U_{Na} \times P_{Cr})/(U_{Cr} \times P_{Na}) \times 100 \end{aligned}$$

In acute renal failure the FE_{Na} is >5% and in prerenal azotemia the FE_{Na} is <1%

A disproportionate rise in blood urea nitrogen concentration compared to creatinine concentration is also a suggestive feature of prerenal azotemia. The rate of urea resorption is urine ßow-dependent, the greater the ßow, the lower the rate of urea uptake. Under normal conditions, the ratio of their respective plasma concentrations approaches 10:1. In low urine ßow conditions such as hypotension or sepsis, the ratio increases, often to greater than 20:1. The BUN/creatinine ratio should only be used as an ancillary clue because non-renal factors often seen in HIV-infected patients, such as nutritional status, liver disease, steroid use and gastrointestinal bleeding alter plasma BUN levels. The diagnosis of either ischemic ARF or prerenal azotemia is almost always made on clinical grounds combined with urinalysis and an analysis of serum and urine electrolytes. A biopsy is rarely, if ever, necessary.

Treatment of prerenal azotemia is aimed at restoring renal hemodynamics to normal as quickly and effectively as possible. In cases of intravascular volume depletion this is easily accomplished with intravenous ßuids. In patients with low cardiac output, early sepsis and third space accumulation of volume due to liver disease, therapeutic options to correct the hemodynamic abnormalities are usually focused on the underlying condition rather than the kidney.

Hepatorenal Syndrome

Advanced liver disease as seen in HIV-infected patients co-infected with hepatitis C or B can lead to acute renal failure which is particularly difÞcult to manage. In cirrhosis, total peripheral vascular resistance is low, mean arterial blood pressure is low and vascular resistance in non-splanchnic vascular beds, including the renal vascular bed, is increased. Clinical conditions that aggravate this underlying renal hypoperfusion, such as diuretic use, large volume paracentesis, GI bleeding or spontaneous bacterial peritonitis, often result in acute renal failure. Prerenal azotemia, ischemic renal failure and nephrotoxic renal injuries are all relatively common in cirrhotic patients as well. Non-steroidal anti-inßammatory drugs commonly cause ARF in patients with liver disease. These agents reduce renal production of prostanoids with vasodilating effects, particularly PGE_2. This leads to unopposed vasoconstriction in the renal vascular bed and a reduced GFR.

The hepatorenal syndrome is a unique form of acute renal failure that occurs in the setting of advanced chronic liver disease with liver failure and portal hypertension. It is similar to prerenal azotemia with low urinary volume, low urinary Na concentration (<10 meq/l) and normal urinary sediment. Unlike prerenal azotemia, however, there is no improvement following a challenge with I.V. ßuids. The hepatorenal syndrome is clinically conÞrmed when infection, nephrotoxic renal injury, ischemic injury and prerenal azotemia have been excluded. It is not restricted to patients with chronic liver disease, and can develop in more acute forms of liver disease, such as in alcoholic hepatitis and acute liver failure (2).

Drug-Related Renal Disease

Nephrotoxicity

Acute renal failure is a well-recognized complication of radiocontrast dyes as well as a number of drugs frequently given to HIV-infected patients (Table 24.1). In general,

when these drugs are used all attempts should be made to limit simultaneous exposure to other nephrotoxic drugs and to optimize the patient's fluid and electolyte status.

Aminoglycosides

Aminoglycosides are selectively filtered then efficiently resorbed into proximal tubule cells where they reach a high concentration and trigger an injury cascade. Toxicity is dose-dependent and renal failure usually occurs 5–10 days after initiation of therapy. It is typically non-oliguric and, in most cases, it reverses with cessation of the drug, although recovery may take weeks to months. Hypokalemia and hypomagnesemia are common, and indicate proximal tubule dysfunction. Potential for toxicity can be minimized by appropriate adjustment of maintenance dosing relative to renal function. Following drug trough levels is the best index for appropriate dosing. Once daily dosing has been shown to be efficacious in most settings and is associated with lower toxicity. As with all nephrotoxic agents, it is important to correct any underlying volume and electrolyte disorders and to avoid the concomitant administration of other nephrotoxic drugs (3,4).

Amphotericin B

Amphotericin B does not accumulate in the kidney but is associated with a dose-related reduction in GFR in most patients by altering renal blood flow. Amphotericin binds to membranes along the renal tubule and causes potassium and magnesium wasting and a distal renal acidosis. Minor elevations of serum creatinine in the range of 2 to 3 mg/dl are common and not an indication to discontinue therapy. Lipid-complexed formulations of amphotericin result in lower renal concentrations, are less nephrotoxic and a reasonable alternative in the setting of altered renal function.

Miscellaneous

Many antivirals are associated with renal toxicity. Nephrotoxicity from acyclovir has been attributed to intratubule obstruction caused by precipitation of the drug, but direct tubule toxicity may occur in the absence of drug crystals. The nucleotide analogue, cidofovir, accumulates in proximal tubule cells where it induces injury. Preadministration of saline and the co-administration of probenecid may lessen toxicity. Reversible renal toxicity is commonly associated with foscarnet therapy and can present as ATN. Appropriate dose adjustments in the setting of renal insufficiency as well as adequate prehydration help minimize nephrotoxicity (5). Nephrotoxicity with or without hyperkalemia appears to be a more frequent complication of pentamidine therapy in AIDS patients than in other patient populations (6). The nucleotide analogue, adefovir was originally developed as an antiretroviral agent. However, at the doses tested in clinical trials (120 mg/day and 60 mg/day), the drug was associated with nephrotoxicity manifested as a Fanconi's syndrome (7). At significantly lower doses (10 mg/day), adefovir appears to be an effective agent against hepatitis B in HIV-infected patients without the associated toxicity (8).

Acute Interstitial Nephritis

Acute interstitial nephritis (AIN) can be associated with a number of drugs often used in the setting of HIV infection. Allergic interstitial nephritis is common with the β-lactam antibiotics; the prototype for the disease is due to methicillin. The disease is rarely seen with the initial use of the drug, but it can occur at almost any other point in the therapeutic course and is not dose-related. Although classically methicillin-associated AIN is associated with fever, skin rash, eosinophilia and eosinophiluria, all elements of the clinical syndrome may not be present. The more commonly used drugs in the setting of HIV infection that might be associated with AIN include trimethoprim-sulfamethoxazole, cephalosporins, phenytoin and rifampin.

A plasmacytic interstitial nephritis has been described in HIV-1 infection that is not clearly related to drug ingestion. The etiology remains unknown, it can be diagnosed only by renal biopsy and responds to steroids.

Rhabdomyolysis and Crystalluria

Two renal syndromes associated with drug ingestion have become more frequently recognized in the setting of HIV, rhabdomyolysis and crystalluria. The higher frequency of rhabdomyolysis is most likely due to the use of statins in the setting of protease inhibitor-induced hyperlipidemia (9). Statins and protease inhibitors (PI) may share a common metabolic pathway (CYP3A4) and their combination may result in high tissue drug levels. Rhabdomyolysis has also been reported with newer agents that are metabolized via alternate pathways (10). The diagnosis should be considered when creatinine phosphokinase (CPK) levels exceed 10,000 IU. Treatment for renal disease secondary to rhabdomyolysis requires discontinuation of the offending drug, increasing urinary flow and increasing the solubility of myoglobin by alkalinization of the urine. This is accomplished by infusing intravenous solutions containing bicarbonate in an attempt to increase urinary ph to levels greater than 7. Rhabdomyolysis may also occur as a direct result of HIV-1 infection, in association with zidovudine or trimethoprim-sulfamethoxazole, and complicating trauma, convulsions, or cocaine use.

Drug induced crystalluria has been reported in 20–40% of patients receiving the protease inhibitor indinavir (11–13). Dehydration secondary to vomiting, diarrhea or poor intake are predisposing factors. Crystalluria may be asymptomatic or associated with dysuria, colic, obstructive uropathy or even a chronic renal insufficiency syndrome due to interstitial fibrosis (14). The urine is characterized by leukocyturia, with needle-shaped, fan or rectangular-shaped crystals by light microscopy. Oral hydration prior to drug ingestion should reduce intratubule concentrations of the drug. Indinavir may be safely continued in the face of asymptomatic disease or even colic, but should be discontinued in patients with chronic renal impairment whose creatinine approaches 2 mg%. Sulfadiazine can also produce crystalluria (15), recognized as "shocks of wheat" under light microscopy. This syndrome usually presents as acute renal failure and should be treated with bicarbonate-containing IV fluids to increase urinary flow and the solubility of sulfadiazine crystals.

Glomerular Diseases

Glomerular diseases, including HIVAN, acute glomerulonephritis and the hemolytic-uremic syndrome, can present as acute renal failure in HIV-infected patients. They can be distinguished from drug-induced disease or ischemic renal failure by the urinary sediment, which is marked by proteinuria and varying degrees of hematuria and red blood cell casts. Acute glomerulonephritis is often due to immune complexes; the more common causes are post-infectious and cryoglobinuric renal disease associated with hepatitis C. Idiopathic renal vasculitis has been described, as has lupus nephritis and IgA nephropathy induced by idiotypic antibodies to HIV-1 envelope proteins. When the clinical picture suggests acute glomerular disease, an evaluation for circulating immune complexes is warranted, but a kidney biopsy is required to make a precise diagnosis and guide therapy. The hemolytic-uremic syndrome and thrombotic thrombocytopenic purpura have been associated with ARF in the setting of HIV infection. The underlying cause is unknown, but several investigators are studying the direct effects of the virus in the pathogenesis. Plasmapheresis is the treatment of choice.

CHRONIC RENAL INSUFFICIENCY

Several glomerular and tubulointerstitial diseases can present as a more chronic disease (Table 24.2).

HIVAN

Epidemiology

The most common cause of chronic renal disease associated with HIV-1 infection is HIVAN. The disease occurs almost exclusively in Blacks, not only from North America, but also in from Europe and Asia (16–18). When Blacks comprise a large proportion of the population under study, as in reports from New York, Washington, D.C., Miami, and the African or Afro-Caribbean communities of Paris and London, HIVAN is the predominant cause of renal disease (16–18). In the United States, HIVAN has become the third leading cause of ESRD in Blacks between the ages of 20 and 64 years old, more common than lupus nephritis, polycystic disease or primary glomerulonephritis (19). When reports of kidney disease in HIV-1 infected subjects come from predominantly Caucasian populations, however, HIVAN is not as frequent a cause of chronic kidney disease (20–23). Because the population of patients at risk for HIVAN continues to expand disproportionately in those living with AIDS, a growth in the number of HIV-infected patients with end stage renal disease is foreseeable in the future (26).

Clinical Presentation

The reported presentation of HIVAN is usually in patients with CD4+ cell counts below 200, however, this may not represent the full spectrum of the disease due to a bias introduced by methods of case identification (24). More recently, HIVAN has been reported in patients with higher CD4+ counts and even during primary HIV infection (25).

HIVAN is defined morphologically as focal segmental glomerulosclerosis with collapse of the glomerular capillary tuft, glomerulosclerosis, and microcystic tubulointerstitial disease (27,28) (Fig. 24.1). Visceral epithelial cell abnormalities include podocyte hypertrophy and hyperplasia. Special stains demonstrate increased markers of proliferation in podocytes as well as loss of markers of differentiation. The important clinical characteristics include the presence of heavy proteinuria, often in the nephrotic-range, the absence of edema or hypertension, and varying degrees of renal insufficiency. Ultrasound evaluation usually reveals enlarged, echogenic kidneys in contrast to most forms of chronic kidney diseases, which are associated with small, shrunken kidneys. The predictive value of these clinical characteristics has not been rigorously tested, and numerous reports suggest that the clinical impression is not always supported by the biopsy findings (29–31). A kidney biopsy is needed to determine the cause of kidney disease in most cases of HIV-associated chronic kidney disease. The clinical course in most patients with HIVAN is marked by rapid progression to ESRD within weeks-to-months, with some recent suggestion of a slowing in the rate of progression since the HAART era. HAART may have reduced the mortality of HIV-infected patients on dialysis as well. Prior to the introduction of HAART, the prognosis of HIV-infected patients on dialysis was very poor with a

TABLE 24.2. *Common causes of chronic renal disease in HIV-1 Infection (Adapted from ref (70))*

Glomerular Disease	Clinical Features
HIVAN	Morphologically de ned by focal segmental glomerulosclerosis with microcystic tubulointerstitial disease. Manifested by proteinuria, absence of an active urinary sediment, absence of hypertension and enlarged kidneys. HIV infection in renal epithelial cells.
Membranoproliferative glomerulonephritis	Associated with hepatitis C infection with or without cryoglobulinemia as well as hepatitis B. Often presents as an acute nephritic syndrome (hypertension, hematuria, red cell casts and azotemia). Hypocomplimentemia is found in approximately 50% of cases. A more subacute form can be clinically indistinguishable from HIVAN.
Minimal Change Disease	Nephrotic syndrome (proteinuria, edema and hypertension), biopsy is required for diagnosis.
Membranous Nephropathy	Nephrotic syndrome, can be secondary to hepatitis B, hepatitis C, lupus nephritis or lymphoma.
Systemic Lupus Erythematosis	Clinical presentation as acute nephritis or nephrotic syndrome. Several cases described in association with HIV-1 infection, but no direct link between these two diseases has been established.
Amyloidosis	Nephrotic syndrome and enlarged kidneys, progressive renal failure, also known as “skin popper’s” amyloid (AAamyloid), associated with injection drug use.
HIV Thrombotic Microangiopathies (Hemolytic Uremic Syndrome and Thrombotic thrombocytopenic Purpura)	Microangiopathic hemolytic anemia, thrombocytopenia and elevated LDH. More common in children.
IgA Nephropathy	Chronic presentation with hypertension and hematuria, however, several cases reported with acute nephritis, IgA anti-HIV idiotypic antibodies.
Focal Necrotizing glomerulonephritis	Necrotizing vasculitis with systemic features (polyarteritis nodosum), reported in HIV-1 infection but no direct link between these two diseases.
Post-infectious glomerulonephritis	Prototype is post streptococcal glomerulonephritis, but may arise as a complication of other bacterial infections.
Tubulointerstitial Diseases	May be idiopathic but consider drug-induced causes; non-speci c urinary ndings, absence of hypertension.

mortality rate of 50% at one year. More recent data from the U.S. Renal Data Service suggests that mortality at one year has declined to 30% (32).

Pathogenesis

Our understanding of the pathogenesis of HIVAN has advanced considerably over the past decade. Transgenic mice expressing a gag/pol deletion mutant of the cloned HIV-1 provirus pNL4Đ3 manifest a renal disease that is indistinguishable from that observed in HIVAN in man. Three lines of transgenic mice with different sites of integration all manifested HIVAN, suggesting a direct role for HIV in the pathogenesis of this disease (33Đ35).

Kidney disease develops when kidneys from transgenic mice are transplanted into normal littermates, whereas normal kidneys transplanted into a transgenic host do not develop the disease demonstrating that in the murine model, expression in kidney is required for HIVAN to develop (36). Renal tubule and glomerular epithelial cells in the murine model as well as in humans with HIVAN express histochemical markers characteristic of an immature phenotype (37,38) along with markers for proliferation and apoptosis (36,39). These data identify the podocytes and renal tubule epithelium as the major targets for HIVAN pathogenesis.

Similar to the murine model, renal epithelial cells appear to be the primary site of pathogenesis and are the cells infected by HIV in man. Proviral DNA has been detected in microdissected glomeruli (40). *In situ* hybridization studies have detected viral mRNA in podocytes, tubule epithelial cells and inÞltrating mononuclear cells (36) (Fig. 24.2). These data provide evidence in man that HIVAN is associated with HIV infection of tubule and glomerular epithelial cells. The mechanisms of viral entry remain unclear. Tubule cells can be infected *in vitro* using co-culture techniques and they have been shown to express low levels of CD4 and chemokine receptors (41). By in

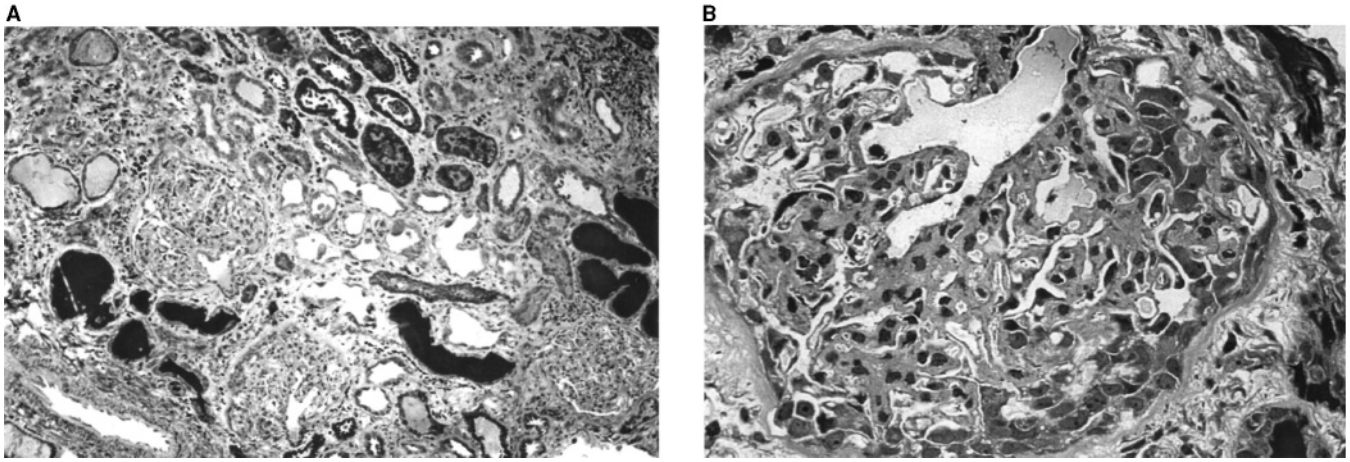

FIG. 24.1. Kidney biopsy of a patient with HIVAN. Panel A and B are low and high power views, respectively. Panel A shows one of three glomeruli with collapsing sclerosis and marked podocyte hyperplasia. The tubules are separated by edema, mild brosis, and patchy interstitial in ammatory in ltrates. Many proximal tubules show degenerative changes and there are focal tubular microcysts containing large casts (Trichrome stain, × 125). Panel B shows a glomerulus with segmental collapse of the glomerular tuft and hyperplasia of the overlying podocytes (Trichrome stain, × 400).

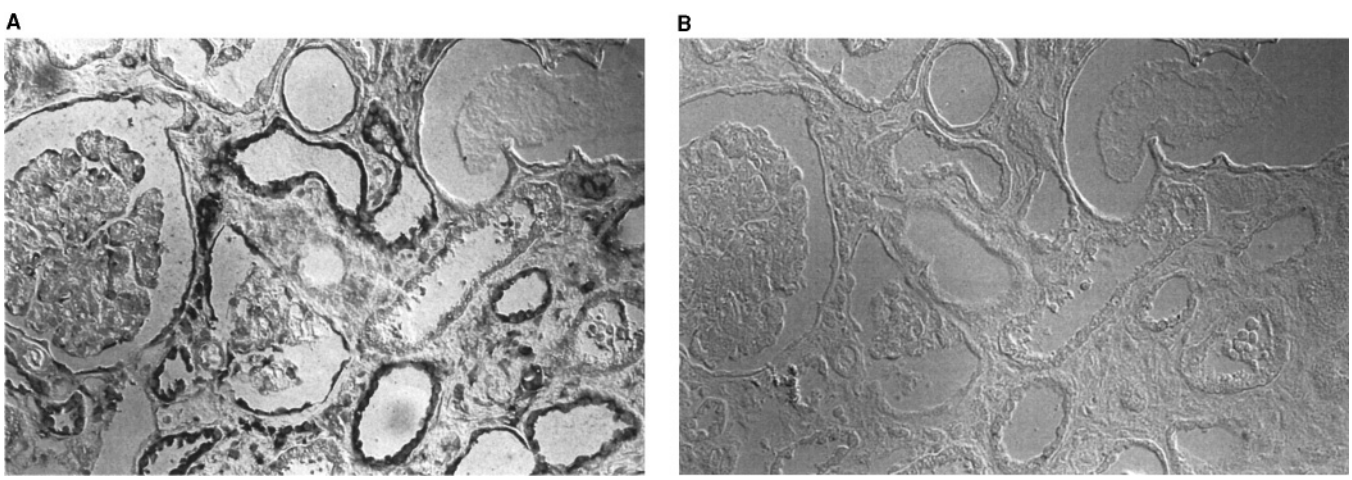

FIG. 24.2. IV RNA *in situ* hybridization of kidney biopsy of a patient with HIVAN. Utilizing an antisense probe generated from the *gag* region, panel A demonstrate the presence of HIV-1 RNA in tubular cells and podocytes as well as in interstitial in ammatory cells. A control sense probe showed no staining of the pretreatment biopsy (Panel B) (Panels A and B, × 50).

situ hybridization techniques, infection of tubules can be shown to be a rare event but once a tubule is infected, most of the cells in the tubule are positive for HIV mRNA (42). Thus, the infection rate of renal cells appears low but once infected, HIV-1 efÞciently moves from cell to cell down the nephron.

Increasing anecdotal evidence supports HAART as the appropriate therapeutic strategy for HIVAN. A recent study (25) presented a patient who developed HIVAN during an acute retroviral syndrome. Prior to initiation of HAART, the patient had nephrotic-range proteinuria and a reduced GFR. HAART reduced plasma viral load to non-detectable levels, and normalized the GFR and urinary protein excretion rate. Kidney biopsies before and after 3 months of therapy demonstrated resolution of the collapsing GN and improved renal function. Viral mRNA in kidney epithelium, however, was detectable both before and after therapy. These observations suggest that while there are clinical and morphological responses to HAART, the kidney is a persistent reservoir for HIV-1 (25).

Therapy

Three classes of drugs have been used to treat HIVAN; steroids, angiotensin converting enzyme inhibitors and antiretrovirals. Most therapeutic reports were in the era prior to HAART when mortality in AIDS was extremely high. Furthermore, the observational nature of the studies and lack of appropriate control groups raises concerns about the ability to extrapolate from the results.

Steroids

Several case reports and small series have described beneficial effect of prednisone for HIVAN (43–46). The largest trial tested the effects of prednisone in 19 patients (47) with HIVAN and found a brief improvement in GFR and/or urinary protein excretion. At the end of the first year, however, only two patients had survived without progressing to end stage renal disease. Another study described an experience using prednisone, 60 mg for one month followed by an eight week taper. Most patients were not treated with HAART (48), and were selected according to individual physicianÕs standards. Kidney function stabilized or improved in steroid-treated patients compared with the untreated group. In contrast to the previous study, treated patients in this study did not experience a greater risk of opportunistic infections, however, this risk may outweigh the benefits of steroids in the absence of HAART.

Angiotensin-Converting Enzyme (ACE) Inhibitors

Ace inhibitors have demonstrated efficacy in preventing progressive kidney failure in other diseases and have been studied in HIVAN (49,50). Their mechanism(s) of action, although still unclear, are ascribed to reducing hydrostatic pressure in the glomerular capillary, altering glomerular basement membrane permeability, or reducing the renal generation of cytokines, such as TGFβ. Similar to the experience with steroids, however, studies of ACE inhibitors were performed prior to the widespread use of antiretrovirals and lacked randomized controls.

Antiretrovirals

Several studies suggest an association between antiviral therapy and a slower progression to ESRD (49,51,52), even prior to the introduction of HAART. In the HAART era, patients with kidney disease have experienced improved survival, as have most patients with AIDS, but there have been no prospective randomized trials to determine the efficacy of HAART for HIVAN. By looking at the end-stage renal disease population as a group, however, some conclusions can be inferred. Since the introduction of HAART, the number of cases of ESRD due to HIVAN has stabilized despite an increase in the pool of patients living with AIDS (26). This indicates that the incidence of HIVAN has fallen, or that the rate of progression to kidney failure has decreased. Retrospective reports indicate that the use of protease inhibitors is associated with a slower progression of HIVAN (53). Two reports demonstrate improvement in kidney structure and function in individual patients with HIVAN treated early in the course of their kidney disease with HAART (25,54). These cases are important because the dramatic effect of treatment was observed when therapy was begun in drug naïve patients almost immediately after the onset of nephropathy. In most clinical situations, HIVAN is diagnosed later in the clinical course at a time when therapy may be less effective. It remains to be tested whether early detection of kidney disease, and initiation of aggressive antiviral therapy along with addition of agents such as ACE inhibitors can further improve overall renal survival.

Other Causes of Chronic Renal Insufficiency

Membranoproliferative Glomerulonephritis

Membranoproliferative glomerulonephritis is characterized by thickening of the glomerular basement membrane, proliferation of mesangial cells, and an influx of mononuclear inflammatory cells. This disease is often the result of immune-complexes and is seen with hepatitis C (HCV) and hepatitis B (HBV) infection (55). Immune complex disease associated with HCV co-infection is the most important cause of MPGN in the setting of HIV-1 infection (56). The presence of cryoglobulins in MPGN is almost exclusively associated with HCV infection. The prevalence of proteinuria has recently been estimated as high 30% in HIV-1 infection (57), and a positive test for hepatitis C antibody increases the risk of kidney disease. It remains to be determined whether the proteinuria is specific for HCV co-infection or whether these are early cases of HIVAN.

The presentation of MPGN as a complication of HCV infection includes nephrotic-range proteinuria, gross or microscopic hematuria, hypocomplementemia and cryoglobulinemia. Renal biopsy findings demonstrate an immune complex glomerulonephritis. Like HIVAN, hepatitis C-associated renal diseases in the setting of HIV progresses rapidly to ESRD, especially in injection drug users. Although the presence of hypertension and cryoglobulins may suggest MPGN rather than HIVAN, a biopsy is often required to distinguish the two entities (56).

Treatment for HCV-induced glomerulonephritis has not been systematically studied in the presence or absence of HIV infection. Prior to identification of HCV, many of these cases were classified as mixed-essential cryoglobulinemia and treated with steroids and plasmapheresis. Antiviral therapy with interferon alpha and ribavirin are considered standards of care for hepatitis C-infected patients, but the role of combination therapy in immune-complex nephritis remains to be established.

Miscellaneous

Several cases of IgA nephropathy associated with immune complexes containing HIV proteins and glomerulonephritis have been described (58,59). Appropriate therapy for this form of immune complex disease remains

uncertain. Both membranous nephropathy and polyarteritis nodosa are known but rare complications of hepatitis C and B infection. Biopsy surveys of HIV-infected patients with chronic renal insufficiency have also revealed findings consistent with diabetes, amyloidosis as well as a lupus-like nephritis. Steroids and alkylating agents are often used to treat lupus nephritis, and reports suggest that this therapy can be reasonably well tolerated in selected HIV seropositive patients (60,61).

Clinical Approach

The clinical approach to patients with chronic renal insufficiency due to HIV infection should not be different from patients who are seronegative. The physician should attempt to make a precise diagnosis by standard tests and by renal biopsy when necessary. The presence of proteinuria and hematuria, particularly with red blood cell casts in the urinary sediment, indicates glomerulonephritis. Serologic tests for circulating immune complexes, complement levels and cryoglobulins should be performed as should hepatitis B and C serologies. While heavy proteinuria in patients who are black or Hispanic may suggest the diagnosis of HIVAN, a biopsy is still required to distinguish the diagnosis of HIVAN from other causes of glomerular disease.

Complications as a result of kidney biopsies are rare (62). Transient microscopic hematuria, with or without computerized tomographic evidence of a hematoma, occurs in the majority of patients although this is rarely associated with clinical morbidity. Transient gross hematuria occurs in 3–10% of cases, requiring transfusions in 0.1–0.3%. Surgery or angiography with embolization of the bleeding vessel has been required in 0.1–0.4% of cases. The latter procedure avoids nephrectomy, estimated to occur in 0.06% of patients.

There are special treatment considerations for patients with chronic renal insufficiency in general. Lipid disorders, secondary hyperparathyroidism and hypertension should be treated early and aggressively. If GFR falls, the therapeutic focus should include preparation for renal replacement therapy. Proper planning for hemodialysis, peritoneal dialysis or kidney transplantation should be made well in advance of uremic symptoms. In those patients who are likely to start hemodialysis, an AV fistula should be created months before an anticipated start date.

In light of the therapeutic success of antiretroviral therapy for the underlying disease, kidney transplantation is an option in selected patients with safety and efficacy currently under study (63). The criteria for considering renal transplantation should include evidence of well controlled HIV infection with sustained undetectable plasma HIV RNA and CD4 cells >200/ul. The long-term prognosis of patients with HIV infection on dialysis is determined by the stage of AIDS (30,64), and these patients should be aggressively treated with HAART. Survival on dialysis is improving and should continue to do so with newer antiviral drug therapies (65,66).

Although data for clearance of some of the antiretrovirals in the setting of renal insufficiency or hemodialysis is incomplete, every attempt should be made to dose for maximal antiviral effects and minimal toxicity. As a class, the nucleoside analogue reverse transcriptase inhibitors (NRTIs) are cleared by the kidney and require dose adjustments to avoid toxicity, while the protease inhibitors are metabolized by the liver and do not generally require dose adjustment. The non-nucleoside reverse transcriptase inhibitors (NNRTIs) are primarily metabolized by the liver as well and therefore dose adjustments are not recommended. In the setting of hemodialysis, NRTIs and NNRTI dosing is recommended after dialysis (67–69).

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Chapter 25

Skin Manifestations of HIV Infection

Miguel Sanchez and Alvin E. Friedman-Kien

The impact of skin disease on the lives of HIV-infected men and women cannot be measured by either financial costs or mortality rates. Early in the AIDS epidemic, HIV-infected men identified Kaposi's sarcoma, as the most dreaded manifestation of their disease, a visible stigma that elicited social prejudice and produced disfigurement unconcealable by even the most opaque available camouflage cosmetics. Even today, despite advances in treatment, the mere suspicion of the "gay cancer" provokes dread in patients who develop the typical violaceous skin lesions. Cutaneous diseases may herald deterioration of immunity, cause intolerable symptoms, heighten isolation, and promote depression. Not only can a skin disease be the initial presentation of the underlying HIV infection, but it may remain a major health problem during the patient's life (1). Disseminated opportunistic infections may present with skin lesions, which permit more expeditious diagnosis of these life-threatening illnesses (2).

With advancing immunosuppression, the prevalence of mucocutaneous diseases requiring treatment becomes nearly universal (3). In a review of medical records of 684 HIV-infected patients insured by the Harvard Community Health Plan, 79% of 540 patients had one or more dermatologic diagnoses, for a total of 2,281 diagnoses, including 188 cutaneous reactions to drugs and 43 hospitalizations for cellulitis (4).

A prospective study of 912 HIV-infected patients followed for 42 months confirmed previous reports that xerosis and seborrheic dermatitis are the most common skin findings. These manifestations as well as dermatophytic infections, common warts and condyloma accuminata are also seen early, although the cases are usually not as severe as in later more advanced HIV disease (5). The prevalences of acne vulgaris and eosinophilic folliculitis peak in mid-stage disease (5). On the other hand, chronic ulcerative herpes simplex infection, herpes zoster, oral candidiasis, molluscum contagiosum, and oral hairy leukoplakia are associated with late-stage disease when immunity is profoundly impaired (5). As patients become increasingly immunocompromised, these diseases tend to become more aggressive, appear more atypical, and respond less readily to treatment.

Widespread use of highly active antiretroviral therapy has not only effectively reduced morbidity and motality rates among HIV-infected persons but has also altered the development and course of many skin diseases (6,7). On the other hand, new dermatologic diseases, some of which are disfiguring and even potentially lethal, continue to be described (7).

Primary HIV Infection

Between 40% to 89% of newly infected cases develop a symptomatic acute retroviral syndrome as early as five days and up to six weeks after HIV contact. The syndrome is associated with high titer HIV replication in blood coupled with a vigorous immunologic response to the virus, and resolves as the plasma level of HIV RNA declines (8). Although initially the acute syndrome was described as a mononucleosis-like illness associated with fever, pharyngitis, and cervical adenopathy, larger studies indicate that this presentation occurs in only 15.6% of patients (9). Symptoms may persist less than 14 days but in a study of 218 cases, the mean duration of acute primary infection was 25.1 days (median, 20.0 days) (9). Rarely, patients may continue to experience symptoms for up to 10 weeks. There appear to be no differences in the duration or type of symptoms according to gender, age, and risk factors (9). The more common symptoms are fever (77–96%), fatigue (70–92%), weight loss (70%), adenopathy (40–70%), pharyngitis (50–70%), fatigue

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(65%), skin eruption (40–80%), myalgia (50–70%), headache (32–70%), nausea (30–60%), nightsweats (40–50%), and aseptic meningitis-like syndrome (9–24%) (8–9). Arthralgias, photophobia, nausea, and abdominal cramps are also common. Mucocutaneous ulcerations can involve the buccal mucosa, gingiva or palate (10–20%), genitals (5–15%), and esophagus or anus (10). Oral thrush was reported in 12% of patients from an early, small study and was associated with early development of opportunistic infections (10).

A skin eruption, usually involving the trunk, occurs in approximately half of persons with symptomatic acute HIV-1 infection. Although often described as morbilliform or maculopapular, the lesions are often discrete, faint, erythematous, 5 to 10 mm, oval macules papules on the chest and upper back, and sometimes the palms and soles (11) (Fig. 25.1). In some cases, papules, vesicles or papulopustules are the predominant lesions and the eruption has been reported to resemble roseola, syphilis, rubella, typhus, pityriasis rosea, drug hypersensitivity, and acne. In persons with darkly pigmented skin, the lesions are more difficult to detect. Histopathological examination shows a mononuclear-cell infiltrate of the superficial dermal vessels, consisting mainly of CD_4 T-lymphocytes, and focal lymphocytic vasculitis (8).

The diagnosis of acute HIV infection is confirmed by detection of serum HIV antigenemia (HIV-RNA) in the absence of HIV antibodies. The course of HIV infection correlates with the HIV-RNA level, which usually stabilizes approximately six months after exposure to the virus. High viral loads ($>10{,}000$ copies) correlate with higher risk for accelerated immune deterioration. Low viral loads (less than 200 copies) are associated with disease stability and a favorable course, and intermediate titers (between 200 and 10,000 copies) with gradual but progressive disease.

A similar syndrome associated with a dramatic rebound in plasma HIV-RNA (the Retroviral Rebound Syndrome) has been reported in patients who have ceased suppressive antiretroviral therapy (12). One case developed an erythematous, generalized, macular eruption, which became papular and pruritic. Neither pharyngitis nor lymphadenopathy was present (12).

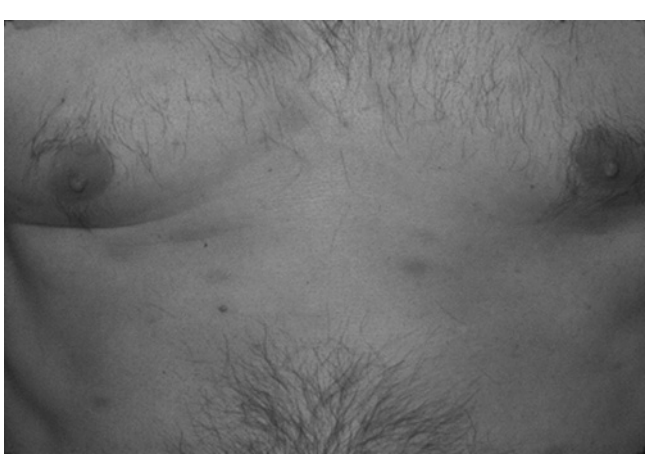

FIG. 25.1. Faintly erythematous, oval, non-scaly patches on the chest of a man with primary HIV infection eruption.

NONINFECTIOUS DISEASES

Seborrheic Dermatitis

In contrast to the 2% to 5% incidence in the general population, seborrheic dermatitis has been detected in 21%, 32% and 46% of HIV-infected persons from various studies, and as high as 83% of severely immunocompromised patients with AIDS (3,13a–13e). This condition is more common and becomes more severe when the CD_4 T-cell count drops below 200 cells/mm^3 and improves or resolves with effective treatment of HIV infection. Patients commonly complain of "dry skin" on the face. The eruption consists of discrete or diffuse, pink patches or plaques. White to yellow oily-looking scales and occasionally crusting may be present on the surface of the lesions (Fig. 25.2). The eruption develops in body regions rich in sebaceous follicles, such as the scalp, eyebrows, nasolabial folds, retroauricular folds, external ears, beard area, and less commonly in the axillae, groin and V-shaped areas of the chest (13). Occasionally, seborrhea is pruritic, and the plaques may become lichenified from rubbing. The severity ranges from scalp flaking to exfoliative erythroderma. Seborrheic dermatitis may be so severe and extensive that it resembles and is sometimes clinically

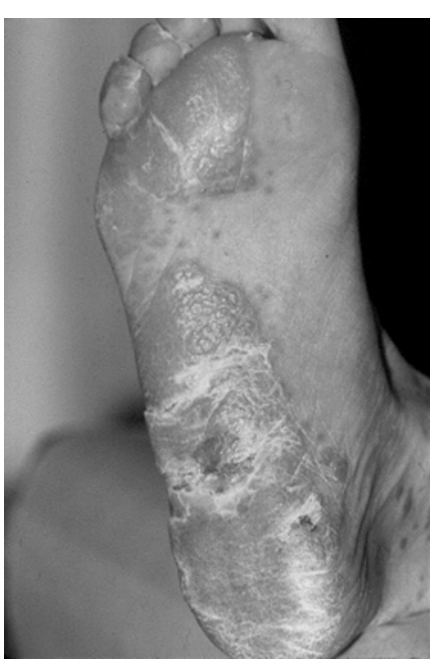

FIG. 25.2. In seborrheic dermatitis, pink-red patches and papules with yellow-white scales are typically present along the nasolabial fold, eyebrows, mustache, beard and forehead.

confused with psoriasis. The pathogenesis remains unelucidated, although an overgrowth of the yeast, *Pityrosporum ovale*, or an exaggerated inßammatory response with cytokine activation in affected areas has been suggested (13f). One study correlated heavy skin colonization with *P. ovale* to speciÞc depressed cellular immunity. In these patients, after exposure to *P. ovale* antigen, peripheral blood mononuclear cells did not proliferate and also produced less interleukin-2 and interferon-gamma but more interleukin-10 (14). One report concluded that the pathogenesis did not depend on the density of *P. ovale* on the skin but rather on the presence of a speciÞc subtype, which was found in 78% of AIDS patients with seborrheic dermatitis (15).

Scalp disease is treated with shampoos containing selenium sulÞde, ketoconazole, zinc pyrithione, sulfur, salicylic acid, or coal tar and also with intermittent courses of topical corticosteroids. Solutions, gels, oils and foams are better-accepted vehicles on densely hairy areas. Some cases respond to calcipotriene lotion. In other body areas, corticosteroid creams or lotions (low-potency on the face and intertriginous areas and mid-potency on the rest of the body) are highly effective. Occasionally, mid-potency corticosteroids are also needed on the face but prolonged use of these agents leads to facial atrophy, acne, and telangiectasias. Pimecrolimus cream and tacrolimus ointment are usually effective without propensity to cutaneous atrophy. Topical imidazole creams, such as ketoconazole, produce resolution of the eruption in approximately 25% of cases but may be used prophylactically. There are reports of marked improvement following treatment with systemic anti-yeast agents, such as ßuconazole, but seborrheic dermatitis can appear even when the patient is being treated with antifungals. Recalcitrant cases often respond to ultraviolet B phototherapy.

Psoriasis

The incidence of psoriasis in HIV-infected persons is between 1% and 2% (16), which is similar to the general population, although prevalences as high as 5% have been reported (17). The disease may develop at any point during the course of HIV infection, but with advancing immunodeÞciency, the lesions become different, more extensive and more therapeutically unresponsive. In approximately one-third of patients, the development of psoriasis precedes HIV-infection but the appearance and extent of the eruption often changes following the marked decline in a patientÕs immune function. In contrast to the well-demarcated, erythematous plaques covered with micaceous, adherent silvery scaling characteristic of classic psoriasis, the lesions seen in HIV-infected individuals tend to be ßatter, larger, more irregular, more widespread, potentially pruritic and are covered with Þner scale and occasionally crusting (Fig. 25.3) (18). Often, the eruption is polymorphous with plaques, guttate papules and pustules simultaneously present in any affected area. Instead of the typical elbows and knees distribution, the face, scalp, intertriginous areas, genitals, palms and soles are often involved (19). In fact, although not pathognomonic, the appearance of a severe form of ÒinverseÓ psoriasis in individuals at risk for HIV infection should prompt consideration of HIV testing. Nail changes, such as onycholysis, nail pitting and subungual hyperkeratosis, are common. The eruption can progress rapidly to generalized exfoliative erythroderma. In fact, psoriasis is the most common cause of erythroderma in HIV-infected persons, although in these cases, histopathological evaluation is necessary to exclude cutaneous T-cell lymphoma, drug eruption, contact dermatitis, atopic dermatitis, or exuberant seborrheic dermatitis. Psoriatic arthritis, present in only 1% of HIV-seronegative cases of psoriasis, has been reported in up to 10% of HIV-infected psoriatics.

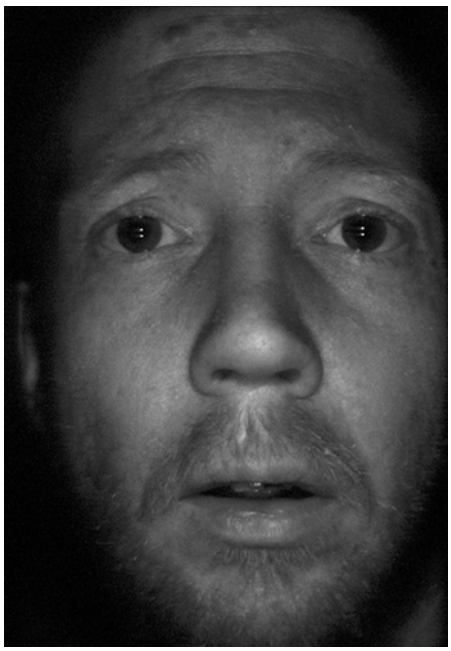

FIG. 25.3. Numerous small erythematous, thin plaques with adherent silvery scale and crusting con uent into a larger geographic plaque on the abdomen of a man with HIV-associated psoriasis.

In contrast to the 0.06% incidence of ReitersÕsyndrome in the general population, this disease has been reported in as many as 6Ð10% of HIV-infected patients, depending on the adherence to the diagnostic criteria (20). In some studies that found high prevalences, several patients were missing the classic triad of arthritis (usually sacroileitis), urethritis, and conjunctivitis. Instead, these patients had incomplete syndromes with arthritis, skin manifestations (pustular psoriasis, balanitis circinata, keratoderma blenorrhagicum, dactilytis, nail dystrophy) and occasionally other noncutaneous associated signs (Fig. 25.4) (21). HLA-B27 has been detected in as many as 70% of HIV-infected patients with ReiterÕs syndrome (22).

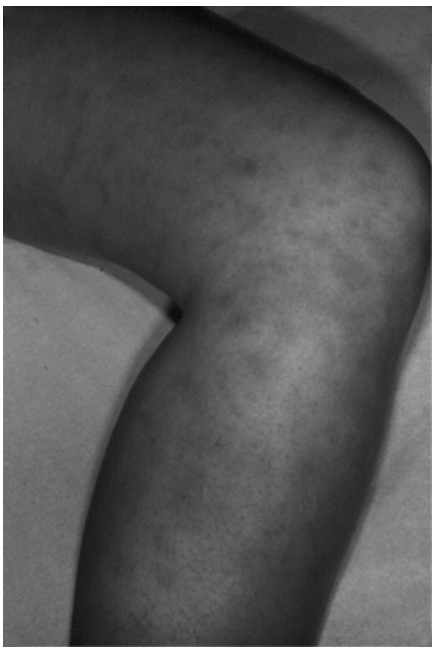

FIG. 25.4. Hyperkeratotic plaques within which crusted papules are present. The plaques are surrounded by an erythematous border with papules and pustules. This is the typical appearance of plantar keratoderma blenorrhagica associated with Reiter's syndrome.

Psoriasis in immunocompromised HIV-infected persons is usually more recalcitrant to traditional treatment regimens. Topical corticosteroids, calcipotriene, pancrolimus, and tazarotene may not sufficiently control the disease so that narrow band or conventional UVB phototherapy, Psoralen and UVA photochemotherapy (PUVA) or oral acitretin are often needed. Despite earlier concerns about safety, phototherapy has been found not to exert a deleterious effect on the course of HIV infection (23) or to increase viral loads significantly (24). Methotrexate and cyclosporine are reserved for severe cases unresponsive to other treatments. These systemic drugs are usually effective and temporarily well-tolerated without a high incidence of complications (17). Although some cases of Reiter's syndrome may respond to acitretin, cyclooxygenase inhibiting non-steroidal anti-inflammatory drugs or etanercept, methotrexate remains the treatment of choice for severe cases. The efficacy of antiretroviral agents in the treatment of HIV-associated psoriasis became evident years ago after high doses of zidovudine were demonstrated to improve psoriasis even in HIV-seronegative patients with, unfortunately, intolerable side effects (25). Although not studied, combinations of other antiretroviral agents appear to be effective either through a direct effect on psoriasis or by improving immunity and reducing HIV viral load. In fact, HIV-associated psoriasis has become less common in patients effectively treated with antiretroviral therapies (26).

Xeroderma

Even before the availability of antiretroviral protease inhibitors, xeroderma ranging from dry skin to severe ichthyosis developed in 23–30% of HIV-infected individuals, and in almost all end-stage, malnourished AIDS patients. Because xeroderma can develop in some patients in the absence of profound immunosuppression, it has been suggested that skin dryness can be caused by direct retroviral effects. Xerodermic skin is diffusely dry and flaky. Ichthyotic skin consists of adherent polygonal, fish-like scales. These changes are most apparent on the lower extremities. Acquired ichthyosis has been correlated with severe helper T-cell depletion, increasing age, and concomitant infection with HTLV-II (27). However, the etiology of such dry skin changes is likely to be multifactorial. Malnutrition, malabsorption, genetic predisposition, antilipidemic drugs, dry climate, and even some antiretroviral agents can cause dry skin. Among the protease inhibitors, indinavir, especially, seems to predispose to xeroderma (28). Some patients treated with protease inhibitors have also developed a desquamative cheilitis and paronychia xeroderma (28). While changes in skin lipids probably occur during the course and treatment of HIV infection, environmental factors and prolonged bathing increases xerosis. Nutritional evaluation may be indicated. Treatment consists of short-duration showers and avoidance of harsh cleansers as well as frequent applications of moisturizers containing alpha-hydroxy acids (lactic acid, glycolic acid), urea, or phospholipids.

Cutaneous Adverse Drug Reactions

Early in the AIDS epidemic, it was recognized that the prevalence of cutaneous eruptions caused by hypersensitivity to drugs was significantly higher in HIV-infected patients than in the general population (29). In one study 27% of 684 patients developed drug eruptions (30). The drugs with the highest rate of cutaneous allergic reactions (per 1,000 courses) included trimethoprim-sulfamethoxazole (22%), sulfadiazine (29%), trimethoprim-dapsone (23%), and aminopenicillins (14%) (30). The majority of drug eruptions are maculopapular or morbilliform.

Cutaneous hypersensitivity eruptions have been reported in 40% of patients on amoxicillin-clavulanic acid, 33% of patients treated for Toxoplasma encephalitis with intravenous clindamycin, 38% of patients treated for aphthous ulcers with thalidomide, and 10% of patients on antituberculous medications (30).

In some studies, 50% to 60% of patients on standard doses of trimethoprim-sulfamethoxazole, the most commonly prescribed prophylaxis and treatment of *Pneumocystis carinii* pneumonia (PCP), developed cutaneous hypersensitivity to the drug (31). The incidence of these reactions is increased in patients with less than

200 CD_4 T-lymphocytes/mm^3, but decreases in late-stage disease when the CD_4 T-lymphocyte counts drop below 25 cells/mm^3. Notably, Black patients in this country or abroad appear to have diminished predisposition to sulfa drug hypersensitivity (30). The eruption, which can be accompanied by fever, appears to involve the activation of mast cells by immunoglobulin E (IgE) but may also be caused by acetylation phenotype and accumulation of a toxic hydroxylamine sulfonamide metabolite which diminish stores of glutathione.

In most patients who develop drug allergy eruptions, widespread pink to bright red macules and papules appear on the trunk, extremities and occasionally the face and mucous membranes (Fig. 25.5). The drug eruption typically appears eight to twelve days after initiation of therapy and reaches its pinnacle of intensity one to two days later. Even with continuation of the drug, the reaction may resolve within three to Þve days. However, it may persist for weeks or require discontinuation of the drug due to severity. Although recurrence of the eruption may not follow rechallenge, readministration of the drug requires careful monitoring for potential anaphylaxis. Gradual dose escalation is also associated with signiÞcantly fewer adverse drug reactions (32). Antihistamines and oral corticosteroids can help to prevent the reaction. Desensitization is indicated when there are no alternatives to trimethoprim-sulfamethoxazole. Although several desensitization protocols have been published, the most extensively studied regimen was developed by Conant and colleagues (33,33a). It consists of increasing dilutions of an oral suspension containing 40 mg of trimethoprim and 200 mg of sulfamethoxazole in 5 mL. A 1:100,0000 dilution is administered on day one followed by dilutions of 1:100,000 (day 2), 1:10,000 (day 3), 1:1,000 (day 4) 1:100 (day 5), 1:10 (day 6), 1:1 (day 7), 1 cc of standard suspension (day 8) and 1 DS tablet (day 9 and thereafter) (33). The regimen has been reported to be successful in up to 83% of studied patients with no fatalities.

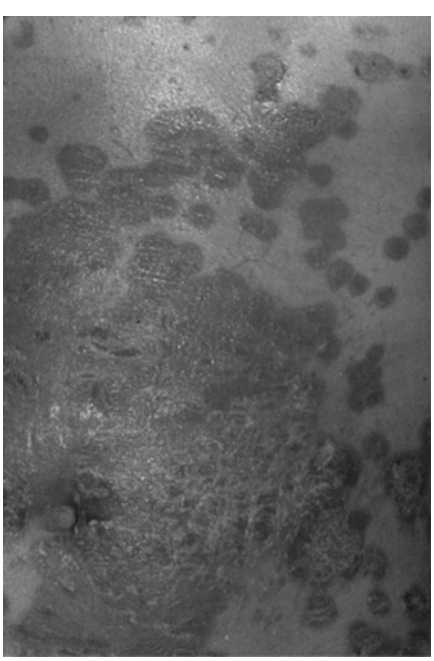

FIG. 25.5. Erythematous, blanching macules and patches with a few papules producing an exanthematous eruption due to hypersensitivity to trimethroprim-sulfamethoxazole.

Other treatments for PCP also have high rates of cutaneous drug reaction. Approximately 20% of patients treated with parenteral pentamidine develop morbilliform or urticarial eruptions (31). Dapsone can also cause morbilliform and scarlatiniform eruptions, as well as the potentially fatal sulfone syndrome which includes exfoliative dermatitis, fever, hepatic necrosis, lymphadenopathy and hemolytic anemia (30).

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe reactions characterized by acute vesicles, bullae and epidermal detachment on the skin and mucous membranes due to necrosis of the epidermis (Fig. 25.6). In SJS, the total extent of skin detachment is less than 10%, whereas in TEN epidermal detachment is found over more than 30% of the body surface area (34). The incidence of toxic epidermal necrolysis in HIV infection is approximately 1.08 per one thousand patients per year, 500 times the rate in the general population. Previously, most cases were caused by sulfonamides in Western countries and thiacetazone in Africa, although other medications, including aminopenicillins, cephalosporins, clindamycin, quinolones, barbiturate, phenytoin and imidazole antifungals have been implicated. However, in a recent review, 83% of SJS/TEN reactions were caused by nevirapine (29). The reaction began 10Ð240 days (median 12 days) after the introduction of nevirapine even though most patients were still taking the recommended initial daily dose of 200 mg (34).

Although with currently prescribed doses, the prevalence of drug eruptions is lower, 1% of patients treated

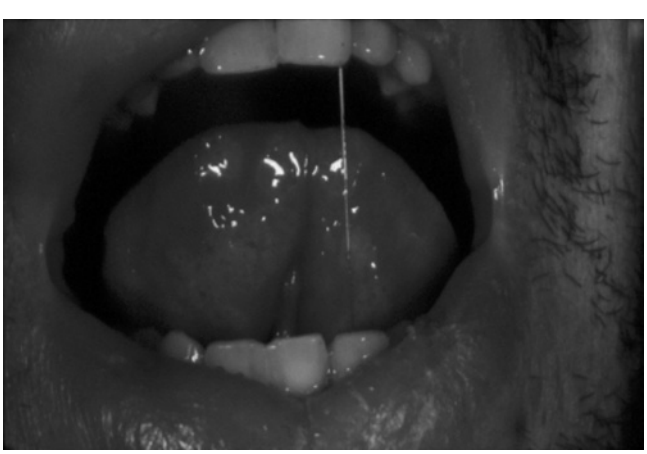

FIG. 25.6. Diffuse erythema and edema of the lips and tongue in a man with Stevens-Johnson syndrome. Well demarcated circular and irregular erosions are present on the undersurface of the tongue.

with zidovudine at high doses require discontinuation of the drug due to severe exanthematous eruptions. Lichenoid eruptions with planar polygonal pink papules on the body and whitish reticulated plaques in the oral mucosa resembling lichen planus, as well as acral and periarticular reticulate erythema that simulates dermatomyositis have also been reported. Zidovudine can cause hyperpigmentation of the nails, skin and mucous membranes. The most common manifestation is the development of pigmented striated nail bands (34a).

Foscarnet has induced painful 1 to 5 cm ulcerations of the urethral meatus, glans penis and scrotum in some individuals receiving high doses due to irritation from the concentrated urine. These ulcers resolve with discontinuation of treatment or dilution of concentrated urine through increased hydration. Foscarnet may also produce a generalized eruption. Rifampin may cause a drug rash that can rapidly progress to erythroderma.

Relatively few serious drug reactions occur from administration of protease inhibitors. However, indinavir has been associated with a number of mucocutaneous manifestations, including in one report, cheilitis (57.1%), diffuse cutaneous dryness and pruritus (40%), asteatotic dermatitis (11.9%), scalp deßuvium (11.9%), fairer, thinner, shedding body hair (7%), digital pyogenic granulomas (5.9%) and severe alopecia (1.2%) (35). Recurrent paronychia, usually of the Þrst toes, and Þssures of the lips have also been reported (36). Although the prevalences may be lower than in this study, all of these effects are, indeed, encountered in clinical practice with the use of indinavir and to a lesser extent other protease inhibitors. Fixed drug eruptions to saquinavir are uncommon.

A case of acute generalized exanthematous pustulosis (AGEP) induced by combination therapy with zidovudine, lamivudine, and a protease inhibitor in the setting of HIV prophylactic treatment in an HIV-seronegative man was reported (37). AGEP is characterized by an acute maculopapular eruption accompanied by skin edema and nonfollicular sterile pustules, and eventual widespread epidermal exfoliation. Fever is invariably higher than 38 degrees C. Purpuric lesions may be present. Most cases are induced by drugs, particularly by beta-lactams and other antibiotics. Cutaneous patch tests are useful when the patient is being treated with multiple medications, and implicate a particular drug in approximately half of the cases.

Some non-nucleoside reverse transcriptase inhibitors have a high incidence of cutaneous reactions. A skin eruption is the most common adverse effect of nevirapine and delavirdine (Fig. 25.7); the incidence is similar with both of these compounds, but lower with efavirenz.

A syndrome consisting of Òdrug rash with eosinophilia and systemic symptomsÓ (DRESS), also called hypersensitive syndrome (HSS), has been associated with the use of navirapine and less often with carbamazepine, sulfonamides, calcium channel blockers, allopurinol, ranitidine, thalidomide, and zalcitabine (38). The onset is usually between two to six weeks after receiving the Þrst dose. The Þrst signs are fever and a maculopapular eruption followed by lymphadenopathy, arthritis, myalgias, visceral manifestations (potentially fulminant hepatitis), hyperleukocytosis, and eosinophilia. The eruption responds well to treatment with topical corticosteroids, but systemic corticosteroids are recommended for internal organ involvement (38).

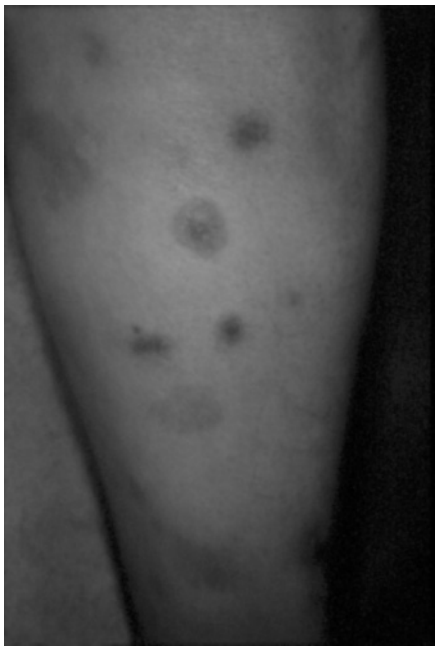

FIG. 25.7. Targetoid erythematous plaques with bullous and necrotic centers caused by hypersensitivity reaction to nevirapine.

Several skin toxic reactions have been reported during administration of liposomal encapsulated doxorubicin for the treatment of AIDS-related KaposiÕs sarcoma. Palmar-plantar erythrodysesthesia syndrome, also known as toxic acral erythema, develops in as many as a third of patients treated with high doses and short schedules. Painful erythema of the palms, soles, and Þngers progresses to diffuse violaceous erythema and edema with or without formation of vesicules or bullae, and eventually to desquamation. Other cutaneous complications include stomatitis, an intertriginous dermatitis, and a scaly erythematous accentuation of the hair follicles (39).

Fat Redistribution Syndrome

In recent years, shortly after the introduction of antiretroviral therapy with protease inhibitors the development of lipoatrophy, lipohypertrophy, or both in localized areas, frequently in association with dyslipidemia and disturbances in glucose homeostasis, was recognized. The largest prevalence surveys found that lipodystrophy was present in 49Ð53% of patients, almost exclusively in patients receiving antiretroviral therapy (40). The severity

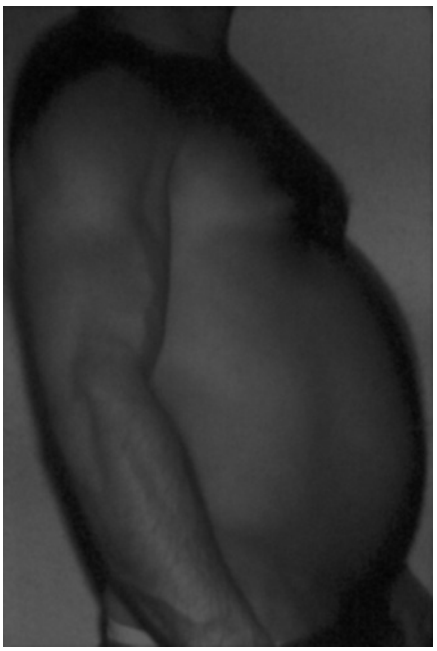

FIG. 25.8. Abnormal lipodystrophic deposition of adipose tissue on the abdomen, cervical area and breasts accompanied by thinning skin over upper extremities resulting in prominence of veins and muscles.

and prevalence of lipodystrophy is higher in patients treated with protease inhibitors, suggesting that these agents accelerate changes of fat redistribution. However, lipodystrophy has also been observed in patients treated with regimens without protease inhibitors, and in a few cases receiving no antiretroviral therapy at all. Notably non-nucleoside reverse transcriptase inhibitors do not appear to promote the development of lipodystrophy. Potential contributing factors include prolonged antiretroviral therapy, long duration of HIV infection, high viral load, genetic predisposition, and increasing age.

Accumulation of fat in the abdomen resulting in increased abdominal girth is the most common manifestation and occurs in 76% of affected men and in 93% of affected women (Fig. 25.8). Women with body changes may be more likely to experience increases in breast size and overall weight gain than men (41). Accumulation of fat also causes enlargement of the dorsocervical fat pad (buffalo hump) and lipomas. In 69% of women, increased fat in breast tissue is present and gynecomastia is common in men. Fat depletion in the arms and legs resulting in prominent cutaneous veins is observed in 67% of men and 59% of women (40). Other changes associated with subcutaneous fat depletion result in sunken facial cheeks and loss of shape in the buttocks (Fig. 25.9).

Dyslipidemia becomes more prevalent with advancing lipodystrophic changes, and includes increased serum levels of triglycerides, total cholesterol and low density lipoprotein (LDL) cholesterol, accompanied by decreased levels of high density lipoprotein (HDL) cholesterol (42). Between 15% and 30% of HIV-infected patients have dyslipidemia, about 1% of patients treated with protease inhibitors develop insulin resistant diabetes, and 47% have impaired glucose tolerance (40). Because of the disÞguring changes, patients may be reluctant to initiate or continue antiretroviral therapy.

Treatment with 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors (atorvastatin, cerivastatin, ßuvastatin, lovastatin, pravastatin, and simvastatin) reduce both triglycerides and cholesterol (40). Fibrates (cloÞbrate, fenoÞbrate, gemÞbrozil) are helpful in reducing triglycerides alone. Metformin reduces serum triglycerides and may diminish deposition of fat. Rosiglitazone and pioglitazone increase insulin sensitivity by increasing glucose transport and adipogenesis. Diets consisting of moderate amounts of fat and substitution of omega-3 and omega-6 fatty acids for saturated fatty acids may reduce lipogenesis and decrease insulin resistance. Exercise programs consisting of resistance training with an aerobic component can reduce abdominal adiposity.

Patients who are switched from HIV protease inhibitor therapy to antiretroviral combinations that include NNRTI agents may have improvement in their lipid proÞles and less intra-abdominal fat (43). Testosterone supplementation may improve abdominal obesity in men. Growth hormone reduces fat buildup around the waist, breasts, and back of the neck. Although it has no effect on wasting, growth hormone may make the loss of fat less noticeable in the legs and arms by promoting muscle growth. Growth hormone improves lipid proÞles but also increases fasting glucose and insulin levels (43). Breast reduction effectively corrects breast enlargement. Liposuction effectively and permanently removes fat cells and has provided

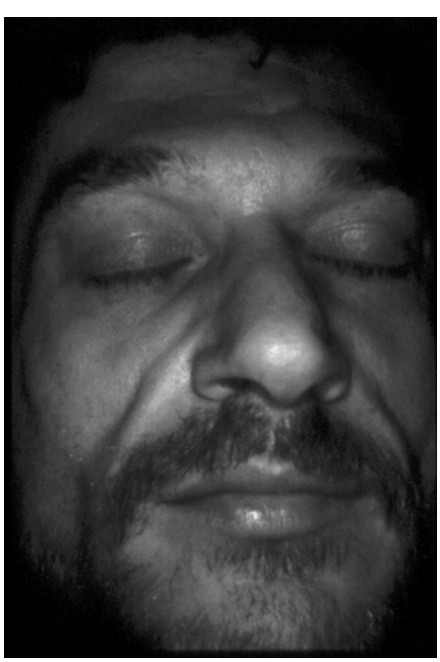

FIG. 25.9. Loss of facial fat resulting in sunken cheeks and depression of nasolabial folds.

cosmetic correction of dorsocervical fat pads. Subcutaneous fat can be suctioned from the abdomen resulting in a more tapered waistline although the presence of peritoneal fat may maintain a protuberant abdomen. Fat implants can temporarily restore a normal appearance to the face and hands. Injections of human collagen (Fascian), hyaluronic acid (Restylane, Perlane), and silicon (Silikon) improve the appearance of lipoatrophic facial changes. Implantation of devices made of expanded polytetraßuoroethylene (Gortex) can improve the appearance of the nasolabial fold furrows. In preliminary studies a polymer of polyactic acid (New-Fill) injected into the deep layers of the skin, where it produces collagen formation, has been shown to improve and correct the subcutaneous depressions in fat depleted regions of the face (43a).

Photosensitivity Disorders

A number of ultraviolet light-induced pathologic changes in the skin of individuals with HIV infection have been reported. Photosensitivity from medications, such as sulfonamides and nonsteroidal antiinßammatory agents, may lead to erythema even after brief exposure to sunlight (44). Chronic actinic dermatitis, a photodistributed, pruritic, eczematous dermatitis with licheniÞed, hyperpigmented plaques on the face, hands, neck, V-area of the chest and arms, has been described in several patients (Fig. 25.10) (45). Hypopigmentaion and hyperpigmentaion secondary to excoriations further contributes to the disÞguring appearance. The reaction persists for months to years. Although affected patients have CD_4 T-cell counts below 200 cells/mm^3, in several cases photosensitivity was the presenting disorder leading to the diagnosis of HIV infection (5). HIV-associated chronic actinic dermatitis predominantly affects patients with very dark skin (type VI). All studied patients have had heightened sensitivity to ultraviolet B light, most to ultraviolet A light, and a rare few to visible light. Treatment consists of avoidance of sunlight and photoallergens, strict sun protection with broad-coverage sunblocks, application of topical corticosteroids to affected areas, oral antihistamines, antimalarials and for the most challenging cases thalidomide (46).

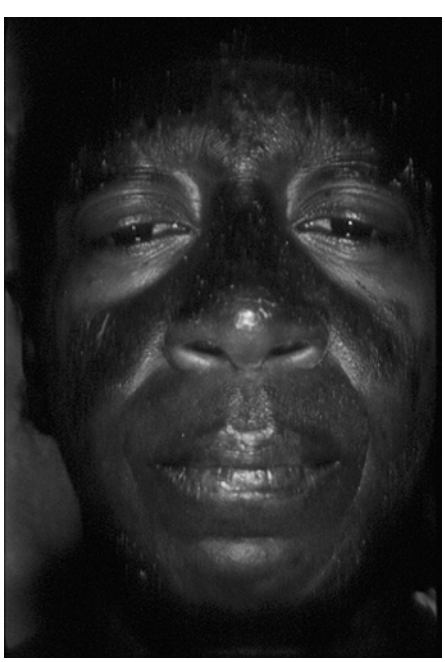

FIG. 25.10. Marked facial hyperpigmentation over sun-exposed areas due to chronic actinic dermatosis.

The occurrence of porphyria cutanea tarda (PCT) has been rarely reported in HIV-infected patients. As in immunocompetent HIV-seronegative patients, vesicles, crusted ulcers, milia, sclerotic skin, and dyspigmentation develops on sun-exposed areas, particularly the face and dorsa of the hands (47). Hypertrichosis and hyperpigmentaion are often present on the malar areas of the face and the dorsa of the hands. Most of the cases have been intravenous drug addicts who were also co-infected with hepatitis C (HCV) or hepatitis B (HBV). Patients may develop the disease at any stage of HIV-infection, but invariably there is hepatic dysfunction. Circulating and urinary uroporphyrin and coproporphyrin levels are elevated. Repeated phlebotomy is the treatment of choice, however antimalarials (hydroxychloroquine, 25 to 200 mg weekly) or erythropoietin are instead prescribed for anemic patients.

Aphthous Ulcers

Although the incidence of aphthous ulcers, also known as canker sores, is similar in HIV-infected and HIV-seronegative persons, immunocompromised HIV-infected individuals with CD_4 T-lymphocyte counts below 100 cells/mm^3 are predisposed to the development of major, recurrent, multiple, slow healing or persistent, symptomatic ulcers (48). The lesions are often preceded by a pricking or burning sensation from 24 to 48 hours before their appearance. The ulcers are characteristically round or oval, crateriform, have a grayish yellow base and are surrounded by an erythematous halo (Fig. 25.11). One or more lesions erupt on the ventral, lateral or underside surface of the tongue, the buccal mucosa, the ßoor of the mouth, or the soft and hard palate. Dysphagia may be severe. Large ulcers may heal with extensive scarring. Herpetiform aphthae erupt in clusters of small 10 to 100 ulcers which coalesce to form large areas of ulceration. These clusters account for 7Ð10% of recurrent lesions (48).

Mixtures of nystatin oral suspension, diphenhydramine elixir, tetracycline suspension, liquid antacids and lidocaine gel are compounded and prescribed for relief of pain. In cases not improved with swish-and-swallow doses of tetracycline suspension, applications of clobetasol or betamethasone gel, covering the ulcer surface with

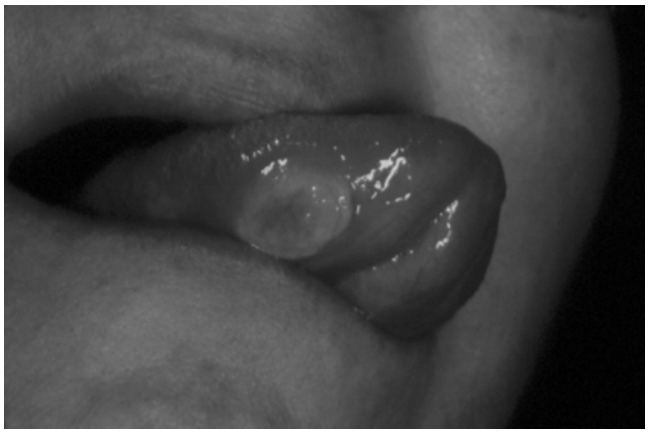

FIG. 25.11. Discrete, well-circumscribed, round apthous ulcer with typical gray slough covering ulcer base.

amlexanox oral paste, ingestion of colchicine and intralesional injections of 5% triamcinolone acetonide solution, or oral corticosteroids may be effective. Approximately 90% of HIV-infected patients with aphthous ulcers of the mouth and oropharynx treated with thalidomide have at least a partial healing of their ulcers (49). In one study, 55% of aphthous ulcers completely healed and 34% partially resolved, as compared to 7% and 18%, respectively, in the placebo group (48).

Granuloma Annulare

Granuloma annulare is a chronic eruption that presents clinically as granulomatous, inÞltrated papules, plaques, or nodules. The disease may occur more commonly in the setting of HIV infection (50). The lesions can be localized, perforating, generalized, erythematous, or subcutaneous. The lesions often form elevated rings with clear centers, hence the name granuloma annulare.

Although the name of the disease implies the lesions are annular, the more frequent lesions in HIV-infected persons are asymptomatic, discrete, skin colored, smooth, inÞltrated papules (Fig. 25.12). In 60% of patients the disease is generalized. The predominant location is the upper extremities, followed by the trunk. Oral mucosa lesions are rare. Some cases are exacerbated by ultraviolet B light. Potential treatments include PUVA, UVA-1; oral, intralesional or topical corticosteroids; systemic retinoids, antimalarials, potassium iodide and cyclosporine.

Vasculitis

Leukocytoclastic (hypersensitivity, necrotizing, hypocomplementemic, immune complex) vasculitis is characterized by the presence of nonblanching purple papules that may become conßuent, forming plaques (51). These lesions, commonly referred to as Òpalpable purpura,Óare gravity dependent and present most often on the lower extremities, although they may erupt on the forearms or any body area. Early lesions usually consist of petechiae, which become conßuent resulting in larger purpuric patches, and occasionally urticarial papules. Late lesions can be pustules, ulcers, and necrotic bullae. Abnormal laboratory tests include elevated erythrocyte sedimentation rates and decreased total complement levels as well as enzyme elevations if systemic organs are affected. Other diseases, including idiopathic thrombocytopenic purpura, drug-induced thrombocytopenia, and septicemia may produce similar purpura. However, the clinical Þndings are usually characteristic, and the histopathologic presence of Þbrin within the walls of small and medium-sized blood vessels as well as neutrophilic debris conÞrm the diagnosis. Leukocytoclastic vasculitis is more common in patients co-infected with HIV and hepatitis B or C. Adverse drug toxicity, connective tissue diseases, cryoglobulinemia and infectious diseases, especially streptococcal infection, must be excluded as potential causes.

Several cases of erythema elevatum diutinun, a chronic variant of leukocytoclastic vasculitis, have been reported (52). The lesions begin as purpuric macules, papules, and plaques which can evolve into indurated erythematous plaques and eventually into nodules. Areas of predilection are the dorsa of the hands, ankles, pretibial regions, knees, and skin overlying other joints. Other types of vasculitis, especially polyarteritis nodosa and microscopic polyangiitis have been reportedly associated with HIV infection (52a-d).

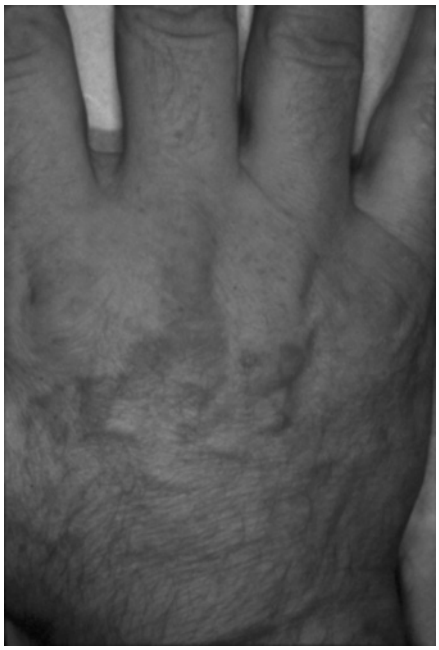

FIG. 25.12. In ltrated plaques and papules with crusted umbilications due to perforating granuloma annulare are present along the metacarpal-phalangeal joints of the hand. In this form of the disease, the characteristic annular lesions are not usually present.

Hair Abnormalities

AIDS patients often develop premature thinning of scalp as well as body hair. Lengthening, softening and discoloration of the scalp hair has been noted in Black patients. Straightening of curly hair has also been reported, as well as curliness of straight hair associated with lipodystrophy during treatment with the protease inhibitor, indinavir. Patients on indinavir may also complain about scalp hair loss due to shedding, as well as fairer and thinner body hair. Scalp hair loss has also been reported during treatment with zidovudine and in association with high viral loads. Febrile illnesses not uncommonly result in diffuse hair loss due to telogen effluvium. During late disease, when nutritional deficiencies are common, the hair may become lusterless, dull, and eventually a diffuse downy alopecia may develop (53). Premature canities (graying) and alopecia areata are rare. Trichomegaly (elongation) of the eyelashes is an interesting finding in some HIV-infected persons (Fig. 25.13).

Nail Disorders

In one study nail changes were present in 67.7% of HIV-infected patients. Clubbing (5.8%), transverse lines (7.1%), onychoschizia (7.1%), leukonychia (14.3%) and longitudinal melanonychia (14.8%) were the most frequent noninfectious abnormalities observed (54).

Most of the nail changes seen in HIV-infected patients are not specific and some have been discussed in other sections of this chapter. "Blue nails" are common in patients treated with zidovudine (AZT), although rare cases of dyschromic nails not associated with zidovudine treatment have been reported (54a). Blue to brown-black longitudinal streaks, transverse bands, diffuse pigmentation or of the nail plates develop in some patients, usually four to eight weeks after initiation of therapy with zidovudine. The "yellow nail syndrome" has been observed in patients with lymphedema and hypoxia. Malnourished patients often have thin, brittle nail plates. Transverse linear depressions of the nail plates, Beau's lines, may develop after a serious opportunistic infection. Muehrcke's lines, transverse white streaks, develop in patients with hypoalbuminemia. All white (Terry's nails) develop in association with hepatic disease and half-white and half-pink nails occur in renal disease. Protease inhibitors, especially indinavir, can cause painful paronychia of one or more toes, usually the first, which may recur even after partial nail avulsion.

HIV-Associated Pruritus

Pruritus is a common symptom of scabies, cutaneous drug hypersensitivity, eosinophilic pustular folliculitis, insect bites and many other skin eruptions. Itching in the absence of primary skin lesions and only excoriations and lichenification, should instigate an evaluation for underlying biliary disease, chronic renal failure, lymphoproliferative disorders, and intestinal parasites. There are cases in which no specific cause is evident and the pruritus is then attributed to HIV infection. However, many cases labeled as HIV-induced pruritus result from xerosis worsened by climactic effects, such as low humidity. In HIV-associated pruritus, only secondary changes, such as excoriations and lichenification are present (Fig. 25.14). The itching usually improves during treatment with emollients containing menthol, camphor, phenol, pramoxine or lidocaine; topical doxepin; capsaicin cream/gel; oral H1 and H2 antihistamines; or ultraviolet B phototherapy. Chronic cases may progress to prurigo nodularis, thick, hyperkeratotic nodular plaques, which may require more

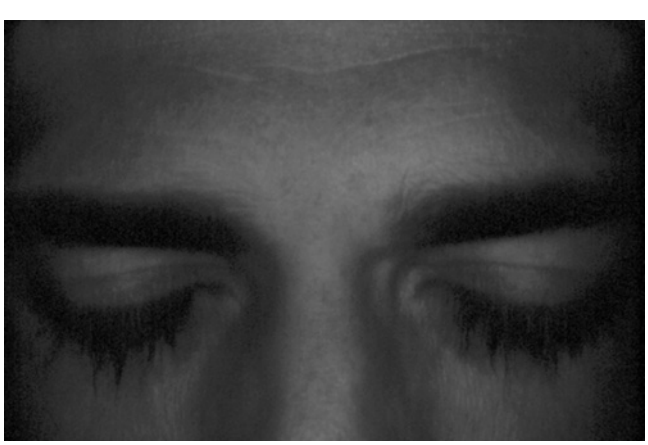

FIG. 25.13. Elongated eyelashes due to stimulated growth during treatment with indinavir.

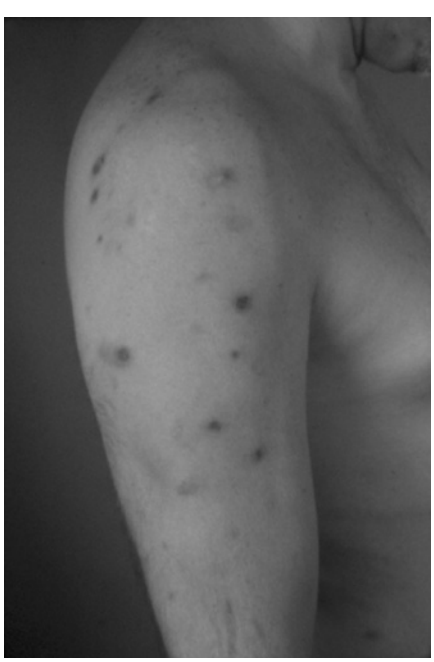

FIG. 25.14. Scattered excoriations in the absence of primary lesions due to HIV-associated pruritus.

aggressive treatments, such as intralesional corticosteroid injections and oral thalidomide.

Pruritic Papular Eruptions

There are a number of pruritic eruptions associated with HIV-infection. Pruritic papular eruption represents an umbrella term for several papular and papulopustular cutaneous eruptions with similar clinical and histologic features and therapeutic response. The causes include arthropod bites; hypersensitivity-induced recall reaction to previous insect bites (papular urticaria); or early lesions of eosinophilic folliculitis which do not yet show histologically the characteristic folliculocentric inÞltrate of eosinophils. Typically, there are pink urticarial papules, each of which resembles an insect bite.

Eosinophilic Pustular Folliculitis (EPF)

This eruption occurs in 5% to 9% of HIV-infected persons with CD_4 lymphocyte counts lower than 250 cells/mm^3, who are not being treated with effective antiretroviral therapy (55). Flares are more common in patients with CD_4 lymphocyte counts between 50 and 100 cells/mm^3. The prevalence of EPF declined sharply after the introduction of highly active antiretroviral therapy. However, in some cases onset has occurred shortly after initiation of antiretrovirals, possibly as a result of sudden immune restoration. Serum IgE levels and peripheral eosinophil counts are usually elevated.

Clinically, 2Ð3 mm follicular, erythematous, urticarial papules and papulopustules, which can be intensely pruritic, erupt on the forehead, face, shoulders, trunk, upper arms, and neck (Fig. 25.15). Often, there is a single vesicle or pustule on the surface of each papule. As a result of scratching, no intact lesions may be present and only potentially disÞguring excoriations and licheniÞcation from rubbing may be apparent. Other body sites can also be affected but the eruption is only rarely generalized. A well-developed, intact lesion is necessary for a histopathologic diagnosis. Alternately, the expressed contents of a pustule can be treated with a Wright stain and examined for a predominance of eosinophils. The etiology remains unknown. The Demodex folliculorum mite has been implicated as a possible cause through induction of an immune response that induces eosinophilia and Ig-E antibodies that bind to mast cells and Langerhans cells. In addition, chemokines and cytokines associated with a Th-2 predominant immune response are important in the pathogenesis (55).

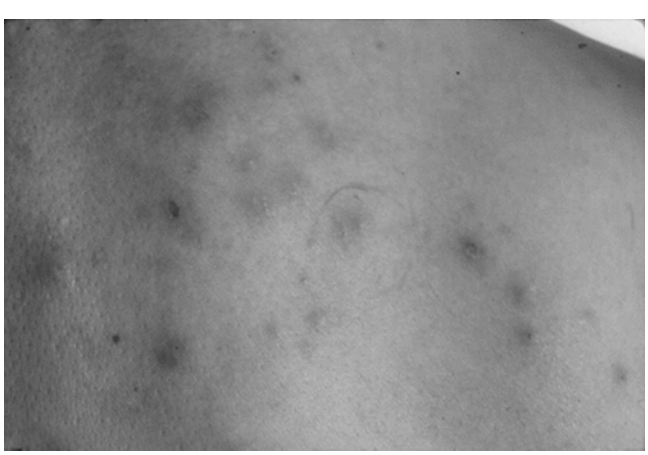

FIG. 25.15. The typical eruption of eosinophilic folliculitis consists of pink and skin colored accuminate papules and papulopustules that resemble arthropod bites.

Milder cases may respond to medium to superhigh potency topical corticosteroids and H_1 antihistamines, such as hydrozyzine. Topical or systemic antibiotics are only beneÞcial if a bacterial folliculitis is concomitantly present, which is occasionally the case. Systemic corticosteroids provide prompt relief, but are not recommended for chronic use. The most consistently efÞcacious therapeutic modality is ultraviolet B phototherapy (56). This previously maligned treatment has been clearly shown not to activate latent HIV infection to any signiÞcant degree (23). Treatment with either isotretinoin (20Ð40 mg daily) (57) or itraconazole (200Ð400 mg daily) (58) is usually more effective than metronidazole (250 mg three times daily for three weeks) (59), dapsone (100 mg daily), or daily applications of permethrin (60).

ARTHROPOD INFESTATION

Insect Bite Reactions

The reaction to bites of ßeas, mites, mosquitoes and other insects may be exaggerated, producing more extensive, exhuberant and symptomatic lesions (61). In these patients, large pruritic, weeping, urticarial papules may erupt on the skin, especially on the extremities. Occasionally, vesicles also appear (61).

The location of the lesions may help to determine the responsible arthropod. Flea bites occur predominantly on the legs, bedbug bites often involve the trunk and extremities and mites and mosquitoes are usually limited to exposed areas. Treatment consists of identiÞcation and avoidance of the offending arthopod, application of insect repellents, topical corticosteroids, oral antihistamines, doxepin cream or topical mentholated lotions.

Demodicidosis, caused by Demodex mites, causes an eruption that may resemble rosacea, acne or miliaria. The eruption consists of numerous tiny papules and papulospustules on the face and occasionally also the neck, scalp, shoulders and presternal area. These mites usually respond to treatment with antiscabicidal agents.

Scabies

In HIV-infected persons, the presentation of infestation with the Sarcoptes scabiei hominis mite may be either typical or exaggerated, and may be crusted and hyperkeratotic (62).

The typical lesions of classic scabies are tiny, intensely pruritic, often excoriated, pink papules (often along a path) and serpiginous or linear burrows. Lesions are frequently found along the Þngers, ßexor aspects of the wrists, antecubital fossae, axillae, umbilicus, buttocks, feet, genitalia and breast areola. The diagnosis of scabies is not routinely an easy one, as only excoriations may be present on examination, the presentation may be variable, and mites are not consistently seen in skin scrapings. Therefore, a high degree of suspicion is warranted in any patient with intense, persistent, unexplained itching. Permethrin 5% cream or gamma benzene hexachloride (lindane) 1% lotion are effective treatments when applied to the whole body from the neck downwards overnight and repeated in one week. All clothing worn during several weeks prior to the diagnosis of scabies, and linens, such as towels and bed sheets, must be carefully laundered.

When immunity is impaired, the clinical picture is exaggerated with generalized scaly papules and extensive excoriations. In these cases, longer duration of treatment is often required and lindane is avoided due to concern about neurotoxicity from absorption through non-intact skin (62a).

Hyperkeratotic (Norwegian) scabies, a fulminant and highly infectious form, occurs in profoundly immunocompromised persons (63). Crusted, hyperkeratotic psoriasiform plaques develop in any part of the body, but especially on the hands, feet, scalp and body folds (Fig. 25.16). Marked periungual and subungual hyperkeratosis can be present in both Þnger and toenails. Weeping Þssures often appear within the plaques. Notably, pruritus may be mild or absent altogether. Hyperkeratotic scabies is highly contagious. It has been estimated that as many as 3,500 mites can be shed into the environment from a single patient every day. This form of scabies may be clinically misdiagnosed as psoriasis, although on skin biopsy or even a simple skin scraping many mites are found, easily establishing the diagnosis. Rarely, Norwegian scabies may present with only thickly crusted localized plaques or nodules on the feet, hands and scalp. The treatment of choice is ivermectin 200 mcg per kg (63). The dose is usually readministered two or three times one week apart. The advantage of concomitant use of topical antiscabiecidal agents is speculative, although it is common practice. Topical scabiecidal agents can be used but more frequent applications are necessary. Salicylic acid 5% ointment should also be prescribed to exfoliate the thickened epidermal scales and facilitate penetration of other topically applied medications.

FIG. 25.16. Diffuse erythema, hyperkeratosis, crusting, excoriations and ssures caused by Norwegian scabies.

BACTERIAL INFECTIONS

Cutaneous Pyoderma

The most common bacterial pathogen cultured from infected skin lesions is *Staphylococcus aureus*. Depending on closeness of inspection and the degree of immunosuppression of the studied patients, *S. aureus* skin infections may be found in 71% of HIV-infected persons (64) and skin bacterial infection may be present in 83% of autopsies (65). Nasal colonization with *S. aureus* has been reported in as many as half of HIV-infected patients (66). Abscesses, furuncles, carbuncles, cellulitis, polymyositis and necrotizing fasciitis occur more commonly in HIV-infected patients with CD_4 T lymphocyte counts below 250 cells/mm^3, and often require aggressive surgical drainage or debridement and intravenous antibiotics (Fig. 25.17). *S. aureus* folliculitis is the most common bacterial pyoderma.

Typically, erythematous follicular and perifollicular pustules are scattered on the trunk and extremities, but lesions may arise on the skin of any body area (Fig. 25.18). When pruritus is present, there may be extensive excoriations and large abrasions with few intact pustules. In intertriginous areas, the eruption may resemble Candidiasis. Atypical Staphylococcal folliculitis in the groin, axilla, or scalp with violaceous crusted plaques up to 10 cm in diameter and superÞcial pustules, has been reported. Botryomycosis appears as crusted, suppurative, verrucous papules or erythematous nodular abscesses, which exude granules composed of clumps of bacteria (66a). The characteristic lesions of ecthyma are chronic, necrotic, deeply seated ulceration. Streptococcal axillary lymphadenitis usually presents as bilateral, painful, tender, swollen lymph nodes. There are reports of nonmenstrual toxic shock syndrome, associated with infected wounds and tampons or packing contaminated by toxigenic strains of *S. aureus*, in immunosuppressed patients (67). Patients with this syndrome present with fever, conjunctivitis, diffuse cutaneous erythema with subsequent desquamation, hypotension and shock. A similar eruption, known as

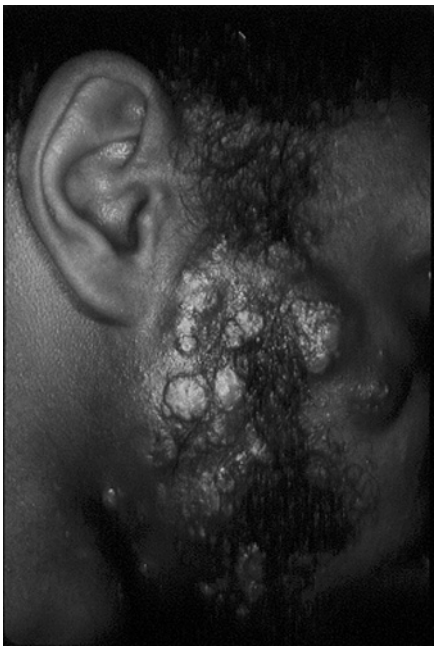

FIG. 25.17. Numerous furuncles, pustules and erosions on the beard area extending to the neck caused by infection with *Morganella morganii*, an uncommon skin pathogen. Abscesses and ulcers were also present on the chest and throughout the back.

the ÒToxic Strep SyndromeÓ caused by exotoxin-producing strains of *Streptococcus pyogenes* has also been reported. Chronic diffuse dermatitis in patients with *S. aureus* infection has been associated with elevated serum IgE levels and eosinophilia.

Haemophilus inßuenzae cellulitis is common in infants, may also occur on the head and neck of adults, has an aggressive course, and may become disseminated or progressively involve deep structures.

Cultures are imperative as skin infections may be caused by a large variety of bacteria, including unusual species, such as *Rhodococcus equi*, which has been cultured from abscesses. *Corynebacterium diphtheriae* may cause bullous lesions that ulcerate and become covered with a grayish pseudomembrane.

Bacillary Angiomatosis

Bacillary angiomatosis is caused by *Bartonella henselae* and, less often by *Bartonella quintana* (68). Epidemiologic evidence has associated infection with *B. henselae* with exposure to ßea-infested cats and infection with *B. quintana* with contact with the human body louse (69).

The most common skin lesions are pyogenic granuloma-like, Þrm, friable, dome-shaped, non-blanching purple to dusky red angiomatous papules and nodules, 0.5 to 10 cm, which may track linearly along veins in the extremities (Fig. 25.19) (70). The lesions may erupt anywhere in the body in numbers ranging from a few to hundreds but usually spare the oral mucosa, and the palms and soles. However, lesions have been reported in the lips, tongue and cervix.

Other lesions include violaceous nodules resembling Kaposiõs sarcoma, lichenoid violaceous plaques and subcutaneous nodules (71). Hyperpigmented indurated

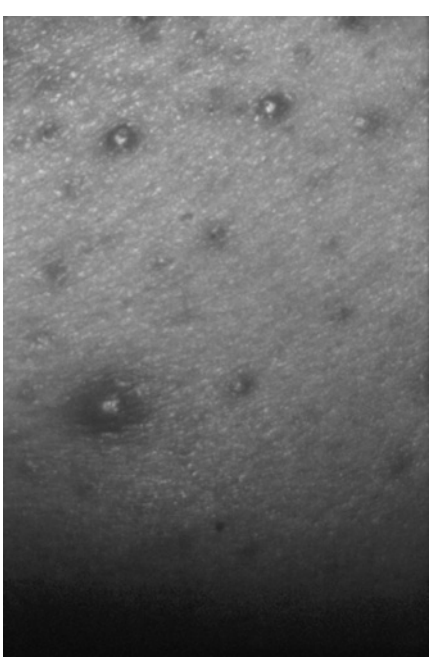

FIG. 25.18. Bacterial folliculitis consisting of follicular pustules with erythematous borders is usually caused by *Staphylococcus aureus*.

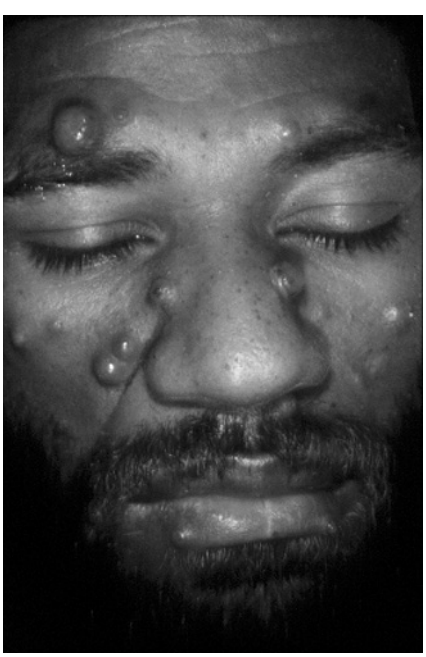

FIG. 25.19. Bright red, exophytic, dome-shaped papules and nodules on the face of a man with bacillary angiomatosis.

ovoid, hyperkeratotic plaques have been described in some dark-skinned patients.

Although skin involvement is the usual presentation, other organs may be affected. Patients may develop fever, chills and weight loss due to Bartonella bacteremia, even in the absence of skin lesions. Peliosis hepatis results from vascular lesions in the liver which typically result in highly elevated alkaline phosphatase levels but normal or only mildly elevated transaminases. Osteomyelitis is characterized by the presence of painful osteolytic lesions usually in the long bones, often beneath soft tissue masses or cellulitic plaques (71). Lymphadenopathy and hepatitis are signiÞcantly more frequent in cases infected with *B. henselae*, while osteomyelitis and central nervous system involvement are more common in *B. quintana*-infected patients. Involvement of the gastrointestinal tract, spleen, lymph nodes, respiratory system, central nervous system, bone marrow, and endocardium is possible (68). In contrast to *B. quintana*, *B. henselae* infection is more often associated with diarrhea.

Histopathologic examination of affected tissue shows vascular proliferation of capillary-sized blood vessels lined with protuberant, cuboidal or polygonal endothelial cells. A dermal mixed inßammatory cell inÞltrate contains numerous neutrophils. In hematoxylin and eosin stained tissue, the pleomorphic bacteria appear as granular or Þbrillary amorphic eosinophilic clumps in close proximity to vascular channels but are best visualized with silver stains (Fig. 25.20) (71). In cases when no bacteria can be identiÞed under light microscopy, electron microscopy demonstrates the characteristic trilaminar cell-walled bacilli (71).

The small, curved, gram-negative rods can be cultured in 5% carbon dioxide with high humidity on freshly poured agar containing 5% deÞbrinated rabbit blood, but growth requires 20Ð40 days (71). However, the diagnosis can be readily conÞrmed with a PCR-enzyme immunoassay (71).

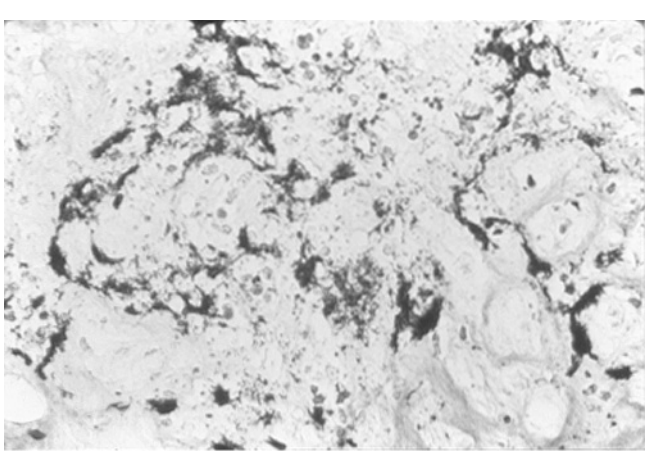

FIG. 25.20. Impregnated silver (Steiner) stain showing irregular clumps of Bartonella bacteria in a lesion of bacillary angiomatosis.

Erythromycin (500 mg orally four times daily) results in dramatic improvement in the skin lesions within one week, and resolution within one month. Intravenous erythromycin is recommended for systemic infections. Although insufÞcient data are available for recommendation, other macrolides such as azithromycin and clarithromycin also appear to be effective. Doxycycline 100 mg twice daily is an alternative treatment. An eight-week course of antibiotics is recommended for isolated cutaneous disease. Relapses are treated with an additional 16-week course. Hepatitis and osteomyelitis require longer treatment courses and possibly even lifelong suppressive antibiotic therapy.

Mycobacterial Infections

Although infection with *M. avium intracellulare* (MAC) rarely involves the skin, Þrm papules and nodules, skin ulcers, perianal erosions, and cellulitis have been described in patients with mycobacteremia (72). The occasional incidental presence of this organism in skin biopsies of other lesions is not surprising considering that 20% to 40% of profoundly immunosuppressed HIV-infected patients not receiving antimycobacterial prophylaxis developed systemic MAC infection (73).

On the other hand, skin lesions are the most common manifestation of infection with *M. haemophilum*. The lesions are erythematous granulomatous papules, exophytic or subcutaneous nodules, and abscesses that drain spontaneously and ulcerate (Fig. 25.21) (74). Lymphadenitis, usually of the submandibular and cervical nodes, and central venous catheter tunnel infections may occur. Infection of bones, joints, lymphatics and lung is common (74). This organism is fastidious and requires supplementation of iron or heme to the culture media which must be incubated at 30Ð32¡C. The infection can be treated with antibiotics to which the microorganism is susceptible, such as minocycline, clarithromycin, ciproßoxacin, clofazimine, isoniazid, rifampin, ethambutol amikacin,

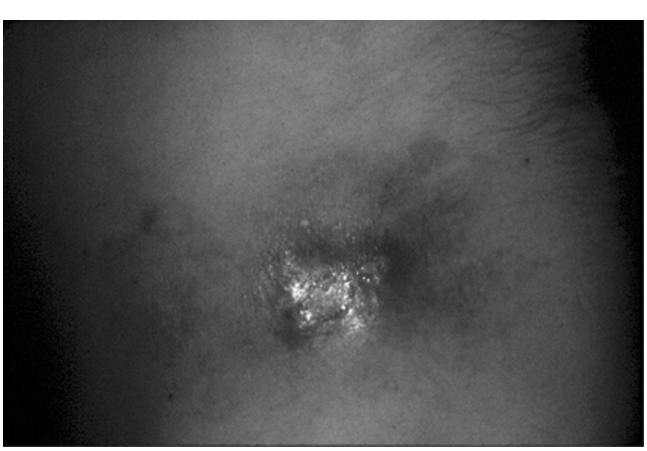

FIG. 25.21. One of several ulcerated nodules in a patient with disseminated *Mycobacterium haemophilum* infection.

streptomycin, and pyrazinamide. Infection with *M. marinum*, *M. kansasii*, *M. ulcerans* and other mycobacteria have been described and cause lesions that are similar to those found in HIV-seronegative individuals. In contrast to tuberculosis, numerous bacilli are readily visible in biopsied, acid fast-stained tissue of lesions caused by atypical mycobacteria (Fig. 25.22).

The skin is rarely involved in *M. tuberculosis* infection. The more common cutaneous presentations are abscesses and scrofuloderma. Lupus vulgaris, which is the most common presentation in HIV-seronegative persons with adequate immunity, is infrequently seen. Miliary tuberculosis is notably uncommon (75). The typical lesions of scrofuloderma are irregular, purulent ulcers and sinuses with a granulation base overlying lymph nodes, and occasionally infected bones or joints.

Although it was suspected that HIV-induced immunosuppression would propel a shift towards the multibacillary (lepromatous) spectrum in patients co-infected with *M. leprae*, studies have failed to document a difference in disease spectrum between HIV-infected and HIV-seronegative Hansen's disease patients. However, in one study 22% of co-infected patients developed new lesions during treatment, suggesting that the disease is less responsive to standard therapeutic regimens.

Syphilis

Numerous studies have demonstrated that syphilis is more common in HIV-infected than in HIV-seronegative persons, however this higher prevalence may be attributable to the increased risk for syphilis in certain groups at risk of acquiring HIV infection (76). It has been stated that the signs and symptoms of primary and secondary syphilis may be more exaggerated and that the course may progress more rapidly towards the tertiary stage. However, prospective studies have shown that the appearance of lesions and the course of disease is not different in the vast majority of HIV-infected patients compared with HIV-seronegative patients (76). The appearance of primary chancres is typical, although patients with primary syphilis present more frequently with multiple ulcers, and chancres may persist more often into the secondary stage (76). The course of secondary syphilis is usually unchanged, although rapidly progressive disease and unusual manifestations have been rarely reported (76). Nodular and noduloulcerative lesions are more common, but most of these cases have no constitutional symptoms associated with malignant syphilis. Although most cases of early syphilis in HIV-infected patients respond to conventional doses of penicillin, serologically defined treatment failures are more common, and for this reason close evaluation after therapy is mandatory. Reactivation of previously thought to be adequately treated latent syphilis has been reported, although many of these patients have serofast titers without clinical manifestations. Clinical relapses, usually with neurosyphilis, occurred in 1% of patients in a prospective study and more frequently in retrospective studies (77). Rarely, patients experience multiple relapses. The prevalence of neurosyphilis is higher in patients coinfected with both syphilis and HIV, and progression to the symptomatic forms of neurosyphilis is accelerated. Uncontrolled production of antibodies may produce extremely elevated titers and a higher rate of biologic false-positive nontreponemal serologic tests. Conversely, an occasional patient may have a nonreactive nontreponemal test during early syphilis, and the sensitivity of treponemal tests appears to be slightly lower in patients treated for past syphilis. Treatment consists of a single injection of benzathine penicillin G, 2.4 million units for primary, secondary or early latent syphilis, and three injections one week apart for late latent or syphilis of indeterminate duration. CSF examination is recommended for patients with early syphilis in the presence of neurologic signs or symptoms, syphilis of indeterminate or more than one year duration, and tertiary disease.

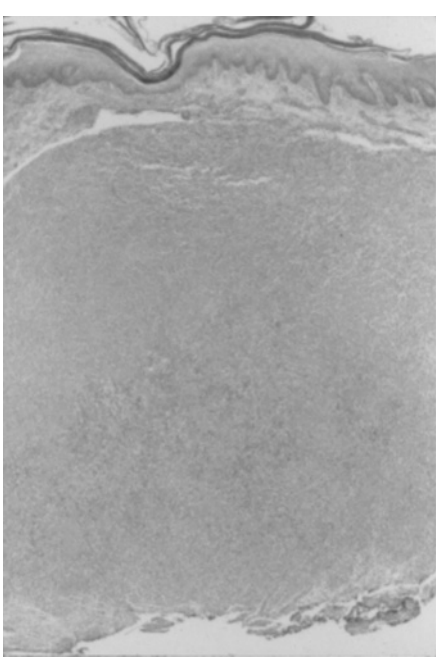

FIG. 25.22. Numerous acid fast bacilli present in the dermal in ltrate are characteristic of infection with atypical mycobacteria.

FUNGAL INFECTIONS

Candidiasis

Oropharyngeal candidiasis (thrush) due to Candida species, usually *Candida albicans*, is the most common opportunistic mucosal infection seen in HIV-infected persons. *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. glabrata* tend to cause infection in patients with advanced HIV infection. Previous to the introduction of highly

aggressive antiretroviral therapy, oropharyngeal candidiasis was found in 7–48% of HIV-infected persons and as many as 93% of profoundly immunocompromised patients (78). The development of "thrush" is an important early marker of progressive immunodeficiency, which implies immune deterioration with progression to symptomatic disease independent of CD_4 counts. Approximately 75% of patients have at least one episode of thrush at some point in the course of HIV infection. In about a third of these, the disease is recurrent and the severity worsens with advancing immunosuppression (79). Candidal esophagitis affects 20–40% of all HIV-infected persons. Mucocutaneous candidal infection is rare with CD_4 T-lymphocyte counts above 500 cells/mm^3 but frequent with counts below 100, and therapeutically challenging when counts drop below 50 cells/mm^3. During treatment with HAART, the prevalence of oropharyngeal candidiasis was reduced from 31% at baseline to 1% at 48 weeks in one study (78). However, the prevalence of oral candidiasis in these patients increases when viral load counts rise above 30,000 copies/ml (80,81).

Oropharyngeal candidiasis is asymptomatic in many patients, but those with inflammation and erosions experience burning pain and altered taste sensation. Dysphagia is more often a symptom of esophagitis. Pseudomembranous candidiasis, the acute and most common form of oral candidiasis, presents with soft, creamy white to yellowish plaques that can be easily scraped off with a tongue depressor, leaving erythematous, eroded, or ulcerated bases, which may be tender and bleed easily. In chronic hyperplastic candidiasis, asymptomatic white plaques or papules that are adherent and unscrapable are most commonly present along the laterodorsal surfaces of the tongue (Fig. 25.23). Chronic atrophic (erythematous) candidiasis appears as an occasionally symptomatic, red patch or velvet textured plaque, usually on the hard palate but also on the dorsal tongue and other mucosal surfaces.

The diagnosis of candidiasis is confirmed by the detection of Candida species in 10% potassium hydroxide preparation or culture. More than 90% of patients have clinical resolution of physical findings and symptoms within seven days of initiation of treatment. Effective agents include fluconazole (100 mg daily), itraconazole (100–200 mg daily), ketoconazole (200 mg daily), or clotrimazole troches (10 mg 4–5 times daily) for 7 to 14 days for thrush or 14 to 21 days for esophageal disease. Azole resistant cases that do not respond to itraconazole or high doses of fluconazole (400–800 mg daily) are treated with amphotericin B suspension (100–500 mg swish and swallow four times daily), or an intravenous amphotericin B preparation. Routine primary prophylaxis is not recommended because of the effectiveness of therapy for acute disease, the low mortality associated with mucosal candidiasis, the potential for resistant Candida strains to develop, the possibility of drug interactions, and the cost of prophylaxis. However, chronic suppression therapy may be required in cases with recurrent episodes.

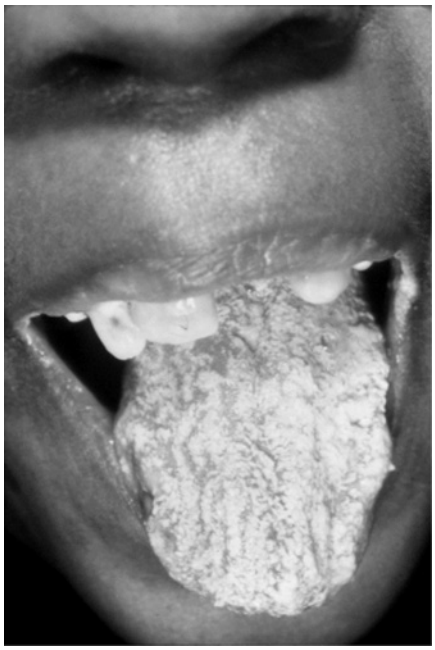

FIG. 25.23. Candida albicans infection (thrush) consisting of creamy white plaques on the dorsal surface of the tongue.

In angular cheilitis due to Candida, either unilateral or bilateral cracking, fissuring, and redness develop at the oral commissures. Candidiasis of glabrous skin is common especially in intertriginous areas, such as the groin, axillae, inframmamary folds, and gluteal cleft (82). In these areas, erythematous, moist, scaly patches and papules become confluent into plaques which are surrounded by tiny satellite reddish papulopustules. In the anal area, pruritus ani is the chief symptom. Vulvovaginal candidiasis is a poorly tolerated complication that develops in 30 to 40% of HIV-infected women. In some women, the infection is chronic and intractable. HIV-infection should be suspected in non-diabetic men with recurrent candidal balanitis. In infants with congenital HIV infection, candidal diaper rashes are more recurrent and severe (82a). Disseminated candidiasis is rare in HIV-infected individuals.

Dermatophytosis

The prevalence and severity of superficial fungal infections increase proportionally with the severity of HIV-induced immunosuppression. These infections are more widespread, more recurrent, require more prolonged treatment and may have atypical appearances. The typical lesions are annular (ringworm) papules and plaques (Fig. 25.24), but scaly planar plaques may be misdiagnosed as psoriasiform or eczematous dermatitis. Several cases of

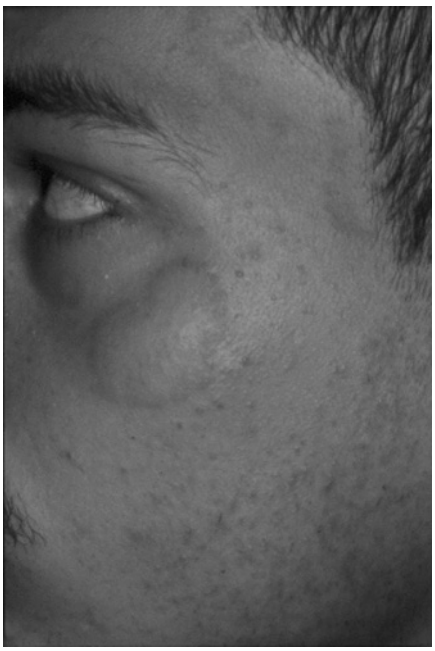

FIG. 25.24. Tinea faciei with erythematous annular lesions covered by ne scaling on the face caused by *Trichophyton rubrum*.

tinea capitis, including kerion formation which is uncommon in adults, have been reported, mainly in women (83).

Onychomycosis

Compared to the general population, HIV-infected persons have an increased susceptibility to onychomycosis. In general, fungal nail infections are more rapidly progressive, involve more nails, respond less favorably to antifungal therapies, and have higher rates of post-treatment relapse. In a prospective study of nails, onychomycosis was the main nail disease and was present in 30.3% of patients as compared with 12.6% of controls (54). *Trichophyton rubrum* was present in 48% of the onychomycoses cases and unusual Candida species were also recovered from some abnormal nails. Multiple fungi were frequently cultured in a single patient. The mean CD_4 T-lymphocyte count was lower in patients with onychomycosis and the frequency of onychomycosis increased with advanced immunosuppression.

Any form of onychomycosis, including distal subungual onychomycosis, proximal subungual onychomycosis and total dystrophic onychomycosis may be present. However, white superÞcial onychomycosis is the most common form of onychomycosis in HIV-immunocompromised patients, and is usually caused by *Trichophyton rubrum*, rather than *T. mentagrophytes*, the fungus usually implicated in most nail infections of this type (84). The fungus invades the dorsal portion of the nail plate. A superÞcial, white, powdery discoloration appears in the dorsal nail plate (Fig. 25.25). As the disease

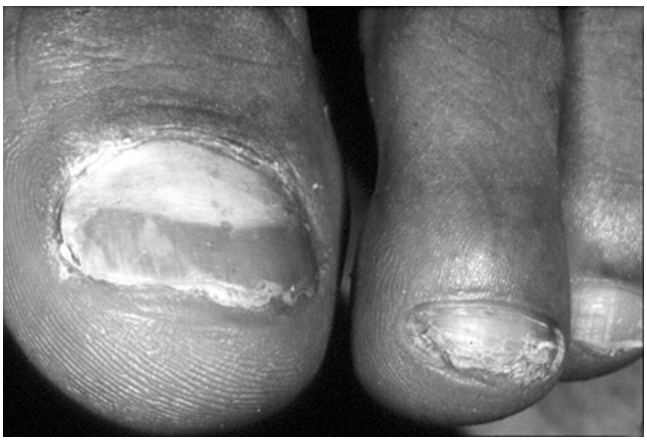

FIG. 25.25. White proximal onychomycosis.

progresses, the coloration merges and spreads. The discolored nail plate becomes rough, soft, and friable. Candida species can invade the nail plate or nail bed directly or infect the nail folds, hyponychium, or nail bed after the nail unit is exposed to trauma, moisture, or contact irritants. Candidal paronychia is characterized by erythema and edema of the proximal and lateral nail folds. *S. aureus* paronychia is associated with greater tenderness, increased rubor, and ßuctuance with purulent discharge. Candidal onychomycosis presents with onycholysis, or brittle, thickened and opaque nail plates.

Cryptococcosis

Without effective antiretroviral therapy, between 6% and 13% of all AIDS patients develop *Cryptococcus neoformans* infection, usually meningitis (85). Approximately 5.9Ð15% of patients with disseminated cryptococcosis manifest skin lesions, the most common of which are skin-colored or pink, umbilicated papules and nodules, many with central hemorrhagic crusts (85). These lesions resemble molluscum contagiosum but there is no central keratotic plug and the surface of the lesions is less smooth (Fig. 25.26). The lesions are most frequently present on the face and neck followed by the trunk and extremities. There could be a single or dozens of lesions. Crater-like ulcers with raised edges resemble basal cell carcinoma (86). Purple nodules and plaques appear similar to Kaposi's sarcoma (85). Cases with cutaneous pustules, abscesses, vegetating plaques, cellulitic areas, panniculitis and palpable purpura are less common (85,86). Rarely, the lesions are scattered erythematous crusted plaques that can be confused with eczema (85). Encapsulated budding yeast forms can be demonstrated in curetted skin scrapings crushed on a glass slide and stained with Giemsa stain or India ink. A skin biopsy specimen stained with PAS, hematoxylin and eosin, or methanamine silver readily shows the organisms. A culture of tissue provides conÞrmation. The treatment of choice is ßuconazole.

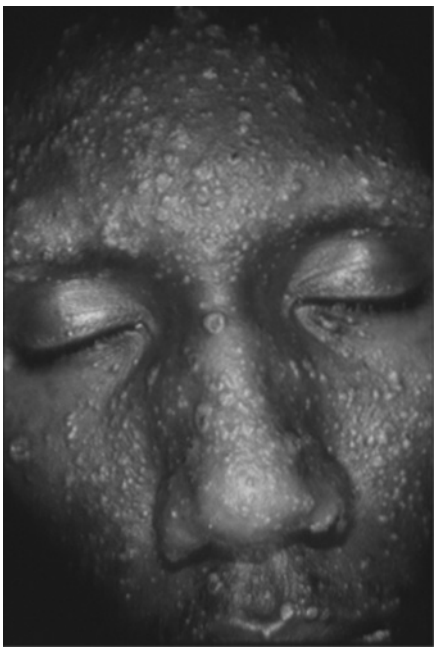

FIG. 25.26. Disseminated cryptococcosis with whitish-pink papules which become centrally eroded, resembling molluscum contagiosum.

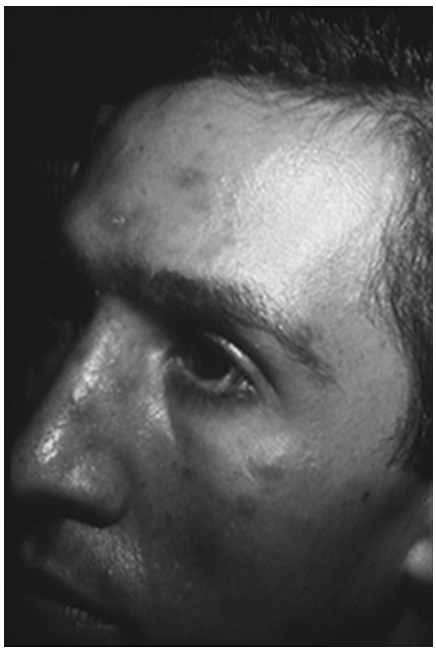

FIG. 25.27. Acneiform erythematous papules and pustules some of which become necrotic due to histoplasmosis.

Itraconazole is also effective. Intravenous amphotericin B with ßuconazole or 5-ßucytosine is given to patients with cryptococcal meningitis or life-threatening infection. Prophylactic treatment has been continued indeÞnitely since over half of cases relapse after discontinuation of antifungals. However, recent reports indicate that discontinuation of prophylaxis may be safe in patients who have responded to HAART with increases in the CD_4 cell count to greater than 100 cells/mm^3 (87).

Histoplasmosis

Disseminated infection with *Histoplasma capsulatum* develops in 4.7% of HIV-infected patients living in endemic areas, especially those who have a history of exposure to chicken coops, serologic evidence of previous infection, and CD_4 T-lymphocyte counts below 150 cells/mm^3. In about 11% (10Ð20%) of systemic cases, mucocutaneous lesions are present. In one study of 32 AIDS patients from South America with disseminated infection, two-thirds presented with mucocutaneous lesionsÑ six exclusively with oral lesions, six only with facial lesions and nine with both orofacial lesions 88. The most common cutaneous presentation is an eruption of scattered erythematous granulomatous papules or pustules that become necrotic on the face, extremities and trunk (Fig. 25.27) (89). However, caseating nodules, ulcers, vegetating plaques, condyloma-like granulomatous papules, intranasal masses, panniculitis and acneiforn, rosacea-like, folliculitis-like and maculopapular eruptions have also been described. Verrucous papules and ulcers are the common oral lesions (88).

Penicilliosis

Infection with the dimorphic fungus *Penicillium marneffei* is the third most common opportunistic infection among HIV-infected persons residing in Southeast Asia and southern China (90). Cases are seen in this country among immigrants or travelers returning from endemic areas. The exact route of infection, although unknown, is assumed to involve inhalation of conidia. The fungus proliferates in macrophages. Patients are profoundly immunosuppressed with CD_4 T-lymphocyte counts below 50 cells/mm^3. The typical presentation consists of fever, anemia, weight loss, lymphadenopathy, hepatomegaly, gastrointestinal symptoms and in 60% of cases cough and dyspnea due to pulmonary inÞltrates (90). In as many as 75% of cases, the skin is involved. Typically, there are one or more umbilicated skin colored or pink papules, usually on the face, pinnae, arms and upper trunk (Figs. 25.28, 25.29). Small genital ulcers measure between 0.5 to 3.0 cm. Shiny oral papules and erosions or shallow ulcers covered with whitish yellow, necrotic sloughing may erupt on the palate, gingiva, labial mucosa, tongue, and oropharynx. A diagnosis can be presumptively established with a positive serologic test and conÞrmed by identiÞcation of the fungus on skin biopsy or culture from blood or other organs. Itraconazole (200 mg) is administered for mild to moderate infection. The treatment of choice in patients with shock or respiratory failure is amphotericin B (0.6 to 1 mg/kg daily). Because relapses are common,

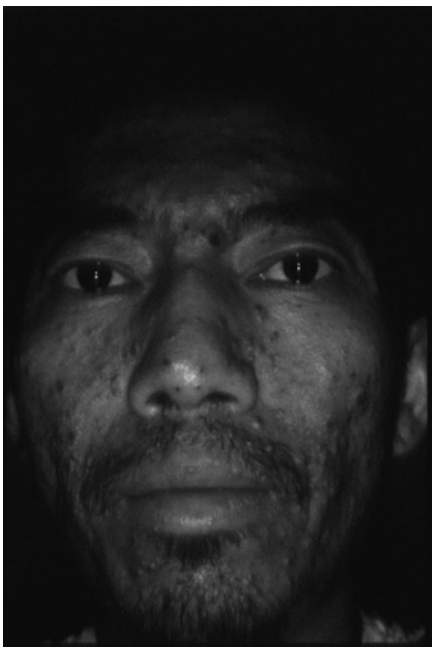

FIG. 25.28. Disseminated penicilliosis with erythematous, umbilicated, skin colored papules of various sizes.

long-term suppressive therapy with itraconazole is recommended.

Coccidioidomycosis

Reactive cutaneous manifestations of acute pulmonary infection with *Coccidioides immitis* include erythema multiforme, erythema nodosum and scarlatiform toxic erythema (91). Chronic primary cutaneous coccidioidomycosis infection consists of erythematous papules, which often ulcerate and become vascular warty granulomatous nodules, usually on the face and neck (92). Disseminated coccidioidomycosis infection is not common and only

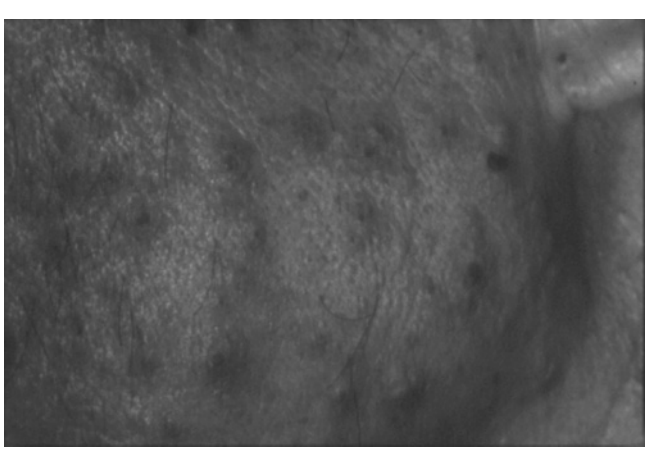

FIG. 25.29. Close up of small, discrete, brownish pink, ulcerating papules on the face of a man with disseminated *Penicillium marnefÞ* infection.

5Ð10% of cases develop skin lesions (91). Verrucous plaques or granulomatous papules may erupt on the face (92). Other lesions are not speciÞc and include pustules, nodules, subcutaneous abscesses, ulcers, sinuses and scars.

Sporotrichosis

Lymphocutaneous infection with the dimorphic fungus, *Sporothrix schenckii*, is usually contracted in North America through traumatic puncture or scratches during outdoor activities, such as rose bush gardening (93). A tender inÞltrated erythematous papule forms at the inoculation site and progressively becomes a pustule and then an ulcerated nodule with ragged undermined borders. In approximately, two to three weeks new subcutaneous nodules may erupt in a linear pattern along the draining lymphatic vessels.

Patients with Þxed cutaneous sporotrichosis usually live in endemic areas and have a high degree of immunity. In Þxed cutaneous infection, pyodermatous erosions or inÞltrated and crusted plaques are localized to one area, most commonly the hand or face. Both forms have been reported in HIV-infected patients (94).

Immunocompromised patients with severe CD_4 T-lymphocyte depletion develop either disseminated cutaneous infection with large, tender, erythematous nodules that ulcerate, or multifocal systemic infection with widespread, purple, crusted ulcerated plaques, often associated with polyarticular arthritis (94). Disseminated osteoarticular sporotrichosis may masquerade as gout.

Sporotrichosis localized to skin and subcutaneous tissues is readily treated with itraconazole, ßuconazole or supersaturated potassium iodide. However, management of osteoarticular and other localized visceral and disseminated forms of sporotrichosis is challenging. Eradication of the organism may not be possible, but clinical resolution can be attained and is subsequently maintained with suppressive antifungal therapy (93).

OTHER OPPORTUNISTIC INFECTIONS

Pneumocystosis

Dissemination of *Pneumocystis carinii* to the skin is rare, but has been reported in some cases receiving pulmonary prophylaxis with aerosolized pentamidine or dapsone (95). Cutaneous pneumocystosis has variable clinical presentations, the most common of which are discrete, friable, erythematous papules or small nodules on the ear canals or the nares (Fig. 25.30) (95). Other lesions have included molluscum contagiosum-like papules on the head and neck, bluish cellulitic plaques in the sternum, polypoid masses in the ears, Þrm hyperpigmented axillary nodules, deep abscesses, and a gangrenous foot (95).

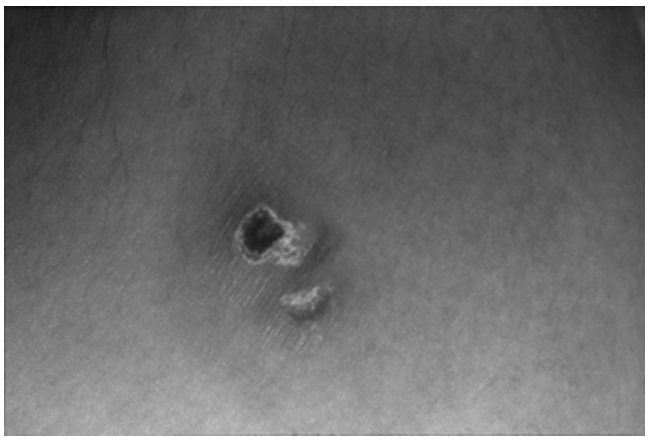

FIG. 25.30. Two ulcerated erythematous papules, one covered with black crust on the face of a patient with disseminated *Pneumocystis carinii* infection.

Amebiasis

Systemic infection with Acanthamoeba or Leptomyxida spread from intestinal infection, usually presents with slowly progressive, invariably fatal granulomatous encephalitis. Skin involvement was present in approximately 75% of reported patients and may be the only manifestation of disseminated disease (96). Notably cutaneous infection may precede other manifestations by weeks or months. Skin lesions include pustules, pruritic and painful erythematous inÞltrated papulonodules, and subcutaneous, occasionally draining, ßuctuant nodules. The usual location is the skin of the extremities and face (96). These lesions often ulcerate forming deep or shallow crusted ulcers, which range in size from 0.5 to 5.0 cm (Fig. 25.31). Ulcer and sinus Þstulas are often present on the hard palate. Histopathologic examination shows a neutrophilic lobular and septal panniculitis with trophozoites. The prognosis is dismal. Various combinations of drugs have been effective in isolated cases. An aggressive multidrug regimen with intravenous pentamidine, oral sulfadiazine, ßuconazole, and ßucytosine, has been advocated as the most promising treatment for disseminated acanthamebiasis (96).

VIRAL INFECTIONS

Human Papilloma Virus Infection

More than 100 types of human papillomavirus (HPV), a DNA virus that can affect the mucocutaneous surface of any body area, have been classiÞed (97). Cutaneous warts account for 2.9Ð27% of all skin diseases in HIV-infected persons. In immunocompromised patients, cutaneous warts tend to be multiple, can grow unusually large, spread over wide areas, and respond less favorably to conventional therapy (Fig. 25.32) (97). Palmoplantar warts may

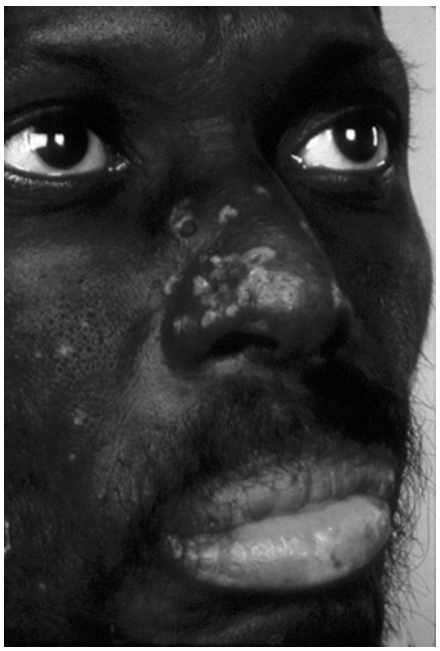

FIG. 25.31. Prothecosis consisting of small erythematous vesicles and papules that ulcerate and scar on the central face and lips.

be so numerous as to become conßuent into keratodermatous plaques (Fig. 25.33). Flat warts may erupt and become conßuent into plaques on the upper extremities, face, and trunk. In such cases type 5 and type 8 HPV, which are associated with epidermodysplasia verruciformis, have been recovered (98).

The prevalence of oral warts has remained at 1 to 4%, a frequency apparently unaltered by the development of new antiretroviral therapies. HPV, most commonly types 7, 13 and 32, can be recovered in clinically normal oral mucosa in up to 60% of HIV-infected individuals versus one-third of the general population. The most common clinical presentation is multiple ßat warts with stippled surfaces on

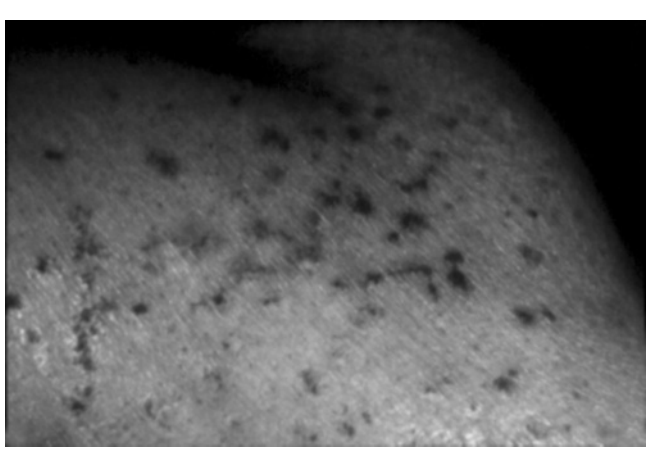

FIG. 25.32. Pigmented, disseminated verrucae plana. Linear arrangement of warts in some areas re ect koebnerization.

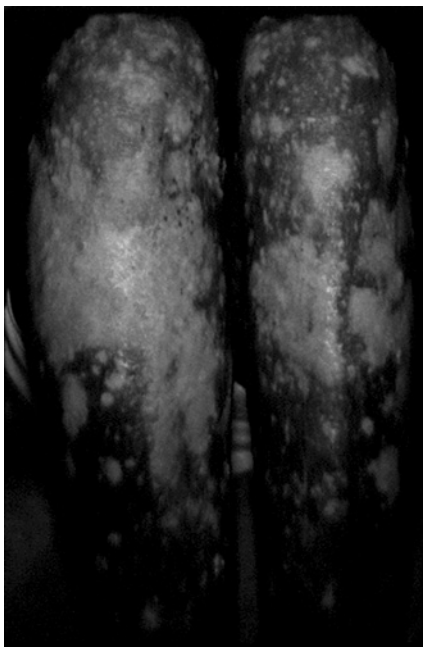

FIG. 25.33. HPV infection with numerous at warts confluent into psoriasiform plaques on the anterior legs. Some lesions had progressed to squamous cell carcinoma.

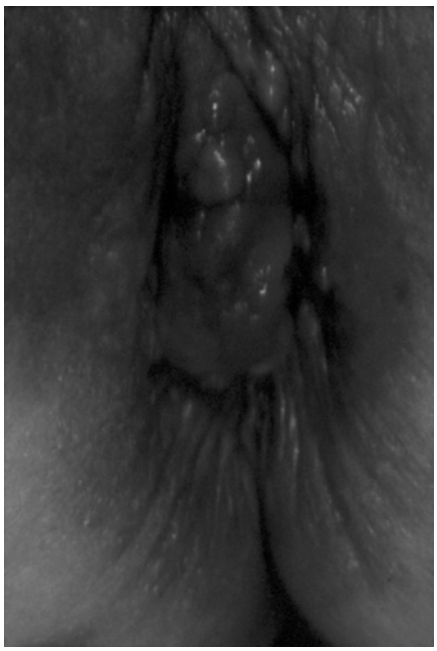

FIG. 25.35. Exophytic, convoluted, rm erythematous nodular invasive anal squamous cell carcinoma.

the lips, labial commissures, buccal mucosa, and tongue (99). Anogenital warts are common, and with increasing immunosuppression become extensive, relapse frequently after treatment, and have higher rates of dysplastic changes (Fig. 25.34). There is evidence that the prevalences of both oral and anogenital warts are increased in patients treated with HAART (99,100).

The prevalence of anal HPV infection is as high as 93% in HIV-infected compared to 23% in HIV-uninfected homosexual or bisexual men (101). In San Francisco 196/269 (73%) of HIV-infected gay men were infected with multiple HPV types, with type 16 being the most common type (101). These statistics are alarming in view of the high incidence of anal cancer in HIV-infected homosexual and bisexual men (at least 70 cases/100,000 persons) in comparison to the general population (0.8/100,000 persons) (101a,101b). The risk of invasive anal cancer was calculated to be 34-fold higher in long-term HIV survivors (Fig. 25.35). HIV-infected men and women have higher rates of high-grade squamous intraepithelial lesions than the otherwise healthy HIV-seronegative population. Some investigators have recommended screening all HIV-infected patients with anal cytologic smears regularly, evaluating abnormal cytology with colposcopic guided biopsies, and obliterating high grade dysplastic lesions (102,103).

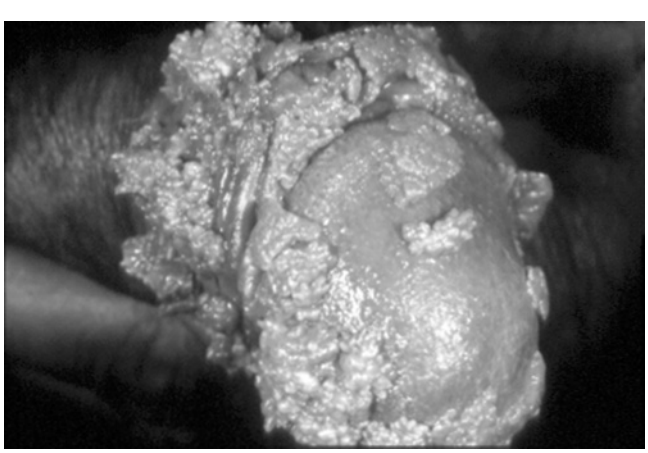

FIG. 25.34. Extensive number of condylomata accuminata on the penile glans and distal shaft.

Cell-mediated immunity is considered to be a critical factor in the regression of warts, and there is an inverse association between CD_4 T-lymphocyte counts and the presence of warts. Indeed, cases of previously treatment unresponsive warts that resolved after initiating effective antiretroviral therapy have been reported (**ref ???**). However, in other patients, cutaneous warts have persisted despite a striking increase in the CD_4 T-lymphocyte counts. It was recently suggested that in patients with a nadir CD_4 cell count below 120 cells/mm^3, or a mean CD_4 cell count below 135 cells/mm^3, at the start of aggressive antiretroviral therapy, cutaneous warts will persist despite a rise in CD_4 T-lymphocyte counts (100).

Common treatments include cryotherapy, electrocautery, CO_2 laser ablation, pulse dye laser treatments, excision, intralesional bleomycin, cantharidin, and imiquimod (97,104). Anogenital lesions can also be treated with podophyllotoxin, trichloroacetic acid, intralesional or intramuscular injections of alpha interferon, and cidofovir (97,105). The use of acitretin at a dose of 25 mg twice

daily has been found to be beneficial in promoting the resolution of even large HPV-related warts, although the lesions return upon discontinuation of this treatment (105a).

Molluscum Contagiosum

Molluscum contagiosum is caused by the largest known human Poxvirus, which replicates in the cytoplasm of infected keratinocytes. Identification of these basophilic intracytoplasmic inclusion bodies in cytological preparations (Molluscum preps) treated with Giemsa stain confirms the diagnosis (106). The usual incubation period ranges form two to seven weeks but data from HIV-infected cases strongly suggest that the virus may remain latent in the skin for years. The virus is usually transmitted from skin to skin contact with a person with active lesions, as well as through contaminated inanimate objects, such as towels and gymnasium equipment. Self-inoculation by picking, rubbing and shaving spreads the infection.

In immunocompetent persons the infection is self-limited with each lesion resolving spontaneously in approximately two to three months, however, in immunocompromised individuals the disease is frequently progressive with increasing number of lesions which persist, disseminate, and grow larger in size (107). The prevalence of molluscum contagiosum in HIV-infected persons ranges between 5% and 18% (107). Infection is common in persons with CD_4 counts below 100 cells/mm^3. Smooth, round, dome-shaped, umbilicated, gray-white, pink or flesh-colored papules erupt as scattered individual lesions or as grouped, often symmetrical, crops (Fig. 25.36). A firm, gray-white core can be expressed from the lesions. The lesions are usually 2–5 mm in size but may grow to 3 cm (giant molluscum). More than one hundred lesions may be present and these may become confluent into cobblestone plaques. When the infection is sexually transmitted the lesions are found in the anogenital and pubic areas, the perineum, upper thighs and lower abdomen. However, in the HIV-infected host, the face, neck, shoulders and upper back are the most commonly affected areas. The presence of molluscum lesions on the eyelids is not uncommon in AIDS patients and has been suggested to be, without supporting evidence, pathognomonic of HIV infection.

Several small reports have documented resolution of molluscum contagiosum five to six months after initiation of highly active antiretroviral therapy. Destructive treatments include curettage, electrodessication, cryotherapy with liquid nitrogen, applications of trichloroacetic acid (50–90%), podophyllin (25–50%), podophyllotoxin, or cantharidin, excision, carbon dioxide laser ablation and pulse dye laser treatments 108. Tretinoin (0.025–1%) cream or gel and 5-fluorouracil, 5% or 1%, are less successful at eradicating lesions than retarding their growth and preventing formation of new ones. Imiquimod used three times a week and even daily has been effective in several cases. Cidofovir 3% in Dermovase successfully cleared some cases which had been recalcitrant to other treatments (109).

Oral Hairy Leukoplakia

The prevalence of oral hairy leukoplakia (OHL) may be as high as 25% in HIV-infected individuals. CD_4 T-lymphocyte counts are below 200 cells/mm^3 in most cases, but may be as high as 500 cells/mm^3. In one study 48% of patients developed AIDS within 16 months of onset of OHL, and 83% within 31 months (110). The lesions usually involute during treatment with effective antiretroviral therapy (111).

Grayish-white, corrugated, shaggy rug-like, verrucous plaques are located continuously or discontinuously along the lateral margins of the tongue (Fig. 25.37). These

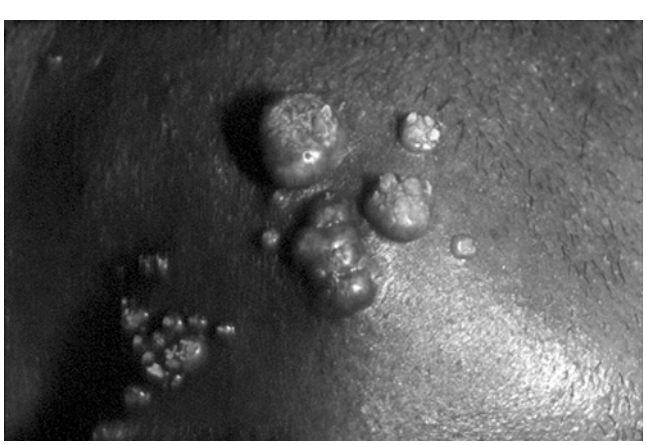

FIG. 25.36. Typical lesions of mim contagiosum consisting of skin-colored, dome-shaped umbilicated papules with central opening (umbilication) from which exudes keratotic material.

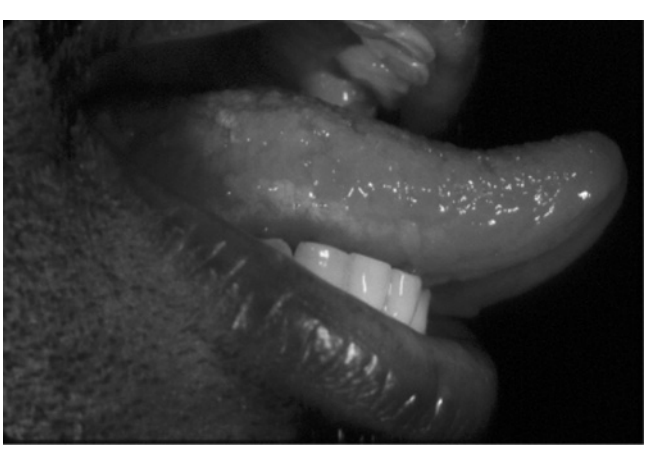

FIG. 25.37. Macerated, gray-white, planar papules confluent into linear corrugated plaques along the side of the tongue are the characteristic lesions of oral hairy leukoplakia.

glossal lesions may extend to the ventral and dorsal surfaces of the tongue. When present in the oral mucosa, gingiva or palate, the lesions are ßat without hairy projections, strongly resembling oral candidiasis. However, the surface cannot be removed by scraping.

The cause is believed to be reactivation of Epstein-Barr virus infection (EBV) (110). Multiple EBV strains can be detected in the lesions. The lesions may disappear spontaneously, presumably when cytotoxic T lymphocyte responses to Epstein-Barr virus are mounted, but may recur.

Although oral hairy leukoplakia is usually asymptomatic, some patients may seek treatment due to mild pain, uncomfortable sensation, taste alteration or unacceptable appearance. Oral acyclovir at high doses has been reported to achieve resolution within two weeks (112). Alternatively, daily application of tretinoin gel, cryotherapy or weekly application of trichloroacetic acid or podophyllin resin have also improved or resolved the lesions. Surgical excision is reserved for painful lesions, which are resistant to other therapeutic modalities (112).

Herpes Simplex Virus

As in the general population, in HIV-infected persons anogenital herpes is the second most common sexually transmitted disease seen (HPV infection is the most common one). A 60Ð80% seroprevalence of HSV-2 infection has been found among HIV-infected homosexual or bisexual men (113). In a study from England, herpetic genital ulcers were found in 43% and 38% of HIV-infected heterosexual women and men respectively (Fig. 25.38). As with other genitoulcerative diseases, especially primary syphilitic chancres, herpetic genital ulcers appear to facilitate the transmission of HIV due to the impaired epidermal barrier and the high density of HIV present in the CD_4 cells in the inßammatory inÞltrate of the ulcerations. Cervical shedding of HSV-DNA is detected in approximately 17% of HIV and HSV-1 or -2 co-infected women by cervical swab samples (114).

Herpetic lesions in HIV-infected patients are caused by reactivation of the latent virus. Although mucocutaneous lesions may appear in any region of the body, the most common sites are the genital, perianal area (Fig. 25.39), lips, oral cavity, and perilabial area (Fig. 25.40). Chronic ulcerations have appeared on the scalp, ears, feet and other unlikely body areas. Necrotic herpetic whitlow has caused destruction of the tip of the digit of some patients. The typical manifestation of herpes simplex is small clusters, each consisting of grouped vesicles on an erythematous base. The vesicles rupture and become small shallow crusted ulcers, which almost always heal without scarring within one to two weeks even in the absence of treatment. The diagnosis of herpes simplex infection can be conÞrmed with Tzanck preparations of a blister roof or the active edge of an ulcer in chronic cases, or by viral culturing or skin biopsy and monoclonal antibody staining of tissue.

As immune deÞciency progresses, clinical reactivation becomes more frequent. Severely immunocompromised patients with CD_4 counts below 100 cells/mm^3 develop erosive, persistent, hypertrophic plaques or ulcers which gradually expand, become progressively destructive, shed virus persistently, and do not heal unless treatment is instituted (2).

Oral acyclovir at a dose of 400 mg three to Þve times a day is usually effective. Intravenous acyclovir at a dose of 5 mg/kg every eight hours, is the treatment of choice for widespread or disseminated lesions. Valacyclovir (500 mg

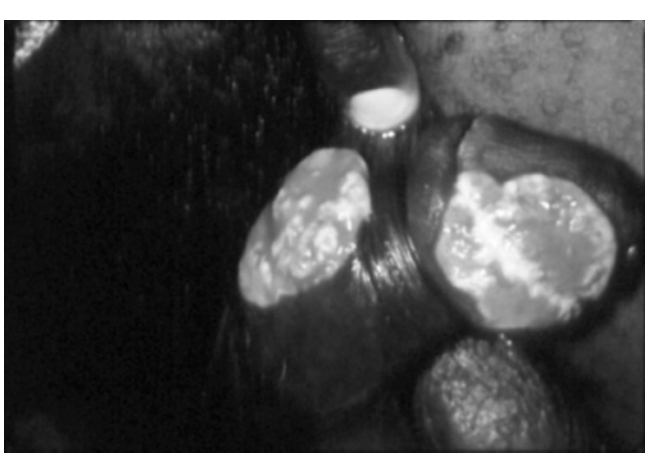

FIG. 25.38. Sharply demarcated, mutulating ulcerations with brinous exudate on the penile shaft due to herpes simplex type 2.

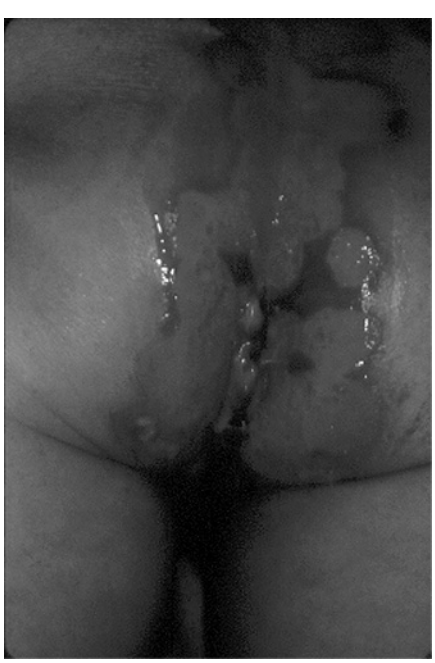

FIG. 25.39. Super cial, weeping, erythematous ulceration with well-de ned but active borders on the anal-perianal area extending towards the sacral and perineal regions caused by herpes simplex.

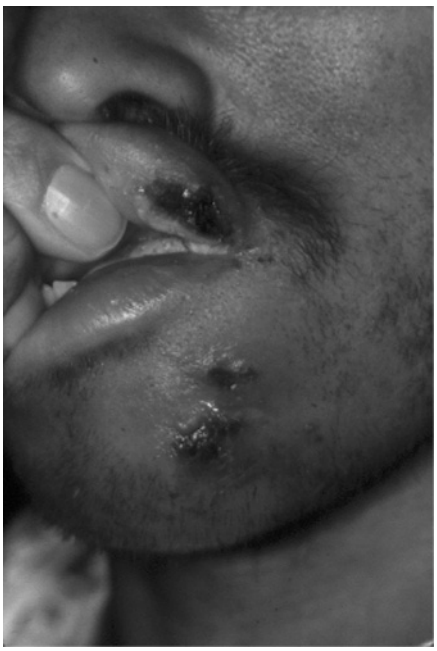

FIG. 25.40. Herpes simplex ulcerations surrounded by erythema and covered with serosanguinous crust on the chin and inner lip.

twice daily) and famciclovir (250 mg twice daily) are also useful in suppressing symptomatic and asymptomatic HSV shedding and recurrences. HSV-2 shedding is decreased 81%, and the frequency of genital signs and symptoms is decreased 65% in patients treated with suppressive doses of famciclovir (115). Thrombotic microangiopathy was reported in immunocompromised patients treated with 8 g of valacyclovir intravenously daily, but this complication does not occur at lower doses of this medication. Resistance to acyclovir, caused by mutations in the viral thymidine kinase or DNA polymerase genes, is found in 6.4% of HSV cases. Resistant strains are more common in patients who receive therapeutic rather than prophylactic antiviral therapy, but may also be found in patients who have never been treated with acyclovir. These cases usually respond to treatment with intravenous foscarnet, cidofovir or vidarabine. Treatment with intravenous foscarnet at a dose of 40 mg/kg every eight hours, should be initiated within 10 days of the onset of the eruption in patients suspected to have acyclovir-resistant herpes simplex infection and should be continued for at least 10 days or until all lesions are completely healed (116). Daily application of cidofovir 1% and 3% gel for at least Þve days has been effective in improving and resolving cases of thymidine kinase inhibitor-resistant herpes (117). A similar preparation can be made by compounding cidofovir solution in a cream vehicle such as Dermovase. The lesions of resistant HSV infection may heal with application of topical foscarnet 2.4% twice daily (118).

Several studies have documented a stimulation of HIV replication following acute or reactivated herpes simplex infection. Because of possible accelerated progression to AIDS in the presence of HSV, some experts recommend suppressive doses of acyclovir to individuals who have frequently recurrent herpes outbreaks. Although HSV outbreaks may be less frequent and less severe in patients taking highly active antiretroviral therapy, these patients continue to have clinical disease and asymptomatic viral shedding.

Herpes Zoster

Herpes zoster infection (shingles) is a reactivation of the varicella zoster virus (VZV), which was dormant in the dorsal root ganglia following the initial episode of varicella (119). The infection is seven times more common in HIV-infected persons and tends to be more widespread, severe and prone to dissemination (120). It may be the harbinger of an underlying HIV infection (119).

Most patients experience localized tingling, burning pain or itching, two to three days prior to the appearance of lesions. The eruption consists of grouped erythematous macules and papules progressing Þrst to vesicles (12 to 24 hours) then pustules (three to four days) and Þnally crusted erosions (seven to ten days) arising in a characteristically unilateral, linear, dermatomal (zosteriform) distribution (Fig. 25.41). Pain can be intense and has been described as throbbing, aching, or sharp and burning like Òelectric jabs.Ó In immunocompromised patients hematogenous dissemination may result in involvement of multiple

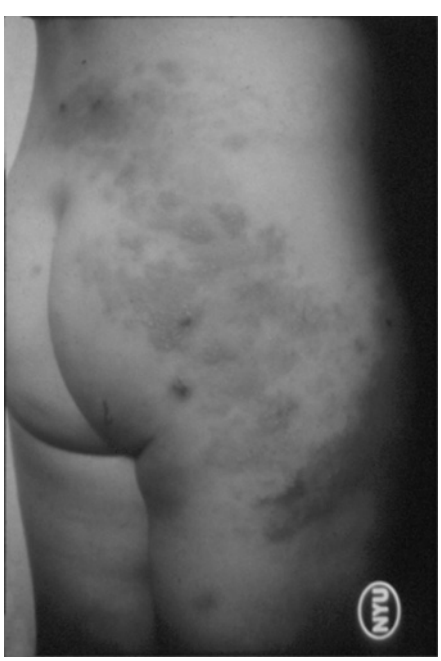

FIG. 25.41. Grouped blisters on erythematous patches linearly distributed along a skin dermatome typical of herpes zoster infection.

dermatomes, and potentially fatal systemic disease, including encephalitis, hepatitis, or pneumonitis. Cutaneous dissemination is deÞned as more than twenty vesicles outside the primary and immediately adjacent dermatomes.

The appearance of lesions on the nose (Hutchinson's sign) indicates involvement of the nasociliary branch of the ophthalmic nerve and requires evaluation for conjunctivitis, keratitis, corneal ulceration, iridocyclitis, and glaucoma. Involvement of the geniculate ganglion is associated with peripheral facial nerve weakness and deafness (Ramsay Hunt syndrome).

Treatment should be started early with famciclovir (500 mg three times daily), valacyclovir (1,000 mg three times daily) or acyclovir (800 mg Þve times daily). Disseminated infection is treated with intravenous acyclovir 10 mg/kg or 500 mg/m^2 every eight hours. Acyclovir resistant cases are more prevalent in the immnocompromised host and are treated with foscarnet, 60 mg/kg q 8 h infused slowly over at least 1 h (116). Studies indicate that these antivirals diminish scarring and reduce the risk of dissemination (121). One study showed that epidural administration of bupivacaine and methylprednisolone was signiÞcantly more effective in preventing neuralgia at 12 months than intravenous acyclovir followed by high dose prednisolone (122).

Postherpetic neuralgia, a common complication, is treated with applications of topical lidocaine patch or capsaicin, intralesional corticosteroids, nerve blocks, and oral analgesics, opioids, tricyclic antidepressants, carbamazepine or gabapentin.

Cytomegalovirus (CMV)

Between 75Ð100% of HIV-infected patients are infected with CMV and active CMV infection occurs in 20% of those with CD_4 counts below 100 cells/mm^3. While the skin is not commonly involved, mucocutaneous lesions may develop even in the absence of other manifestations, such as chorioretinitis, esophagitis, and enterocolitis. Still, the presence of CMV lesions on the skin usually indicates concomitant generalized infection.

The most common lesions are ulcers and erosive erythematous papules localized on the perianal, genital, perigenital areas or oral cavity, usually as part of polymicrobial infections, particularly herpes simplex and varicella-zoster virus. In these areas, the ulcers are usually round or oval, small, and have erythematous, weeping bases, occasionally with exhuberant granulation tissue (Fig. 25.47). However, the manifestations of CMV infection are protean and include large painful ulcerations, erosive vesiculobullous lesions, or chronic, asymptomatic or pruritic hyperkeratotic papules and plaques resembling prurigo nodularis on the extremities. The diagnosis is established with skin biopsy. Many inclusion bodies which stain immunohistologically with anticytomegalovirus antibodies, are present around dermal blood vessels. On electron microscopy, many viral particles are found in the areas with inclusion bodies. Treatment consists of intravenous ganciclovir, valganciclovir, cidofovir or foscarnet.

MALIGNANT NEOPLASMS

Kaposi's Sarcoma

Up until 1981, almost all cases of Kaposi's sarcoma in the United States were of the "classic" type, which affected elderly men and women of Eastern European and Mediterranean ancestry in a ratio of twelve males to one female (123). In general, these patients developed few slowly-growing lesions on their lower extremities and the disease had a rather indolent course. The most common complication was venous stasis and lymphedema of the involved lower extremities; visceral involvement occurred in approximately 10% of patients. A fulminant form of Kaposi's sarcoma seen in young, previously healthy homosexual men with rapidly progressive mucocutaneous and visceral lesions was Þrst recognized in 1981. That observation heralded the AIDS epidemic. Remarkably, few cases of Kaposi's sarcoma have been seen in non-gay HIV-infected individuals who contracted HIV infection through intravenous drug use, heterosexual sexual intercourse, or blood transfusions.

Patients with AIDS-related Kaposi's sarcoma present with mucocutaneous lesions consisting of pink, violaceous or deep purple, usually nontender patches, plaques, exophytic nodules and subcutaneous nodules, often with symmetrical distribution (Figs. 25.42, 25.43). Although

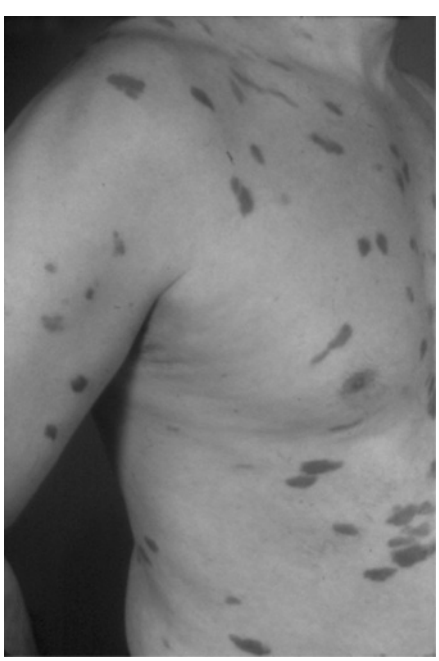

FIG. 25.42. Typical Kaposi's sarcoma violaceous papules and plaques oriented along skin lines of the trunk, neck and upper extremity.

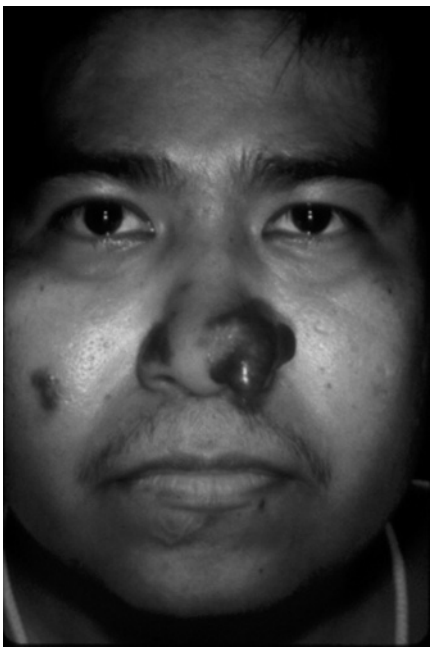

FIG. 25.43. Purple in ltrated oval plaques and a large dis guring nodule on the nose of a man with Kaposi's sarcoma.

early patches can blanch after direct pressure, the lesions soon become non-blanchable (124). In patients with dark skin, the tumors are hyperpigmented (Fig. 25.44). The most commonly affected areas are the head (tip of nose, periorbital area, ears, scalp), trunk, penis, legs, palms, soles, and oral mucosa (Fig. 25.45) (125). Approximately

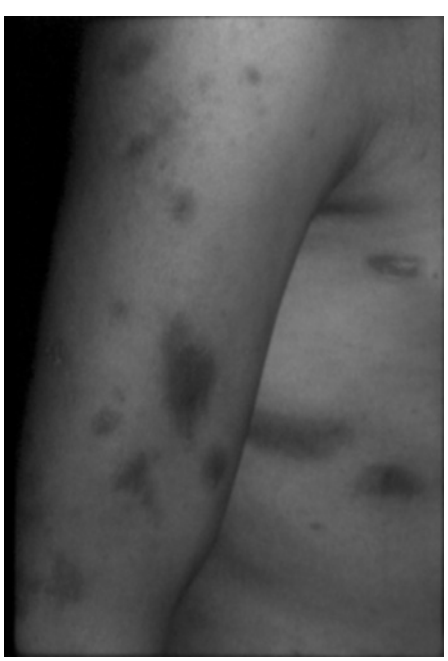

FIG. 25.44. In persons with dark skin shades Kaposi's sarcoma plaques and nodules are often dark-brown and may lack a violaceous hue.

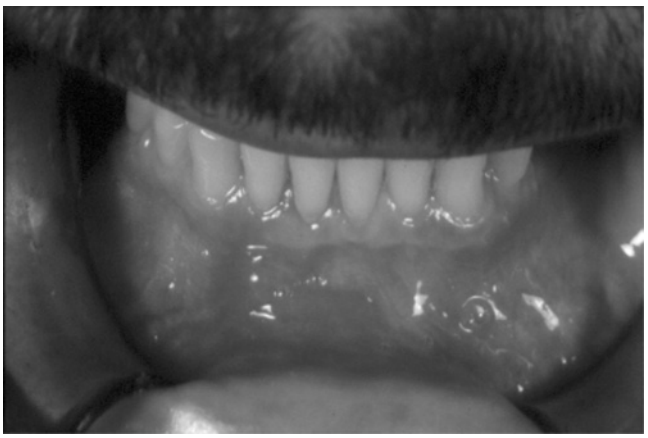

FIG. 25.45. Brightly red nodule of Kaposi's sarcoma in l-trating the gingiva.

80% of patients eventually develop visceral involvement in the gastrointestinal tract (75%), lymph nodes (30%), pulmonary, liver, urogenital tract, kidney and other organs. The course is progressive and disseminated with systemic involvement. In this country, approximately 95% of all the cases of this epidemic type of Kaposiõ sarcoma have been diagnosed in homosexual or bisexual men. However, in Central Africa AIDS-related Kaposiõ sarcoma is commonly seen in heterosexual men, women and children with HIV infection. In contrast, in Southeast Asian countries, such as Thailand, Kaposiõ sarcoma is extremely rare, even in HIV-infected gay men.

Kaposiõ sarcoma was the presenting manifestation in 40% of gay men with AIDS from New York and California in 1981. In the early years of the AIDS epidemic, approximately 26% of all HIV-infected homosexual men but only 3% of heterosexual intravenous drug users developed Kaposiõ sarcoma at some point during the course of their illness (125). Following widespread treatment with highly active antiretroviral therapy, the adjusted incidence declined from 15.2 in 1992 through 1996 to 4.9 in 1997 through 1999. However, Kaposiõ sarcoma remains the most common AIDS-associated malignant neoplasm in the world (124). Even during its peak prevalence, patients usually died from other opportunistic infections rather than from the effects of Kaposiõ sarcoma. The exception was patients with pleuropulmonary Kaposiõ sarcoma, who without treatment died in an average of six months.

Epidemiological evidence strongly suggests that a novel virus discovered in 1994 known as the Kaposiõ sarcoma-associated herpesvirus (KSHV) or Human Herpesvirus-8 (HHV-8), causes Kaposiõ sarcoma (126). Fragments of this virus have been detected by PCR in more than 95% of Kaposiõ sarcoma lesions. Furthermore, serum antibodies are detectable in 70Ð90% of all patients and almost 100% of immunocompetent patients with Kaposiõ sarcoma

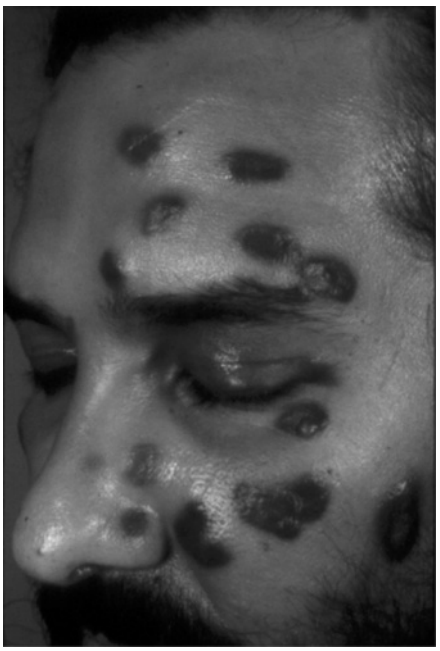

FIG. 25.46. Dark-purple in ltrated plaques resulting in marked facial dis gurement. Note a bright red dome-shaped Kaposi's sarcoma nodule resembling bacillary angiomatosis.

(127). The virus is recoverable from blood, saliva and semen (128).

Due to intense dread of disÞgurement and social discrimination associated with the readily noticeable lesion of KaposiÕs sarcoma, treatment is sought by many HIV-infected patients, even when the nature of the disease is not life-threatening (Fig. 25.46).

The type of treatment depends on the location, size, and extent of the tumors. Intralesional vinblastine or localized cryotherapy using liquid nitrogen are effective for small, superÞcial mucocutaneous lesions. An overall response rate of 87% has been reported in thin tumors treated with cryosurgery for a variable duration of thaw time depending on the depth of the lesion. Usually there is signiÞcant cosmetic improvement, but the treatment may not eradicate neoplastic cells in the deep reticular dermis, resulting in a high rate of recurrences. A dose of 0.1mg/cm^2 of vinblastine is injected into each tumor. The overall response rate in appropriately selected lesions is 70%, however partial regression rather than complete resolution is more common. Side effects with both of these treatment modalities include mild to severe local inßammation, ulceration, pain, and pigmentation. Application of 0.1% Alitretinoin gel (9-cis-retinoic acid, Panretin) to the tumors for four to eight weeks results in a 34% overall response rate in patch stage lesions (129). Dermal irritation is common but generally mild to moderate in severity.

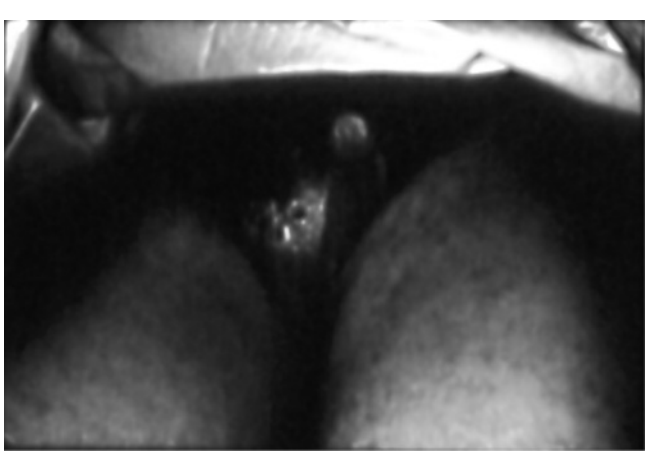

FIG. 25.47. Well-demarcated circular, discrete ulcerations with raised borders and necrotic slough secondary to cytomegalovirus infection.

Although KaposiÕs sarcoma lesions are very responsive to radiotherapy, this treatment is avoided due to concern about chronic radiation dermatitits now that the lifetime of patients with AIDS has been extended by more effective antiretroviral treatment combinations (130). Radiotherapy is especially discouraged in the oral mucosa where it can cause painful residual mucositis. Surgical excision is practical when one or few lesions are present. There was an overall response of 91% (complete response rate, 80%) in 36 patients with tumors of the feet treated with a fractionation schedule of three fractions a week at 3.5 Gy per fraction to a total dose of 21.0 Gy.

Systemic chemotherapy is used to treat extensive or symptomatic visceral involvement and widespread, disÞguring or disabling mucocutaneous disease. Regression and resolution of both cutaneous lesions and visceral tumors follows initiation of effective antiretroviral therapy.

Either liposomal encapsulated doxorubicin (Doxil) at a dose of 20 mg/m^2, liposomal daunorubicin (DaunoXome) at a dose of 40 mg/m^2, or paclitaxel (Taxol) at a dose of 100 mg/m^2 every two weeks or 135 mg/m^2 every three weeks, are more effective and less toxic than previous chemotherapeutic regimens, which included combinations of adriamycin, bleomycin and vincristine (ÒABVÓ) (131,132). The most common adverse effect of liposomal doxorubicin is neutropenia. Liposomal doxorubicin achieves responses in up to 59% of treated cases. Even in previously refractory cases, paclitaxel achieves objective response rates of 59Ð71%, which persist for a median duration of approximately 10 months (132,133).

Recombinant interferon alfa-2a and interferon alfa-2b result in a 40% objective response rate in patients with epidemic KS with CD_4 lymphocyte counts above 200 cells/mm^3, with lower response rates in more immunosuppressed patients (134). Compared to chemotherapy, the response is indolent. Interferon-alfa in doses of 1 to 18 million units may be given in combination with aggressive antiretroviral therapy. However, the side effects of interferon have reduced the use of this drug in favor of Doxil.

One study suggested that low does (1 million units daily) and moderate dose (10 million units daily) interferon produce similar response rates when each is combined with antiretroviral agents.

OTHER NEOPLASMS

Epidermal skin cancer

Both extra-anogenital squamous cell carcinomas (SCC) and basal cell carcinomas (BCC) appear to be more prevalent and more aggressive in HIV-infected persons (Fig. 25.48). Risk factors include fair skin, actinic damage, and immunosuppression. As patients live longer with AIDS, the number of HIV-infected cases with ultraviolet radiation-induced skin epidermal skin cancer is expected to increase. Some HIV-infected patients develop multiple and occasionally synchronous basal cell carcinomas (Fig. 25.49) (135). The typical appearance of basal cell carcinoma is a dome-shaped pearly papule or nodule with a tendency toward slow enlargement and central ulceration. Telangiectasias are common. SuperÞcial spreading basal cell carcinoma, a variant that is common in HIV-infected persons with multiple tumors, appears as an erythematous patch with thin pearly borders.

Squamous cell carcinomas have a variety of morphologies, but usually an erythematous, scaly patch or plaque, or an ulcer are the most common lesions. In contrast to the usual appearance of these tumors on sun-exposed areas, especially the face, SCC arises more commonly on the scalp while BCC occurs more commonly on the trunk.

Although it is common practice to treat basal cell carcinomas with electrodessication and curettage or surgical excision, the recurrence rate with these modalities in HIV-infected persons is 5.4% for tumors followed for more than 12 months (135). The rate of recurrence for squamous cell carcinomas after curettage and electrodessication is 20% (135). Therefore, MohsÕmicrographic surgery is the treatment of choice for these neoplasms. Photodynamic therapy using tin ethyl etiopurpurin has been used successfully in cases of multifocal basal cell carcinomas. Experience with topical treatments, such as imiquimod and 5-ßuorouracil, is limited.

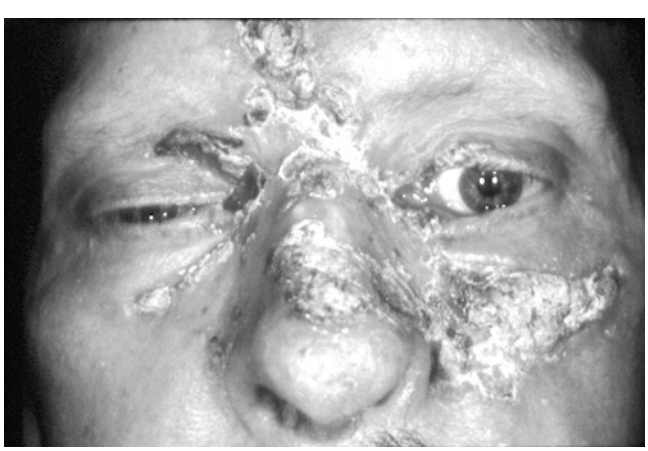

FIG. 25.48. Unusually aggressive and rapidly growing basal cell carcinoma invading nose and eyes.

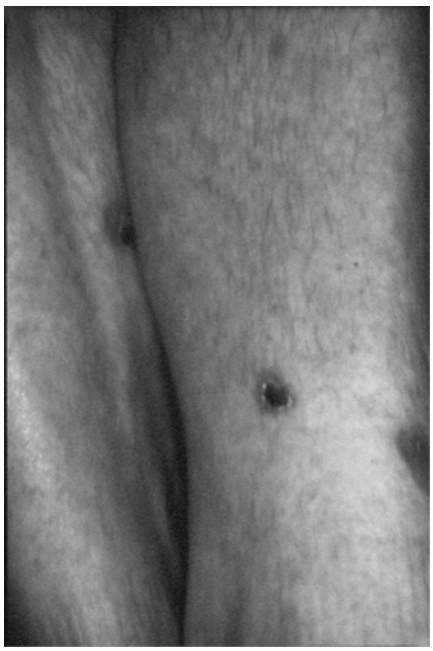

FIG. 25.49. Multiple super cial spreading basal cell carcinomas on the lower extremities.

Melanoma

Although epidemiologic conÞrmation is lacking, the incidence of malignant melanoma appears to be increased in HIV-infected persons. Available data suggest that the prognosis is graver for HIV-infected men than for immunocompetent HIV-seronegative men for melanomas with similar thickness, especially for tumors thicker than 3 mm (135). Cases with multiple melanomas have been reported. In one series of twelve cases of melanoma, six patients presented with metastatic disease (135). The mean age of the HIV-infected men at the time of diagnosis is 35.6 years. The number of CD_4 T-lymphocyte correlates inversely with melanoma thickness and prognosis (135).

Cutaneous Lymphoma

In HIV-infected patients, cutaneous lymphomas are more aggressive, are usually diagnosed at a more advanced stage, respond less favorably to therapy and more frequently involve extranodal sites. Although most lymphomas are of the B-cell type, cutaneous T-cell lymphoma

(CTCL) has also been reported. In one large recent survey of nine HIV-infected patients, seven had B-cell lymphomas, one had an HTLV-I-positive CD4+ T-cell lymphoma, and one patient infected with HIV-2 had a CD4+ T-cell lymphoma that was HTLV negative (136).

On the skin, the typical presentation of B-cell lymphomas is one or more discrete ulcers, nodules or ulcerated plaques or nodules (Fig. 25.50). In some cases, the immunohistologic pattern suggest a T-cell proliferation, and the correct diagnosis is only established after gene rearrangement studies demonstrate a clonal B-cell population.

HIV-infected patients with cutaneous T-cell lymphomas have a dismal prognosis, because intact immunity is an essential factor in preventing progression of the disease. In late stages of lymphoma, profound T-cell deficits compound those resulting from AIDS to increase the patient's susceptibility to opportunistic infections, the most common direct cause of death from both diseases. The clinical findings range from skin colored, dry-looking patches with fine scale, to plaques, to nodules, to exfoliative erythroderma (138). The lesions may be pruritic.

Some patients with inflammatory diseases manifest erythematous plaques and even erythroderma with lymphadenopathy, and have been misdiagnosed as CD8+, suppressor cutaneous T-cell lymphomas (137). In advanced HIV infection with CD_4 lymphopenia, inflammatory processes may be result in a massive cutaneous influx of CD8+ T-lymphocytes which result in histopathologic patterns which mimic those of cutaneous T-cell lymphoma. However, Southern blot and polymerase-chain-reaction (PCR) studies show that these lymphocyte aggregations have polyclonal patterns. Despite the benign nature of the infiltrate, the prognosis in these patients is poor.

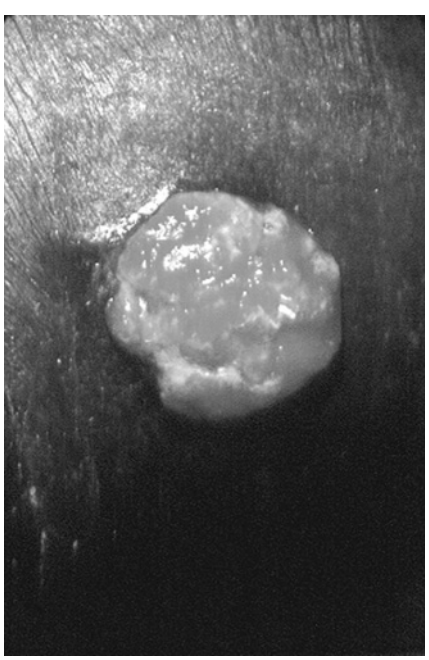

FIG. 25.50. B-cell lympoma presenting as an exophytic ulcerated nodule with exuberant granulation tissue.

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Chapter 26

Ophthalmologic Aspects of HIV Infection

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Ever since the original description of eye changes by Holland et al. (1) in 1982, it has become evident that ocular manifestations are often seen in patients with the acquired immunodeficiency syndrome (AIDS). Such conditions are generally classified into five areas: (1) a non-infectious microangiopathy, most often seen in the retina, and sometimes called "AIDS retinopathy;" (2) opportunistic ocular infections, particularly cytomegalovirus (CMV) retinitis; (3) conjunctival, eyelid, or orbital involvement by those neoplasms seen in patients with AIDS (e.g. Kaposi's sarcoma and lymphoma); (4) neuro-ophthalmic lesions; and (5) drug-induced manifestations (Table 26.1). The retinal microangiopathy is the most frequent ocular manifestation, and CMV retinitis is the most frequent opportunistic intraocular infection. Recent advances in the treatment of human immunodeficiency virus (HIV) infection, specifically the development of highly active antiretroviral therapy (HAART) (2–5), have altered the frequency and management of opportunistic ocular infections (6–8). HAART has been shown to increase CD4+ T-cell counts (3,5,9,10), decrease HIV viral load, (5,9) and increase survival (6,8,9,11,12). Even though HAART has decreased the incidence of opportunistic infections in the developed world, ocular complications of AIDS continue to occur, albeit at a decreased rate.

AIDS RETINOPATHY

"AIDS retinopathy," also known as "HIV retinopathy" "noninfectious HIV retinopathy" or "HIV-related retinal microangiopathy syndrome," has been the most frequent form of ocular involvement reported in patients with AIDS. Multiple series (1,13–30) from the pre-HAART era have reported an abnormal eye exam in 35% to 100% of patients with AIDS. In the Johns Hopkins series of 754 patients with AIDS from the pre-HAART era (31), retinopathy was present in 51% of the patients. It generally is asymptomatic and there is no indication for treatment (29,32).

Ophthalmoscopic features of HIV retinopathy include cotton wool spots, intraretinal hemorrhages, and less frequently, perivascular sheathing. Cotton wool spots are microinfarcts of the nerve fiber layer of the retina caused by ischemic disruption of axonal transport. Axonal swelling occurs which produces these characteristic white, opaque lesions. Cotton wool spots (Fig. 26.1) are the most common feature of HIV retinopathy and in the pre-HAART era were reported in 22–92% of patients with

TABLE 26.1. *Ocular complications of aids in the pre-Haart era (modified from jabs)*

HIV Retinopathy	50%
Cotton Wool Spots	46%
Intraretinal Hemorrhages	10%
Opportunistic Infections	
Cytomegalovirus retinitis	37%
Toxoplasmic retinitis	1%
Varicella Zoster retinitis	< 1%
Pneumocystis carinii choroiditis	< 1%
Fungal retinitis or endophthalmitis	< 1%
Infectious keratitis	< 1%
Kaposi's sarcoma	
Conjunctival	1%
Cutaneous lid	2%
Orbital Lymphoma	< 1%
Neuro-ophthalmic lesions	6%
Drug-induced manifestations	unknown

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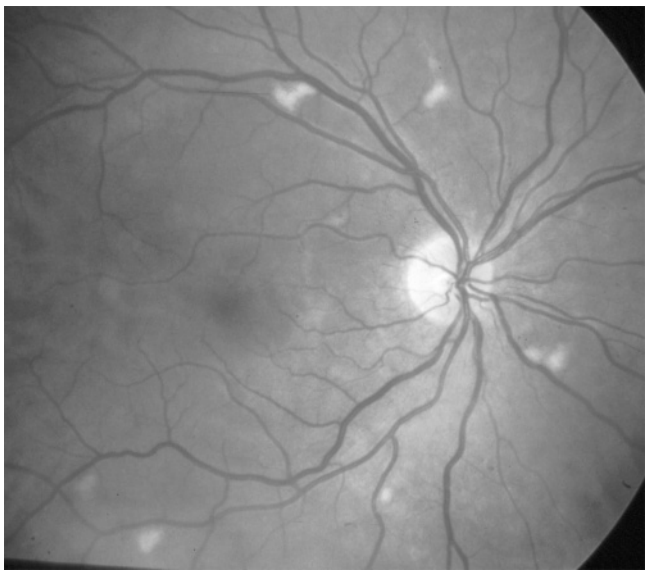

FIG. 26.1. AIDS retinopathy (cotton wool spots).

AIDS, with most series reporting that over 50% of patients were affected (14,16,18–21,23,26,27,30). Intraretinal hemorrhages were less frequent and were reported in 0–54% of patients, with most series reporting their presence in less than 20% of patients. In the Johns Hopkins series (31), cotton wool spots were present in 46% of patients and intraretinal hemorrhages in 10% of patients. Perivascular sheathing without an infectious retinitis has been reported (14,26) but is uncommon in the United States. Most often, perivascular sheathing is seen in association with CMV retinitis or other infections (26,33). Perivascular sheathing has been reported to occur in 15% of African patients with AIDS and 60% of African children with AIDS-related complex (ARC) (22,25). The reasons for the regional difference in perivascular sheathing are unclear.

Fluorescein angiographic and autopsy studies of AIDS retinopathy in the pre-HAART era showed evidence of a widespread microangiopathy. The ßuorescein angiographic study by Newsome et al. (19) found microaneurysms, telangiectatic vessels, and other microangiopathic changes in all twelve patients studied. A single case report (34) described signiÞcant visual loss associated with angiographic macular ischemia. The autopsy series of Pepose et al. (21) found evidence of a retinal microangiopathy in 89% of cases. Histologic Þndings in the retina in this and other series include loss of pericytes, microaneurysm formation, thickened vascular walls with deposition of periodic acid-schiff-positive material, and lumenal narrowing (19–21). Ultrastructural studies have shown swelling of the endothelial cells, occlusion of the vascular lumina, and thickening of the vascular basal lamina (35). These vascular lesions result in the microinfarcts that produce cotton wool spots.

Although retinopathy is common in patients with AIDS, it is uncommon in patients with asymptomatic HIV infection. In the Johns Hopkins experience from the pre-HAART era (31), retinopathy was present in 50% of patients with AIDS, 34% of patients with earlier but symptomatic HIV infection (formerly known as AIDS-related complex), and 3% of patients with asymptomatic HIV infection. It was not identiÞed in a cohort of non-HIV-infected homosexual men (26).

Retinopathy is more common in patients with lower CD4+ T cell counts (27). In the series of Spaide et al. (36), 93% of patients with cotton wool spots had CD4+ T cell counts less than 200 cells/µL. In the series of Kupperman et al. (28), 45% of patients with CD4+ T cell counts less than 50 cells/µL had HIV retinopathy. The mean CD4+ T cell counts in patients with cotton wool spots in these two series were 36 cells/µL and 31 cells/µL, respectively. Freeman et al. (27) also reported that patients with HIV infection and retinopathy have lower CD4+ T cell counts than do patients without retinopathy. These results suggest that the frequency of the retinopathy parallels the decline in the immune system.

In addition to the association of AIDS retinopathy with HIV stage and lower CD4+ T cell counts, other risk factors have been identiÞed, such as concurrent infections and method of HIV exposure. In one report (31), concurrent *Mycobacterium avium* complex infection was associated with the presence of HIV retinopathy with an odds ratio of 1.67. Age, race, sex, other opportunistic infection and concurrent neoplasia were not associated (31). In the series of Spaide et al. (36), cotton wool spots were more common in the group which acquired HIV through sexual contact compared to that group which acquired it through injection drug use (24% vs. 13%) despite similar CD4+ T cell counts.

Pathogenesis

The pathogenesis of AIDS microangiopathy is unknown and is likely to be multifactorial. Three hypotheses have been proposed: (1) immune complex disease; (2) infection of the retinal vascular endothelium; and (3) rheologic abnormalities.

The earliest hypothesis for the etiology of the microangiopathy was Type III hypersensitivity. Holland et al. (14) noted circulating immune complexes in 10 of 12 patients studied, and Gupta and Licorice (37) in 6 of 10 patients with AIDS and 8 of 10 patients with earlier but symptomatic HIV infection. Engstrom et al. (38) were unable to conÞrm this association, although they did report an association between circulating immune complexes and the conjunctival microvasculopathy present in some patients with HIV infection. Pepose et al. (21) demonstrated immunoglobulin deposition within arterial walls, and others (37,39) have reported that AIDS is characterized by polyclonal B cell activation and

hypergammaglobulinemia. Diseases such as collagen vascular diseases, which also have polyclonal B cell activation and circulating immune complexes, have an indistinguishable microangiopathy (19). Therefore, immune complex disease seems a plausible mechanism, although whether it is present in all cases is uncertain.

Direct infection of the retinal vascular endothelium by HIV or other pathogens has also been suggested as a cause of AIDS retinopathy but the evidence is conßicting. This hypothesis contends that the microbial insult to the vascular endothelium or the subsequent cascade of immunologic events results in the development of a vascular occlusion and cotton wool spot formation. HIV itself has been suggested to infect retinal blood vessels. Pomerantz et al. (35) have cultured HIV from the retina of patients with AIDS and have localized HIV proteins to the retinal vascular endothelial cells using immunohistochemical techniques. Shuman et al. (24) have reported electron micrographic evidence of HIV infection of the retina. Since the central nervous system is commonly affected by HIV (40Ð42), demonstration of HIV infection in the retina is not surprising. Conversely, Faber et al. (43) were unable to Þnd multinucleated giant cells in trypsin digests of autopsy retinas or immunohistochemical evidence of HIV-1 p24 antigen at sites of cotton wool spots. Gonzalez et al. were unable to Þnd an association between HIV DNA and cotton wool spots. Thus, direct involvement of HIV with cotton wool spot formation is debatable.

The evidence supporting infections other than HIV as being linked directly to cotton wool spot formation is similarly contradictory. Although Kwok et al. (45) reported one case of *Pneumocystis carinii* infection possibly causing cotton wool spots, multiple other studies have demonstrated no associated opportunistic organisms (19Ð21,26,43). *Pneumocystis carinii* infection has not been associated with cotton wool spots in any of the large autopsy series of eyes of patients with AIDS. Faber et al. (43) were unable to identify histologic evidence of CMV, *Toxoplasma gondii*, fungal infection, or *Pneumocystis carinii* in their autopsy series. More recently, however, CMV DNAÑ but not HIV DNAÑ was identiÞed in 90% of cotton wool spots in patients without CMV retinitis (46). That some patients with CMV retinitis never develop cotton wool spots may argue against viral pathogenicity (46). It has been suggested (46) that the vascular disruption at the site of the cotton wool spot merely facilitates entrance into the eye of the hematogenously spread CMV.

Lastly, systemic rheologic abnormalities have been suggested to be associated with AIDS retinopathy. Abnormal blood ßow has been identiÞed in patients with HIV (38,47,48). Engstrom et al. (38) found conjunctival changes consisting of blood column sludging, alterations in blood ßow, capillary dilatation, microaneurysms, and irregular vascular caliber. These changes, as well as cotton wool spots, were found to be associated with increased Þbrinogen levels. Some of these changes were found associated with anemia. It has been suggested that Þbrinogen, which is a large molecule, might induce red blood cell aggregation and sludging thus producing vascular damage. The vascular damage in the setting of anemia may then lead to ischemia and cotton wool spot formation. An increased frequency HIV retinopathy in patients coinfected with hepatitis C virus (HCV) has been attributed to increased serum viscosity and immunoglobulin deposition resulting from the hypergammaglobulinemia of HCV (30). The relative contributions of immune complex disease, direct infection, and blood ßow abnormalities to the development of HIV retinopathy remain to be determined.

OPPORTUNISTIC OCULAR INFECTIONS

Multiple opportunistic infections have been documented to infect the eye of patients with AIDS (Table 26.1). The most common of these is CMV retinitis, but other opportunistic ocular infections include herpes zoster ophthalmicus, *Pneumocystis carinii* choroiditis, varicella zoster retinitis, toxoplasmic retinitis, and fungal chorioretinitis. Multiple infectious agents can coinfect the same eye (31,49Ð52). The correct diagnosis and treatment may have life-saving beneÞts. In one autopsy series (51), 8% of patients with AIDS studied had an infectious choroiditis. Eighty-three percent of the patients with infectious choroiditis died due to dissemination of the same organism identiÞed in the choroid, yet only 27% of the patients had the diagnosis made before death (51).

Cytomegalovirus Retinitis

Cytomegalovirus is a ubiquitous herpes family virus of the Betaherpesvirus subfamily (53). Its four structural elements include an outer glycoprotein-containing envelope, an amorphous matrix (tegument), a nucleocapsid, and an internal core with linear double-stranded DNA (46,53). Approximately 50% of the general population have antibodies to CMV, suggesting previous exposure (53,54), while nearly 100% of homosexual men have antibodies to CMV (46,55). Transmission of CMV occurs by direct contact, and humans are believed to be the only reservoir for human CMV (53). Generally, CMV exists in a latent state; however, when the patientÕs immune system declines, CMV can reactivate. It is thought that CMV is hematogenously disseminated to the retina, invades the retinal cells, and establishes a productive infection. The autopsy series of Rao et al. (56) identiÞed the retinal vascular endothelial cells as the initial target of CMV infection in eyes with a clinical diagnosis of CMV retinitis. Over 90% of patients with AIDS and CMV retinitis will have positive cultures for CMV from a nonocular source, such as the blood or urine (57).

Epidemiology

CMV retinitis is the most common intraocular infection in patients with AIDS (26) and the most common end organ disease in CMV infection (58–60). In a multicenter observational cohort of 1,002 patients with CD4+ T cell counts less than 250 cells/μL, AIDS or earlier but symptomatic HIV infection, CMV retinitis developed in 85% of the 109 patients who developed CMV end organ disease (59). In the study by Hoover et al. (61), CMV end organ disease increased in incidence from 25–45% with the advent of routine prophylaxis for *Pneumocystis carinii* pneumonia (PCP) which has delayed the onset of AIDS defining illnesses in patients with HIV infection. Risk factors for the development of CMV retinitis include white race (31), sexual transmission (31,36), *Mycobacterium avium* complex infection (31), extraocular CMV disease (31), AIDS retinopathy (31), high HIV viral load (46), high CMV viral load (46), lack of protease inhibitor-containing anti-retroviral regimen (46), and low CD4+ T cell counts (36,60,62). Hoover et al. (60) reported that the incidence of CMV retinitis in the four years following a decline in CD4+ T-cell counts to less than 100 cells/μL was 25%. Pertel et al. (62) showed that at 27 months the incidence of CMV retinitis was 42% for patients with CD4+ counts less than 50 cells/μL, 26% for patients with CD4+ counts between 50 and 100 cells/μL and 15% for patients with CD4+ from 101–250 cells/μL. Approximately one-third of patients will present with bilateral ocular involvement and of those patients who present with unilateral disease, 60% will ultimately develop bilateral disease unless treated (57).

Estimates of the frequency of CMV retinitis in patients with AIDS from the pre-HAART era have varied from 6–38% (14–20,26,28,36,57,60,63) with higher estimates coming from surveys of inpatients and autopsy series (21,64). The variability in these estimates is probably due to a variety of factors, including differences in the patient populations studied, underdetection in non-ophthalmic series, referral bias in ophthalmic series, and the limitations of retrospective data analysis. Lower CMV retinitis incidences in more recent series have been attributed to the widespread use of HAART (2,8,65,66). Recognizing the potential for ascertainment bias, Jabs (57) estimated a minimum frequency of 11% among all patients with AIDS seen at the Johns Hopkins Hospital prior to the routine use of HAART (2). The reported frequency at that time among patients seen in the AIDS Ophthalmology Clinic at the same institution was 37% (31).

Estimates of the frequency of CMV retinitis since the advent of HAART are much lower than in the pre-HAART era. A 60–75% reduction in the incidence of new cases has been reported (67). At the Johns Hopkins hospital alone, the frequency of CMV retinitis has declined by over 50% (2). The University of California, Davis, similarly has reported a profound reduction in cases of CMV retinitis (65,68). This reduction has been mirrored internationally as well (66). In the series of Varani et al. (66), from Northern Italy, the frequencies of CMV retinitis, CMV visceral disease, and CMV reactivation in 1995 prior to the widespread introduction of HAART were 5.8%, 12.4%, and 38% respectively. Following the introduction of HAART in 1998, these incidences uniformly decreased to $< 1\%$, 1.5%, and 0% respectively (66).

The decline in the frequency of CMV retinitis following the use of HAART (11) has been attributed to the recovery of immunity to CMV load due to HAART-induced immune reconstitution (6). In the series of Deayton et al. (6), patients with preexisting CMV viremia identified by PCR, but without documented CMV-related end organ disease, were followed after initiation of HAART. All 16 patients became CMV PCR negative in a median time of 13.5 weeks (range 5–40) (6). Fourteen remained CMV PCR negative during a median follow-up of 21 months, and no patient developed CMV retinitis (6). In the report by Varani et al. (66), the frequency of a positive CMV antigenemia assay, an assay that quantitates the expression of CMV lower matrix phosphoprotein pp65, in patients with AIDS, declined from 25.9% in 1995 to 2.4% in 1998 following the widespread use of HAART.

Although it initially was suggested that CMV retinitis was a preterminal event (14), it has been recognized that CMV retinitis may occur at any time during the course of AIDS (57,69,70). The median interval from the diagnosis of AIDS to that of CMV retinitis in the pre-HAART era varied from 9–14 months, with ranges of 0 to 81 months (31,62,70). With the advent of the 1993 revised definition of AIDS by the Centers for Disease Control, which includes patients with CD4+ counts less than 200 cells/μL (71), almost all patients will have AIDS before the diagnosis of CMV retinitis.

CMV retinitis is associated with profound immunodeficiency (20,72), and prior to widespread use of HAART, very low CD4+ T-cell counts were characteristic of patients with CMV retinitis. Several series have reported mean CD4+ T-cell counts of less than 25 cells/μL (20,28,31,36,72). In the pre-HAART era, rare cases of CMV retinitis with CD4+ T cell counts greater than 200 cells/μL, existed (73), however, these cases have become less rare (4,74). Prior to widespread use of HAART, only 4% of patients with newly diagnosed CMV retinitis had CD4+ T-cell counts greater than 100 cells/μL compared to 14% following HAART acceptance (46).

CMV infection itself may potentiate the degree of immunosuppression in HIV-infected patients with CMV end organ disease. CMV has been shown to be a cofactor in HIV-I infection *in vitro*, possibly mediated through tumor necrosis factor alpha (75). Bilateral CMV retinitis has been associated with HIV encephalitis (43). Furthermore, patients with CMV retinitis have been shown to have a more rapid decline in their CD4+ T cell counts than patients without CMV retinitis (62).

Since CMV retinitis is associated with profound immunodeficiency, survival after the diagnosis of CMV

retinitis historically had been brief, in the range of six to thirty months (31,57,60,70,76–81) in the pre-HAART era. Survival had been shown to differ based on some regimens utilized for CMV retinitis (79,82), but not on other regimens (83). In one large prospective trial, a positive blood or urine culture at the time of diagnosis of CMV retinitis decreased median survival by five to seven months (84). Quantitative CMV PCR also has been shown to be predictive of mortality (85). With the advent of HAART, the potential for more significant longevity has been achieved.

Diagnosis and Natural History

Symptoms

The symptoms associated with CMV retinitis may be minimal or nonexistent. In one series of 62 patients who underwent routine ophthalmologic screening (86), 10% were found to have previously undiagnosed CMV retinitis. The CMV retinitis in almost half of these patients was bilateral and in more than half of the patients it was immediately sight threatening. None of the 14 patients identified with CMV retinitis in a medical HIV clinic were symptomatic, and almost 30% of these patients were found to have macular lesions (28). Eleven percent of the patients with CMV retinitis in the series of Pertel et al. (62) were asymptomatic.

One reason for the lack of symptoms is that the retinitis may start peripherally and spread circumferentially around the fovea thus sparing central vision until late in the course of the disease (87). Peripheral retinal lesions are typically larger than more posteriorly located lesions since they are less symptomatic (88). Another reason for a lack of patient symptoms is that patients often do not monitor the vision in each eye separately. Of the patients that are symptomatic, the complaints include a vague sense of blurred vision, floaters, photopsia (flashing lights), and constriction in the peripheral vision. For posteriorly located lesions, patients may complain of scotomata or a loss of vision. Because patients with advanced disease can be asymptomatic, routine screening of patients at high risk through dilated ophthalmoscopy is recommended.

Screening

Although large fluctuations in a patient's CD4+ T cell count have been identified (89), the CD4+ T cell count has been used as the basis for routine screening recommendations (2,62,67,86,90–93). In the series of Pertel et al. (62), the mean time from the first CD4+ count less than 50 cells/μL to the diagnosis of CMV retinitis was 13.1 months (range 0–40). It has been recommended that that dilated ophthalmoscopy be performed every four months for patients with CD4+ T cell counts less than 50 cells/μL, every six months for patients with CD4+ T cell counts between 50 and 100 cells/μL, and yearly for patients with CD4+ T cell counts greater than 100 cells/μL (2). The cost effectiveness of this approach, however, has never been validated. Patients with any symptoms potentially attributable to CMV should be seen by an ophthalmologist as soon as possible.

The utility of CD4+ T cell count-based screening recommendations has been influenced by the use of HAART since there is a time lag before protective immunity has been restored (2,4,7,29,94,95). A few cases of have been described in which CMV retinitis developed in the setting of CD4+ T-cell counts > 100 cells/μL early in the course of HAART (74). This incomplete immune reconstitution despite elevated CD4+ T-cell counts has been attributed to the preferential expansion of memory CD4+ T-cells compared to naïve CD4+ T-cells in patients who have had very low CD4+ T-cell counts (46). The naïve CD4+ T cells are thought to expand later in the course of immune reconstitution (46). Lymphocyte proliferative responses to recall antigens have been shown not to increase *in vitro* during the first year of antiretroviral therapy (9), and one patient with recurrent CMV retinitis with a CD4+ T cell count >200 cells/μL on HAART did not have a CMV-specific lymphocyte proliferation response despite having responses to other antigens (96). Thus, some patients who have lost CMV-specific clones may not be able to generate a significant immune response to CMV or other "new" antigens until later in the course of immune recovery. As a result of this HAART effect, a lag of several months may be required for a patient to develop the level of immune function predicted by their CD4+ T-cell count, and a single CD4+ T-cell count measurement in the early phase of immune reconstitution should be interpreted with caution.

Certain laboratory tests have been helpful in stratification of patients at risk for developing end organ disease from cytomegalovirus and may ultimately facilitate directed examinations or primary prophylaxis. Current investigations suggest that plasma CMV DNA (92,93), quantitative CMV PCR on plasma or white blood cells (85,93), quantitative hybrid capture (93), and CMV antigen (p. 65) (92,93) testing may predict the development of CMV-related end organ disease. Shinkai et al. (97) reported prospectively that qualitative PCR had a sensitivity, specificity, positive predictive value, and negative predictive value of 89%, 75%, 58%, and 94% respectively in identifying patients with CMV-related end organ disease. Quantitative PCR (QC-PCR) in the same study had a sensitivity, specificity, positive predictive value, and negative predictive value of 73%, 90%, 73%, and 90% respectively. Retinitis accounted for 73% of the end organ disease in this study; however, routine dilated eye examinations were not a part of the protocol suggesting that retinitis may have been underdiagnosed.

Other investigations similarly have shown a potential benefit of CMV PCR testing and have linked testing to

screening recommendations (93,98). In the Roche oral ganciclovir prophylaxis study (93), the risk of CMV retinitis was directly proportional to the baseline quantitative CMV viral load (93). The 12 month risks of CMV retinitis for patients with baseline CMV viral loads (in copies/ml) of zero, less than 2,500, 2,500–50,000, and 50,000–100,000 were 14%, 22%, 50%, and 75% respectively (93). Unfortunately, the sensitivity of this method has allowed detection of CMV in many patients who do not develop end organ disease (99). Furthermore, up to 25% of patients at the time of diagnosis of CMV retinitis may have negative PCR studies (93), and with the marked decrease in CMV retinitis due to HAART, such laboratory screening typically is not performed.

Blood and urine cultures also have been investigated regarding their ability to identify patients at risk for CMV-related end organ disease. In the prospective Cytomegalovirus Retinitis and Viral Resistance Study (CRVR) (84), patients who developed positive blood, urine, or blood and urine cultures at follow up had increased risk of CMV retinitis developing in the contralateral eye with odds ratios of 6.5, 6.4, and 5.7 respectively. Positive blood and urine cultures, and lower CD8+ T-cell counts in a separate large prospective trial (100) were associated with progressive retinitis despite therapy in previously untreated patients. The sensitivity, speciÞcity, positive predictive value, and negative predictive value of urine cultures were reported by Shinkai et al. (97) to be 85%, 29%, 31%, and 83% respectively, whereas the corresponding values for blood leukocyte cultures were 38%, 74%, 69%, and 81% respectively. Thus, although correlation with disease exists, the predictive value of blood and urine cultures is considered too low to be used as a basis for routine screening (58).

Other methods studied for their usefulness to predict those at highest risk of CMV end organ disease include CMV immune reactivity (53), conjunctival swabs for CMV (101), laser ßare photometry (102), and entoptic perimetry (103). None of these methods to date has supplanted CD4+ T-cell count based screening recommendations for the evaluation of asymptomatic HIV-infected patients for CMV retinitis.

Clinical Appearance

The diagnosis of CMV retinitis usually can be made by ophthalmoscopy by an experienced observer. The most characteristic feature of CMV retinitis is a yellowish-white area of retinal necrosis with a granular border that extends into the surrounding retina. Although CMV retinitis often has been described as hemorrhagic, hemorrhages may or may not be present. Lesions that have extensive necrosis and hemorrhage are often described as ÒfulminantÓ whereas those without hemorrhage are sometimes described as ÒgranularÓ or ÒindolentÓ (Fig. 26.2). Lesion location may be a factor in lesion appearance. In one large series (104), 51% of the lesions were considered fulminant, 45% granular, and 4% indeterminate. In that series, two-thirds of the fulminant lesions involved zone 1 (deÞned as the area within two disc diameters of the foveal center or one disc diameter of the optic nerve). Conversely, only 36% of granular lesions had zone 1 involvement. There was no difference in the rate of spread of the two lesion types (87,104); however, granular lesions have been associated with better visual acuity, presumably due to this lower frequency of macular involvement (104).

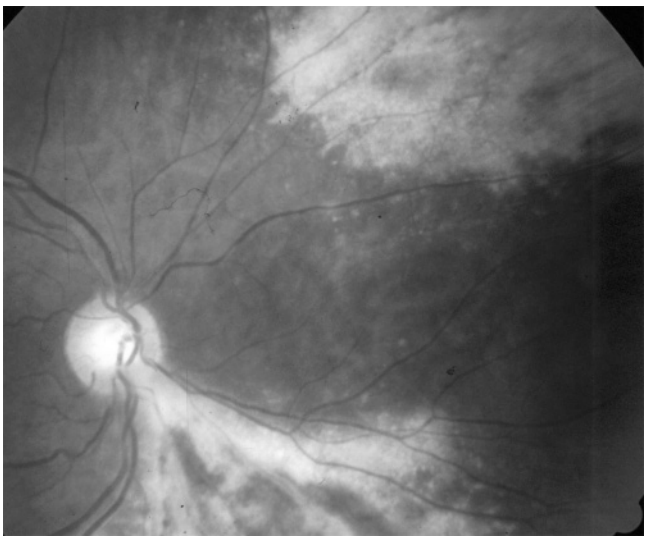

FIG. 26.2. CMV retinitis in a patient with AIDS. Note the granular (non-hemorrhagic) and fulminant (hemorrhagic) areas.

At times, CMV retinitis can be associated with mild anterior uveitis (90,105,106), elevated laser ßare photometry measurements (102), Þne refractile stellate corneal endothelial deposits (105), retinal periphlebitis (33), papillitis (64), disc neovascularization (107), exudative maculopathy (108), large vessel occlusion (108), and bilateral serous retinal detachments (109). Occasionally, a very early and small lesion of CMV retinitis may be difÞcult to distinguish from a cotton wool spot; however, follow up examination generally reveals the diagnosis since CMV retinitis will progress and enlarge over time, whereas a cotton wool spot will resolve. Rarely, CMV has been identiÞed as a cause of a necrotizing retinitis with vasculitis and vitreous inßammation indistinguishable from the acute retinal necrosis syndrome (110,111). The diagnosis in this and other atypical presentations of CMV retinitis can be conÞrmed through PCR studies of intraocular ßuid samples (99,110,111).

Natural History

Untreated CMV retinitis is a progressive and potentially blinding disease (57). Natural history series of CMV retinitis report progression of the disease in virtually 100% of patients (57,112). The borders of the infection harbor active CMV, and the retinitis characteristically spreads from the periphery of the process outward, reminiscent of a brush-fire, and such lesions are often referred to as "brush-fire" lesions. In the series by Holland et al. (87), 17 patients with untreated retinitis had mean border advancement of 24.4 μm/day (range 0–164 μm/day).

Clinical trials often evaluate a drug's efficacy by its ability to slow this advancement of the retinitis, generally measured as time-to-progression. Time-to-progression is the time for the retinitis to move a specified distance, and the definition of progression in several large series has been the movement of the border of retinitis by 750 μm over a span of 750 μm or the development of new lesions (79,80,104,113–116). Untreated patients typically are shown to have progression identified in 15 to 21 days when photographs are evaluated by a reading center (80,115,116). Time to progression as assessed by clinical examination is typically longer for treatment groups than that assessed by fundus photography reading centers (83,117).

Areas of retina previously infected with CMV show total destruction of the retinal architecture and replacement by a thin gliotic scar (21). Often there is hyperpigmentation of the scarred lesion or areas of atrophic retina with holes. Lipid and calcium may be deposited as well (119). Some areas of retinitis exhibit a "persistent white edge" (120) that does not advance but can be mistaken for active retinitis unless compared with prior photographs. Histopathology of one eye with a "persistent white edge" revealed necrotic, devitalized, nonviable cells without evidence of superinfection (120). The clinical picture of treated CMV retinitis where only an atrophic and gliotic scar can be detected and there is no evidence of an active process, is often called a "complete response" or "remission." (Fig. 26.3).

Effect of HAART

HAART has modified the clinical manifestations of CMV retinitis in some patients (4,7,29). Excessive inflammation and atypical features of CMV retinitis, previously described in patients with CMV disease from iatrogenic immunosuppression (121), have now become less rare in patients with HIV on HAART (4,7,8,29,74,122,123). In the series of Deayton et al. (8), of patients with CMV retinitis, 5% developed vitreitis and 4% developed cystoid macular edema (CME) following institution of HAART (8). Neither the vitreitis nor the CME in this series was related to anti-CMV treatment, CD4+ T-cell count, or HIV viral load (8). Bilateral optic disc neovascularization occurred in 2.4% of patients in the series of Curi et al. (122) as well as in at least one additional case report (123). These features were rarely identified in HIV-infected patients before the widespread use of HAART.

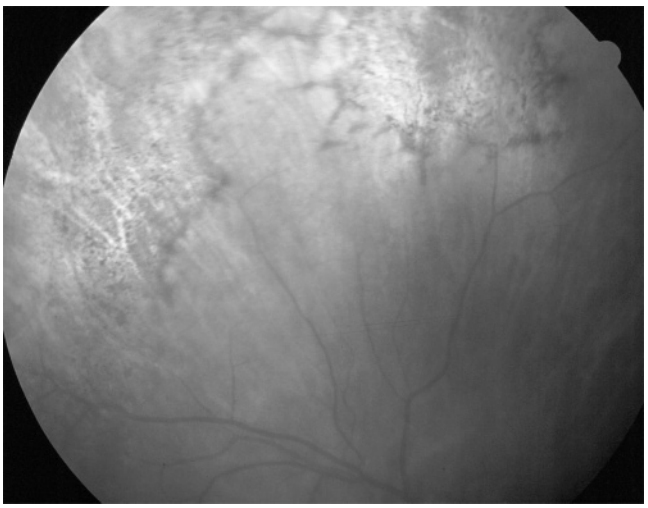

FIG. 26.3. CMV retinitis following successful treatment in a patient with AIDS.

Treatment

The goal of treatment of CMV retinitis is to arrest the progression of the disease, prevent further spread of infection in the eye, and maximize visual function. Treatment of CMV retinitis is generally performed in a two-step fashion. Initially a high dose of an anti-CMV drug is given to control the infection (induction therapy), and subsequently long-term lower dose therapy is given to prevent relapse (maintenance therapy). The phenomenon of relapse when an antibiotic is discontinued was characteristic of patients with AIDS in the pre-HAART era and of patients who do not have immune reconstitution from HAART. This phenomenon occurred in other infections such as *Pneumocystis carinii* pneumonia, cryptococcal meningitis, and *Toxoplasma gondii* encephalitis. Because relapse of CMV retinitis was frequently seen when induction therapy was discontinued, maintenance therapy became used early in the AIDS epidemic. Since maintenance therapy must be continued indefinitely unless there is immune reconstitution, placement of a permanent indwelling central venous catheter often is necessary for patients treated with intravenous therapy. Monitoring clinical response with high quality fundus photographs is important in order to recognize subtle progression of the retinitis (83,117,118).

Prior to HAART, cases of resolution of CMV retinitis without specific treatment for CMV were reported, but

were rare. Two case reports describe the resolution of CMV retinitis after the initiation of zidovudine (also known as azidothymidine or AZT) therapy in patients with AIDS (57,124). Since widespread application of HAART to patients with HIV disease, the frequency of arrested CMV retinitis in the absence of speciÞc CMV therapy has increased (4,68,92,125,126) and has led to recommendations regarding the cessation of anti-CMV maintenance therapy once immune reconstitution has occurred (92) (below). The efÞcacies of some treatments appear to have improved when given with HAART (74).

Intravenous Medication

Ganciclovir

The Þrst drug approved by the Food and Drug Administration (FDA) for the treatment of CMV retinitis in immunocompromised patients, ganciclovir, is still used. Ganciclovir (Cytovene, Roche Bioscience) was previously known as 9-(1,3-dihydroxy-2-proproxymethyl) guanine or DHPG. It is a nucleoside analog, which is taken up by the infected cell, phosphorylated by a viral phosphotransferase, and inhibits viral DNA replication through its effect on viral DNA polymerase (127,128). Autopsy studies of patients treated with ganciclovir have demonstrated the presence of viral DNA at the border of the lesion but the absence of a productive infection and the absence of intact virions (129).

Induction therapy with ganciclovir consists of 5 mg/kg every 12 hours intravenously for 14 to 21 days (10 mg/kg/day). There have been no reported differences between the 14 and 21-day induction courses. The dosing schedule recommended for maintenance ganciclovir is 5 mg/kg/day intravenously once daily (35 mg/kg/week), although 6 mg/kg/day, Þve of seven days (30 mg/kg/week), has been used in the past. Lower doses of maintenance and every other day dosing schedules were associated with unacceptably high rates of early relapse. Because ganciclovir is excreted in the urine, the dose must be adjusted for renal function.

Multiple series have reported the efÞcacy of ganciclovir for the treatment of CMV retinitis. Response rates have ranged from 80% to 100% (57,63,76,77,130ÐI135). Sixty to 80% of patients will achieve quiescence of the infection while on therapy (57,63,76,77,130ÐI135). In patients treated with ganciclovir, the median time to quiescence is 21 to 38 days (57,77). Prior to the use of maintenance therapy, patients given only induction ganciclovir often achieved quiescence of retinitis at one month after starting therapy only to subsequently suffer relapse of the active retinitis (130). This lag between the institution of therapy for CMV retinitis and the resolution of the ophthalmoscopic evidence of active disease represents the time required for areas of necrotic retinitis to resolve and leave a scar.

The most frequent side effect reported for ganciclovir is reversible granulocytopenia. Other side effects include thrombocytopenia, poorly characterized neurologic side effects, and abnormal liver function tests. Approximately one-third of patients receiving ganciclovir will experience granulocytopenia at sometime during the course of their disease (57,114) with an event rate of 1.30 per person-year at risk (79). The fall in the absolute neutrophil count (ANC) often occurs during or just after the completion of induction therapy with 46% of the events occurring within the Þrst month of treatment (114). Historically, physicians treating patients with ganciclovir needed temporarily to discontinue ganciclovir if the ANC fell below 500 cells/µL. Colony stimulating agents now available such as granulocyte colony stimulating factor (G-CSF, Þlgrastim) or granulocyte-macrophage colony stimulating factor (GM-CSF, sargramostim), however, have allowed ganciclovir therapy to continue by boosting the white blood cell count above this dose limiting cutoff. Before the widespread use of these agents, 16% of patients treated with ganciclovir were unable to tolerate the drug because of recurrent granulocytopenia. A later series reported that only 6.5% of patients treated with ganciclovir needed to change therapy due to this side effect (114). Thrombocytopenia occurs in approximately 10% of patients, and may also limit the dose tolerated. Other side effects rarely lead to discontinuation of therapy. Since an indwelling catheter is often placed, line-related complications occur (136,137). Thorne et al. (137) reported a rate of catheter complications of 1.2 complications per person-year or 0.33 complications per 100 catheter-days (137). In this series, mortality from the Þrst complication was 5.8%, 40% of patients required catheter removal, and 87% of these required a new catheter (137). The use of subdermal port catheters and current injection drug use increased the risk of catheter infections (137).

Two mechanisms of CMV resistance to ganciclovir have been identiÞed *in vitro*. The Þrst involves structural changes to the viral UL97 gene that encodes a viral phosphotransferase (53,138,139). Multiple mutations have been identiÞed (139). As a result, the ganciclovir, once taken up by the infected cell, is not activated through the initial phosphorylation. Point mutations and deletions have been identiÞed; however, CMV strains with more than one mutation in the UL97 gene were not more resistant than those strains with only one mutation (138). Resistant mutants with wild type UL97 genes have been identiÞed which led to the suggestion of an additional mechanism of resistance (138). This second mechanism has been related to changes in the *pol* gene coding for the viral DNA polymerase (gene UL54) (53,127,139). The identiÞed *pol* point mutation encodes a structurally altered DNA polymerase with decreased ganciclovir binding afÞnity (127). Consequently, the ganciclovir is activated through phosphorylation but cannot affect viral replication. As with UL97 mutations, multiple UL54 mutations have been identiÞed (139). Mutants with resistance from both mechanisms have been identiÞed *in vitro* (140,141). Smith et al. (141) characterized these mutants with resistance

from both mechanisms as high-level, and reported that they were associated with prior prolonged ganciclovir therapy.

The frequency of *in vivo* ganciclovir resistance has been characterized, and although low at time of diagnosis, it increases with duration of therapy (142). In the prospective series of Jabs et al. (143,144), 0.9% and 2.7% of baseline blood and urine culture isolates, respectively, showed ganciclovir resistance. After nine months of therapy for patients treated initially with ganciclovir, the frequency of blood or urine isolate resistance increased to 27.5%. In this series, the development of resistance correlated with the development of CMV retinitis in the contralateral eyes of patients initially affected unilaterally (143,144).

Foscarnet

The second drug reported to be effective against CMV retinitis is foscarnet (Foscavir, Astra Phramaceuticals) (128). Foscarnet, also known as trisodium phosphonoformate hexahydrate, is a pyrophosphate analog which, like ganciclovir, inhibits DNA polymerase in infected cells. Foscarnet is also used in a two-step fashion with the initial induction dose being 90 mg/kg/day every 12 hours (or 60 mg/kg/day every eight hours) for two weeks and the maintenance dose being 90–120 mg/kg/day. Some researchers have used three week induction courses (116); however, there have been no reports directly comparing the duration of induction therapy.

Preliminary studies have demonstrated a similar response rate with foscarnet as with ganciclovir, with 80–100% of patients showing some response to the drug (145–147). In a dose ranging study of patients with previously untreated CMV retinitis, Jacobson et al. (82) found that the median time to retinitis progression for patients treated with 180 mg/kg/day divided every eight hours for two weeks followed by maintenance of 90 mg/kg/day was 56 days by clinical exam and 31 days when evaluated by the more sensitive masked fundus photograph reading center. Higher dose maintenance was more effective in delaying relapse (82,148), but there was a trend toward a higher creatinine level and a higher incidence of proteinuria (82). In the randomized controlled clinical trial by Palestine et al. (116), mean time to progression was 13.3 weeks (93 days) by masked fundus photograph reading center for patients treated with 180 mg/kg/day divided every eight hours for three weeks followed by 90 mg/kg/day maintenance compared to 3.2 weeks (22 days) for patients in the treatment deferred control group.

In contrast to ganciclovir, foscarnet is not significantly bone marrow toxic. Its primary toxicities are renal and metabolic, which occur in 13% of patients (82,114,116, 149). In the series by Jacobson et al. (82), 9% of 54 patients were unable to tolerate maintenance foscarnet due to these adverse events. Nephrotoxicity is usually reversible (116). In a large prospective series with close monitoring, frequent dose changes based on renal function, and concomitant hydration, the incidence was only 13% at six months (114). Despite its renal toxicity, foscarnet has been used in a patient with renal failure (150).

The metabolic side effects of foscarnet include abnormalities of calcium, phosphorus, and magnesium. Often, supplementation will be required for hypocalcemia or hypomagnesemia. Paresthesias related to temporary metabolic aberrations are not uncommon. Infusion related nausea has been reported in 26–50% of patients (114,116). Uncommon side effects include oral and genital ulcerations (114,151–154), which generally resolve within two weeks of discontinuation of the foscarnet. Hygeine has been implicated as a factor in the genital ulcers since foscarnet is excreted in the urine and many of the affected patients were uncircumcised, however, the explanation of the oral ulcerations is less clear (153). Seizures, previously thought to be associated with foscarnet therapy, occurred at the same frequency in patients on ganciclovir or foscarnet in one large comparative trial (114); hence, seizures appear to be unassociated with foscarnet therapy.

Despite low baseline foscarnet resistance (142,155), resistance increases with treatment duration (155,156). In one large prospective study, blood and urine culture foscarnet-resistant CMV strains increased from 2.4% and 0.8% respectively at baseline to 22% and 3% respectively at six months with foscarnet therapy (157). As with ganciclovir, *in vitro* foscarnet resistance has been linked to *pol* gene mutations (158); however, a single *pol* mutation does not necessarily confer resistance to both drugs (127). Resistance to foscarnet and to both foscarnet and ganciclovir has been identified in clinical isolates (119,156,159, 160). One foscarnet resistant isolate was from a patient who had never been exposed to foscarnet (156). Dunn et al. (156) found that continued treatment with either foscarnet or ganciclovir was associated with an increase in the IC50 for that drug.

The relative efficacy and safety of ganciclovir and foscarnet were evaluated in the multicenter randomized Foscarnet-Ganciclovir Cytomegalovirus Retinitis Trial (FGCRT) (79,104,113,114), which showed no difference in median time to progression of the CMV retinitis. The FGCRT and other studies (80,82,149,161) identified a survival benefit in patients treated with foscarnet, which was attributed to inhibition of HIV-1 replication *in vitro* (162,163), as well as a synergistic (162) or additive (163) effect against HIV-1 reverse transcriptase when used with zidovudine. Unlike the combination of foscarnet and zidovudine which has been shown to be beneficial, ganciclovir has been shown to reduce the antiretroviral effect of zidovudine when HIV p24 antigen production, reverse transcriptase activity, and infectious viral yield were used as measures of HIV replication (164). Currently, the survival difference of foscarnet and ganciclovir

may be less important due to the increased number of available antiretroviral medications, their application via HAART, and the diminished concern regarding HIV resistance to zidovudine monotherapy.

Although survival was increased for patients randomized to treatment with foscarnet, treatments were changed in 36% of patients assigned to foscarnet and 11% of patients assigned to ganciclovir (79,104), indicating that foscarnet was not as well tolerated as ganciclovir. The rates of hypocalcemia, hypomagnesemia, hypophosphatemia, hypokalemia, nephrotoxicity, genital lesions, infusion related nausea, and infusion time were higher with foscarnet (114). The incidence of neutropenia was expectedly higher in the ganciclovir group (114). The incidence of catheter related infections (79), anemia (114), opportunistic infections (79), hospitalization (79), extraocular CMV (114), and seizures (114) were not signiÞcantly different between the two groups. In the era of HAART, the modest survival beneÞt of foscarnet therapy appears to have little signiÞcance.

Cidofovir (HPMPC)

In addition to ganciclovir and foscarnet, cidofovir or (S)-1-(3-hydroxy-2-(phosphonylmethoxy)propyl)cytosine has obtained FDA approval for the treatment of CMV retinitis. It is a long-acting nucleotide analogue, which does not require a viral-encoded phosphotransferase for its initial phosphorylation to cidofovir diphosphate (HPMPCpp), the active metabolite (165Ð167). Its target, like that of ganciclovir and foscarnet, is viral DNA polymerase (140), and altered DNA polymerase via *pol* gene mutation is one method of resistance to this agent (53,127,140,168,169). Mutations to DNA polymerase capable of causing ganciclovir resistance may show resistance to cidofovir (141,166,168). Prior ganciclovir and cidofovir use have been associated with resistance formation (141,170). As with ganciclovir and foscarnet, resistance increases with duration of drug exposure (157). In one large prospective study, 4.1% and 6.6% of baseline blood and urine cultures contained cidofovir resistant CMV strains respectively, but after six months of therapy, 29% of blood or urine cultures contained resistant organisms (155).

One advantage of cidofovir over ganciclovir and foscarnet is its long duration of effect which abrogates the need for, and risks of, chronic indwelling catheters (137,160,171). This long duration of effect has been attributed to the long intracellular half-lives of two of its metabolites, cidofovir diphosphate (17Ð65 hours) and cidofovir phosphate-choline (87 hours) (46,166,171). In a phase I/II study of patients with asymptomatic CMV excretion, negative semen cultures persisted for 37 days after the last dose of cidofovir (167). Human studies evaluating the effect of cidofovir on asymptomatic viral shedding showed efÞcacy at doses of greater than 3.0 mg/kg/week (167) or 5.0 mg/kg/week (165). Several studies have shown efÞcacy for both untreated and previously treated CMV retinitis (166).

The multicenter Phase II/III HPMPC Peripheral Cytomegalovirus Retinitis Trial (HPCRT) (81,83) which compared low dose (3 mg/kg) and high dose (5 mg/kg) intravenous maintenance injections to no treatment (deferred group) for previously untreated peripheral CMV retinitis showed that both doses were effective in delaying time to progression of retinitis. Each treatment group received the same induction dose of 5 mg/kg once weekly for two weeks, then the individual maintenance therapy doses every two weeks (83). The median time to progression in the low dose group was 64 days versus 21 days in the deferred group (83). The median time to progression in the high dose group was not reached but was 20 days in its corresponding deferral group (83). Although the median time to progression was longer in the high dose group compared to the low dose group, the study was not designed to compare the two dosing regimens, so such a conclusion could not be made (83). There was no difference in mortality among the three groups.

The multicenter controlled trial of cidofovir for previously treated relapsing CMV retinitis of Lalezari et al. (172), in which patients were randomized to receive induction cidofovir 5 mg/kg weekly for two weeks, then either 3 mg/kg or 5 mg/kg maintenance therapy every two weeks, showed a median time to progression (as determined by masked fundus photo reading center) of 49 days for the 3 mg/kg group. The second group did not reach the median time to progression (172). The relative risk for progression in the 3 mg/kg group was 3.05 compared to the high dose maintenance group (172). The Kaplan-Meier survival estimate for this series in 1996 was 4.9 to 5.9 months (172).

Dose and schedule dependent nephrotoxicity is the principal side effect as the drug is concentrated by active transport into the proximal convoluted tubule (92,128,167) and typically becomes evident Þrst as proteinuria and then as an elevated serum creatinine level (128). Reported frequencies of proteinuria and elevated creatinine are 39% and 24% respectively (172). Prior treatment with foscarnet has been shown to be a risk factor for cidofovir-induced nephrotoxicity (172), and death from cidofovir-associated renal failure (in two patients with additional nephrotoxic drugs) has been reported (173). In the HPCRT (81,83), proteinuria occurred at rates of 2.6, 2.8, and 6.8 per person-year for the deferred, low dose (3mg/kg), and high dose (5 mg/kg) groups respectively suggesting a dose relationship. For patients with adequate follow-up to assess toxicity resolution, 80Ð90% had subsequent normalization of renal function (81). One case report describes a patient who developed nephrogenic diabetes insipidus without signiÞcant preceding change in creatinine or proteinuria (174). Other side effects of cidofovir include nausea (13Ð48%), fever (14Ð35%), alopecia

(16–18%), myalgia (16%), neutropenia (13.7–25%), headache (11%), cutaneous hypersensitivity (7.8%), anemia (2%), peripheral neuropathy, Fanconi syndrome, cardiomyopathy, and erythroderma (128,165–167,172,173,175).

The ocular side effects of intravenous cidofovir are potentially sight-threatening. The two most concerning are hypotony and anterior uveitis. Both have been reported in patients taking intravenous cidofovir for non-ocular CMV infections (176). Hypotony has been reported to occur in approximately 10% of patients in some series (92,128,172,177); however, in the HPCRT (83), there was no difference in frequency of hypotony between the low dose, high dose, and deferred treatment arms. This HPCRT result has been attributed to low patient numbers (83) and concurrent use of probenecid, which has been shown to reduce this toxicity (178).

The frequency of anterior uveitis following intravenous cidofovir has been reported to occur in 12–42% of patients (81,92,128,172,173,177,179–182) at a rate 0.2 per person-year (81). It typically occurs in the week following the cidofovir infusion and between four and 10 weeks following induction therapy (177,179–181). Patients complain of pain, redness, and photophobia. Features of the anterior uveitis include hypopyon (20%) (179), non-granulomatous keratic precipitates (12%), posterior synechiae (50–60%) (177,179,180), iris transillumination defects (50%) (177), and corneal edema (20%) (179).

Uveitis associated with cidofovir use is related to the immune status of the patient. In the series of Ambati et al. (179), which reported an anterior uveitis frequency of 59%, stepwise linear regression suggested that one-third of cases were related to immune reconstitution as evidenced by a more significant increase in CD4+ T-cell counts in patients with anterior uveitis compared to those without anterior uveitis. All patients in this series received probenecid prophylaxis. Since 25% of the patients with unilateral CMV retinitis developed anterior uveitis bilaterally, CMV retinitis itself was not a pathogenetic requirement (179). Berenguer et al. (173) similarly found an association between immune reconstitution with HAART and iritis development since, in their series of 51 patients, median CD4+ T-cell counts were three times higher in the 41% of patients who developed iritis than in those patients without iritis. Age, sex, HIV risk factors, retinitis severity, and renal insufficiency were not associated with iritis development in this series (173), although an association with renal dysfunction was suggested in one small case series (181). In the HPCRT (81), the relative risk of developing uveitis associated with cidofovir use was 6.9 if protease inhibitors were used concomitantly; however, nearly one-half of the cases of cidofovir uveitis occurred in patients not receiving a protease inhibitor. Bainbridge et al. (177) however, found no association of anterior uveitis following intravenous cidofovir with HAART, rifabutin use, or absolute CD4+ T-cell count. Thus, while some studies are contradictory, it appears that immune reconstitution may increase the rate of development of inflammation associated with cidofovir use.

Probenecid, a benzoic acid derivative with a sulfa moiety, has been used competitively to block cidofovir concentration in the proximal convoluted tubule in an attempt to minimize toxicity (53,92,165,167); however, probenecid allergy is not uncommon (167) and more than half of patients treated with this drug will develop side effects (46). Probenecid reactions of nausea and constitutional symptoms occur in 43% of patients and are treatment-limiting in some patients (172). Other reactions include fever, hypotension, and angioedema (81). In the HPCRT (81,83), probenecid reactions occurred at a rate of 0.7 per person-year, and in the 3mg/kg/dose maintenance group, the median time to discontinuing therapy due to side effects increased from 7.4 months to 16.3 months when probenecid intolerance was excluded. In spite of these side effects, use of cidofovir along with probenecid, hydration, and careful monitoring for proteinuria (92,167), may be a viable alternative to the currently available intravenous preparations.

Oral Preparations

The first oral anti-CMV agent, which has received FDA approval for maintenance therapy and primary prophylaxis of CMV end organ disease, is ganciclovir. The bioavailability of oral ganciclovir of 2.6–8.8% is poor (128,183,184); however, bioavailability is enhanced by food (183,185). Pharmacokinetic studies by Anderson et al. have shown that oral ganciclovir at doses of 500 mg six times a day or 1,000 mg three times a day provided area under the time-concentration curves equivalent to 70% of that provided by an intravenous regimen of 5 mg/kg/day. The peak ganciclovir concentration in serum by oral administration was 16% of the peak IV concentration; however, the steady state concentration by the oral route was higher than that obtained by IV for 12 hours of the day (183). Since the serum concentrations approximate the IC50 for CMV, the exacerbation of drug resistance is a concern, although there has been no increased incidence of drug resistance in patients treated with oral ganciclovir when compared with intravenous ganciclovir according to one report (186).

The efficacy of oral ganciclovir maintenance was compared to intravenous ganciclovir maintenance in several trials (187–189). In a study by Drew et al. (187) which randomized patients to oral ganciclovir (500 mg six times a day) or intravenous ganciclovir (5 mg/kg/day), following three weeks of intravenous ganciclovir (5 mg/kg every 12 hours for two weeks then 5 mg/kg/day for one week), found no significant difference in the time to progression of retinitis by masked fundus photograph reading center. The mean time to progression by masked fundus photograph reading center was 62 days for intravenous ganciclovir and 57 days for oral ganciclovir.

By unmasked clinician examination, however, there was a signiÞcant difference favoring intravenous ganciclovir: 96 days versus 68 days, respectively (187). Physician bias was invoked to explain the difference between the masked reading center and clinical examination discrepancies.

Other studies have similarly supported the efÞcacy of oral ganciclovir (184,188,189); however, when all studies are collectively evaluated, there is a suggestion that oral ganciclovir may be slightly less effective than intravenous ganciclovir for maintenance therapy and the recommendation has been made to avoid oral ganciclovir for induction monotherapy for any patient or for maintenance monotherapy for a patient with immediately sight-threatening disease (92,128).

The relative side effects of the oral and intravenous preparations generally favor the use of oral ganciclovir. The incidences of neutropenia (187Ð190), fever (189), sepsis (46,188Ð190), anemia (187,190), and catheter-associated problems (187,188,190) were higher in patients treated with intravenous ganciclovir compared to oral ganciclovir. The incidence of rash was higher in patients treated with oral compared to intravenous ganciclovir (188), and there are conßicting reports regarding the relative incidence of gastrointestinal problems between the two routes of administration (187Ð189). Survival does not seem to be affected by the different treatment modalities (187).

A second oral agent approved by the FDA for CMV infections is valganciclovir (46,85,93,128,191,192). Valganciclovir, or L-Valine, 2-((2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy)-3-hydroxypropyl ester monohydride, is an L-valyl ester prodrug of ganciclovir which is rapidly converted by hepatic and intestinal esterases into ganciclovir. Like ganciclovir, valganciclovir requires dose adjustment for patients with renal impairment. In a randomized, open-label, controlled study of patients with AIDS and newly diagnosed CMV retinitis, valganciclovir (900 mg bid induction for 21 days followed by 900 mg qd maintenance) was comparable to intravenous ganciclovir. Although no clinical data directly compare oral valganciclovir (900 mg qd) to intravenous ganciclovir (5 mg/kg/day) as maintenance therapy, pharmacologic data suggest equivalency. Side effects of valganciclovir include gastrointestinal complaints, pyrexia, neutropenia, anemia, and insomnia.

Local Therapy

Given the morbidity associated with central venous catheters (103,136,137,193Ð195), there has been an effort to develop local treatment of CMV (196). At least one case report documents successful control of bilateral CMV retinitis using local therapy alone for over three years (197). The principal disadvantage of local therapy is the absence of systemic protection against CMV disease (196,198,199). Contralateral retinitis develops in 50% of previously uninvolved contralateral eyes in patients with unilateral disease treated locally at six months (115). Visceral CMV disease occurs in 27Ð31% of patients treated locally at six months (115,200). Local therapy for unilateral disease in combination with oral ganciclovir or valganciclovir for systemic prophylaxis may be an attractive approach.

Early attempts at local therapy have utilized intraocular injections of ganciclovir (197,201Ð205) and/or foscarnet (206). Both require six injections for induction over two to three weeks (202,203,206) or until the retinitis is inactive (204), then weekly injections for maintenance. The 0.1 ml injections generally are given via a trans-pars plana approach 4 mm from the limbus with a 30-gauge needle on a tuberculin (1 cc) syringe (202,206). Ganciclovir doses of 200 μg to 2,000 μg have been well tolerated (202Ð205) as have foscarnet doses of up to 2,400 μg (206). Velez et al. (207) report the successful use of combined high-dose ganciclovir and foscarnet at doses of up to 3.0 mg and 2.4 mg, respectively, twice a week for a patient with resistant CMV retinitis. The efÞcacy of both drugs given intravitreally has been reported to be similar to that of the intravenous preparations (202,204Ð206). Since multiple injections are required, it is not surprising that complications of local therapy have included endophthalmitis (202,203,205), retinal detachment (203,204), vitreous hemorrhage (204), optic atrophy (204), keratitis (204), and subconjunctival hemorrhage (202). Administering topical povidone iodide (206) before the injection or performing the injections in an operating room (204) may decrease the risk of endophthalmitis, but cannot eliminate it completely.

Methods to increase the length of action of locally injected drugs or using longer acting preparations may increase the acceptance by patients and physicians. Loading biodegradable 50:50 poly(DL-lactide-co-glycolide) microspheres with ganciclovir and injecting them intravitreally has shown some promise in a rabbit model of CMV retinitis, vitreitis, and optic neuritis (208); however, it is not yet available for human use.

The long duration of action of cidofovir may Þll this need since less frequent injections are required (171). Kirsch et al. (209) in a phase I/II un-masked, uncontrolled, case series reported that the median time to progression after a single 20 μg injection of cidofovir was 64 days. Typically, dosing regimens of 20 μg every Þve to six weeks are administered (46). In the non-randomized series of Taskintuna et al. (210) of patients with previously treated and untreated CMV retinitis, a dose of 10 μg was shown to be less effective than a dose of 20 μg and possibly associated with the development of drug resistance.

The ocular side effects of intravitreal injections of cidofovir can be sight-threatening. Kirsch et al. (209) found that a 20 μg intravitreal dose was well tolerated but that a 100 μg dose was associated irreversible visual loss, due to cidofovir toxicity (uveitis and hypotony). Doses of

10 μg have been shown to cause fewer side effects; however, as noted earlier, they are less effective (210).

Like intravenous cidofovir, intravitreal cidofovir has been associated with iritis and hypotony (178,200). In the prospective series by Chavez-de la Paz et al. (178) in which 46 eyes received 130 injections of cidofovir at a dose of 20 μg, the frequency of anterior uveitis after the first injection was 26%. The anterior uveitis typically occurred between five and 12 days after the injection, was symptomatic, nongranulomatous and at times associated with posterior synechiae formation, miosis, perilimbal flush, and hypotony (178). Iritis occurring in one eye increased the likelihood of iritis in the second eye when treated by injection (178). Taskintuna et al. (200) in his series of 115 eyes that received 296 injections of cidofovir (20 μg) with probenecid prophylaxis, reported a frequency of nongranulomatous anterior uveitis of 32%. Posterior synechiae, cataracts, and retinal detachment developed in 19%, 11%, and 6% of eyes respectively (200).

Hypotony can be equally sight-threatening. Taskintuna et al. (200), reported that transient hypotony occurred in 14% of eyes and chronic hypotony in 1% of eyes. The risk of chronic hypotony with permanent visual loss was increased in eyes in which the fellow eye had transient hypotony, which led to the recommendation of waiting at least two weeks between injections in the two eyes (200). The frequency of chronic hypotony with visual loss in the series of Banker et al. (211) was similarly 1%.

The hypotony and intraocular inflammation associated with intravitreal cidofovir use has been attributed to ciliary body toxicity. Aqueous fluorophotometry has shown a reduction in aqueous humor flow rate following intravitreal cidofovir injections (211). *In vivo* ultrasound biomicroscopy of eyes with severe hypotony following intravitreal injections of cidofovir has identified ciliary body atrophy (211). Histopathology of human eyes that have received multiple injections of intravitreal cidofovir (cumulative doses of 40–60 μg) has shown mild to moderate atrophy of the nonpigmented ciliary epithelium (200). Electron microscopy of these same eyes showed vacuolization of the nonpigmented ciliary epithelium (200). Histopathology of the retina, choroid, and remainder of the ciliary body showed no abnormalities (200). Concurrent HAART has been suggested to increase cidofovir toxicity either by increasing circulating cidofovir levels or by increasing the number of circulating pro-inflammatory CD4+ T cells (212). Probenecid prophylaxis has reduced the frequency of anterior uveitis from 71% to 18%, presumably by reducing cidofovir absorption into the ciliary body (178). Pretreatment with topical steroids (prednisolone acetate 1% q.i.d.) and cycloplegics does not alter the frequency of hypotony or uveitis (178). The routine use of intravitreous injections of cidofovir is neither recommended nor approved (196).

Encapsulating cidofovir in phospholipid liposomes prior to intravitreal injection in rabbit eyes has shown antiviral efficacy and allowed the injection of higher doses of cidofovir without the toxic effects (213). The liposome acts as a sustained release depot similar to the ganciclovir microspheres (214) mentioned earlier. A single injection of liposome-encapsulated cidofovir provided protection against an intraocular HSV-1 challenge for 240 days in this model (213). Akula et al. (215) reported one patient in whom liposome encapsulated ganciclovir halted progression of retinitis for four months. Additional studies will be required to further delineate the utility and side effects of this treatment.

Fomiversen sodium (Vitravene, formerly ISIS 2922, Isis Pharmaceuticals, Inc., Carlsbad CA, and CIBA Vision Ophthalmics, Atlanta, GA) was approved by the FDA for the local treatment of CMV retinitis in patients with AIDS in August 1998 (53,91,216–218). The first "antisense" drug approved for clinical use, Fomiversen is an oligonucleotide which binds to the "sense" strand of messenger RNA (mRNA) for CMV immediate early genes UL36 and UL37, thus inhibiting translation and subsequent protein formation (53,216,217). The fomiversen-mRNA complex is then degraded by RNAse H liberating a degraded mRNA strand and an unaltered fomiversen oligonucleotide which can then bind to another strand of mRNA (216). DNA replication in human CMV can be inhibited by 99% with use of this agent (53). The recommended intravitreal dose is 330 mcg on day 1 and 15 for induction followed by monthly maintenance injections (216). Fomiversen requires storage between 2 and 25 degrees Celsius and protection from light (216). Activity against ganciclovir-, foscarnet-, and cidofovir-resistant CMV strains has been identified (53).

Phase III trials of fomiversen showed median time to progression of 71–80 days compared to 13 days in the deferred treatment group (216,217). Side effects included temporary elevations in intraocular pressure and intraocular inflammation (46,216). As a result of the inflammatory potential of fomiversen, it should not be used in patients treated with cidofovir in the prior two to four weeks (216). Other side effects include peripheral retinopathy and a reversible, non-visually significant "Bull's eye"-type maculopathy (218). No systemic side effects have been observed (217).

The longest-acting form of local therapy is the sustained-release ganciclovir intraocular implant (Vitrasert) which was approved by the FDA for use against CMV retinitis in March 1996 (196). The implanted device is 5–6 mm long, 4 mm wide and 3.5 mm high (219). It is composed of a 4.5–6.4 mg pellet of ganciclovir coated on all sides but one by impermeable ethylene vinyl acetate then completely recoated with permeable polyvinyl alcohol (196,220,221). Intraocular implantation occurs in an operating room and involves placement of the device through a pars plana incision with the pellet facing the lens to facilitate observation (221). Its benefit compared to other local therapies is the ability to deliver ganciclovir steadily for prolonged periods of time. Calculated release rates from explanted devices range from 1.40 to 1.89 μg/

hour (115,221) which are constant until 90% of the drug has been released (220). Mean vitreous concentrations range from 4.1 to 8.37 μg/ml (115,221) which are well above the IC50 for many CMV isolates and higher than that obtained following intravenous administration (128). The duration of delivery is approximately Þve to eight months (222) and the device can be replaced as needed (223) typically every six to eight months (2,92,103,128, 196,197).

Clinical studies have shown efÞcacy of the ganciclovir implant against CMV retinitis (115,221,223). In the study by Martin et al. (115), in which patients with newly diagnosed peripheral retinitis were randomized to deferred treatment or immediate treatment with a device, the median time to progression by masked fundus photograph reading center was 15 days for the deferred group and 226 days for the device group. Progressive retinitis generally occurs with the implant is empty, resulting in low or nondetectable vitreous concentrations of ganciclovir (115,223). In the randomized controlled trial of Musch et al. (199), patients were treated with either a ganciclovir implant with a release rate of 1 microgram per hour, an implant with a release rate of 2 micrograms per hour, or intravenous ganciclovir. The median times to progression of retinitis were 221 days, 191 days, and 71 days respectively for each of the groups. The risk of progression was 2.8 times higher for patients treated with intravenous ganciclovir than for patients treated with the implants. In the randomized controlled trial of Martin et al. (198), patients received either a ganciclovir implant plus oral ganciclovir (4.5 mg/day), a ganciclovir implant plus placebo, or intravenous ganciclovir monotherapy. Retinitis progression was delayed in patients who received the implant plus oral ganciclovir compared to the implant plus placebo.

The implant may be less effective in relapsed disease than in primary disease (92,103,224). In the review of Roth et al. (224), in which 54 patients with active, previously treated, sight-threatening, CMV retinitis and poor venous access or prior failure of intravenous ganciclovir and foscarnet, were given a ganciclovir implant, a positive initial response was seen in 67.4% of patients. The median time to progression in eyes with a positive initial response was 8.0 months in the group with poor intravenous access versus 2.0 months in the group that previously had failed intravenous ganciclovir and foscarnet (224). The relative risk of early implant failure was 6.1 for each additional six month period of treatment with ganciclovir (224). In the series of Marx et al. (225), of the 91 eyes of 70 patients with recurrent CMV retinitis that were treated with the ganciclovir implant, a positive initial response occurred in 76% of eyes. The median time to progression in eyes with a positive initial response was seven months (225). Some, but not all, investigators recommend intravitreal injections of high dose ganciclovir (2mg) prior to placement of the ganciclovir implant in eyes with refractory disease (two relapses in a 10 week period despite two induction/ maintenance cycles) to determine efÞcacy (92).

A prospective randomized multicenter clinical trial, the Ganciclovir Cidofovir Cytomegalovirus Retinitis Trial (GCCRT) (74), compared the ganciclovir implant combined with oral ganciclovir (1 gram tid) to parenteral cidofovir (5 mg/kg weekly for two weeks, followed by 5 mg/kg every two weeks) in patients with AIDS and newly diagnosed or recurrent CMV retinitis. There was no signiÞcant difference in rate of retinitis progression (0.67 versus 0.71 per person-year), rate of visual loss (0.78 versus 0.47 per person-year), and mortality (0.41 versus 0.49 per person-year) between the ganciclovir and cidofovir groups (74). There was a higher rate of visual Þeld loss in the patients treated with the ganciclovir implant which was attributed to the implant blocking a portion of the visual Þeld (74). Quality of life questionnaire indices such as mental health score, and energy score, favored cidofovir use (74). Side effect proÞles of each of the treatments were different, as expected.

The ganciclovir implant is well tolerated. Histopathologic studies in a rabbit model and in autopsy human eyes have shown no signiÞcant inßammatory effect (219Ð221). Implantation of uncoated ganciclovir pellets into rabbit eyes showed no ophthalmoscopically evident toxicity; however, there were some changes by electrophysiologic testing (220).

As with any surgical procedure, complications occur. Posterior segment complications such as retinal detachment, vitreous hemorrhage, endophthalmitis, cystoid macular edema, and epiretinal membrane have been reported in 12% of patients and are often associated with permanent visual loss (226). Reported frequencies of retinal detachment are variable (115,223); however, in the large prospective series of Kempen et al. (227), there was no increased risk of retinal detachment following placement of a ganciclovir implant compared to systemic therapy with ganciclovir, foscarnet, or cidofovir, a Þnding which has been attributed to better control of the intraocular CMV. Cases of separation of the ganciclovir pellet from the suspension strut during removal have been reported and recommendations regarding the technique of implant removal have been made (228). Other complications include transient astigmatism, corneal dellen, hypotony, and temporary decrease in visual acuity (92,115,128,221,223,224).

Investigational Therapy

Additional agents have been evaluated for the treatment of CMV disease including MSL-109, benzimidazole riboside derivatives, and others; however, the low incidence of CMV disease due to HAART has hindered these investigations. MSL-109 (Protein Design Laboratories, Inc, Mountainview, CA) is a human IgG1-κ class monoclonal antibody directed against the CMV gH glycoprotein

with *in vitro* neutralizing activity against CMV (46,53,229–237). Although a phase I/II investigation suggested a therapeutic benefit of MSL-109 (53), a larger phase II/III trial (229) showed that there was no difference between MSL-109 and placebo with respect to rates of retinitis progression, visual acuity outcomes, and quality of life indices (229).

Two benzimidazole riboside compounds have been investigated as therapy for CMV infection (53,99,128, 238). Derivatives of 5,6-dichloro-1-b-D-ribofuranosyl benzimidazole (DRB), 2-bromo-DRB (BDCRB) and 1263W94 inhibit CMV replication via mechanisms unlike ganciclovir, foscarnet, and cidofovir (238). As a result, cross resistance is not a concern (238). BDCRB is thought to inhibit viral DNA maturation by interacting with the products of genes UL89 (possible terminase) and UL56; however, it is rapidly inactivated thus making its clinical application difficult (238).

The second benzimidazole compound, 1236W94, also inhibits viral DNA synthesis, but by an as yet uncharacterized mechanism, which is distinct even from that of BDCRB (238). 1236W94 shows activity against ganciclovir, foscarnet, cidofovir, and BDCRB-resistant mutants (238). It has very good oral bioavailability, limited metabolism, and an adequate half-life (238). It does not require phosphorylation for activity, is eliminated by biliary excretion, and its albumin binding is readily reversible (238). Phase 1 trials (238) in healthy and HIV infected volunteers have shown that it is well tolerated with altered taste being the most common but not dose-limiting side effect. Efficacy studies on asymptomatic CMV shedding in urine and semen in patients with HIV are under way (238).

Relapsed Retinitis

Although many agents have been shown to be effective against CMV retinitis, if anti-CMV therapy is interrupted for a sufficiently prolonged period of time, relapse is essentially universal. Active, immature, replicative intermediates of human CMV have been shown to persist in retinal glial cells despite treatment (239). Whether the human retina is a normal site of viral latency is unclear (239). The time to relapse among patients who have had ganciclovir discontinued is approximately three to four weeks (57,135). In addition, despite the use of maintenance therapy, CMV will often relapse while on maintenance, a phenomenon sometimes called "breakthrough" retinitis (79,104,113,114). Given enough time nearly all patients on systemic therapy will experience recurrent retinitis. Although relapse of retinitis while on maintenance therapy may occasionally be fulminant, it often appears to be a slow "smoldering" intermittent movement of the border, suggesting a partial effect of the maintenance drug.

Relapsed retinitis has been attributed to both progressive immunosuppression from AIDS (92), less intraocular drug penetration due to progressive arterial occlusion with advancing CMV disease (196), and drug resistance (119). The efficacy of the ganciclovir implant when compared to intravenous ganciclovir supports the belief that reduced drug penetration may account for some treatment failures (161,224).

The ability to predict which patients are likely to develop a relapse of their retinitis may allow for a change in therapy to prevent the progressive loss of visual function that occurs with each reactivation. This ability would be especially beneficial for patients with peripapillary or perifoveal lesions whose visual function would not tolerate a relapse. Viral culture sensitivities have provided mixed results in terms of predicting those patients at risk for recurrent CMV retinitis since viral strains identified by blood and urine cultures may not be the same as those responsible for ocular disease (156,229). In addition, the identification of CMV viremia and viruria by culture is not always possible despite active retinitis (58,119,240). One alternative to viral culture that has been investigated is polymerase chain reaction (PCR) detection of CMV DNA from blood (139,144,241,242). None of these methods to date has proven sufficiently reliable for general use.

Treatment of Relapsed Retinitis

The treatment of relapsed or resistant retinitis has included continued monotherapy with the same or different agent (119,120,128,149,156,159) and combination therapy with different agents or different routes of administration (119,128,240,243–245). The Cytomegalovirus Retinitis Retreatment Trial (CRRT) was a multicenter randomized clinical trial which enrolled 279 patients with persistently active or relapsed retinitis to treatment with either foscarnet (90 mg/kg every 12 hours for two weeks then 120 mg/kg/day high dose maintenance), ganciclovir (5 mg/kg every 12 hours for two weeks followed by 10 mg/kg/day high dose maintenance), or ganciclovir and foscarnet (same induction doses as above but 5 mg/kg/day maintenance with ganciclovir and 90 mg/kg/day maintenance with foscarnet) (240). Median time to progression was 1.3 months for the foscarnet group, 2.0 months for the ganciclovir group, and 4.3 months for the combination group when determined by masked fundus photographic reading center. Changing treatment from one monotherapy drug to the other at randomization made no difference in outcome suggesting that there was no benefit in switching treatment as opposed to re-induction with the same medication for early relapses. Treatment impact on a quality of life questionnaire (246) was more negatively affected in the combination group than in either monotherapy group, which was probably related to the increased infusion time. There was no significant difference in morbidity or mortality among the three groups. Although this combination therapy generally no longer is used, the principle of

combination therapy for relapsed CMV disease such as foscarnet plus the ganciclovir implant has been used.

Primary Prophylaxis

Three studies have evaluated the safety and efficacy of selected antiviral agents for the prevention of CMV retinitis (46,192). A multicenter, randomized, double-blind study by the AIDS Clinical Trial Group and the Glaxo Wellcome International CMV Prophylaxis Study Group (53,192), compared valacyclovir 8 g/day with acyclovir 3.2 or 0.8 g/day for prevention of CMV end organ disease. Baseline and semi-annual dilated eye exams were performed. The study was terminated early due to a significant albeit, unexplained, early mortality increase in patients treated with valacyclovir (192). Despite the early termination, valacyclovir was shown to reduce the frequency of CMV end organ disease by 33% (192). CMV retinitis accounted for 79.3% of CMV-related end organ disease and occurred in 9.75% of patients treated with valacyclovir compared to 13.5% of patients treated with acyclovir (192). Oral ganciclovir (1,000mg po tid) was evaluated in two randomized, double-masked, placebo-controlled trials (53). The Roche Bioscience/ Syntex Study 1654 included HIV positive patients with CD4+ T-cell counts less than 100/μL and an AIDS defining opportunistic infection or patients with a CD4+ T-cell count less than 50/μL with positive CMV serology (53). Dilated eye exams were performed at baseline and every two months (53). A 49% reduction in the frequency of CMV retinitis from 39% in the placebo group to 18% in the treatment group was found which was not restricted to patients in the lower CD4+ T-cell group (53). The prophylactic benefit of oral ganciclovir was related to baseline CMV viral load with no benefit identified if the baseline viral load was greater than 50,000 copies/ml (93). The Community Programs for Clinical Research on AIDS (CPCRA) study (Trial 023) included HIV-infected patients with CD4+ T-cell counts less than 100/μL, and positive CMV serology, but no evidence of CMV disease (53). No reduction in the frequency of CMV retinitis was identified in the treatment group (relative risk, oral ganciclovir versus placebo, 0.92); however, patients in the study did not undergo routine dilated eye exams which suggests that the true incidence of retinitis may not have been identified (53). These conflicting results, combined with drug expense, and the low incidence of CMV retinitis in the HAART era have limited the use of primary prophylaxis for CMV retinitis.

Discontinuation of Anti-Cytomegalovirus Therapy While on HAART

The renewed ability of some HAART-reconstituted immune systems to control CMV retinitis effectively and the publication of several case series where anti-CMV agents were successfully discontinued without relapse in patients with immune reconstitution has led to the development of recommendations for when discontinuation of anti-CMV maintenance therapy may be appropriate (4,7,92,247–249). In a review of 41 patients with CMV retinitis and immune reconstitution following HAART, Curi et al. (122) found no CMV retinitis reactivation following discontinuation of maintenance anti-CMV therapy with a mean follow up of 20 months. In the series of Macdonald et al. (249), of 22 patients with immune reconstitution following HAART who discontinued anti-CMV therapy, only three developed recurrent CMV retinitis during the median follow up of 72 weeks. The three patients who had reactivation of the CMV retinitis had simultaneous failures of HAART with CD4+ T-cell count declines to less than 50 cells/μL. Reed et al. (126) and Whitcup (250) similarly found that CMV reactivation in patients on HAART was associated with HAART failure. In the series of Jabs et al. (248) in which 15 patients with CMV retinitis and immune reconstitution following HAART were monitored off maintenance CMV therapy, no CMV relapses were identified during the median follow-up of eight months. Median CD4+ T-cell nadir before stopping HAART was 20 cells/μL and at time of discontinuing CMV maintenance therapy it was 297 cells/μL (248). As a result, some patients who have had immune reconstitution may safely have maintenance anti-CMV therapy discontinued.

The recommendations for discontinuing anti-CMV therapy typically are based on the CD4+ T-cell count, or change in the CD4+ T-cell count, in combination with a time factor (248–250). Absolute CD4+ T cell count alone may not be reliable as previously discussed since it is the existence of memory CD4+ T-cell clones directed against specific antigens that more directly identifies the disease-specific immune reconstitution (251). The U.S. Public Health Service recommends that cessation of maintenance anti-CMV therapy (95) be considered only for patients with an increase in CD4+ T-cell counts to greater than 100–150 cells/μL for three to six months on HAART while other factors such as duration of CD4+ T-cell count rise, location of retinitis, vision in contralateral eye, and ability to continue regular ophthalmologic monitoring are considered as well.

Retinal Detachments

Retinal detachments are a substantial cause of ocular morbidity in patients with CMV retinitis. The prevalence of retinal detachments at the time of diagnosis of CMV retinitis is 3.4–6% (104,252). In one report, the median time from the diagnosis of CMV retinitis to retinal detachment was 10.6 months (31); however, detachment may occur at any time following the diagnosis of CMV retinitis. In retrospective series done prior to the widespread use of HAART, the cumulative probabilities of

retinal detachment at six and 12 months from the diagnosis of CMV retinitis were 11–23% and 24–57% respectively (31,225,252,253). In the prospective FGCRT, the cumulative probability of retinal detachment in at least one eye at six months was 27–28% (104), and in a series of patients with recurrent CMV retinitis treated with the ganciclovir implant, the six month risk of retinal detachment was 23% (225). The rate of retinal detachments in two large prospective trials was 0.28 per person-year (81,192). Of patients with a retinal detachment in one eye, 28–46% develop detachments in the contralateral eye (31,254). As a result, retinal detachment is a common complication of CMV retinitis.

Since the widespread use of HAART, a 60% reduction in the rate of retinal detachments has been reported (227). This reduction has been attributed to improved control of CMV replication by the patient's immune system, increased inflammation producing stronger retinal-retinal pigment epithelial adhesion in areas of retinitis, and/or alterations in the course of posterior vitreous detachments in patients with CMV retinitis on HAART (227).

Risk factors for the development of retinal detachments include peripheral retinitis, extent of retinitis, and the presence of detachment in the contralateral eye (252). The presence of active retinitis may (252), or may not (253) be associated with an increased risk. Freeman et al. (252) reported that the relative risk of retinal detachment in an eye with active retinitis and more than 25% retinal involvement in zones 2 and 3 was over 20 times that of an eye with no activity and less than 5% retinal involvement in zones 2 and 3. Treatment of the CMV retinitis delays development of the retinal detachment (253), and treatment with a ganciclovir implant, despite requiring intraocular surgery, does not increase the rate of retinal detachment when compared to systemic therapy (227).

Retinal detachments associated with CMV retinitis are difficult to repair, but the prognosis for eyes with unrepaired detachments is poor (253,254). The causative retinal breaks are identified at the location of the normal and atrophic retinal areas in 66% of eyes, although the retinal breaks are not identified in 15% of eyes (254). Prophylactic laser has been suggested for small or peripheral detachments. The detachments often progress despite this treatment but the treatment may delay the need for more invasive surgery. Since the median survival following retinal detachment was from 7.6 to 9 months in the pre-HAART era (253,255), with 25% of patients living longer than two years (254), and given the high incidence of contralateral CMV retinitis or retinal detachments, the surgical repair of CMV-associated detachments is indicated.

In the pre-HAART era, the surgical management of retinal detachments associated with CMV retinitis typically required the use of silicone oil, which provided a better tamponade of the areas of necrosis, atrophy, and frequently developing proliferative vitreoretinopathy than did gas (253,254). Retinal reattachment using silicone oil could be accomplished in 70–100% of eyes (254,256,257). Visual acuities of eyes with retinal detachments treated with silicone oil initially had been poor with only 20% of patients achieving ambulatory visual acuity (253); however, as surgical technique and CMV therapy improved, 75–86% of patients could achieve ambulatory visual acuity postoperatively (256,257).

In the HAART era, with the improved control of CMV retinitis, the increased life expectancy of patients, and the corneal complications of silicone oil, attempts to repair CMV-related detachments without the use of silicone oil have been investigated (258). In the study of Canzano et al. (258), retinal reattachment was achieved in 83% and macular reattachment in 100% of six eyes following pars plana vitrectomy, peeling of the posterior hyaloid, laser photocoagulation, scleral buckle placement and intraocular gas tamponade without the use of silicone oil. Mean postoperative visual acuity was 20/40 at a mean follow up of 12 months (258). Thus, improved anti-retroviral and anticytomegaloviral control may allow for improved surgical outcome.

Cataract formation has been one complication of retinal detachment repair, and in the setting of increased longevity due to HAART, surgical repair is indicated. Capsular fibrosis, hyphema, and lens calculation errors have been reported following cataract surgery following CMV related retinal detachment repair (157). Lens power errors have been attributed to differing indices of refraction between silicone oil and vitreous and the attendant alteration of the refractive effect of the back surface of the intraocular lens due to its juxtaposition with the silicone oil (157). Use of convexplano intraocular lenses and modifications of the intraocular lens power formulas have improved postoperative refractive predictability (157).

Immune Recovery Uveitis

Patients with immune reconstitution following HAART have been reported to develop more significant intraocular inflammation, especially vitreous inflammation, than patients prior to HAART (7,8,29,46,259,260). This immune recovery uveitis (IRU), also known as immune recovery vitreitis (IRV), is a potential cause of vision loss in patients with AIDS and CMV retinitis (261). There is as yet no standard definition of immune recovery uveitis with definition variations related to severity of inflammation, loss of vision, symptomatology, level of CMV retinitis activity, and antiretroviral therapy (261).

Immune recovery uveitis is caused by a latent response to intraocular CMV infection in patients who respond to HAART (250,260,262). It does not occur in HAART non-responders or in eyes without a history of CMV retinitis (250,260,262,263). The CMV retinitis does not need to be active to precipitate the inflammation (264). In the series of Zegans et al. (260), vitreous inflammation occurred only in eyes with active or inactive CMV retinitis and not

in contralateral eyes of patients with unilateral CMV disease. In that series, CMV retinitis did not recur following resolution of the inßammation (260) suggesting that the inßammation had some anti-CMV activity. Nussenblatt and Lane (251) suggested that the intraocular inßammation occurs when the expansion of speciÞc CMV CD4+ T cell clones reaches a level at which an inßammatory response can be generated but at which viral protein production continues. The inßammatory response then diminishes once viral protein production ceases. The level of intraocular inßammation may be related to the aggressiveness of anticytomegalovirus therapy, (261) since the less active the infection, the smaller the antigenic load stimulating the immune response. The breakdown of the blood-ocular barrier by the CMV retinitis may facilitate the development of inßammation by allowing migration of inßammatory cells into the eye (263) or by allowing CMV antigen to exit the eye and contact the lymphoid organs (250).

Immune recovery uveitis is common and may occur from one month to several years following the onset of HAART (259,262,263). In the series of Karavellas et al. (262), 63% of 30 patients who responded to HAART developed symptomatic vitreitis with an incidence rate of 0.83/person-years. There were no signiÞcant differences in frequency whether patients with pro-inßammatory cidofovir use were included or not included (262). In the series of Nguyen et al. (265), six (18%) of 33 HAART responders developed IRU with an incidence rate of 0.109/person-year. The Longitudinal Studies of the Ocular Complications of AIDS (LSOCA) (67) reported a frequency of IRU of 15.5% among prevalent cases of CMV retinitis. The cause of the variability of these frequency estimates is uncertain but appears to be due to variable deÞnitions of immune recovery.

Ocular morbidity associated with immune recovery uveitis is high. In the series of Robinson et al. (263) 50% of patients developed a visual acuity of 20/40 or worse in at least one eye and 12% had a visual acuity of less than 20/800 in one eye. Anterior segment complications include cataracts, chronic anterior uveitis, posterior synechiae, and intraocular lens deposits (250,263,266). Severe and/or prolonged intraocular inßammation following cataract surgery in eyes with immune recovery uveitis has been reported prompting the recommendation of aggressive perioperative corticosteroid use (266). Posterior segment complications include proliferative vitreoretinopathy (266), spontaneous vitreous hemorrhage (266), papillitis (250,262,263), optic disc neovascularization (265), vitreomacular traction syndrome (259), cystoid macular edema (250,262,263,265), and epiretinal membrane formation (250,262,263,265,266).

The treatment of IRU often requires topical, regional, and/or oral corticosteroids (46,260). Clinical improvement typically occurs within six weeks of onset. It may resolve without treatment (260), or may develop a chronic course (266). Response rates to treatment are approximately 50%.

Other Opportunistic Infections of the Posterior Segment

Necrotizing Herpetic Retinopathy

Infection of the retina with the varicella-zoster virus has been described (18,267–280). It is the second most common infectious retinitis after CMV at tertiary referral centers (29,268,278), accounting for 4% of ophthalmologic cases in one series (278). The frequency among 1,163 HIV-infected patients at Johns Hopkins was 0.6% (31). Two variants of necrotizing herpetic retinopathy have been identiÞed, acute retinal necrosis (ARN) syndrome, and progressive outer retinal necrosis (268–279).

Acute retinal necrosis is characterized by prominent anterior chamber reaction, marked vitreitis, occlusive retinal and chorodial vasculitis, and full thickness retinal necrosis (273,281). The lesions usually begin peripherally and extend rapidly and circumferentially (273). Pain, optic atrophy and scleritis may be associated (273), and retinal detachment is common (281). Affected patients may have a history of cutaneous herpes zoster occurring either simultaneously with the retinitis or several months before (29,280). The ARN syndrome may occur in both immunologically normal and immune compromised patients and in patients with AIDS it may occur at any level of CD4+ T cell counts.

ARN can be managed successfully with intravenous acyclovir at a dose of 500 mg/M^2 every eight hours for 10 to 14 days (282), and, in patients with AIDS, is followed by long-term suppression with oral acyclovir. Palay et al. (283) reported that treatment with acyclovir reduces the risk of bilateral involvement in immunocompetent patients. Monitoring renal function in patients taking intravenous acyclovir is recommended given the rare complication of acyclovir-induced acute renal failure, which has been attributed to acyclovir crystallization in the collecting ducts and to acute tubular necrosis (284). Luu et al. (285) reported successful management of ARN with adjunctive intravitreal injections of ganciclovir and/or foscarnet in three immunocompetent patients with retinitis refractory to systemic therapy. Ramsay et al. (286) caution that immune recovery alone with HAART may be insufÞcient to control ARN in patients with HIV.

Although VZV is likely the most common cause of clinically deÞned ARN, PCR studies have identiÞed CMV, HSV1, and HSV2 as pathogens as well (29,110,111,287,288). In a retrospective series of 28 cases, Ganatra et al. (111) found that ARN in a patient younger than 25 years more commonly is associated with HSV2 whereas in a patient older than 25, the underlying etiology is likely HZV or HSV1. Prior or concurrent central nervous system involvement in a patient with ARN suggests HSV as a

cause of the inßammation: HSV1 if the patient had encephalitis and HSV2 if the patient had meningitis (111). Some investigators have noted that vascular involvement by HSV1 is typically arteriolar with widespread occlusive disease, whereas the vascular involvement seen with HSV2 is venular and associated with an exudative retinal detachment (287). Whether these clinical differences hold for larger numbers of involved cases remains to be determined.

Unlike ARN, eyes with the progressive outer retinal necrosis variant have much less clinical inßammation. The differential appearance of the two variants of necrotizing herpetic retinopathy is thought related to differences in the degree of immune deÞciency among those at risk (271). Patients with progressive outer retinal necrosis generally have profound immune deÞciency (CD4+ T-cell counts < 50 cells/μL), whereas patients with ARN are often healthier (268,278,288), or even immunologically normal. In the series of Engstrom et al. (268), the median CD4+ T cell count of 38 patients with progressive outer retinal necrosis was 21 cells/μL with only one patient having a CD4+ T cell count greater than 100 cells/μL. As with ARN, patients with progressive outer retinal necrosis often, but not always, have a history of active or antecedent cutaneous herpes zoster (268,271,278).

Progressive outer retinal necrosis is characterized by rapidly progressive multifocal retinal opaciÞcation associated with little or no inßammation (268,270,271,273). Although the patients may present while the process is unilateral, approximately two-thirds will develop bilateral disease (271,277,278). The retinitis often begins in the posterior pole and can involve the optic nerve and vessels (268,270,271). Previously involved retina may have a Òcracked mudÓ appearance due to clearing of necrotic material and edema from around the vessels. Although the diagnosis often can be made on clinical appearance alone, retinal biopsy may be necessary (268,270,271,278).

Numerous medications have been used for the treatment of progressive outer retinal necrosis including acyclovir, ganciclovir (intravenous and intravitreal), vidarabine, foscarnet, and sorivudine alone, and in varying combinations, with inconsistent results (268,271,272,276–279, 289,290). There have been no controlled trials comparing these treatment regimens. Intravenous acyclovir alone usually is not effective. Some authors avoid acyclovir monotherapy given the identiÞcation of acyclovir-resistant varicella zoster virus mutants and that many patients with progressive outer retinal necrosis have had prior acyclovir exposure (268,279). We have had success with combination foscarnet (90 mg/kg twice a day IV induction for two weeks followed by 120 mg/kg/day IV maintenance) and acyclovir (500 mg/M^2 every eight hours IV induction followed by 800mg Þve times a day orally); however, the combination of foscarnet with valacyclovir may reduce the intravenous requirements without sacriÞcing therapeutic efÞcacy. It is unclear whether discontinuation of maintenance therapy may be an option for patients who respond to HAART.

Despite treatment, eyes with progressive outer retinal necrosis often do not do well visually. In one series, 67% of 61 eyes lost light perception within four weeks of diagnosis (268). Treatment did not prevent second eye involvement. Retinal detachment occurs in approximately 70% of involved eyes (268,278). Prophylactic laser photocoagulation has not been shown to be effective at preventing retinal detachments (268,279), possibly due to the posterior location of many of the lesions, although it may preserve vision for several weeks and has been recommended by some investigators (289).

Ocular Toxoplasmosis

Disease due to ocular infection by the protozoan *Toxoplasma gondii* in the pre-HAART era occurred in Western countries in up to 3% of patients with HIV infection (31,278,291). One autopsy series of patients with AIDS reported a frequency of 6% (52). The incidence declined due to widespread use of trimethoprim-sulfamethoxazole prophylaxis for *Pneumocystis carinii* pneumonia and HAART. Seventy-six to 100% of patients with HIV and ocular toxoplasmosis will have AIDS (31,291); the rest will have earlier stages of HIV infection. Correct diagnosis is important due to the high incidence of concurrent central nervous system (CNS) toxoplasmosis. Twenty-nine to 56% of patients with ocular toxoplasmosis have associated CNS toxoplasmosis (31,291), and in one study, the relative risk of CNS disease for patients with ocular involvement was 20 (31). Thus, the diagnosis of ocular toxoplasmosis should prompt neuroimaging in HIV-infected patients (291).

Unlike immunocompetent patients who generally develop ocular toxoplasmosis from reactivation of congenitally acquired infection, patients with HIV may develop ocular toxoplamosis as a primary infection or as a metastatic focus from another source (291–294). Only 4% of HIV patients with toxoplasmic retinitis in one series had retinal scars suggesting local reactivation, and 12% had positive IgM titers suggesting primary infection (291).

The appearance of ocular toxoplasmosis in patients with AIDS is protean, from a pauci- or unifocal homogeneous yellow-white, full-thickness, necrotizing retinitis with ßuffy borders, sheathing and an occasional hemorrhage (51,291,295,296) to a multifocal, Òmiliary Ódeep retinitis. The disease is predominantly unilateral although it can be bilateral (291). Vitreitis and anterior uveitis are common (291), but unlike ocular toxoplasmosis in immunocompetent patients not universal. The presence of signiÞcant vitreitis, anterior uveitis and the lack of granular borders can help to distinguish ocular toxoplasmosis from CMV retinitis, with which it may occasionally be confused (31,295). Patients with ocular toxoplasmosis historically

had higher CD4+ T cell counts than patients with CMV retinitis, typically over 100 cells/μL. Both CMV and toxoplasmosis can coexist in the same eye (31,51,291). The absence of serum antibodies to *Toxoplasma gondii* make the diagnosis of ocular toxoplasmosis unlikely, but their presence does not conÞrm the diagnosis due to the high incidence of these antibodies in the general population (291,295).

Most lesions respond to standard anti-toxoplasma treatment within six weeks (291). Induction regimens have included pyrimethamine, sulfadiazine, and clindamycin (31,291,295,297). Atovaquone, a lipophilic hydroxynaphthoquinone, which selectively inhibits mitochondrial electron transport in protozoa, has shown beneÞt in patients intolerant of standard treatment (296,298), but can be associated with a non-visually signiÞcant vortex keratopathy (299). Unlike ocular toxoplasmosis in immunocompetent patients, systemic steroids are not needed in patients with HIV and ocular toxoplasmosis (297). As with other infections in patients with HIV, unless there is immune reconstitution, long-term maintenance therapy generally is required to prevent relapse of the disease. The best maintenance therapy for ocular toxoplasmosis in patients with AIDS currently is unknown, but any of the antibiotics effective against toxoplasmosis may be tried. In one retrospective series, the 24-month relapse rate was 0.18Ð0.20 for patients maintained on pyrimethamine and either sulfadiazine or clindamycin (291). We have successfully maintained patients on pyrimethamine and sulfadiazine or clindamycin alone (31).

Intraocular Cryptococcal Infection

Intraocular infection with *Cryptococcus neoformans* is rare, and in the pre-HAART era, occurred in less than 1% of patients with AIDS (31), 2.5% of patients with systemic cryptococcosis (300), and 6.25% of patients with cryptococcal meningitis (301). Autopsy series have reported higher frequencies (22,51). The primary site of cryptococcal infection is the lung; however, hematogenous dissemination to the eye can occur (300). Case studies have identiÞed cryptococcal lesions of the choroid which clinically appear deep, hypopigmented or yellow-white and range from 1/5 to 1 disc diameter in size (300,301). They can be unifocal or multifocal and bilateral (51,301,302) and generally are distinguishable from *Pneumocystis carinii* choroiditis; however, they may be difÞcult to distinguish from other infectious choroiditides. A less commonly identiÞed clinical appearance is that of a ÒcloudyÓ choroiditis in which the choroidal lesions are larger, more blotchy, and have the appearance of clouds (303). Histopathologically, the lesions involve the choriocapillaris and inner choroidal vessels (51). One clinicopathologic study described a cryptococcal iris mass with anterior uveitis and trabecular inÞltration (302). Treatment of cryptococcal infection usually requires amphotericin B, ßuconazole, or itraconazole. Choroidal granulomata have been shown to decrease in size and fade with adequate treatment (300,301). Late pigmentation can occur (300).

Other Fungal Infections

Several fungal pathogens have been identiÞed clinically and in autopsy series. One autopsy series has identiÞed isolated cases of choroidal infection with Candida sp., *Histoplasma capsulatum*, and *Aspergillus fumigatus* (51); however, these infections rarely are identiÞed clinically. Candida has been identiÞed in the choriocapillaris in a multifocal distribution with several organisms extending through Bruch's membrane (51). Candida retinitis and/or endophthalmitis has been reported clinically in patients with AIDS but the frequency in the pre-HAART era was less than 1% (16). *Histoplasma capsulatum* has been identiÞed in scleral vessels (304), the optic nerve (305), choroidal vessels (51,304,305), all layers of the retina (305), the ciliary body (305), trabecular meshwork, and Schlemm's canal (304). Case reports of disseminated bilateral chorioretinitis due to *Histoplasma capsulatum* do exist but are rare (26,305,306). The chorioretinal lesions have been described as creamy-white 1/6Ð1/4 disc diameter in size with distinct borders and occasionally tan halos (305). Some may have surrounding hemorrhage (305). Glasgow et al. (195) reported a case of bilateral endogenous endophthalmitis from *Fusarium*.

Bacterial Infections

Syphilis

Syphilis is the most common intraocular bacterial infection in patients with HIV infection (29,307Ð310). Ocular involvement can occur in patients at any stage of HIV infection (290) and can be the Þrst manifestation of HIV disease (311). Ophthalmic disease associated with syphilis was the presenting sign of HIV in four of the nine patients in the series of McLeish et al. (310); however the true frequency is likely signiÞcantly lower. Conventional syphilis staging is unrewarding in patients with ocular syphilis (312).

All parts of the eye can be affected by *Treponema pallidum*. Anterior segment involvement can include episcleritis (311), scleritis (311), interstitial keratitis (311), granulomatous or non-granulomatous keratic precipitates (311,312), and iridocyclitis (310). Elevated intraocular pressure on presentation occurs in only 4% of eyes (312). Posterior segment complications include vitreitis (isolated or in association with other intraocular inßammation) (311,313), chorioretinitis (313), sectoral retinochoroiditis (312), retinitis (310), serous retinal detachments (311,312), neuroretinitis (310), acute retinal necrosis

(313), retinal vasculitis (313), posterior placoid retinitis (313), and pseudoretinitis pigmentosa (313). Neuro-ophthalmic manifestations include papillitis (310), optic perineuritis (310), papillary light-near dissociation (311), and retrobulbar optic neuritis (310).

Controversy exists regarding the indication for lumbar puncture in patients with ocular syphilis (312); however, we recommend lumbar puncture since, if positive, a post-treatment lumbar puncture can be used to conÞrm therapeutic success (311). In the series of McLeish et al. (310), six of nine patients had neurosyphilis, and when the authors added their cases to other published reports, 15 of 19 patients with ocular syphilis had CNS involvement.

Syphilis in association with HIV coinfection may cause signiÞcant morbidity (214,314). In addition, serologic testing may be unreliable (310,315). Haas et al. (315) reported that the sensitivity of the treponemal tests (FTA and MHA-TP) dropped from 93% in patients with asymptomatic HIV infection to 62% in patients with symptomatic HIV infection. In BrowningÕs (312) series of nine patients with HIV and ocular syphilis, the range of RPR titers was 1:1 to 1:2,048 with a median titer of 1:128. Fourteen per cent of patients in BrowningÕs series (312) had a negative non-treponemal study, and up to 75% of patients with ocular syphilis have been reported to have a negative venereal disease research laboratory (VDRL) study (312). Despite the limitations of conventional treponemal and non-treponemal tests, they are likely the most commonly used method for screening. Polymerase chain reaction (PCR) studies are not widely available and require intraocular specimens to make an ocular diagnosis.

Ocular syphilis associated with HIV coinfection generally is treated with 12Ð24 million units of aqueous penicillin daily for 10Ð14 days (310,314). Less aggressive regimens are ineffective (312); and primary treatment failures can occur even with this regimen (313). In the series of Ormerod et al. (313) neurosyphilis dose treatment failures were managed with a second course of the IV penicillin followed by three weekly intramuscular injections of benzathine penicillin. Other agents have been used in patients with penicillin allergies such as tetracycline, erythromycin, and chloramphenicol; however, desensitization with penicillin followed by neurosyphilis level dosing should be considered (310,312).

Miscellaneous Bacterial Infections

Like *T. pallidum*, other bacteria can infect the eye, typically as a result of hematogenous spread in the setting of systemic infections (316). One case documents endogenous *Staphylococcus aureus* endophthalmitis from bacteremia from the intravenous catheter used to administer ganciclovir for CMV retinitis (136). Metastatic choroidal abscesses from *Staphylococcus aureus* have been reported in a patient with AIDS who succumbed to severe bilateral polymicrobial infectious pneumonitis (317). Mycobacterial infection of the choroid with *M. avium* complex or *M. tuberculosis* has been demonstrated at autopsy (22,26,50Ð52). One case report describes a white non-tuberculous choroidal granuloma with an associated exudative retinal detachment and iridocyclitis that responded to clofazimine, ciproßoxacin, ethambutol and amikacin (52). Unusual bacterial retinitis has been reported in two patients with AIDS (318). The bacteria were identiÞed morphologically on an endoretinal biopsy specimen but not in culture and responded to tetracycline therapy. Overall, these infections are rare, occurring in less than 1% of patients with HIV in the pre-HAART era (31).

Pneumocystis carinii Choroiditis

Choroidal infection with the unicellular *Pneumocystis carinii* is uncommon, occurring in less than 1% of patients with AIDS (31) in the pre-HAART era but accounting for 22% of infectious choroidopathies identiÞed in one autopsy series (51). First described by Rao et al. in 1989 (319), several additional series subsequently have been reported (319Ð322). Most, but not all, patients with *Pneumocystis carinii* choroiditis have a history of *Pneumocystis carinii* pneumonia (PCP) (31,321). Eighty-six percent of patients with *Pneumocystis carinii* choroiditis in one series had received aerosolized pentamidine for PCP prophylaxis (321). *Pneumocystis carinii* choroiditis may be the initial or only sign of disseminated *Pneumocystis carinii* infection (320).

Clinically, the lesions are 1/3 to 2 disc diameters in size, creamy-white, round or oval, and located at the level of the choroid (Fig. 26.4) (319Ð321). They are usually multifocal and bilateral, although they can be unilateral and unifocal (321,322). They are usually found in the posterior pole or midperiphery but not anterior to the equator (321). Some

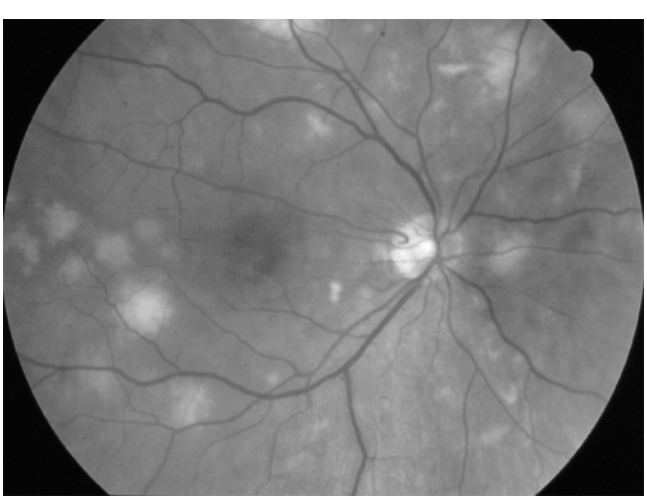

FIG. 26.4. Pneumocystis choroiditis in a patient with AIDS.

can become confluent and appear multilobulated (319). The retinal pigment epithelium is normal although it can be mottled or appear granular if the lesion is large (319). There is no associated vitreitis. The lesions are generally asymptomatic (319,321), although patients may complain of blurry vision (320). Pathologically, an eosinophilic, amorphic, acellular, foamy infiltrate is identified in the inner choroid and choriocapillaris (51,319,321). Organisms can be identified with electron microscopy (319). *Pneumocystis carinii* choroiditis often responds to systemic trimethoprim/sulfamethoxazole, pentamidine, or dapsone, alone or in combination (320–322). Atovaquone has been used for pulmonary pneumocystosis, however, its role for the management of ocular involvement remains to be determined (299). Lifelong maintenance historically has been needed to prevent recurrence, however, with the advent of HAART, such continued treatment may not be needed. It has been recommended that maintenance therapy be continued at least until the patient's CD4+ T-cell count has been higher than 200 cells/μL for at least three to six months (95).

Other Ocular Infections

Herpes zoster ophthalmicus

Herpes zoster ophthalmicus (HZO), a vesiculobullous dermatitis caused by varicella-zoster virus, was reported to occur in approximately 3% of patients with AIDS and 4% of patients with earlier stages of HIV infection in the United States in the pre-HAART era (31). The process typically involves the distribution of the ophthalmic branch of the trigeminal nerve alone; however, in patients with HIV infection, up to 29% can have involvement of multiple dermatomes simultaneously (323). Zoster *sine herpete* in which there is no observable dermatomal involvement, has been reported (323,324).

Ocular complications occur in 49% of patients with HZO and HIV infection and can involve all portions of the eye (31). Corneal involvement can be either epithelial or stromal and occurs in approximately 20% of patients with HIV and HZO (323). The stromal keratitis can be disciform, non-disciform, or manifest as avascular stromal infiltrates (323). An uncommon but particularly painful chronic infectious pseudodendritic epithelial keratitis is characterized by elevated, gray, pleomorphic pseudodendritiform lesions distributed over the corneal, limbal, and conjunctival epithelium which stain intensely with rose bengal but only moderately with fluorescein (323,324). In the series of 16 patients reported by Chern et al. (324), the interval between onset of herpes zoster and keratitis ranged from 0 days to 6 years. Most of the patients had a history of herpes zoster ophthalmicus; however, two patients had thoracic zoster alone, two had no history of a rash, and one had primary varicella infection. The diagnosis of chronic infectious pseudodendritic keratitis can be made by culture, direct fluorescent antibody testing, or polymerase chain reaction (324). The latter two methods have been reported to be more sensitive (324). Antiviral agents are usually effective against the process. Intravenous foscarnet has been used successfully to control the chronic infectious pseudodendritic keratitis; however, discontinuation of therapy was associated with relapse (323). For patients with chronic infectious pseudodendritic keratitis, pain is a prominent feature and pain management is typically the most important issue. Other complications of HZO include blepharitis, conjunctivitis, ischemic optic neuropathy, encephalitis, and a post-infectious, chronic pain syndrome, post-herpetic neuralgia (29,325).

In the series by Margolis et al. (323), mild or no ocular involvement was seen in 31% of 48 patients HIV infection and HZO. Ocular involvement in the remaining patients took the form of iritis (50%), stromal keratitis (35%), conjunctivitis (21%), elevated intraocular pressure (6%), chronic infectious pseudodentritic keratitis (4%), necrotizing retinitis (4%), post-herpetic neuralgia (4%), scleritis (2%), and eyelid scarring (2%). Four percent of patients in the series developed central nervous system involvement (323). A relationship between CD4+ T-cell count and specific ocular complications has not been identified (323).

In appropriate populations, HZO in a young man may be a marker for HIV infection (326–328), even without other manifestations of HIV. In immunocompetent patients, HZO can be treated successfully with oral acyclovir (329). The dose most commonly used is 800 mg five times daily. In immunocompromised patients, the initial treatment is often with intravenous acyclovir at a dosage of 500 mg/M^2 every eight hours followed by oral maintenance therapy of 800 mg three to five times per day (29). This form of therapy decreases the incidence of ocular side effects.

Alternative agents for HZO include valacyclovir and famciclovir, which have the advantage of less frequent dosing (330). Valacyclovir, a L-valine ester of acyclovir, has been shown to have excellent oral bioavailability and similar efficacy to acyclovir with respect to ocular complications of keratitis, uveitis, and episcleritis, time to lesion healing, and pain for patients with HZO (331). Famciclovir (Famvir) is an oral prodrug of penciclovir, a nucleoside deoxygaunosine analog, with excellent bioavailability (332). The bioavailability of penciclovir (following oral administration of famciclovir) is 77% compared to the oral bioavailability of acyclovir of 10–20% (332). Following phosphorylation by viral and cellular thymidine kinases, penciclovir triphosphate inhibits viral DNA synthesis (332). Penciclovir is eliminated in the urine, requires dose adjustment for patients with renal disease, and has an excellent safety profile. Side effects include headache (similar frequency as placebo), abdominal discomfort, elevated serum lipase, and hyperbilirubinemia, however, there have been no cases of thrombotic

thrombocytopenic purpura (TTP) or hemolytic uremic syndrome (HUS) as has been a concern with valacyclovir at doses of 2 grams qid, much greater than the 1 gram tid dose used for HZO. The use of intravenous foscarnet for induction and maintenance may be considered in patients that do not respond to acyclovir or famciclovir.

Infections of the Anterior Segment

Other ocular infections reported in patients with AIDS include infectious keratitis (29,333,334), microsporidial keratoconjunctivitis (335–338), and molluscum contagiosum (339,340).

Bacterial, viral, and fungal corneal infections have been reported in HIV-infected patients, and although for most pathogens the infection rates among patients with and without HIV infection appear similar, patients with HIV infection may have more severe involvement (341). The frequency of Herpes simplex virus keratitis has not been shown to be more common in HIV-infected patients than the general population; however, when it does occur, it may be atypical, more severe, and take longer to heal (325,341).

Microsporidial keratoconjunctivitis is caused by ubiquitous eukaryotic obligate intracellular protozoa of several species of the Microspora phylum (337,338,342–345). Of the five genera, Nosema and Encephalitizoon are the only two known to cause ocular disease, specifically *Encephalitizoon hellum*, *Encephalitizoon cuniculi*, *Nosema corneum*, and *Nosema ocularum* (337,338,342–345). The mode of infection is unclear, however, direct inoculation such as from trauma or poor hygiene has been suggested (345).

In patients with HIV, the microsporidial ocular appearance is characterized by a fine to coarse corneal punctate epitheliopathy with associated conjunctival hyperemia and papillary conjunctivitis and is usually caused by *Encephalitizoon* species (345). Superficial stromal infiltrates and conjunctival fluorescein staining are inconsistent findings. In contrast, immunocompetent patients typically develop ulcerative keratitis with deep stromal involvement usually in association with *Nosema corneum* (341,345). Symptomatically, patients report foreign body sensation, dryness, epiphora, photophobia, and decreased vision.

Although it is difficult to culture microsporidia, the diagnosis can be made by identifying the intra-cellular, oval, organisms with Gram (positive), Giemsa (341,346), periodic acid-Schiff (345), Grocott-methenamine silver (345), acid-fast (345), and Weber modified trichrome stains (344), and confocal microscopy (343). Speciation can be accomplished by transmission electron microscopy (345) or immunocytochemistry (341,342,344). Microsporidial infection also can occur in the lungs, sinuses, urogenital, renal, and gastrointestinal tracts as well as in bone (341,342). Agents with activity against this parasite include fumagillin, itraconazole, and propamidine isethionate (341–344). An oral benzimidazole, albendazole, has shown inconsistent efficacy (341,342,344–346). Martins et al. (347) reported one case of microsporidial keratoconjunctivitis in a patient with HIV that was effectively managed with HAART consisting of indinavir, lamivudine, and stavudine but no specific anti-microsporidial agents. Penetrating keratoplasty was curative in at least one immunocompetent patient with deep stromal involvement following the failure of medical therapy (345).

Molluscum contagiosum, a highly infectious pox virus, is capable of producing multiple small umbilicated lesions on skin and mucous membranes. It occurs on the eyelids of up to 5% of patients with HIV (29,341,348). The process is more severe in patients with HIV infection, but unlike in immunocompetent patients, it typically does not produce a follicular conjunctivitis or superficial keratitis when involving the eyelid (29). Cryotherapy, curettage, excision, incision, and topical chemotherapy are therapeutic (29,341).

Cryptococcus neoformans has been described in one case report by Coccia et al. (349) as a cause of an eyelid nodule in a patient on HAART. Surgical excision of this papular lesion allowed histologic identification of the encapsulated saprophytic yeast (349). Waddell et al. (350) described a case of a 6×10 mm conjunctival mass mimicking a squamous cell carcinoma which similarly was identified histopathologically as caused by *Cryptococcus neoformans* (350). In this patient, the conjunctival mass led to the subsequent identification of systemic cryptococcosis.

OCULAR NEOPLASMS

Kaposi's sarcoma is a vascularized tumor of skin and mucous membranes possibly associated with human herpesvirus 8 (29,341). In the pre-HAART era, ocular involvement was reported in 2% of patients with AIDS. Of patients with AIDS and Kaposi's sarcoma, 15–22% (26,351) had ocular involvement. Either the eyelids or the conjunctiva may be involved. The lesions are typically painless and may mimic a subconjunctival hemorrhage or chalazion (29). Dugel et al. (352) have correlated the histological and clinical appearances of these lesions. They identified a continuum of features from thin dilated vascular channels with flat endothelial cells to densely packed spindle cells depending on the size, shape and duration of the lesion.

Conjunctival Kaposi's sarcoma (Fig. 26.5) usually does not require treatment. The lesions are slowly growing, do not invade the eye and often do not compromise vision. When removal is necessary, small, early lesions of the conjunctiva do well with surgical excision with a clear margin of 1–2 mm (353). Larger, older lesions of the conjunctiva often recur with simple excision (353). Eyelid involvement by Kaposi's sarcoma may require therapy if it causes functional problems with the eyelid. Some lesions of the eyelid can be treated with cryotherapy; however,

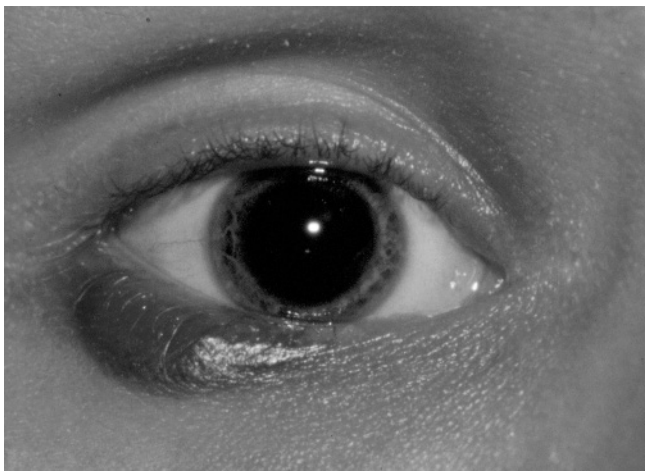

FIG. 26.5. Kaposi's sarcoma of the lower eyelid in a patient with AIDS.

others may require radiation therapy (341,353). Intralesional vinblastine (29) or interferon alpha (341) may prove a viable option for conjunctival and lid involvement. Systemic or extensive disease may require chemotherapy with doxorubicin hydrochloride, bleomycin sulfate, and vinblastine sulfate alone or in combination (351). Local therapy should be deferred for patients undergoing chemotherapy for systemic involvement until the response of the ocular adnexal lesions is known (351,353). HAART alone has been reported to produce spontaneous and sustained remission of Kaposi's sarcoma (341).

In patients with HIV infection, high grade lymphoma is an AIDS defining disorder (354). Orbital involvement by lymphoma has been reported (26,355), but even in the pre-HAART era it occurred in less than 1% of patients with AIDS (26). Intraocular malignant large cell lymphoma (356,357), primary eyelid, and conjunctival non-Hodgkins lymphoma (341) also have been reported, but are uncommon. Other neoplastic lesions identified in patients with HIV include squamous cell carcinoma, conjunctival intraepithelial neoplasia, and basal cell carcinoma (341).

NEURO-OPHTHALMIC LESIONS

In the pre-HAART era, neuro-ophthalmic lesions were reported in 6–8% of patients with AIDS (26,31). These lesions included cranial nerve palsies, papilledema, optic neuropathy, hemianopsias, and cortical blindness (26,291,300,310,358–365). The most common etiology for neuro-ophthalmic lesions was cryptococcal meningitis, accounting for 54% of the neuro-ophthalmic lesions reported in one series (26,31,300). Of patients with AIDS and cryptococcal meningitis, 25% had neuro-ophthalmic lesions on careful ophthalmologic examination (31). Papilledema occurred in 31% of patients with cryptococcal meningitis (300). Disc elevation without hemorrhage, focal blurring of the disc margin or loss of the cup are the rule in 84% of patients with cryptococcal meningitis and papilledema according to Kestelyn et al. (300). Visual loss from cryptococcal meningitis occurs in 1–9% of patients (300,365). This loss may occur from direct invasion of the optic nerve by *C. neoformans*, elevated intracranial pressure, or adhesive arachnoiditis (365).

Other causes of neuro-ophthalmic lesions in patients with HIV include herpes zoster ophthalmicus, toxoplasmosis, syphilis, viral encephalitis (29,364), other opportunistic infections, progressive multifocal leukoencephalopathy associated with JC polyomavirus, and CNS lymphoma. *Histoplasma capsulatum* was cultured from the optic nerve sheath of a patient with optic neuritis (366).

HIV may be responsible for some cases of optic neuritis or optic neuropathy. Case reports of optic neuropathies in the setting of HIV infection but without other identified cause have been described by Sweeney et al. (367) and Newman and Lessell (368). Tenhula et al. (369) found that optic nerves from patients with AIDS but no associated ocular opportunistic infections have 42% fewer axons than those from age matched controls without AIDS. Quiceno et al. (370) found that patients with AIDS and no ocular opportunistic infections had deficits in Farnsworth-Munsell 100-hue color vision testing and in contrast sensitivity compared to controls without AIDS. In addition, Plummer et al. (371) found that patients with HIV have an increased frequency of localized visual field defects and an increased mean defect on perimetry compared to age-matched healthy controls. Whether these findings are related to the cumulative effect of nerve fiber layer infarcts from AIDS retinopathy or due to a direct effect of HIV on the optic nerve or CNS is unknown (369,370). Subtle ocular motility defects also can be detected in patients with AIDS by eye movement recordings using infrared oculography (360,361). These defects include slowed saccades, fixational instability, and abnormal pursuit and appear to be more directly related to HIV infection than to opportunistic ocular or neurological infections. They may correlate with the severity of the AIDS-dementia complex.

DRUG RELATED OCULAR EFFECTS

Several drugs used to treat HIV and its complications have been associated with adverse ocular effects. Clofazimine, an iminophenazine dye with antimicrobial activity used to treat *M. avium* complex infection, has been associated with pigmented corneal verticillata and in one case a bull's eye maculopathy (372). Dideoxyinosine (didanosine), a purine nucleoside analogue used to treat HIV-1, was reported to cause well-circumscribed areas of RPE atrophy in three of 43 children in one study (373). No such toxicity has been reported in adults treated with this agent. Intravenous and intravitreous cidofovir has been associated with hypotony and intraocular inflammation

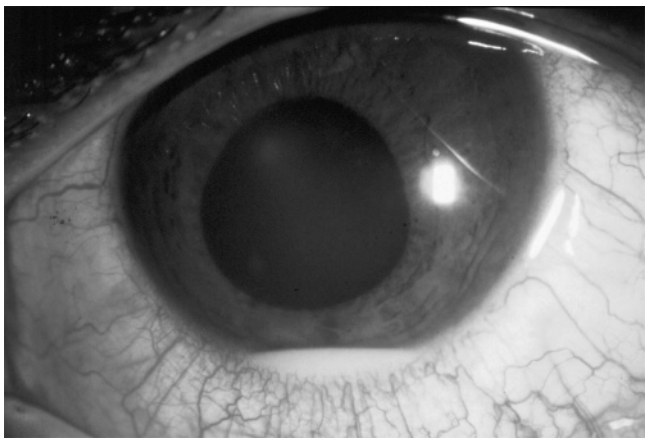

FIG. 26.6. Hypopyon secondary to rifabutin use in a patient with AIDS.

(29,212). Ganciclovir and acyclovir have been reported to be associated with the development of a corneal epitheliopathy due to the accumulation of lipid vacuoles and lipid containing inclusions in the corneal and conjunctival epithelial cytoplasm in two patients (374). Whether one, both, or the combination of these two agent(s) is responsible is unclear; however, dose reduction or discontinuation of the agents has led to resolution of the process. Atovaquone used in the treatment of Toxoplasmosis and *Pneumocystis carinii* pneumonitis has been associated with subepithelial corneal deposits and vortex keratopathy which may or may not affect vision (29,298,299,341). Rifabutin, an antimycobacterial agent, has been linked to the development of fulminant anterior uveitis which can mimic infectious endophthalmitis (Fig. 26.6) (375–381). Two of nine subjects in one study who developed a symmetric polyarthralgia/polyarthritis syndrome while taking 1,800 mg/day or more of rifabutin also developed acute anterior uveitis (379). Twenty-three of 59 patients randomized to receive rifabutin 600 mg/day, clarithromycin 1,000 mg twice a day and ethambutol 15 mg/kg/day for treatment of MAC infection as part of the MAC Study Group of the Canadian HIV Trials Network developed iridocyclitis (380). The process can be unilateral or bilateral with 55–100% of patients presenting with a hypopyon (375,378). The use of concurrent ßuconazole and/or clarithromycin has been suggested as a factor in the onset of this condition due to the inhibition of the hepatic microsomal cytochrome P-450 system by these agents which has the pharmacokinetic effect of raising serum levels of rifabutin (375,378,381). Reports of rifabutin-associated uveitis occurring in patients with iatrogenic immunosuppression and in patients with no immunosuppression suggests that HIV infection is not a prerequisite for this drug-related complication (381,382). Dose reduction, drug discontinuation, with or without topical steroids often is effective. Drugs associated with ocular toxicity used in non-immunosuppressed patients, such as ethambutol, are likely to have similar toxicity proÞles in patients with HIV infection.

MISCELLANOUS OCULAR INVOLVEMENT

HIV has been isolated from the tears (383,384), conjunctiva (383,384), cornea (385–387), aqueous and vitreous of patients with HIV infection (388–392). As such, all potential material for corneal transplantation is screened for the presence of HIV infection, and all infected material not transplanted. Whether viable virus can be aerosolized during laser corneal refractive surgery is under investigation. Hagen et al. (393) were unable to infect tissue culture plates from the plume generated following excimer laser photoablation of a culture plate infected with pseudorabies virus, a porcine herpesvirus similar to HIV, and suggested that photoablation of HIV infected human corneas during photorefractive keratectomy (PRK) and laser *in situ* keratomileusis (LASIK) is unlikely to pose a health hazard to the surgeon. Nonetheless, HIV infection is considered a contraindication to laser eye surgery by at least one laser manufacturer. Due to the potential presence of HIV in tears, it is recommended that ophthalmologists performing eye exams utilize appropriate universal precautions (394).

HIV-associated keratoconjunctivitis sicca has been reported to occur in 10–20% of patients with HIV infection (29,341). Potential causes include direct involvement of HIV with the lacrimal gland, accessory lacrimal glands, or conjunctiva, abnormal blink reßex due to encephalopathy or ocular surface tumors, abnormal tear composition, and drug-related side effects (29,341). Treatment with artiÞcial tear drops or ointments, punctual occlusion, and management of contributing eyelid abnormalities is appropriate.

Anterior uveitis has been identiÞed in HIV-infected patients with intraocular infections such as cytomegalovirus, varicella-zoster, *Toxoplasma gondii*, *Treponema pallidum*, bacterial, and fungal infections (29,106). One autopsy report identiÞed cytomegalic and acute inßammatory cells in the iris stroma of a patient with HIV suggesting that CMV can cause a direct infectious anterior uveitis (106). As discussed previously, some patients taking rifabutin or cidofovir develop intraocular inßammation (29). Cases of HIV-associated uveitis, which responded to treatment with zidovudine have been reported (389,392), however, this process appears to be an infrequent occurrence, and was not related to immune recovery uveitis since they did not involve HAART, immune reconstitution, or CMV retinitis. Cases of immune uveitis associated with systemic conditions such as ReiterÕs syndrome may be more common in patients with HIV infection (29,341).

Levinson et al. (395) described an inßammatory syndrome consisting of multifocal retinal inÞltrates, and mild

anterior chamber and vitreous cellular reaction in 50 eyes of 26 patients with HIV infection, none of whom received HAART. Clinical or angiographic retinal vascular sheathing and keratic precipitates were inconsistent findings. The retinal lesions were irregular, poorly-demarcated, gray-white or yellow, less than 200 μm in diameter, and located in the midperipheral or anterior retina. The lesions remained stable in size or slowly enlarged over a period of months and were felt to respond to zidovudine but not acyclovir or ganciclovir. Infectious causes of multifocal retinitis were excluded, however, HIV was cultured from the vitreous of one patient. Whether this syndrome is caused by direct HIV infection, an associated autoimmune phenomenon, or some other cause remains to be determined. Profound immunosuppression was not a prerequisite for this relatively benign condition since the median CD4+ T-cell count among affected patients was 272 cells per μL with a maximum count of greater than 2,000 cells per μL in one patient.

Cases of other uncommon ocular complications have been reported in patients with HIV infection, but whether these complications were of higher frequency than in the general population remains to be determined (341, 396–398).

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PATHOLOGY OF HIV INFECTION

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Chapter 27

General Pathology of HIV Infection

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Since the initial cases of what was subsequently labeled as acquired immunodeÞciency syndrome (AIDS) (1,2), pathologic diagnosis of the major indicator diseases, opportunistic infections and neoplastic disorders according to the Centers for Disease Control (CDC) deÞnition of AIDS (3), has played a major role in recognition of the syndrome. In addition to diagnosis of known lesions, new or previously undescribed lesions were also recognized in biopsy material from AIDS patients (e.g. pulmonary lymphoid lesions in children with AIDS, bacillary angiomatosis of skin). Similarly, autopsy studies on fatal cases of AIDS extended the clinical spectrum by demonstrating involvement of clinically unsuspected organs by opportunistic infections and neoplastic disorders. Familiarity with the clinical spectrum of AIDS and dissemination of information regarding its various clinicopathologic Þndings had reached a plateau with respect to new or unsuspected pathologic observations about the infectious and neoplastic complications of AIDS. With longer patient survival due to effective antiretroviral drug treatment, in conjunction with prophylactic and supportive therapies, a variety of processes have been identiÞed for which the pathogenesis is obscure, though clearly associated with human immunodeÞciency virus (HIV) infection (i.e. nephropathy, cardiomyopathy, arteriopathy, etc.). We are also seeing examples of tissue damage associated with toxic reactions to different drugs, with diagnostic and therapeutic procedures, and with the chronic debilitating disease process of AIDS. Thus, pathologic lesions seen in patients with AIDS are broadly classiÞed into three major categories (4): (1) primary lesions directly due to HIV infection itself (lymphoreticular system and brain); (2) associated lesions due to direct or indirect sequelae of HIV infection (opportunistic infections due to defective cell-mediated immunity, iatrogenic lesions, lesions associated with chronic debilitation disease); and (3) lesions of undetermined pathogenesis (cardiomyopathy, nephropathy, arteriopathy, etc.). Finally, it is recognized that many lesions are the result of more than one pathogenic mechanism and are modiÞed by attempts to inßuence the disease process with chemotherapy or immunotherapy.

Most pathology reviews of this subject have employed either an organ systems approach or have concentrated on speciÞc infections and neoplasms. As the pathology of diseases associated with AIDS represents a broad spectrum of lesions affecting virtually all tissues and organs, we have endeavored to integrate both approaches to avoid redundancy and stress important clinicopathologic correlations.

The Þrst sections in this chapter address the diagnosis of AIDS-related opportunistic infections in cytology and biopsy material by use of both conventional and more recently described immunologic and molecular methods. This is followed by an overview of common neoplasms and some of the poorly understood complications of the disease. Pathology of the nervous system and skeletal muscle in HIV infection, which is described in a separate chapter, is excluded. The pathology of AIDS in children emphasizes characteristic lesions of this age group and highlights important similarities and differences as compared with adults. The chapter concludes with a discussion of the risks of nosocomial transmission of HIV and recommendations to laboratory personnel on procedures to prevent mucosal and parenteral exposure to infectious agents.

GENERAL ASPECTS OF THE PATHOLOGY OF AIDS

Because of the profound deÞciency in cell-mediated immunity, the pathologic lesions related to infections in AIDS patients have certain unusual features. The lesions tend to be larger than usual (e.g. molluscum contagiosum of skin); the inßammatory reaction is minimal or absent

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(e.g. cryptococcosis); granuloma formation is indistinct or absent (e.g. *Mycobacterium avium-intracellulare* (MAI) infection); lesions contain large numbers of organisms (e.g. MAI); infection with two or more organisms may involve the same site (e.g. *Pneumocystis carinii*, cryptococcal and cytomegalovirus (CMV) pneumonitis); necrotizing lesions may be present (e.g. CMV lesions in gastrointestinal tract or adrenal glands); and wide dissemination of organisms (e.g. *Pneumocystis carinii* infection) may occur. Concerning neoplastic lesions, malignant lymphomas are usually of high grade and occur more frequently in extranodal sites. Similarly, HIV-related Kaposi's sarcoma is clinically distinct from the endemic form and often shows wide cutaneous dissemination, early visceral spread, and incomplete brief response to therapy.

DIAGNOSIS OF AIDS-RELATED OPPORTUNISTIC INFECTIONS

Opportunistic infections are the most common initial manifestations of AIDS, and the etiologic diagnosis of these infections is generally made through examination of tissue biopsies or through cytologic techniques. Many of these organisms either cannot be cultured (e.g. *Pneumocystis carinii*) or require several days for positive culture results. Therefore, morphologic identiÞcation of the etiologic agents of infections is of prime importance in these patients. Besides the routine histologic procedures, immunologic and molecular biologic methods can be used as ancillary diagnostic techniques.

Cytologic Specimens and Techniques for Diagnosis of Infectious Diseases

The rapidity with which a diagnosis can be rendered is one of the major strengths of cytologic techniques. Respiratory specimens (bronchial washings, brushings, transtracheal aspirates, and bronchoalveolar lavage), Þne-needle aspiration biopsies, cerebrospinal ßuid, effusions, and gastrointestinal brushings are the principle sources of material for diagnostic cytologic studies in these patients. The more common respiratory cytopreparatory methods include the Saccomanno technique, cytocentrifugation, thin-layer, and membrane Þltration. Most specimens are received in the Þxed state due to their infectious nature, but fresh material gives laboratory personnel greater ßexibility if ancillary techniques, such as immunocytochemistry or molecular diagnostic methods, are to be employed. From cerebrospinal ßuid and effusions, slides are prepared from cytocentrifuged material or membrane preparations. For gastrointestinal brushings, direct smears are usually employed. Attention to the precautions outlined later in this chapter make the cytopreparatory techniques safe.

Fine-needle aspiration biopsy (FNAB) has assumed an important role in the diagnosis of opportunistic infections and neoplasms in AIDS patients. Aspiration biopsy of superÞcial lesions is easily performed using a 22 or 25-gauge needle attached to a 20-ml syringe. Percutaneous FNAB of deep lesions is generally performed by a radiologist using a 22-gauge Chiba needle employing ultrasound, computerized tomography (CT), or ßuoroscopic guidance. Smears are prepared by spreading the material over a small area with another slide (similar to the preparation of a bone marrow aspirate) and some are air-dried and some are alcohol-Þxed. The rapid staining and interpretation of air-dried smears using a modiÞed Wright-Giemsa stain (Diff-Quick, Baxter, Healthcare Corp.) determines the adequacy of a specimen and can often provide prompt information to clinicians, in addition to identifying those cases requiring more material for ancillary studies such as immunohistochemistry, culture, electron microscopy, and ßow cytometry. FNAB is also an excellent source of material for bacterial, fungal, or viral cultures. Alcohol-Þxed smears are stained by a modiÞed Papanicolaou technique.

From the sources described above, a variety of special stains are selectively employed to detect microbiologic organisms. These include Gomoris methenamine silver (GMS) for fungi, Nocardia, and *Pneumocystis carinii*, periodic acid-Schiff (PAS) for fungi; mucicarmine to demonstrate the capsule of cryptococcus; modiÞed Ziehl-Neelsen, Fite or Kinyoun for mycobacteria; Brown-Brenn and Brown-Hoff for bacteria; Giemsa for certain protozoans; and Dieterle for Legionella. Calcoßuor white, a nonspeciÞc ßuorochrome with afÞnity for chitin and cellulose, has been used to delineate fungal elements and cysts of acanthamoeba in clinical specimens (5,6). While not widely utilized, ßuorescence microscopy performed on Papanicolaou-stained material can identify several genera of fungi such as Aspergillus, Blastomyces, Histoplasma, Cryptococcus, and Coccidioides that demonstrate autoßuorescence (7). The choice of Þxed or unÞxed material employed for immunocytochemistry, immunoßuorescence, and DNA probe studies for microbiologic organisms must be tailored to the particular antibody or probe.

Surgical Biopsy Specimens

The most common surgical biopsy specimens for diagnosis of infections and neoplasms in AIDS patients are those from lung, lymph nodes, alimentary tract, skin, liver, bone marrow, and brain. The proper handling of this material is dependent on the disease process suspected. Frozen section examination plays a pivotal role by allowing the pathologist to triage the specimen for appropriate diagnostic studies in a timely and cost-effective manner similar to that provided by an immediate examination of an FNAB specimen. Recognition of an infectious process in a frozen section of lung or lymph node would prompt submitting tissue for appropriate

microbiologic cultures, molecular biology studies, and preparing touch imprints or frozen sections for histochemical stains. If a neoplastic process were suggested, routine light microscopy could be supplemented with immunocytochemical studies, cytogenetics, and ßow cytometry.

Immunologic and Molecular Biologic Procedures in the Diagnosis of Infectious Agents

When traditional methods fail to establish an unequivocal diagnosis, newer immunologic and molecular biologic procedures may improve recovery rates and detect fastidious organisms. These include immunoperoxidase and immunoßuorescence methods, RNA/DNA hybridization, and molecular diagnostic techniques. Commercially available polyclonal and monoclonal antibodies can be used to detect a variety of antigens and infectious agents including many fungi, viruses, bacteria, and protozoa (8). DNA/RNA hybridization techniques identify speciÞc microbial DNA or RNA sequences in tissue sections, smears, or culture specimens (9). Commercially available probes useful in AIDS include those to Epstein-Barr virus, cytomegalovirus, mycobacteria, Legionella, Campylobacter, herpes simplex virus, and HIV (8Ð14). Polymerase chain reaction results in the rapid replication of target DNA sequences providing dramatic improvement in sensitivity. Because of the extreme sensitivity of this technique, control studies are essential and specimen/reagent contamination must be meticulously avoided. A wide variety of infectious agents, including mycobacteria, cytomegalovirus, Epstein-Barr virus, *Pneumocystis carinii* and HIV have been detected using this method (15Ð19). A case of HIV infection occurring in 1959 was identiÞed by using this technique in the DNA extracted from paraffin blocks of autopsy tissues in a 25-year-old sailor (20).

IMPORTANT OPPORTUNISTIC INFECTIONS IN AIDS PATIENTS

Parasitic Infections

Pneumocystis carinii

Pneumocystis carinii (PC) pneumonia is an important indicator of severe immunodeÞciency due to HIV infection and is a criterion for the diagnosis of AIDS as deÞned by the Centers for Disease Control (CDC) (3). Since 1987, there has been a signiÞcant decrease in the incidence of PC pneumonia as an initial AIDS-deÞning diagnosis among HIV-infected homosexual men, which is most likely due to increasing use of chemoprophylaxis for PC prior to the onset of AIDS (21). Despite the decreased frequency of PC infections in these patients, it does remain a common cause of death in AIDS (22). While PC infections are most commonly conÞned to the lungs, an increasing number of patients with extrapulmonary spread, including widely disseminated disease, are being reported (23Ð27). Resistance to sulfonamide therapy has been detected in some cases of PC infection. Mutations in dihydropteroate synthetase genes of PC have been identiÞed which are responsible for this sulfonamide resistance. These mutations may develop following exposure to sulfa drugs and have been found to be a poor prognostic factor in PC pneumonia (28).

Although traditionally classiÞed as a sporozoan protozoal parasite, recent molecular biological and ultrastructral studies have demonstrated that PC is more closely related to fungi than to protozoa (26). There is homology of mitchondrial DNA and ribosomal RNA in PC and fungi, and a gene found exclusively in fungi, translation elongation factor 3 gene, has also been demonstrated in PC (29). The organismÕs natural reservoir and life cycle are poorly understood. Current studies support four stages in the life cycle: precyst, cyst, sporozoites within cysts, and freestanding trophozoites (30,31). The cyst, presumed to be the proliferative form, contains up to eight small, nucleated structures termed sporozoites. After rupture of the cyst wall, sporozoites are released and mature into trophozoites, the vegetative form. Large trophozoites develop into precysts, which undergo further maturation to cysts containing sporozoites.

PC has been recovered from a large number of animal species as well as humans (32). Serologic investigations in humans imply that many infections are acquired early in childhood (33) and disease develops through reactivation of latent infection. Alternatively, de novo infections probably also occur and transmission is by the aerosol route (34,35).

Diagnosis

Pulmonary specimens including induced sputum, bronchial washings and brushings, bronchoalveolar lavage, FNAB, transtracheal aspirate, transbronchial biopsy, and open lung biopsy are used for diagnosis. However, with the recognition of disseminated pneumocystosis, FNAB and surgical biopsies of tissues from a variety of sites are also employed. In our experience and from a number of studies, bronchoalveolar lavage has shown the greatest sensitivity in the diagnosis of lung infections when compared with other noninvasive methods. Bedrossian (36), in reviewing 17 studies comparing various techniques, reported yields of 83% for transbronchial biopsy, 82% for bronchoalveolar lavage, and 53% for washing and brushing cytology. When bronchoalveolar lavage is combined with other methods such as bronchial washings and brushings, diagnostic yields approach 100%. Open lung biopsy and transbronchial biopsy remain the gold standard but are rarely necessary to secure a diagnosis. Spontaneous and induced sputum yield the worst results as compared with other methods. CT-guided FNAB is usually reserved

for focal or cavitary lung lesions (37) but may also have a role in the evaluation of pediatric patients (38).

Staining Methods

While the Grocott or GMS stains are the most commonly used methods, Gram-Weigert, PAS and toluidine blue also detect the cyst wall. Sporozoites and trophozoites are best demonstrated with Diff-Quick, cresyl violet, Giemsa, May-Grunwald-Giemsa, PAS, and Wright-Giemsa stains (39–41). The cyst wall stains can be used to evaluate tissue sections, imprints, and cytology specimens, whereas the trophozoite/sporozoite stains are applicable only on imprints and cytology specimens. We utilize the GMS stain with a microwave modiÞcation and have found this to be the single best method as it can be rapidly performed and has the advantage of staining other pathogens such as fungi, bacteria, and the cytoplasmic inclusions of cytomegalovirus (42). With this stain, cyst forms are spherical, crescentic, or cup-shaped and measure 5–7 μm in diameter. Single or paired capsule dots correspond to the internal capsular thickening seen ultrastructurally. Ghali et al. (43) have shown ßuorescence of the cysts in Papanicolaou-stained material when it is examined under UV light.

Other methodologies for detection of PC in cytology specimens and tissue biopsies include immunoßuorescence and immunoperoxidase techniques (44–46). Genomic DNA fragments of PC have been used as probes in the Southern blot technique and ribosomal RNA sequences employed as probes for in situ hybridization (47,48). PCR appears to be a speciÞc and sensitive method for detection of PC in HIV-infected patients (19). While antibody and probe techniques have been shown to be speciÞc, demonstrating little or no cross reactivity with common fungi, bacteria, or parasites, they offer few advantages over traditional staining methods.

Histopathologic Features

As compared with sporadic and endemic forms, AIDS-associated PC lung infections generally show less interstitial widening and lymphoplasmacytic inÞltrate and more atypical features (49). The pattern of reaction also differs from that described in animal models in which cortisone and low protein diet are used for immunosuppression (50). The characteristic and speciÞc feature is a foamy refractile intra-alveolar exudate in which variable numbers of organisms are demonstrated with the cyst wall stains (Fig. 27.1). This is accompanied not infrequently by nonspeciÞc features including diffuse alveolar damage with hyaline membrane formation, hyperplasia and atypia of type II pneumocytes, and sparse septal inßammation. Less common manifestations include epithelioid granulomas, giant cell formation, necrotizing bronchopneumonia,

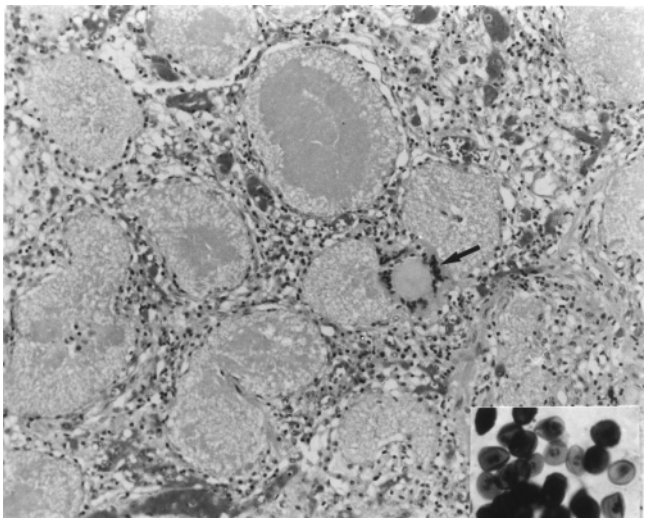

FIG. 27.1. *Pneumocystis carinii* pneumonitis characterized by foamy alveolar exudate and mild interstitial mononuclear cell in ltrate. Giant cell reaction (arrow) is an unusual feature (hematoxylin and eosin, × 100). Inset: GMS stain demonstrating cup-shaped and spherical cysts with dot-like or double comma-shaped capsule thickenings (Gomori's methenamine silver, × 1,000).

interstitial Þbrosis, a desquamative interstitial pneumonitis like pneumonia, pneumothorax with pleural spread of PC, and cavitary lesions showing central necrosis (37, 51–59). In a study of 123 lung biopsies from 76 AIDS patients with PC pneumonitis, Travis et al. (51) additionally noted (in a small percentage of patients) parenchymal cysts, interstitial microcalciÞcations, vasculitis, and vascular permeation by PC; the latter Þnding was also described by Liu et al. (54) and was suggested as a possible mechanism for dissemination. The characteristic alveolar inÞltrate was not present in 19% of PC-positive biopsies, emphasizing the importance of performing special stains in the absence of typical features (51). Concomitant polymicrobial infections with a variety of viruses, fungi, and bacteria are common. The cysts of PC are cleared slowly in patients treated for acute PC pneumonia, with cysts still detectable in sputum of 76% of patients three weeks after diagnosis (at the time of completion of therapy), and cysts detectable in 24% of patients 6 weeks after diagnosis (60).

Extrapulmonary Spread

An increasing number of extrapulmonary infections, including widely disseminated disease, are being reported (23–27,61). In a study by Telzak et al. (24), 2.5% of patients with AIDS showed evidence of extrapulmonary PC infection. There is an increasing incidence of extrapulmonary PC (21,62). The most common extrapulmonary site of spread is the lymph node; however, involvement of bone marrow, spleen, liver, GI tract, pancreas, palate, pericardium, thymus, central nervous system, ear and eye

have also been reported. These patterns suggest lymphatic and hematogenous spread, but the risk factors for dissemination are not well understood. Rarely, patients with AIDS may present with extrapulmonary disease without a documented lung infection (24, 63). Sites of involvement in this setting have included spleen, thyroid, external auditory canal, middle ear, and skin. The histopathology of extrapulmonary PC infections is generally similar to that described in typical pulmonary disease. Frothy eosinophilic exudates containing numerous cysts and trophozoites are usually associated with necrosis and scant inßammatory inÞltrates.

Toxoplasma gondii

Transmission of *Toxoplasma gondii* (TG) to humans occurs primarily by ingesting oocysts from cat feces or by eating meat that contains tissue cysts (64). When the oocysts are ingested by secondary hosts (possibly all mammals), the organisms excyst, cross the bowel wall, and invade cells of many organs. Before immunity develops, they divide rapidly, giving rise to tachyzoites in a pseudocyst that eventually Þlls the cell, ruptures it, and enters contiguous cells. In chronic and latent infections, bradyzoites develop slowly and form within true cysts.

Toxoplasmosis is the most common opportunistic infection of the central nervous system in AIDS. Toxoplasmosis is the presenting/index AIDS diagnosis in up to 51% of HIV-infected patients (65). The overall incidence of clinical toxoplasmosis among AIDS patients with neurological disease is about 30% and it is particularly common in Haitian patients with AIDS (66). Toxoplasmosis appears to represent reactivation of latent infection rather than primary infection, as serologic studies have shown detectable toxoplasma IgG antibodies in patients several months prior to development of clinical disease (67).

Chorioretinitis is an infrequent manifestation, but may appear associated with or independent of encephalitis (68). Extraneural toxoplasmosis has been reported in the heart, lungs, testis, and skin of AIDS patients with concurrent central nervous system (CNS) disease (69Ð72). In autopsy studies, the organisms have been detected in many other organs, including stomach, adrenals, skeletal muscle, and pancreas, but infections in these sites are usually of little clinical signiÞcance (73,74).

Diagnosis and Histopathology

The primary method of diagnosing toxoplasmosis is by demonstrating the tachyzoites and cysts in clinical specimens including ßuids, smears, and tissue biopsies. While serologic studies are considered reliable in the normal host, in an immunocompromised individual they play a limited role in establishing the diagnosis of acute infection. Even with severe toxoplasmosis, it is unusual for AIDS patients to demonstrate positive IgM titers or appropriate four-fold increases in IgG titers (73). However, the absence of antibodies against TG is strong evidence against a diagnosis of toxoplasmosis (75). Methods to detect TG antigens (dot-blot and enzyme-linked immunosorbent assay (ELISA)) are under investigation and may prove more reliable than antibody tests (76,77). TG antigen can be detected by PCR in specimens such as CSF (65). TG can also be detected in peripheral blood specimens using indirect immunoßuorescence in cell culture systems (78).

TG visualized in ßuids, tissue imprints, and histologic sections show the characteristic crescentic or oval tachyzoites, pseudocysts, and true cysts. In Giemsa or Wright-stained material, the tachyzoite cytoplasm stains blue and the eccentric nucleus appears dark red. The organisms are small, measuring 3Ð4 μm in length and 1Ð2 μm in width. They are generally broader at the end containing the nucleus. Cysts are variable in size, measuring up to 40 μm in diameter and contain numerous tightly aggregated bradyzoites surrounded by a thin eosinophilic membrane that is weakly positive with silver and PAS stains (Fig. 27.2). Organisms may be more easily demonstrated with a commercially available immunohistochemical stain for TG (79). The lack of a kinetoplast and the presence of a distinct nucleus differentiate TG from Leishmania and *Trypanosoma cruzi* (80).

The gross and microscopic pathology of TG infection, which is described in another chapter devoted to the central nervous system pathology of HIV infection, will be brießy reviewed here since TG can produce lesions outside the CNS. In the brain, the principle microscopic features are a diffuse meningoencephalitis or a focal necrotizing encephalitis with associated arteritis and thrombosis (81, 82). Tachyzoites and cysts can be demonstrated in brain tissue surrounding areas of necrosis although organisms may not be seen in over 50% of cases.

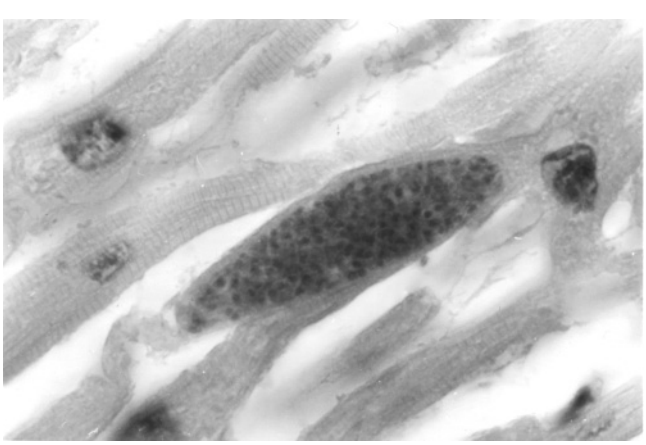

FIG. 27.2. Chronic myocardial infection with *Toxoplasma gondii*. Elongated cyst contains numerous tightly aggregated bradyzoites unassociated with in ammation (hematoxylin and eosin, × 200).

Ocular toxoplasmosis often produces a granulomatous inßammatory reaction of the retina and choroid, which is in part related to a hypersensitivity reaction. There may be retinal necrosis with hemorrhage and vascular thrombosis. The parasites may be numerous or difÞcult to demonstrate (83).

Toxoplasma pneumonitis shows a variable histology often consisting of an interstitial and intra-alveolar inÞltration by mononuclear cells of a focal or diffuse nature. Abundant tachyzoites are usually demonstrable (69).

Involvement of many other organs such as heart, liver, adrenal gland, and stomach has been documented in AIDS patients with these lesions usually showing focal necrosis and variable numbers of organisms and inßammatory cells (73,74). Rarely, tachyzoites can be found in peripheral blood smears (Figs. 27.3aÐc).

Cryptosporidia and Other Protozoa

Cryptosporidium, a sporozoan parasite taxonomically related to Toxoplasma, Isospora, and Plasmodium species, has become recognized as a common cause of enteritis in the immunocompromised host (84Ð89). The life cycle of Cryptosporidium is similar to other coccidia in that asexual and sexual stages lead to production of oocysts that are released into the gastrointestinal tract and discharged in the feces. The organism gains access to humans through inhalation or ingestion and thus can be sexually transmitted through the oral-fecal route (90,91). As the oocysts are resistant to most common disinfectants, strict hand washing and enteric precautions are necessary to prevent nosocomial transmission.

In the United States, cryptosporidia infects approximately 5% of HIV-infected men (92). Cryptosporidia is found in the stool of 10Ð20% of patients with AIDS associated diarrhea (93), while in African patients with enteropathic AIDS, they are implicated in up to 48% of cases (94). These infections can involve the entire gastrointestinal tract from esophagus to rectum (84,95). Less common sites of infection include gallbladder, pancreas, biliary and pancreatic ducts, and respiratory tract (87,88,96,97). The biliary and pulmonary forms have been

FIG. 27.3a. Disseminated acute toxoplasma infection. Gastric ulcer containing numerous free tachyzoites within lamina propria (inset) (hematoxylin and eosin, × 100). Inset: (hematoxylin and eosin, × 400).

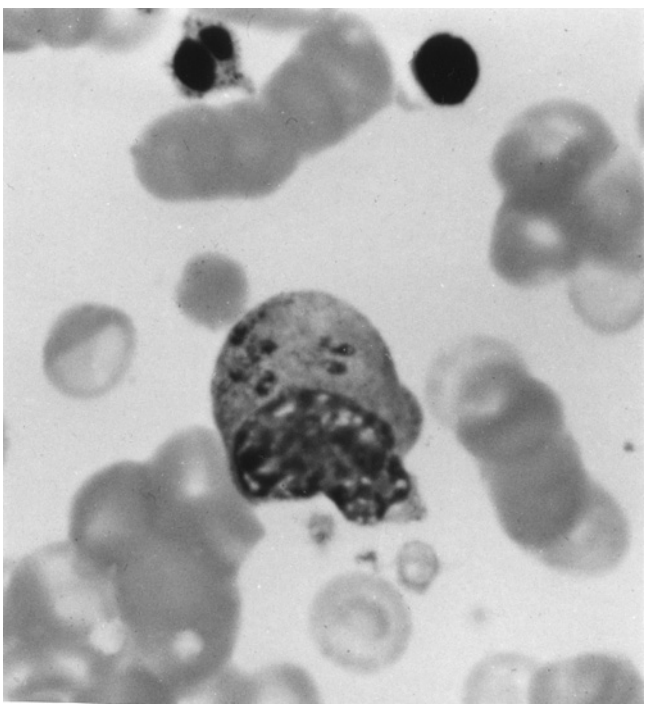

FIG. 27.3b. Disseminated acute toxoplasma infection. Tachyzoites within peripheral blood mononuclear cells and cells of adrenal cortex (hematoxylin and eosin, × 630).

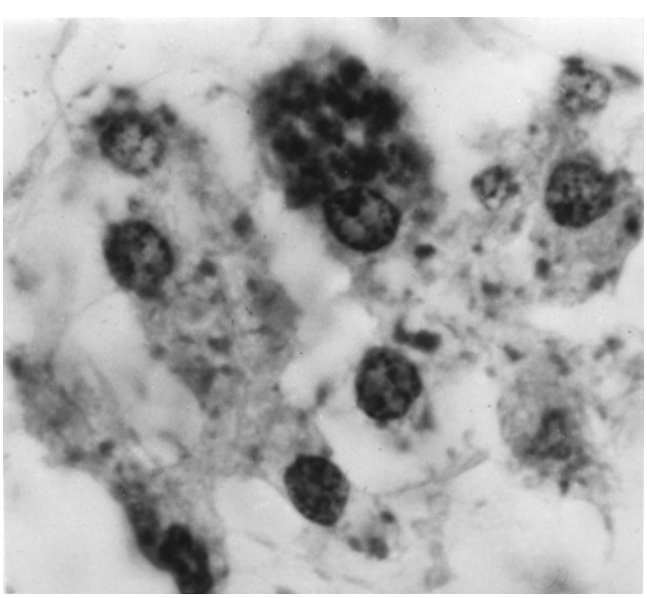

FIG. 27.3c. Disseminated acute toxoplasma infection. Tachyzoites within peripheral blood mononuclear cells and cells of adrenal cortex (hematoxylin and eosin, × 400).

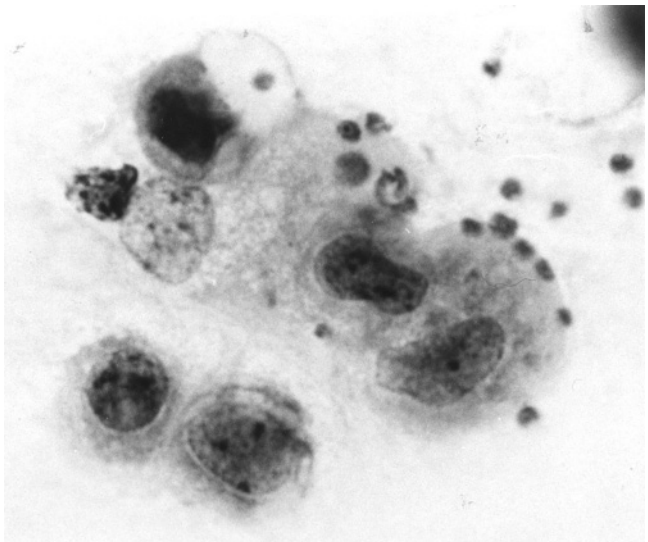

FIG. 27.4. Small intestinal brushing cytology demonstrating loosely cohesive group of epithelial cells with cryptosporidia aligned along luminal surface (Diff-Quik, × 630).

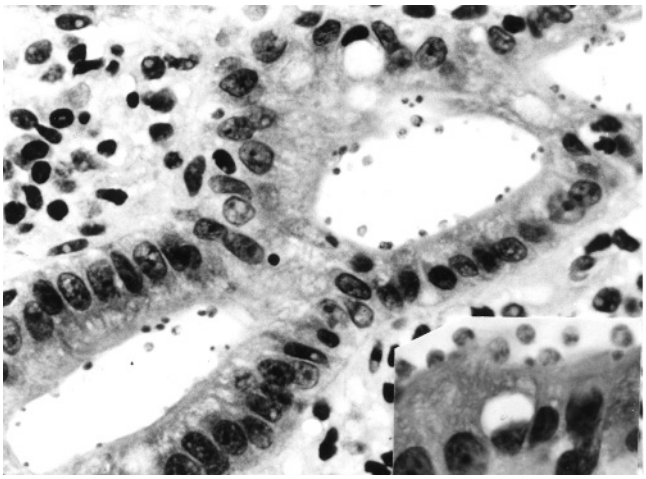

FIG. 27.5. Concomitant gastric mucosal biopsy with cryptosporidium along surface of epithelium (hematoxylin and eosin, × 250). Inset: (hematoxylin and eosin, × 1,000).

described almost exclusively in the immunocompromised host.

Diagnosis of cryptosporidiosis is usually based on fecal examination or small intestinal biopsy, but in our experience, the parasite can also be recognized in cytology preparations of small intestinal aspirates or brushings (98) (Fig. 27.4). The organisms are readily detected in the stool by a Kinyoun modiÞed acid-fast stain in which 4Ð5 μm bright red oocysts are present in background fecal material (99,100). The acid-fastness allows differentiation from yeast (100). As oocyst excretion can be sporadic, multiple stool examinations are sometimes necessary. In infections shedding low numbers of organisms, concentrating techniques such as SheatherÕs sucrose or zinc sulfate ßotation improve rates of detection. A speciÞc and sensitive direct immunoßuorescence method has been described and can be used on smears and parafÞn-embedded tissue sections (101).

In small intestinal mucosal biopsies, histologic changes do not correlate well with the degree of infection or clinical symptoms. Small intestinal villi exhibit mild atrophy and a nonspeciÞc increase in lamina propria chronic inßammation. The diagnostic feature in hematoxylin and eosin-stained sections is the presence of basophilic organisms located along the brush border or within parasitophorous vacuoles (86) (Fig. 27.5). Organisms are best demonstrated with the Giemsa or Gram stains and are inconsistently positive with the Kinyoun acid-fast stain in tissue sections (86,102). Cryptosporidia are differentiated from Isospora by their smaller size and location along the brush border.

Cryptosporidial infections of the gallbladder and extrahepatic biliary tree are observed in up to 10% of AIDS patients and present as acalculous and rarely gangrenous cholecystitis (87). The gallbladder shows luminal exudates and ulceration, and as in other sites, the organisms are visible lining the mucosa. Liver biopsies reveal pericholangitis and organisms attached to the epithelium of large bile ducts (97). Respiratory tract infections are less well characterized and probably result from aspiration of gastroduodenal material. Cryptosporidia are demonstrated lining bronchiolar epithelium associated with mild chronic inßammation (88). About one-third of patients with cryptosporidiosis have other gastrointestinal pathogens including herpes, Candida, cytomegalovirus, Mycobacterium, Giardia, *Entamoeba histolytica*, Shigella, and Salmonella (103).

Isospora belli, a protozoan related to the Cryptosporidium species, is an uncommon pathogen in AIDS patients in the United States only being found in approximately 2% of patients with AIDS-related diarrhea (93). *Isospora belli* is found in approximately 10% of patients with AIDS and diarrhea in Brazil, and in 12% of such patients in Zaire and Haiti (93). Clinical manifestations closely resemble those of cryptosporidial infection, most commonly chronic or relapsing enteritis (104,105). Diagnosis is made by demonstrating oocysts in stool or duodenal aspirates with the Kinyoun acid-fast or auramine-rhodamine stains (113, 114). Concentration methods similar to those described for Cryptosporidium enhance recovery and identiÞcation (104). In small intestinal mucosal biopsies, the organisms are seen in an intracellular location within clear vacuoles (106). Giemsa and PAS stains with diastase pretreatment enhance the ability to identify the organism (107). In gastrointestinal infection, Isospora may disseminate to regional lymph nodes and rarely to extraintestinal lymph nodes (lymphadenopathic isosporiasis) (104,107).

Only a limited number of human microsporidial infections have been recognized in AIDS patients, usually associated with gastrointestinal involvement or sinonasal

disease (108Ð112). The predominant clinical manifestations of these infections are weight loss, chronic diarrhea, or refractory sinusitis (110). Microspordia are found in 6Ð50% of patients with chronic diarrhea and AIDS (93). Small intestinal biopsy is currently the best method to demonstrate the organisms (111). They appear as small refractile bodies in a supranuclear location in epithelial cells of the villi. While difficult to visualize in hematoxylin and eosin-stained sections, some species are variably gram and Giemsa-positive and acid-fast (113,114). Electron microscopy has been used diagnostically and ultrastructural characteristics allow species identification (108,112). These organisms have also been reported to infect other tissues including liver, peritoneum, and skeletal muscle (109).

Other protozoan parasites including Leishmania, *Giardia lamblia*, *Blastocystis hominis*, and *Cyclospora cayetanensis* have also been infrequently reported to cause enteric infections in AIDS (115Ð118).

Fungal Infections

Fungal infections have been recognized as important AIDS-related opportunistic infections dating from early clinical reports. Initially, mucosal candidiasis and cryptococcosis were identified as the most prominent AIDS-related opportunistic pathogens while histoplasmosis and coccidioidomycosis were recognized much less frequently. While this trend still holds true today, in certain endemic locations, histoplasmosis and coccidioidomycosis are now being diagnosed with increasing frequency. Other fungi reported less commonly in these patients include Aspergillus, Sporothrix, Alternaria, Zygomycetes, and the superficial/deep dermatophytes.

Detection

Fungal serologic tests, with the exception of cryptococcal antigen testing and histoplasma antigen testing, are generally not useful in making a specific diagnosis of fungal infections, and culture methods may require extended periods of time, so direct microscopic examination of clinical specimens plays an important role in the management of AIDS patients providing prompt and useful information to clinicians.

In our laboratory, cytology preparations from fluids, smears, or FNAB are initially stained with the Papanicolaou technique and a modified Wright-Giemsa (Diff-Quick) stain. We have found the GMS and PAS stains in combination with Calcofluor-white to be the best general purpose fungal stains. In the microbiology laboratory, prompt handling of freshly collected specimens and use of a battery of enriched culture media with and without antibiotics are essential for recovering pathogenic fungi. Table **27.1???** outlines the characteristic morphologic features of fungi seen in clinical specimens discussed in this section.

Candida

Candida is a genus of yeast that is a well-recognized cause of mucocutaneous and invasive disease in patients with AIDS. Oral and esophageal candidiasis, the most common fungal infections in HIV-infected patients, are often the first indications of advancing immune dysfunction (119,120).

Oral candidiasis occurs as four distinct clinical variants: (1) pseudomembranous; (2) erythematous; (3) hyperplastic; and (4) angular cheilitis (121,122). The pseudomembranous type is the most frequent variant and presents as small yellow patches often on the tongue and buccal mucosa. Scrapings of these lesions contain epithelial cells mixed with necrotic debris, inflammatory exudates, and fungi consisting of yeast and pseudohyphae (Fig. 27.6).

Gastrointestinal candidiasis most frequently involves the esophagus but is also commonly seen in the stomach and to a lesser extent the small and large intestine (123). In AIDS patients, esophageal candidiasis almost always coexists with oral candidiasis (124). The clinical diagnosis is made by esophagoscopy, in which the characteristic white patches overlay inflamed mucosa. Endoscopic biopsy demonstrates yeast and pseudohyphae within the epithelium. Positive culture alone from biopsy specimens is not definitive evidence of infection (because the microorganism is part of the normal oral flora).

While oral and esophageal candidiasis is extremely common in patients with AIDS, systemic Candida infections are less frequently encountered. Recently, however, because of the more widespread use of indwelling catheters and parenteral hyperalimentation, increasing

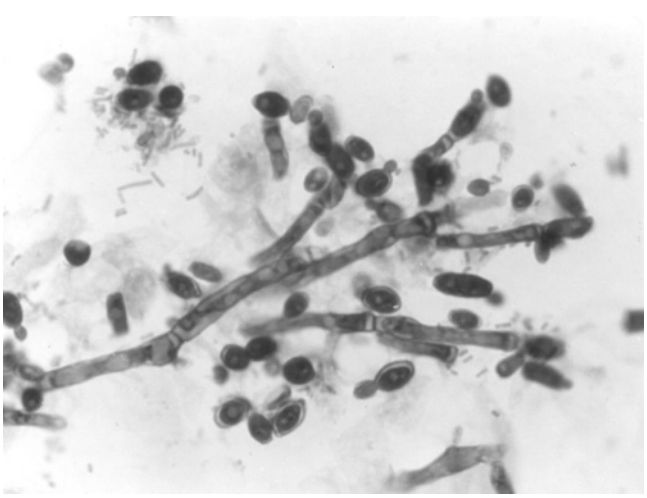

FIG. 27.6. Candida in esophageal brushing. Pseudohyphae and budding yeast differentiate this from other fungi (Papanicolaou, × 400).

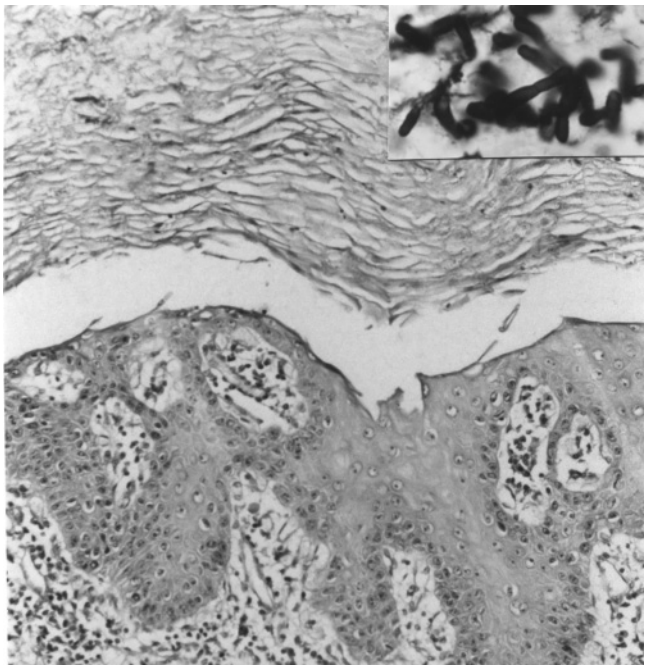

FIG. 27.7. Candida esophagitis, hyperplastic type. Hyperkeratotic layer above epithelium contains numerous yeast and pseudohyphae (hematoxylin and eosin, × 200). Inset: GMS stain demonstrating pseudohyphae and budding yeast (Gomori's methenamine silver, × 400).

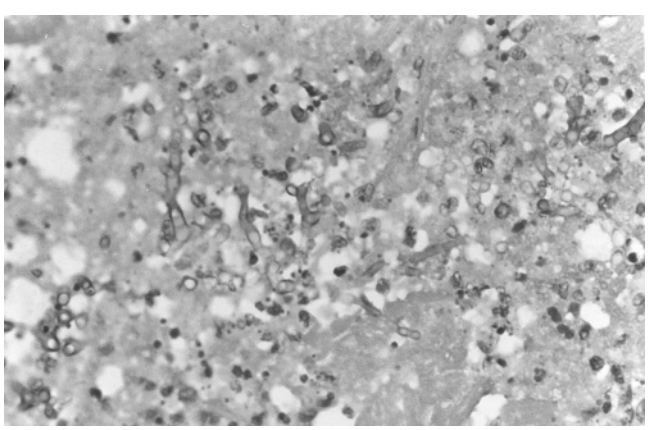

FIG. 27.8. Necrotizing candida pneumonitis. Pseudohyphae admixed with brin and in ammatory cells in destroyed lung parenchyma (hematoxylin and eosin, × 250).

numbers of reports of candidemia have appeared. Depending upon the cause and mechanism of entry, candidemia may spontaneously resolve, or the patient may succumb to overwhelming sepsis or develop visceral candidiasis. Widespread visceral dissemination usually involves the central nervous system, kidneys, heart, bone, skin, and lung (Fig. 27.8) (125–128). Unusual cutaneous presentations of mixed infections include coexistent Candida within lesions of molluscum contagiosum and Candida with herpes simplex (129).

Cryptococcus

Cryptococcosis is the fourth most common infection in AIDS patients after *Pneumocystis carinii*, cytomegalovirus, and mycobacterial disease (130). This life-threatening opportunistic infection is observed in between 5% and 10% of AIDS patients in the United States and has three major clinical presentations of disease: CNS, pulmonary, and disseminated infection (131,132). As many as 10–20% of patients with cryptococcosis have cutaneous involvement (papules, pustules, nodules, in ltrative plaques, ulcers, bullae, and cellulitis) as a component of disseminated disease, although there are reports of isolated cutaneous infection in the absence of systemic involvement (133). The vesicular and ulcerative forms may be mistaken for a herpetic infection or pyoderma gangrenosum, respectively, while the umbilicated papules may resemble molluscum contagiosum (134,135). Rarely, cutaneous lesions can clinically mimic Kaposi's sarcoma (136). Disseminated infections frequently involve lung, lymph node, adrenal gland, skin, and kidney (137,138). Unusual manifestations of systemic infections include myocarditis, massive peripheral and mediastinal lymphadenopathy mimicking malignant lymphoma, isolated pleural effusion, and biliary tract obstruction secondary to cryptococcal lymphadenitis (139–142). Simultaneous infections with other opportunistic organisms including *Pneumocystis carinii* in the lung and Toxoplasma in the brain have been found in up to 13% of cases (131,138,143). Cryptococcus has also been reported within lesions of Kaposi's sarcoma (144).

Diagnosis

The diagnosis of cryptococcosis requires isolation of the organism from body uids, biopsy demonstration of the encapsulated yeast, or detection of the polysaccharide capsular antigen in serum or cerebrospinal uid (CSF).

In cytology specimens such as CSF, bronchial washings and lavage, and joint uid, the diagnosis is made by identifying the 4–6 μm in diameter yeast with doubly refractile cell walls, a distinctly outlined capsule, and narrow based budding. Organisms are highlighted by the PAS or methenamine silver stains. The mucicarmine stain, speci c for the mucopolysaccharide capsule, and the characteristic variability in size, distinguish cryptococcus from other fungi.

The India ink method, in which nigrosin or India ink is added to CSF (or other body uid) and examined by light microscopy, is a commonly used technique to detect Cryptococcus. While the method is simple, the test suffers from poor sensitivity and requires strict attention to criteria for identi cation, as encapsulated forms of *Klebsiella pneumoniae*, Rhodotorula, Candida, and Prototheca can be confused with Cryptococcus. It is preferable to utilize routine cytologic stains supplemented by methenamine silver, PAS, and Mayer's mucicarmine stains to

establish the diagnosis. Although not unique to AIDS patients, recent reports have suggested that poorly encapsulated or capsule-deÞcient strains may be more commonly isolated from AIDS patients (145,146). Capsule-deÞcient strains can be presumptively identiÞed by demonstrating a melanin-like substance in the cell wall with a Fontana-Masson stain (147).

The reaction to cryptococcus in the skin has been classiÞed as gelatinous or granulomatous, but both reaction patterns may be seen in the same lesion. The gelatinous type shows numerous organisms surrounded by abundant capsular polysaccharide and little inßammation (Fig. 27.9). The granulomatous lesion shows fewer Cryptococci, predominantly in an intracellular location with greater inßammation consisting primarily of lymphocytes, histiocytes, and occasional giant cell (148).

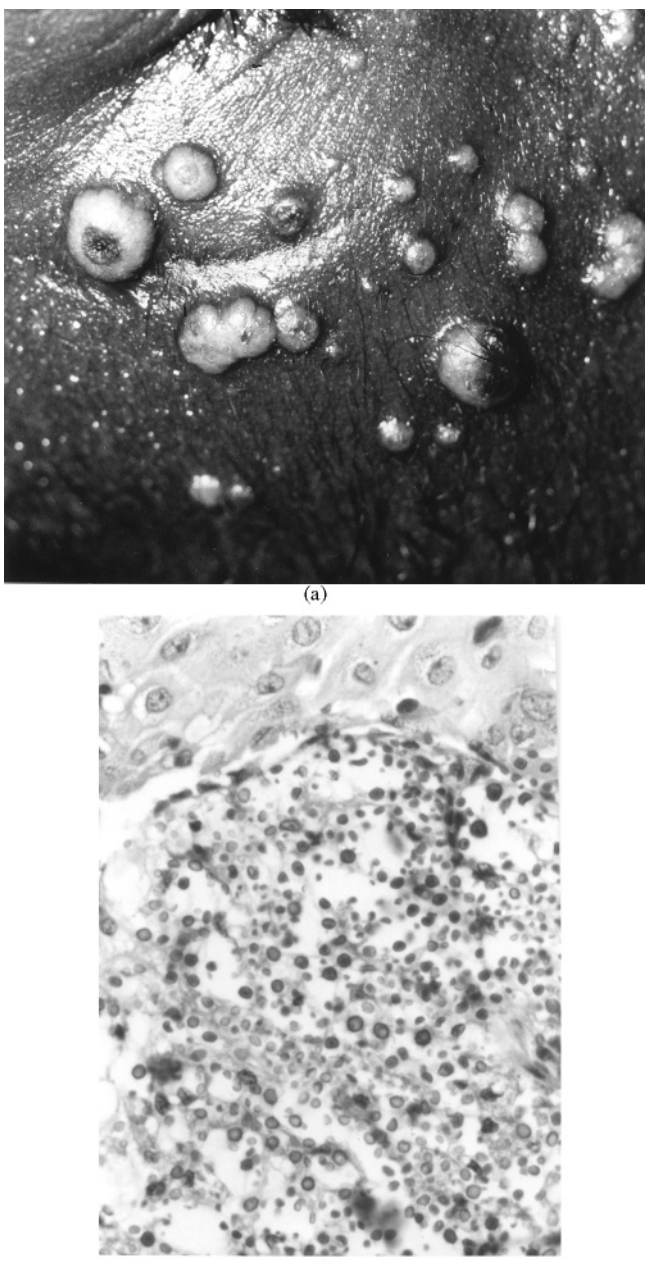

FIG. 27.9. a: Cryptococcus of skin: Multiple umbilicated nodules involving the face. b: Skin biopsy demonstrating gelatinous dermal reaction. Numerous organisms surrounded by abundant capsular material with a paucity of in ammation (PAS, × 250).

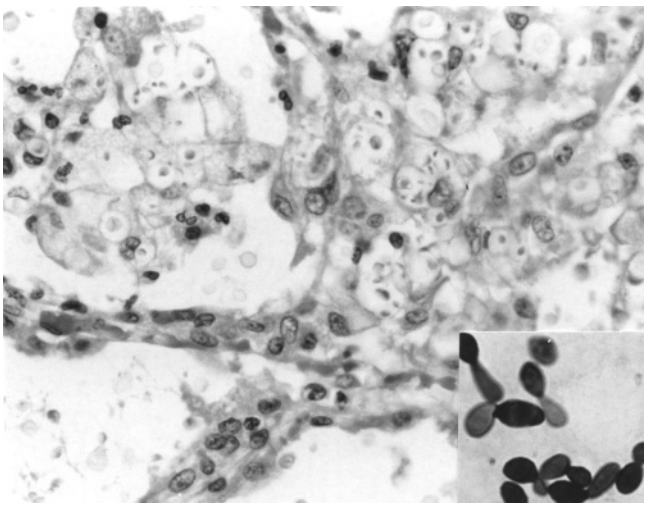

FIG. 27.10. *Cryptococcus neoformans* in lung. Organisms within alveoli and interstitium demonstrate characteristic variability in size, surrounding clear zones occupied by mucopolysaccharide material and narrow based budding (inset) (hematoxylin and eosin, × 250). Inset: (Gomori's methenamine silver, × 1,000).

In transbronchial lung biopsies, a variety of histopathologic abnormalities can be seen. Most commonly, fungi are present in an interstitial location with minimal accompanying inßammatory inÞltrate (Fig. 27.10). There may also be alveolar invasion and rarely vascular invasion (138). The organisms often coexist with other pathogens including Pneumocystis and cytomegalovirus.

Infections by Cryptococcus have been demonstrated in a variety of other sites, particularly lymph node, bone marrow, adrenal gland, and kidney. The inßammatory response to infection can be quite variable, ranging from absent to ßorid. As Cryptococcus elaborates no known exotoxins, tissue necrosis is minimal and inßammatory reactions are usually sparse. Occasionally, some inßammatory cells of mixed types accompany the proliferating organisms, but well-formed granulomas are generally not present (137,149).

Endemic MycosesÑHistoplasmosis and Coccidiodomycosis

The endemic mycoses are designated as such because of their characteristic geographic ranges. While these pathogens can produce disease in normal individuals, they generally do not cause progressive disseminated infections in hosts with intact cellular immunity. However, patients with AIDS who live in or have traveled from endemic

areas are at an increased risk for disseminated multisystem disease. Major organisms in this group include *Histoplasma capsulatum*, *Coccidioides immitis*, and *Blastomyces dermatitidis*.

Histoplasma Capsulatum

Histoplasma capsulatum, a dimorphic fungus, exists in soil contaminated by bird feces. The major endemic area is the central United States and most normal persons living in that region show skin test evidence of past infection (150,151). Reactivation of latent infection and newly acquired primary infection are both important modes of acquiring disease in immunocompromised patients. As expected, the frequency of histoplasmosis in AIDS patients shows marked geographic variability. In highly endemic regions, *H. capsulatum* has been reported in up to 27% of AIDS patients, while in some autopsy series from nonendemic areas, the infection is documented very rarely (152,153). The clinical manifestations, including high fever, maculopapular rash or necrotic papules, hepatomegaly, splenomegaly, lymphadenopathy, anemia, and pulmonary inÞltrates, reßect a disseminated process.

Diagnosis

The diagnosis is made by the identiÞcation or culture of the fungus in clinical specimens and tissue biopsies and by urine Histoplasma antigen detection assays (154). Because of the disseminated nature of infection in AIDS patients, a variety of specimens including bone marrow, blood, respiratory specimens (lavage, washings, and bronchial biopsy), CSF, lymph node, and skin can be diagnostically useful (Figs 27.11 and 27.12). Culture of bone marrow specimens is highly sensitive, but because of the organismÕs slow growth, up to six weeks may be required for identiÞcation (155). Positive cultures from peripheral blood have been reported in about 40% of published cases in AIDS patients, with the lysis centrifugation technique being the most sensitive method to detect the organism (152,155Ð157). Yeast will be identiÞed occasionally in Wright-stained buffy coat smears and in up to 46% of peripheral blood smears (158,159).

Biopsy of focal sites of disease such as skin, lymph nodes, or liver will reveal the typical 2Ð5 μm oval to round budding yeast in clusters within histiocytes. Special stains including methenamine silver or PAS are usually necessary to visualize the organisms because of their small size. Bone marrow biopsy is particularly useful and positive in most cases of disseminated disease. Four patterns of involvement are recognized in marrow biopsies: (1) no evidence of infection (positive culture without morphologic abnormality); (2) discrete granulomas; (3) lymphohistiocytic aggregates; and (4) diffuse macrophage inÞltration. Organisms are found within and outside of macrophages. Other common Þndings reported, which are probably unrelated to infection with this organism, include megaloblastoid erythropoiesis and dysplastic myeloid and megakaryocytic maturation (160).

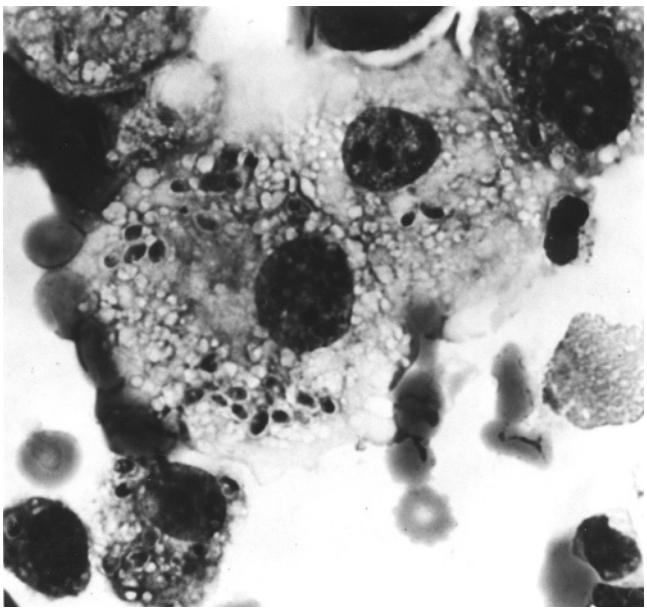

FIG. 27.11. *Histoplasma capsulatum* in bronchial washing. Small oval to round yeast are primarily within macrophages. Narrow based budding, intracellular location, and absence of cup-shaped organisms differentiates this from *Pneumocystis carinii* (Papanicolaou, × 1,000).

Coccidioides Immitis

Coccidioidomycosis, a systemic mycosis endemic to arid regions of North and South America, is becoming recognized as a signiÞcant cause of morbidity and mortality in AIDS patients, particularly in endemic regions (161). Symptomatic disease may occur from reactivation of latent infection or by progressive primary infection (162). The immunocompromised host is prone to develop severe pulmonary and widely disseminated disease (163).

Diagnosis

The diagnosis of coccidioidomycosis is made by serologic methods, skin testing, microbiologic culture, and identiÞcation of the endosporulating spherules in body ßuids or tissue biopsies. The most effective method for diagnosis is evaluation of respiratory tract specimens (bronchial washings, bronchoalveolar lavage, and transbronchial biopsy) using culture and special stains including GrocottÕs or GMS and PAS. In tissue sections, *C. immitis* is identiÞed as large spherules measuring 30Ð60 μm in diameter containing endospores 2Ð5 μm in diameter. Because of the large spherule size, they are often visualized in routine hematoxylin and eosin-stained sections. Hyphal forms consisting of barrel shaped

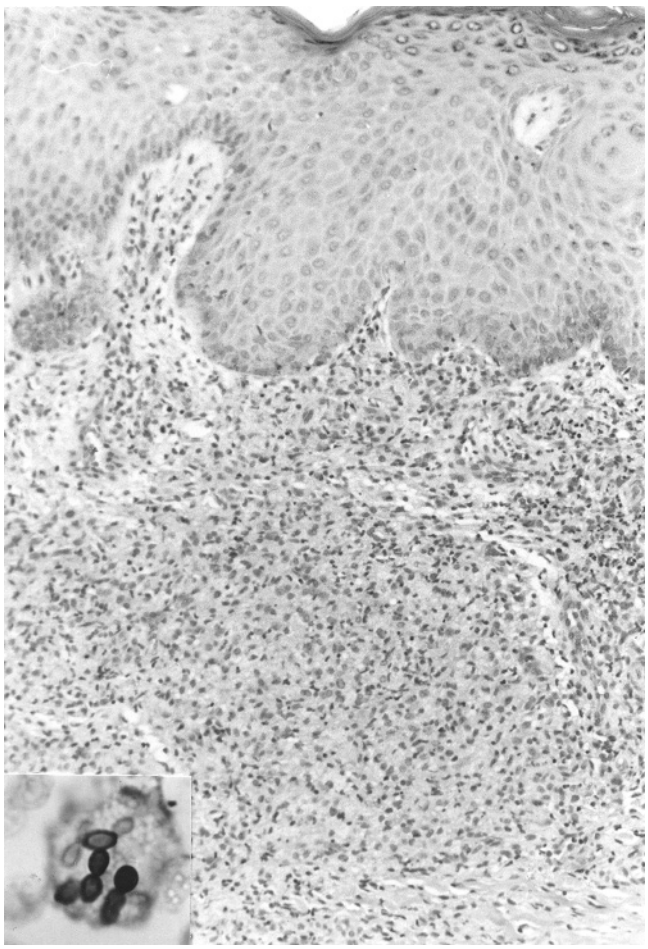

FIG. 27.12. Cutaneous histoplasmosis. Dermal infiltrate of histiocytes and poorly formed granulomas containing numerous yeast in an intracellular location (inset) (hematoxylin and eosin, × 200). Inset: (Gomori's methenamine silver, × 400).

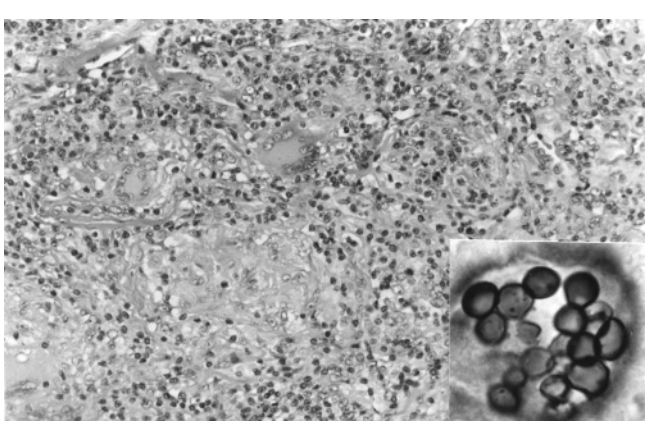

FIG. 27.13. Lung biopsy with *Coccidioides immitis*. Diffuse granulomatous reaction with giant cells. Inset: Endosporulating spherule diagnostic for *C. immitis* (hematoxylin and eosin, × 100). Inset: (Gomori's methenamine silver, × 400).

arthroconidia are also seen in more than 50% of pathologic specimens (164). The inflammatory response to the organism in lung biopsies usually consists of poorly formed granulomas and lymphohistiocytic aggregates with few multinucleated giant cells (Fig. 27.13). Older lesions may show areas of organizing pneumonitis. AIDS patients show greater numbers of spherules compared with non-AIDS patients, and the fungus often coexists with other opportunistic pathogens including *Pneumocystis carinii*, cytomegalovirus, and MAI (163). In keeping with the disseminated nature of the infection, organisms have also been cultured or identified histologically in lymph node, liver, spleen, bone marrow, kidney, skin, and blood (161,163,165–167).

Other Fungi

While considerably less common than Candida, Cryptococcus, Histoplasma, and Coccidioides, a variety of other fungi have infrequently been described in AIDS patients. These include *Sporothrix schenckii* (168,169), Aspergillus (170,171), Alternaria (172), and *Blastomyces dermatitidis* (173).

Bacterial Infections

Tuberculous and Nontuberculous Mycobacteria

Up to 50% of AIDS patients will develop a mycobacterial infection at some stage of their disease (174). The pathogenicity of mycobacteria is modulated by the host's immune response to determine the type and extent of infection (175). About 10% of isolates are *Mycobacterium tuberculosis* (MTB) and the remaining are nontuberculous mycobacteria (NTM), which include MAI, *M. kansasii*, and *M. scrofulaceum* (176). Infections with other mycobacteria, especially *M. gordonae*, *M. xenopi*, and *M. fortuitum*, are also being recognized with increasing frequency (177–178).

As environmental saprophytes, nontuberculous mycobacteria gain access through the aerosol route or by ingestion of food or water and may temporarily colonize the nasopharynx or intestinal mucosa (179). In immunodeficient individuals, once established, these opportunistic pathogens can extensively disseminate and proliferate to enormous numbers before causing overt disease (gastrointestinal, pulmonary, soft tissue, and systemic lesions are seen in these infections) (180). NTM infections may be clinically silent, the diagnosis being made incidentally in a lymph node biopsy, in endoscopic biopsy of gastrointestinal tract, or at autopsy. When clinical manifestations occur they may be nonspecific (fever, weight loss, anorexia, abdominal pain, etc.). A case of disseminated *M. bovis* infection following bacillin Calmette-Guérin (BCG) vaccination highlights the importance of not using live

vaccines in individuals with HIV infection (181). MTB among patients with AIDS is seen most frequently in those groups with historically high rates of infection (intravenous drug users, Haitians, Hispanics, and blacks) and tends to occur early in the course of HIV infection, often in a disseminated extrapulmonary form (182).

Histopathologic Diagnosis

Although microbiologic culture is required for precise species identiÞcation, rapid diagnosis of mycobacterial infections rests on morphologic demonstration of the organisms in cytologic preparations and tissue sections. Gastrointestinal tract, lymph node, lung, liver, and bone marrow are the most common biopsy specimens. The cellular response to the organisms is variable and includes well or poorly formed granulomas, lymphohistiocytic reactions, histoid pattern, and paucity of immune response.

MAI Infections

Disseminated MAI infection is the most common bacterial infection in AIDS patients (183). In endoscopic biopsies of the small intestine, MAI infections show villous atrophy with diffuse inÞltration of the lamina propria by foamy or granular histiocytes (Fig. 27.14). Necrosis is unusual, and granulomas are generally not present or are poorly formed. Acid-fast bacilli (AFB) stains demonstrating the abundant beaded magenta rods within these histiocytes differentiate this lesion from Whipple's disease, which can have similar clinical and histologic features, and from intestinal *Rhodococcus equi* infection (184,185). Focal involvement of gastric or colonic mucosa may be overlooked, and special stains are appropriate even in the absence of an obvious inÞltrate.

Lymph node involvement with NTM can take several forms. The cortex and part of the medulla are replaced by sheets of histiocytes containing many organisms. Granulomas, necrosis, Þbrosis, and calciÞcation are usually absent. An unusual spindle cell proliferation, described by Wood and similar to the histoid reaction of lepromatous leprosy, may suggest a connective tissue tumor or Kaposi's sarcoma, but special stains demonstrate abundant bacilli within the spindle cells (186) (Fig. 27.15).

In the lungs, NTM infections manifest minimal tissue response. In the interstitium, rare clusters of histiocytes containing numerous organisms associated with minimal chronic inßammation are often the only evidence of infection. Special stains may demonstrate abundant organisms throughout the interstitium in the absence of reaction.

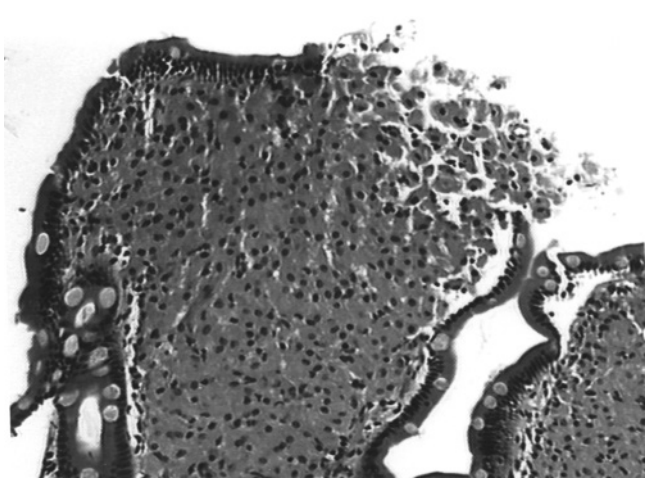

FIG. 27.14. MAI enteritis. Small intestinal biopsy demonstrating blunting and expansion of villi with a histiocytic in Itrate in the lamina propria. An acid fast stain showed large numbers of acid-fast bacilli within the histiocytes. The differential diagnosis includes Whipple's disease and infection by *Rhodococcus equi* (hematoxylin and eosin, × 200).

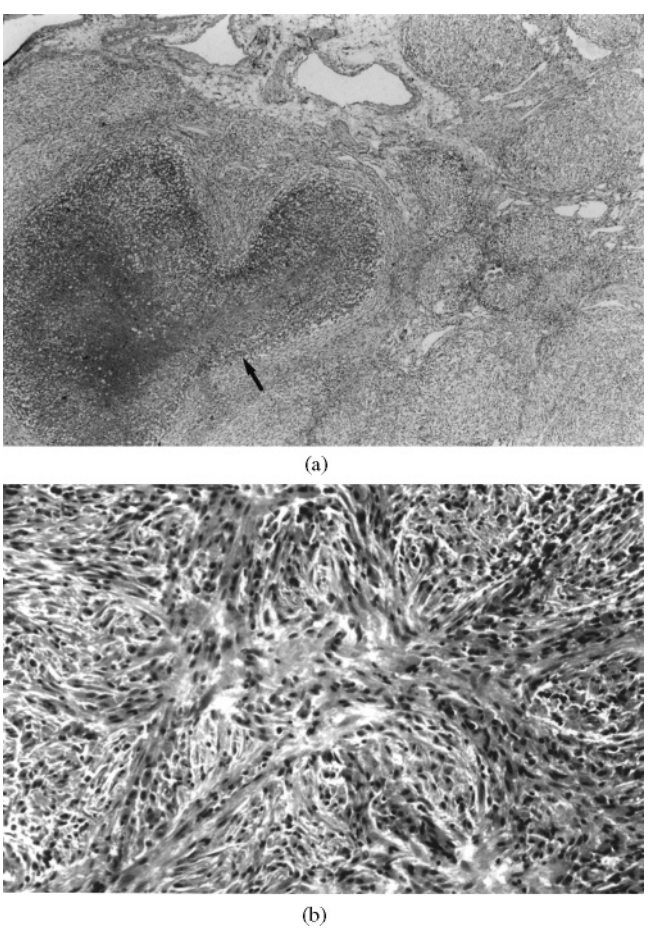

FIG. 27.15. Necrotizing lymphadenitis and "histoid" reaction. a: Atypical MAI infection of lymphoid tissue. Culture proven MAI infection demonstrating unusual feature of zonal necrosis (arrow) (hematoxylin and eosin, × 25). b: Histoid reaction with spindle-shaped histiocytes arranged in interlacing fascicles. Ziehl-Neelsen stain showed acid-fast bacilli within spindle cells (hematoxylin and eosin, × 250).

Hepatic involvement of MAI is seen in about 70% of liver biopsies in patients with systemic MAI infection and has been reported in up to as many as 83% of those patients coming to autopsy (187–188). The histologic appearance can be deceptively bland, ranging from widely dispersed histiocytes to focal granuloma-like collections lacking necrosis and giant cells. AFB stains show numerous organisms within histiocytes and rarely within Kupffer cells (189). Occasionally organisms are cultured from biopsies demonstrating no tissue reaction (190).

Bone marrow involvement by mycobacteria manifests as lymphohistiocytic aggregates and poorly formed granulomas that tend to be very small and focal in distribution. AFB stains demonstrate organisms within granulomas and also within more widely dispersed histiocytes (191). As formic acid decalciÞcation may interfere with AFB stains, EDTA decalciÞcation solutions are recommended since it is desirable to perform acid-fast stains routinely on bone marrow biopsies from HIV-infected patients (192).

The ßuorescent stain auramine-rhodamine can be used on cytology preparations and adapted to tissue sections to facilitate demonstration of mycobacteria. This method requires less time to screen the slides as it can be read at lower magniÞcation and has been shown to be about twice as sensitive as the Ziehl-Neelsen stain in demonstrating AFB in lymph node biopsies (193). Several methods of rapid mycobacterial culture and identiÞcation are now commercially available, with the BACTEC system being employed by many laboratories. Ribosomal RNA probes for the identiÞcation of MTB and MAI provide rapid results and are now the gold standard. Methods such as PCR for the direct identiÞcation of mycobacteria in clinical specimens are being developed, but are not yet appropriate for routine clinical diagnosis (194).

MTB Infections

HIV-associated MTB infections of any site generally present a more variable tissue response, ranging from well-formed granulomas to loose clusters of histiocytes with lesser numbers of organisms than are seen in NTM infections. However, in our experience, MTB infections in AIDS patients cannot be reliably distinguished from MAI infections solely on the basis of morphologic features such as necrosis, type of inßammatory response, presence of granulomas, or numbers of organisms. In the lungs, miliary patterns are common and typical necrotizing cavitary lesions infrequent (195). Some cases of pulmonary MTB infection in AIDS patients show abscess formation with numerous neutrophils, necrosis and few or no epithelioid histiocytes (196). Dual pulmonary infections with MTB and MAI have been reported (197). Involvement of other organs shows similar histologic features.

In summary, the tissue reactions to mycobacterial infections are varied and depend on factors such as the inherent virulence of the particular mycobacterial species, degree of immunodeÞciency when the infection occurs, the length of time the lesion is present prior to biopsy, and the inßuence of concomitant or prior chemotherapeutic agents. Because of this variability and differences in the response and treatment of NTM and MTB, stains to demonstrate the bacteria should always be supplemented with methods to culture the organisms and determine chemotherapeutic sensitivities.

Other Bacteria

Infections with a variety of bacteria such as *Streptococcus pneumoniae*, *Haemophilus inßuenzae*, *Staphylococcus aureus*, *Neisseria meningitides*, *Pseudomonas aeruginosa*, Salmonella, Shigella, Campylobacter, and *Treponema pallidum* are important causes of morbidity and mortality in HIV-infected children and adults (198–214). The multiplicity of immunologic defects of cellular and humoral types predisposes these patients to persistent infections and bacteremia (215). In addition, chemotherapeutic and antineoplastic agents used to treat opportunistic infections and neoplasms may cause neutropenia, further compromising the immune status. These infections often show atypical presentations including infections with more than one organism and a high relapse rate despite appropriate antibiotic therapy. Infections with *Streptococcus pneumoniae* and *Haemophilus inßuenzae* are particularly frequent in children with AIDS and the latter organism is responsible for about 10% of pneumonias in adult AIDS patients (198,202–203). Bacterial pneumonia is a very common pulmonary Þnding in AIDS patients at autopsy, with most cases caused by *Pseudomonas aeruginosa*, *Staphylococcus aureus* or *Klebsiella pneumoniae* (216). Important enteric pathogens in HIV-infected patients include Salmonella, Campylobacter, and Shigella. Salmonellosis may present initially as acute gastroenteritis or less commonly as bacteremia. The infections often have a severe clinical course with bacteremia in up to 45% of AIDS patients and show a tendency to relapse despite antibiotic therapy (200–201,203). Cultures of blood and stool usually conÞrm the diagnosis, but Salmonella may be recovered from many sources including brain, bone marrow, urine, and spleen. Campylobacter species have been recognized as important pathogens in the general population as well as in homosexual men and immunocompromised patients. Most infections in AIDS patients are associated with gastroenteritis while bacteremia and cholecystitis are infrequently reported (208,209). *Shigella ßexneri* is the most common species isolated from AIDS patients with Shigella gastroenteritis (205–207). Bacteremia is infrequently seen and diagnosis is principally made by culture of stool.

A number of studies have suggested that HIV infection may be associated with rapid progression of secondary

syphilis or tertiary neurosyphilis, even following appropriate antibiotic therapy (210,211). Unusual clinical presentations, atypical serologic responses after appropriate treatment and false-negative serologic tests, make the diagnosis of syphilis in HIV-infected patients difficult (212,214,217). Darkfield examination or direct fluorescent antibody staining of scrapings or exudates from suspicious primary lesions will help to establish the diagnosis even with negative rapid plasma reagin (RPR) or Venereal Disease Research Laboratory (VDRL) serologic studies. Silver stains such as the Steiner or Warthin-Starry can be performed on tissue biopsies but in the author's experience are difficult to interpret (218). Overall, the diagnosis of syphilis can be problematic in HIV-infected patients, and many clinicians will presumptively treat patients for early syphilis and closely monitor serial serologic tests to detect a delayed antibody response.

Bacterial infections are seen more commonly at autopsy than in biopsy specimens. Few pathologic studies, however, describe the microscopic features of pure bacterial enteric infections uncomplicated by the more common pathogens seen in AIDS patients. In typical enteric infections by most of these organisms, the initial phase is an invasion of the epithelium with propagation of the bacteria in epithelial cells followed by entry into the lamina propria. The subsequent reaction is acute and pyogenic with superficial mucosal necrosis and pseudomembrane formation beginning as a focal process and in severe infections becoming confluent. Organisms can be demonstrated within epithelial cells, lamina propria, and adherent membranes by Gram, Giemsa, and silver stains. Microbiologic culture will confirm the species of the enteropathic bacteria. This typical reaction pattern is modified by the host's ability to mount an immunologic response based on the degree of immune suppression. Dissemination to other organs will result in necrotizing lesions associated with scan inflammatory infiltrates and an abundance of organisms, particularly in neutropenic patients.

Viral Infections

Cytomegalovirus

Cytomegalovirus (CMV) is a common opportunistic pathogen found in cytology and biopsy specimens from AIDS patients surpassed in frequency only by Pneumocystis and MAI (219,220). Nearly all AIDS patients have serologic evidence of prior exposure, suggesting these infections almost always represent reactivation and dissemination of latent virus (221).

The gastrointestinal tract is a common site of CMV infection in AIDS. Colitis is the most frequent clinical presentation of enteric disease and these lesions appear as focal or diffuse areas of ulceration and hemorrhage commonly involving the more distal colon (219). Multiple biopsies are usually required to establish a diagnosis. A CMV-related occlusive vasculitis which may be complicated by intestinal perforation, has also been described (222). Esophageal involvement results in ulceration predominantly involving the distal portion (223). In the stomach, CMV appears as a nonspecific gastritis with focal ulceration or as a submucosal mass (224). Other alimentary tract sites less commonly involved include the duodenum, pancreas, biliary tree, and gallbladder (225). In patients with AIDS, CMV pneumonitis is not a well-characterized syndrome and is frequently found to coexist with other opportunistic pulmonary pathogens, especially *Pneumocystis carinii* (226). The histopathologic findings reported in lung biopsies or autopsy tissues from AIDS patients with CMV pneumonitis range from focal and mild interstitial pneumonitis to severe diffuse alveolar damage with necrosis. Infections in a variety of other organs including brain, lymph nodes, skin, endocrine organs (especially adrenal gland), genitourinary system, liver, and heart have also been described (227,228).

Diagnosis

The most widely utilized method of diagnosing active CMV infection is by demonstrating the characteristic inclusion bodies in biopsies or cytology specimens. The significance of a positive CMV culture in the absence of clinical or pathologic evidence of tissue injury or viral cytopathic effects, remains unclear. The identification of infection based on the presence of cytopathic changes, while specific, is a relatively insensitive technique. The diagnostic accuracy in biopsy material can be enhanced by immunohistochemical stains for CMV early and late antigens and in situ hybridization for CMV nucleic acids (229,230). Recently the polymerase chain reaction has been used to demonstrate CMV DNA in clinical specimens and paraffin-embedded tissues. However, the presence of CMV DNA does not necessarily indicate active disease as the virus is latent in a very high proportion of the HIV-infected population (231).

Cells actively infected by CMV show characteristic cytologic changes including cellular and nuclear enlargement, acidophilic intranuclear inclusions, and granular basophilic cytoplasmic inclusions (Fig. 27.16a). Gomori's methenamine silver and PAS stain the viral glycoproteins of the cytoplasmic inclusions because of their high carbohydrate content (42). Mesenchymal cells, especially endothelium, are preferentially involved, while epithelial cell involvement is seen less frequently (Fig. 27.16b). CMV infections display wide variability in the degree of associated inflammation, necrosis, and tissue damage, which ranges from focal and mild to extensive with organ

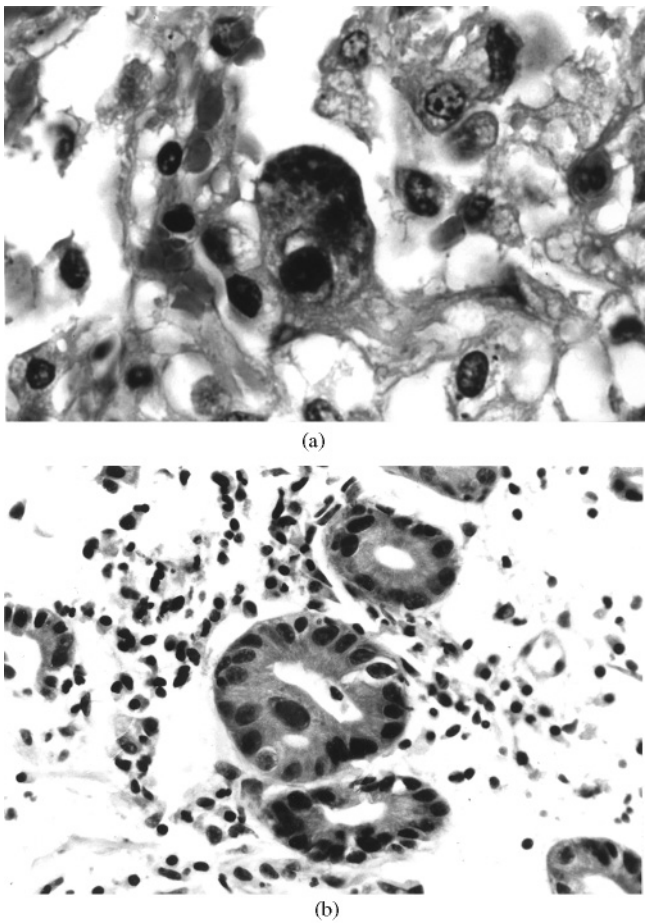

FIG. 27.16. Cytomegalovirus pneumonitis and gastritis. a: Pneumocytes showing cytomegaly and large intranuclear inclusions (hematoxylin and eosin, × 250). b: Gastric biopsy with cytomegalic inclusion in epithelial cell (hematoxylin and eosin, × 100).

perforation (232). Rarely, CMV vasculitis is associated with severe infections (233).

Herpes Simplex and Varicella-Zoster

Primary infections with herpes simplex virus (HSV) are unusual in adults with AIDS because of the high rates of previous infection. However, these patients are prone to severe recurrent infections, which show delayed healing, prolonged virus shedding, and frequent relapses (234,235). HSV produces oral, labial, genital, and anal lesions, retinal infections/necrosis (236), esophagitis, pneumonitis, and encephalitis, while the lesions of varicella-zoster (HZ) occur in a dermatomal distribution or show extensive cutaneous dissemination (237). During recurrences, patients may develop visceral disease (238). In most studies from western countries, cases reported in association with HIV infection usually have disease progression to AIDS prior to the onset of disseminated HZ infection (235). In African patients, HZ is highly predictive of HIV infection; in one study 91% of African patients with HZ were HIV-seropositive (239).

Diagnosis

Serologic tests for herpes antibody are rarely useful in AIDS patients because antibody formation occurs late in primary infection and is usually unchanged during recurrent infection. DeÞnitive diagnosis requires demonstrating viral cytopathic effects in cytology specimens or surgical biopsies, culturing the virus from tissues, or detecting viral antigens. Cultures of fresh vesicles show high recovery rates whereas specimens from crusted lesions are usually negative. Biopsy specimens or swabs from vesicles are inoculated onto tissue monolayers capable of supporting virus growth. Viral cytopathic changes or staining of the monolayer with speciÞc monoclonal antibodies permit rapid identiÞcation usually within four to Þve days (240).

Cytologic preparations from scrapings of fresh vesicles provide a rapid and inexpensive method to identify virally infected cells but are a relatively insensitive technique and cannot differentiate HSV types 1 and 2 from varicella-zoster infections. This procedure (Tzanck preparation) consists of unrooÞng a fresh vesicle and gently scraping the base of the lesion. The scrapings are transferred to a glass slide and stained with any of the routine cytologic methods (usually Wright-Giemsa or Papanicolaou). Typical viral cytopathic changes include nuclear enlargement and multinucleation with nuclear molding. The nuclear features consist of a diffuse homogenization giving the chromatin a Òground glassÓ appearance (Fig. 27.17). Alternatively, nuclei may contain prominent eosinophilic inclusions surrounded by a clear halo and prominent nuclear membrane.

Tissue biopsies of skin or mucosal lesions show vesicle formation or ulceration with dermal or submucosal necrosis and inßammation. The virally transformed cells

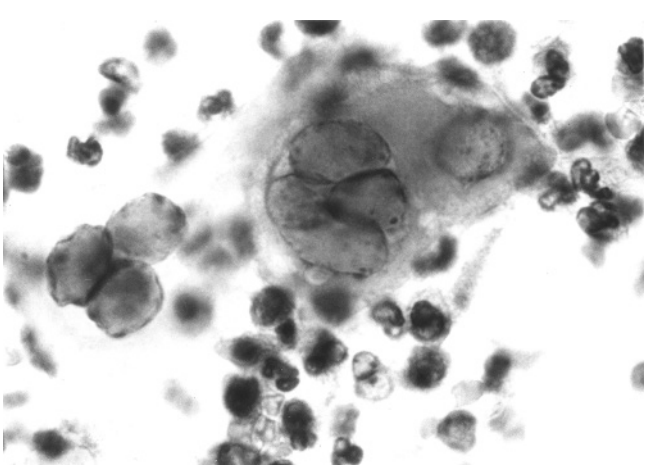

FIG. 27.17. Tzanck preparation demonstrating characteristic viral changes of herpes infection. Epithelial cells show multinucleation, nuclear molding, and “ground glass” nuclear chromatin (Diff-Quik, × 400).

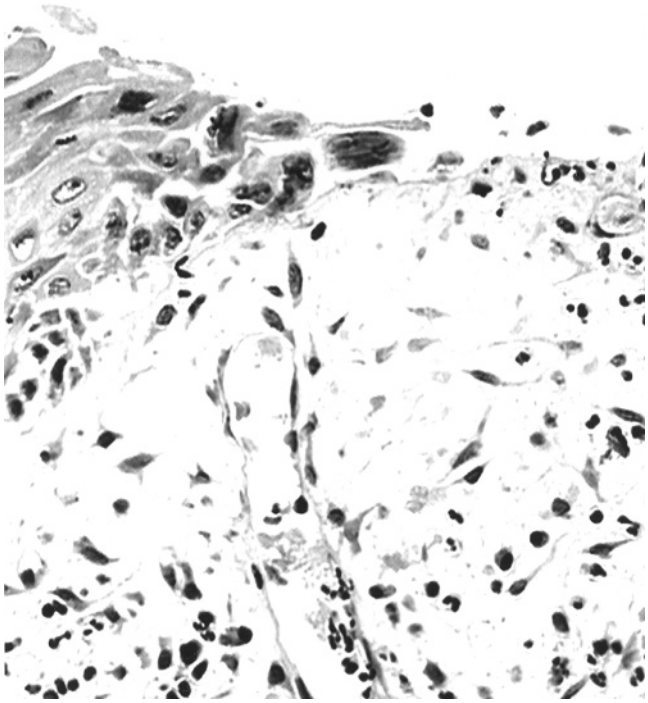

FIG. 27.18. Persistent herpes skin infection. Focus of ulceration with acantholysis and multinucleated giant cells (hematoxylin and eosin, × 200).

described previously are frequently seen at the margins of the ulcer (Fig. 27.18). In contrast to CMV, HSV preferentially infects squamous epithelial cells, whereas CMV shows a propensity for mesenchymal and endothelial cells. HSV-infected cells do not show marked cytomegaly or produce the intracytoplasmic inclusions seen in CMV-infected cells. The diagnosis in smears and in tissue sections can be conÞrmed by immunoperoxidase methods to identify viral proteins or by in situ hybridization techniques that demonstrate HSV DNA (241,242).

Herpes simplex and varicella-zoster viral pneumonitis are often antedated by oral or labial lesions. Histologically, this type of viral pneumonitis usually produces parenchymal necrosis, lymphohistiocytic inßammatory inÞltrates, multinucleated giant cells, and intranuclear inclusions. There may be associated necrotizing tracheobronchitis. Evidence of disseminated herpetic infections can be detected in many visceral organs and lymph nodes. Biopsies from these sites show necrotizing changes with scattered virally transformed cells.

Other Viruses

A number of other viral infections have been described in patients with AIDS. Epstein-Barr virus (EBV), another member of the herpes virus group, is endemic in humans and serologic studies show it to be a frequent latent infection in AIDS patients. There is considerable evidence associating EBV with malignancies including lymphoma and nasopharyngeal carcinoma (243Ð245). Oral hairy leukoplakia, a benign papillomatous epithelial proliferation usually involving the tongue or buccal mucosa and described predominantly in AIDS patients, has been shown to contain EBV DNA sequences in most cases (246). It has been theorized that oral hairy leukoplakia is due to reactivation of latent lingual infection with EBV (247). Some children with AIDS develop a lymphocytic interstitial pneumonitis also shown to contain EBV capsid antigen and viral DNA (244). Infections with this virus can be demonstrated using DNA probes and serologic techniques (244,248,249). Histopathologic features of acute EBV infections of lymph nodes show characteristic but nonspeciÞc hyperplastic patterns and are sometimes confused with Hodgkin's disease (250).

Other viral diseases described in AIDS or AIDS-related complex include the pox virus associated with molluscum contagiosum and human papillomavirus associated with verruca vulgaris and cervical/vaginal neoplasia (251,252). Molluscum contagiosum may be generalized and severe in patients with AIDS and can clinically mimic cutaneous cryptococcosis (135). Lesions of condyloma accuminata may be large and extensive in patients with AIDS, and one study has suggested a high prevalence of cervical and vaginal cytologic abnormalities of squamous epithelium in HIV-infected women (252). Progressive multifocal leukoencephalopathy caused by a papovavirus is described in a separate chapter.

NEOPLASMS ASSOCIATED WITH HIV

Kaposi's Sarcoma

Kaposi's sarcoma (KS), the most common neoplasm occurring in association with AIDS, has been recognized clinically in up to 25% of patients at some point during their illness and had been reported in between 50% and 95% of AIDS patients at autopsy (171,253,254). Epidemiologic studies have shown the highest prevalence to be in white male homosexuals with AIDS (255). Since the advent of antiretroviral therapy, the incidence of KS has declined precipitously in North America and Europe, but not in third world countries where this therapy is largely unavailable (256).

AIDS-associated KS differs from the classical form seen in elderly individuals of Mediterranean or Jewish extraction in the sites of involvement (arms, oral cavity, trunk, and face rather than of lower extremities) and appearance (multicentric pink to violet patches, inÞltrated plaques, or small angiomatoid nodules in contrast to purple macules, papules, or nodules). Although these characteristics enable a clinical diagnosis to be made, a biopsy diagnosis, particularly of early lesions, is recommended. Most AIDS patients with KS have cutaneous and

visceral involvement, but between 5% and 29% show visceral involvement in the absence of cutaneous lesions (257,258). In autopsy studies, many organs have been reported to be involved; however, lymph nodes, gastrointestinal tract, and lung are the most frequent sites of extracutaneous disease (171,257,256). Most patients with KS die as a result of opportunistic infections and only rarely as a direct consequence of this tumor (257,259).

The histogenesis and pathogenesis of KS have been extensively studied but are poorly understood. The proposed cell of origin has included most cells of mesodermal origin, but recent research has focused on the vascular (capillary) endothelium, lymphatic endothelium, or a combination of both (260–264). Immunohistochemical comparisons between the AIDS-related KS and sporadic KS have shown no signiþcant differences between these two forms despite the marked clinical dissimilarities (263,265). The presence of angioproliferative changes of nonlesional skin from AIDS patients, diploid DNA content, multicentric nature, and occasional reversibility have led some investigators to hypothesize that KS is a reactive proliferation rather than a true sarcoma (266). A recently described virus, human herpesvirus 8 (HHV8), also known as KS associated herpesvirus, is present in KS lesions of both AIDS patients and all forms of HIV-negative KS (267). It has been suggested that HHV8 may be an etiologic agent of KS. HHV8 has also been detected in body cavity based lymphoma (also known as primary effusion lymphoma), a rare form of B cell AIDS-related lymphoma occurring in HIV patients, anaplastic large cell lymphoma, as well as in multicentric Castlemanõ disease (267,268).

Clinical Characteristics

Cutaneous lesions of KS are often þrst recognized on the face, oral cavity (especially hard palate), arms, and soles of feet, and can be quite subtle (269,270). They may present as asymptomatic pigmented pink to violaceous macules, papules or small angiomatoid nodules being mistaken for hemangioma, pyogenic granuloma, dermatoþbroma, melanoma, molluscum contagiosum, or lichen planus (271,272). Gastrointestinal KS may cause bleeding, obstruction, and perforation, but is more commonly asymptomatic (259,273,274). Pulmonary involvement can result in cough, shortness of breath, hemoptysis, and respiratory failure as a consequence of intra-alveolar hemorrhage, tracheal and bronchial obstruction, and hemorrhagic pleural effusions (275,276). Lymph node KS can be seen in a high proportion of cases with estimates varying from 30–95% (254). As lymph node enlargement from a variety of causes is commonly encountered in patients with AIDS, conþrmation of lymphadenopathic KS requires excisional biopsy.

Diagnosis

Aggressive diagnostic studies of patients who have this condition are essential as chemotherapy and radiation therapy may provide signiþcant palliation, particularly if used in conjunction with antiretroviral therapy (256). Any cutaneous or mucous membrane pigmented lesion in a member of a high-risk group for HIV infection should raise the suspicion of KS and prompt tissue biopsy to establish a histological diagnosis. In small biopsy specimens of early skin lesions, it may be necessary to examine multiple levels, and the diagnosis of patch KS should be make cautiously in the presence of healed ulceration or at sites of previous trauma (277). Gastrointestinal tract lesions can be seen with or without cutaneous disease and usually present as either small red macules with associated submucosal hemorrhage or violaceous nodules involving the stomach and duodenum most commonly (274). Endoscopic biopsies will sometimes yield diagnostic material, although yields tend to be low because of the submucosal location of these lesions (274). Pulmonary KS occurs in between 20% and 50% of cases and clinically can be difþcult to distinguish from opportunistic infections, especially *Pneumocystis carinii*, as both can have similar symptoms and show diffuse interstitial inþltrates. The bronchoscopic appearance is that of multiple discrete raised red to violaceous mucosal plaques of the trachea or bronchial tree. KS is usually conþrmed by bronchoscopy without biopsy, but involvement of the parenchyma in the absence of bronchial disease may require an open lung biopsy for conþrmation (275). Bone marrow involvement has rarely been reported in core biopsies (278). Fine-needle aspiration biopsies from a variety of sites infrequently yield diagnostic material (279).

Histopathology

The histopathologic appearance of AIDS-associated KS in skin does not differ signiþcantly from the classical, endemic, and allograft-associated types despite the marked differences in clinical behavior among these groups. Two microscopic features are seen in the lesions: vascular spaces and spindle cells. The basic histologic patterns as described by Ackerman (280) include the patch, plaque, and nodular types, although mixed patterns are commonly observed. In the early patch stage, anastomosing thin-walled vascular spaces with irregular outlines involve the upper dermis and dissect collagen bundles (281). The lining endothelial cells display little nuclear atypia, and luminal erythrocytes are infrequent. These lesions, resembling granulation tissue, reaction to trauma, or areas of recently healed ulceration, can be quite subtle and difþcult to diagnose. Thin-walled anastomosing angulated and

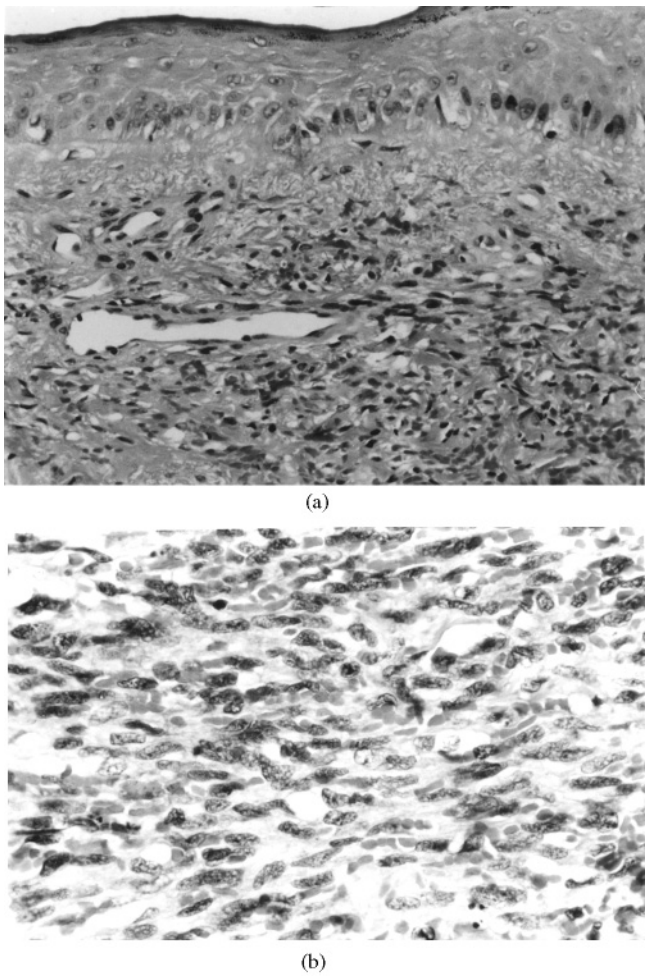

FIG. 27.19. Cutaneous Kaposi's sarcoma. a: Vascular spaces and spindle cells characterize the plaque lesion (hematoxylin and eosin, × 100). b: Late stage lesion with predominantly spindle cell proliferation and extravasated erythrocytes (hematoxylin and eosin, × 200).

irregularly outlined vascular spaces admixed with spindle cells are the hallmark of the plaque lesion (Fig. 27.19a). Nodular lesions contain a predominance of spindle-shaped cells, usually in well-deÞned aggregates that involve the dermis but may extend into the subcutaneous tissue (Fig. 27.19b). Vascular slits containing erythrocytes and hemosiderin deposits between the spindle cells help distinguish KS from other sarcomas. The spindle cells frequently show erythrophagocytosis and may contain eosinophilic inclusions, the latter shown ultrastructurally to represent lysosomal residual bodies (282). In the late stages, the histologic picture may resemble Þbrosarcoma with minimal nuclear atypia and occasional mitotic Þgures. Features said to be useful in differentiating KS from other vasoproliferative lesions include the promontory sign and the angiomatoid lesion. The former is a proliferation of jagged irregular endothelial lined spaces arising around a normal dermal vessel, while the latter is a collection of small vascular spaces lined by prominent ÒhobnailÓ endothelial cells surrounded by small numbers of proliferating spindle cells (277).

Extracutaneous KS

The histopathology of lymphadenopathic KS is that of a focal or diffuse expansion of the sinuses by a vascular and spindle cell proliferation, which may progress to the classic nodular pattern. The subcapsular sinusoids, medulla, and T cells zones are usually involved Þrst with eventual involvement of germinal centers. Eosinophilic globules can be found in macrophages or spindle cells. The lesions may co-exist with infectious processes, malignant lymphoma, and the various reactive patterns described in AIDS associated lymphadenopathy. The main differential diagnosis of early node involvement is with nodal angiomatosis.

AIDS associated bronchopulmonary KS may be the Þrst site of involvement recognized, but more commonly the diagnosis has been established by prior skin or oral biopsy. KS grows Þrst along lympathic pathways and involves intralobular septae, pleura, and forms distinct proliferations around bronchi and pulmonary vessels (275). The lesions consist predominantly of spindle cells and to a lesser extent, small vascular lumina and variable inßammation, that grow in a diffuse fashion rather than as nodules (283).

Endoscopic biopsies of the gastrointestinal tract can show diffuse mucosal plaque-like involvement or polypoid proliferations whose histologic appearance is similar to that described in skin. The tumors usually Þrst involve the submucosa but later intramucosal lesions also occur (Fig. 27.20). Involvement of the muscularis propria is uncommon (274).

Involvement of other organs including liver, spleen, heart, adrenal gland, and kidney is seen less frequently (284,285). The lesions can present as distinct parenchymal

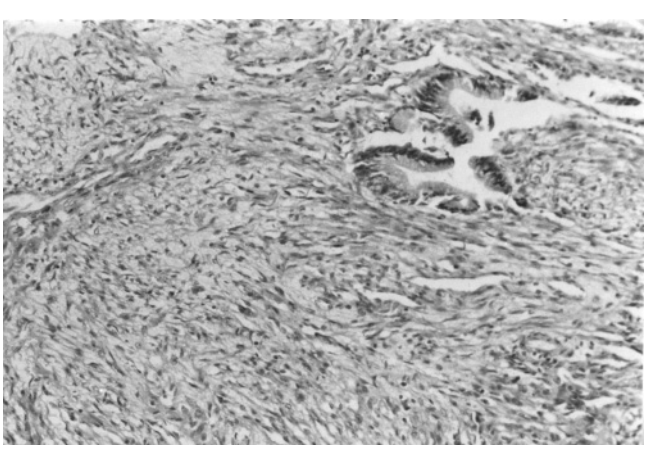

FIG. 27.20. Kaposi's sarcoma involving the stomach. The lamina propria is extensively in ltrated by a spindle cell proliferation with few vascular spaces. Residual gland is seen in upper right (hematoxylin and eosin, × 100).

nodules or inÞltrate along pre-existing lymphatic and vascular pathways. Microscopically, the classic mixture of spindle cells and vascular spaces are recapitulated regardless of the site of involvement.

Differential Diagnosis

Bacillary angiomatosis (BA) is a lesion occurring predominantly in AIDS patients that may be mistaken clinically and histologically for KS (285Ð287). BA can involve the skin, lymph nodes, mucosal surfaces including those of the gastrointestinal tract and respiratory tract, and causes peliosis of the spleen and liver (288). The cutaneous lesions usually present as erythematous papules, dome-shaped papules and nodules, indurated plaques or more deeply located circumscribed tumors. The majority of cases of BA are caused by *Bartonella henselae*, with a minority due to *B. quintana* (288). *B. henselae* is also the major cause of cat scratch disease. HHV8 has not been detected in the lesions of bacillary angiomatosis using polymerase chain reaction methods (289). Cutaneous BA is characterized histologically by lobular proliferations of capillaries lined by protuberant endothelial cells surrounded by neutophils, neutrophilic debris, macrophages and aggregates of gram-negative bacilli, visible on H and E stained section as granular, faintly basophilic material. The bacilli can be demonstrated in these lesions by the Warthin-Starry silver stain and by electron microscopy. The presence of neutrophils, granular debris, and clumps of bacteria in the absence of spindle cells, bizarrely shaped vascular channels, and eosinophilic inclusions help to distinguish BA from KS. The microscopic features of BA resemble the cutaneous form of bartonellosisÑÒverruga peruanaÓÑa benign cutaneous vascular proliferation associated with systemic infections with *Bartonella bacilliformis* (290). The differential diagnosis also includes angiosarcoma, pyogenic granuloma, hemangioma and angiolymphoid hyperplasia with eosinophilia. Angiosarcomas generally exhibit greater endothelial atypia and are less well circumscribed than BA. Hemangioma and pyogenic granuloma are usually more circumscribed, lack the neutrophilic debris and bacteria are not demonstrable. Accurate diagnosis of BA is important as treatment with erythromycin has resulted in complete and rapid resolution of the lesions (291). **Table 27.2???** highlights the important histologic features of BA and KS.

Malignant Lymphoma and Lymphadenopathy

AIDS-associated malignant lymphomas may be preceded by multifocal lymph node enlargement termed persistent generalized lymphadenopathy (PGL) (292,293). PGL is deÞned by the CDC as palpable lymphadenoopathy (>1.0 cm) at two or more extrainguinal sites persisting for more than three months in the absence of a concurrent illness or conditions other than HIV infection to explain the Þndings (294). Biopsies of lymph nodes from patients with PGL show three characteristic, although nonspeciÞc, histopathologic patterns of reaction (295,296). These patterns have been shown to correlate with the progression of immune dysfunction and the clinical course (297Ð299).

Histologic Patterns Seen in Lymphadenopathy

In ßorid follicular hyperplasia (type I or A pattern), the normal architecture is effaced by a marked proliferation of irregularly shaped follicles in the cortex and medulla. The follicles may show conßuence and attenuation of the mantle zones. The germinal centers contain a mixture of cell types, including large and small lymphocytes, immunoblasts, tingible body macrophages, nuclear debris, and numerous mitoses (Fig. 27.21). Invagination of mantle zone lymphocytes into the germinal centersÑ termed follicular lysis, results in islands of large transformed germinal center lymphocytes and disruption of follicles. Interfollicular zones show a prominent vasculature with a mixture of small lymphocytes, immunoblasts, plasma cells, and histiocytes. Neutrophilic aggregrates and multinucleated giant cells similar to the Warthin-Finkeldey giant cells of measles are frequently onserved. Changes resembling dermatopathic lymphadenitis and prominent histiocytic proliferation with erythrophagocytosis may also be seen.

Lymphoid depletion (type III or pattern C) shows atrophic Òburned outÓ follicles with depletion of lymphocytes, absent or vestigial germinal centers, and prominent vasculature. Interfollicular zones show loss of lymphocytes and excessive vascularization. Sinuses are dilated and usually contain numerous histiocytes, some of which show erythrophagocytosis. Multinucleated giant cells may also be seen. This pattern, which has the worst prognosis, is associated with the shortest intervals of progression to AIDS and the shortest patient survivals.

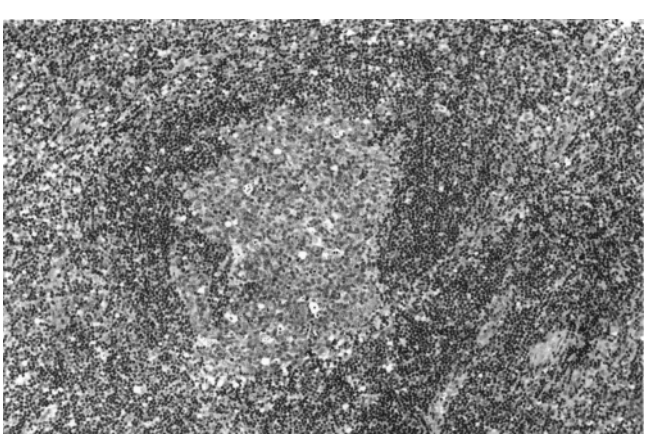

FIG. 27.21. Lymph node biopsy demonstrating marked follicular and interfollicular hyperplasia (type I pattern) (hematoxylin and eosin, $\times$ 100).

Mixed or intermediate patterns of reaction (type II or pattern B) include features of both ßorid hyperplasia and lymphoid depletion. This pattern is strongly associated with the impending development of AIDS (295,296).

At the present time lymph node biopsy is rarely done in HIV-infected patients with PGL. It is selectively performed to diagnose treatable opportunistic infections, lymphadenopathic Kaposi's sarcoma, and malignant lymphoma.

Malignant Lymphoma

It is estimated that 8–10% of HIV-infected individuals will develop a non-Hodgkin's lymphoma (NHL) (300). NHL is the second most common neoplasm associated with AIDS. Most cases have been reported in homosexual men, hemophiliacs, and intravenous drug abusers, although lymphomas can occur in individuals belonging to any AIDS risk group. PGL precedes the development of NHL in about one-third of patients (292,293). Most AIDS-associated NHL present with widely disseminated disease usually involving extranodal sites, particularly the gastrointestinal tract, central nervous system, liver, bone marrow, and skin. These tumors belong to the aggressive histopathologic subtypes: small cell noncleaved (Burkitt-type), large cell immunoblastic, and large cell noncleaved (Fig. 27.22) (301). However, a variety of low and intermediate-grade lymphomas including maltomas have also been reported.

About 95% of AIDS-associated NHL are of B-cell origin, and display various genetic lesions including Epstein-Barr virus infection, c-myc and bcl-6 gene rearrangements, ras gene mutations and p53 gene mutations/deletions (302,303,304). HIV does not appear to be directly involved in malignant transformation, as HIV DNA sequences have not been found in the genome of these neoplastic lymphoid cells (305). About one-third of AIDS-associated NHL have detectable Epstein-Barr virus DNA sequences or proteins (302,306). Body cavity-based lymphomas (BCBL), also known as primary effusion lymphomas, are a rare form of B-cell non-Hodgkin's lymphoma, which occur in HIV-infected patients and display a propensity to grow as ascites tumors in the pleural or peritoneal cavities (267). HHV8 has also been detected in cases of BCBL (267). Hodgkin's malignant lymphoma has been described in HIV-infected individuals, and like AIDS-associated NHL, the majority of patients present with extensive disseminated disease that often involves extranodal sites, including bone marrow, liver, skin, and soft tissues. In contrast with classic Hodgkin's disease, most patients with AIDS-associated Hodgkin's disease have an aggressive clinical course and a rapidly fatal outcome. The distribution of histopathologic categories includes mixed cellularity, nodular sclerosis, and lymphocyte-depleted types (Fig. 27.23) (301,307,308).

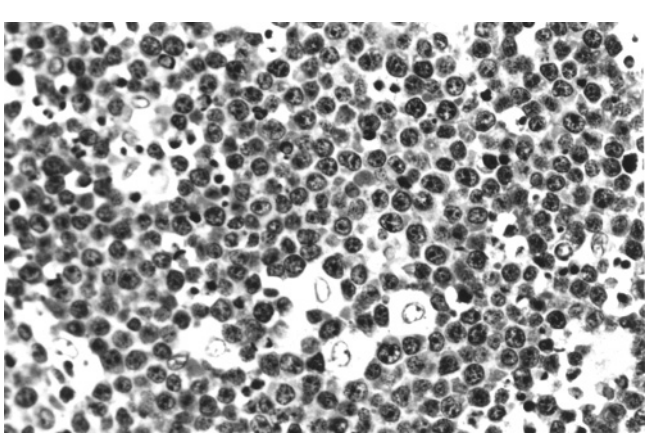

FIG. 27.22. AIDS-associated small noncleaved cell lymphoma involving lymph node. The presence of benign histiocytes within tumor imparts a "starry sky" appearance (hematoxylin and eosin, × 250).

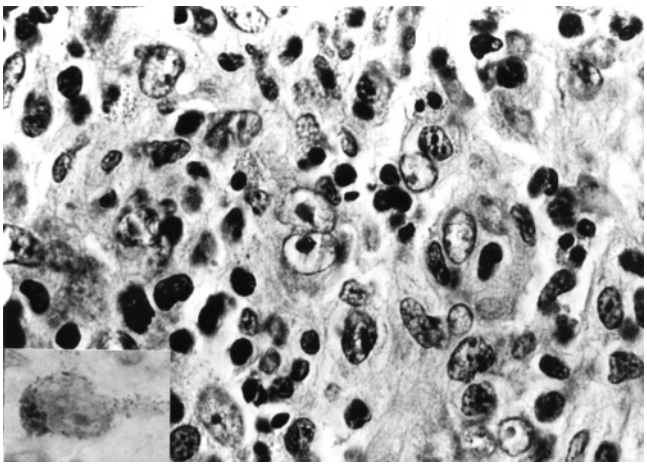

FIG. 27.23. AIDS-associated mixed cellularity Hodgkin's disease. Polymorphous in Itrate including the presence of Reed-Sternberg cell (hematoxylin and eosin, × 200). Inset is Leu M-1 (CD-15) immunoperoxidase stain of atypical mononuclear variant (Avidin-Biotin complex, hematoxylin counterstain, × 250).

Other Malignancies

Other hematopoietic and nonhematopoietic neoplasms have been reported in HIV-infected individuals (**Table 27.3**) (252,309–342). While the occurrence of many of these tumors is probably coincidental, some investigators believe tumor development is facilitated by the immunosuppression of HIV infection (343). These include low-grade B-cell NHL, T-cell leukemias and lymphomas, plasmacytomas, acute lymphoblastic leukemia, multiple myeloma, squamous cell carcinomas of several sites, adenocarcinomas of pancreas and colon, malignant melanomas, metastasizing basal cell carcinomas, thymomas, mesenchymal tumors including Þbrosarcomas, liposarcomas, leiomyosarcomas, leiomyomas, angiolipomas, and germ cell tumors including seminomas and embryonal

carcinomas. Anorectal squamous carcinomas and squamous cell carcinomas of the oral cavity are the most frequently reported malignancies after Kaposi's sarcoma and malignant lymphomas (309,310). Some of the squamous neoplasms have been associated with infections by human papillomavirus (313,341,343,344). A large National Cancer Institute study found that of a total of 38 malignant disorders other than KS and NHL, only angiosarcoma, Hodgkin's disease, multiple myeloma, brain cancer, and seminoma were increasing significantly from the pre-AIDS to the post-AIDS period (345).

POORLY UNDERSTOOD COMPLICATIONS OF AIDS

Cardiac Pathology

Early reports of cardiac pathology in autopsy studies focused on opportunistic infections and neoplasms involving the heart, and later reports focused on other cardiac lesions. Clinical studies subsequently established the presence of cardiac dysfunction in patients with HIV infection in the absence of opportunistic infection (346). The incidence of symptomatic heart disease in AIDS patients is estimated to be approximately 7% (347).

Infections and neoplastic involvement is usually seen in the setting of systemic disease. The findings of cardiac disease unrelated to opportunistic infections and neoplasia emerged in a report of dilated cardiomyopathy in 1986 (348). Since that report, several patterns of cardiac dysfunction in AIDS patients have been described that may be grouped into pericardial effusions and tamponade, dilated cardiomyopathy, heart failure without ventricular dilatation, refractory ventricular tachycardia, sudden death, and systemic disease (347).

Pericardial effusions are frequently detected in AIDS patients (349,350). Most often these effusions, which may result in cardiac tamponade, are seen in association with opportunistic infections (e.g. toxplasmosis, cytomegalovirus infection) and malignancies (e.g. Kaposi's sarcoma, malignant lymphoma) (72,351). When cardiac tamponade is present, infectious or neoplastic involvement is found in 88% of the reported cases.

AIDS-related dilated cardiomyopathy has been associated with ventricular dilatation and idiopathic myocarditis. It has been found that 6% of HIV-infected patients develop symptomatic cardiomyopathy (352). In one pediatric autopsy study, cardiac disease, including cardiomyopathy was a major contributor to mortality, being the underlying cause or an important contributing factor in almost one-third of patients (353). Ventricular dilatation may involve the left or right ventricles. Isolated right ventricular dilatation usually reflects severe pulmonary disease. The myocardium grossly appears normal in thickness, flaccid, and pale. Enlargement of valvular annuli is seen in biventricular dilatation. Cardiac hypertrophy (heart weight >400g) is usually absent. Microscopic findings, with the exclusion of cases with myocarditis, include enlarged myocyte nuclei with bizarre shapes, lipochrome pigment deposits, variable interstitial fibrosis, and edema (348,354). Myocyte fiber loss is variable.

Myocarditis, defined as myocyte degeneration or necrosis associated with adjacent inflammatory infiltrates, has been observed in some cases of dilated cardiomyopathy, ventricular tachycardia and sudden death, and pericardial effusions (354). Clinical symptoms were seen in 58% of the cases that were detected at autopsy (355). Idiopathic myocarditis was diagnosed by endomyocardial biopsy in a patient with refractory ventricular tachycardia (356). The cause of myocarditiis in HIV infection remains obscure in the majority of cases (354). No correlation has been found between therapy and the occurrence of myocarditis (354,355). Viral-induced injury either directly or by activation of latent viral infections by immunosuppression has been proposed. Acute global left ventricular dysfunction occurs infrequently in AIDS patients and can be associated with myocarditis or acute onset of cardiomyopathy, both of which have a uniformly fatal outcome, or can be associated with toxic myocardial damage, in which case it is potentially reversible (357).

Nonbacterial thrombotic endocarditis is usually a right-sided, asymptomatic finding at autopsy. The vegetations are small masses of fibrin and blood that collect on the valve leaflets along the lines of closure. Either side of the heart may be affected. The lesions are commonly seen in chronic debilitating conditions such as metastatic cancer, renal failure, or chronic sepsis. Their presence in AIDS patients probably reflects the underlying severe illness. Involvement of all four cardiac valves has been reported. Complications are rare, but one patient with cerebral infarctions has been reported (351).

HIV-Associated Enteropathy

Diarrhea, weight loss, and malabsorption have been recognized as major manifestations of HIV infection and may precede the AIDS-related complex (ARC) or AIDS stage of HIV infection (358). Mucosal injury may result from immunosuppression leading to the development of opportunistic infections or AIDS-associated malignancies. Some of the opportunistic infections, such as cryptosporidiosis, are primary for the gastrointestinal tract (86,180, 188,359). Alternatively, evidence for direct mucosal injury by HIV itself has been recognized as HIV-associated enteropathy (360).

HIV-associated enteropathy has been postulated in those patients who do not have a demonstrable specific infectious agent. HIV antigen and virus have been demonstrated in the lamina propria of intestinal biopsies (361,362). Diarrhea and malabsorption have been documented in 29% of patients who are HIV-seropositive but have negative stool cultures and other examinations for the

infectious agents common to AIDS patients (363). It is postulated that depletion of CD4+ T-cells leads to altered IgA B-cell development and decreased IgA production (364). HIV-infected patients have also been reported to have impaired gastric acid secretion which, together with reduced IgA production, may lead to bacterial overgrowth of the small intestine. These bacteria may contribute to mucosal inßammation, villous atrophy, and malabsorption (364). Duodenal mucosal biopsies in these patients may show nonspeciÞc changes of mucosal atrophy, normal crypt depth, and decreased mitotic activity (361). Jejunal biopsies have demonstrated an increase in intraepithelial lymphocytes over control subjects (363). Rectal Þndings include focal epithelial cell degeneration (apoptosis) with intranuclear virus inclusions (360). Electron microscopy studies demonstrate contact between intraepithelial lymphocytes and apoptotic cells, which suggests a cell-mediated immune response. Apoptosis is not a typical feature of infectious enteritides, but has been observed in acute graft-versus-host disease of the colon following bone marrow transplantation. It has been postulated that viral infection induces changes in the cell membranes that triggers a host response and apoptosis (360). In those patients with diarrhea the changes seen are most pronounced. Mucosal atrophy, crypt hyperplasia, and lymphoplasmacytic inÞltration of the lamina propria are not speciÞc for HIV enteropathy. Similar changes may be seen in other enteric infections (365).

Damage to autonomic nerve bundles in the jejunal mucosal lamina propria has been described and is similar to that reported in laxative abuse, diabetic autonomic neuropathy, inßammatory bowel disease, and amyloidosis (366).

The carrier rate for enteric pathogens increases for the population of HIV-infected patients with AIDS. In these patients with diarrhea and malabsorption, one-third will be found to have a treatable infection, which underscores the importance of searching for enteric pathogens.

Renal Pathology

The renal manifestations of HIV infection may be the result of indirect or direct mechanisms (367Ð369). These include acute tubular necrosis, allergic interstitial nephritis (AIN), immune complex-associated glomerulonephritis, focal segmental glomerulosclerosis/collapsing glomerulopathy, thrombotic microangiopathy, microbial infection from a variety of pathogens (370), and crystal nephropathy (371).

Acute tubular necrosis is not usually directly attributable to HIV but occurs as a result of episodes of hypovolemic shock and sepsis, or because of exposure to radiocontrast agents. Acute interstitial nephritis or acute tubular necrosis may be the result of therapy with trimethoprim-sulfamethoxazole, gentamicin, pentamidine, acyclovir, and amphotericin B. Biopsies are infrequently performed in the typical clinical setting of AIN but may reveal interstitial edema, epithelial damage, and inßammatory inÞltrates. Eosinophils may be present in variable numbers. Toxic or ischemic injuries occur in a clinical setting, which likewise infrequently lead to a biopsy. The histopathologic Þndings at biopsy include low ßattened tubular epithelium with sloughing, loss of brush border, dilatation of tubules, mild interstitial edema, and sparse cellular inÞltrates. The acute renal failure experienced by these patients often contributes to their death because of underlying serious illness.

Immune complex-mediated glomerulonephritis has been reported in sporadic cases, often related to various infectious diseases in these patients. Mesangial proliferation and diffuse proliferative glomerulonephritis have been described (372,373). Immunoglobulins and complement proteins are usually seen in the mesangial areas and along capillary loops by immunoßuorescence.

Fluid and electrolyte abnormalities are frequently seen in these critically ill patients, with hyponatremia being one of the most common. Gastrointestinal ßuid loss, before hospital admission, is the etiology of the hyponatremia in 40% of patients with AIDS. Mortality in hospitalized hyponatremic patients with AIDS is twice as high as in normonatremic patients (374).

HIV-Associated Nephropathy

In 1984, Rao and associates (375) recognized a renal disorder characterized by heavy proteinuria and progression to renal failure in AIDS patients. Since then, HIV-associated nephropathy has been recognized in many medical centers but has been controversial for several reasons (374). Initially, the lesion was described in AIDS patients with a high proportion of drug abuse cases. Controversy was spawned because some centers, notable those in San Francisco (376), did not see this lesion with the same frequency. Later, this renal lesion was shown to be present in children with AIDS, which strengthened the argument for a primary causal relationship (377). A second controversial point was whether this lesion was secondary to immunological changes that accompany AIDS or was just associated with HIV infection. Again, recently the lesion has been described in patients with HIV infection, but no manifestations of AIDS (378). So, in spite of initial controversies, acceptance of HIV nephropathy is generally agreed as valid.

The clinical features of HIV-associated nephropathy have the following characteristics. The lesion may be found in asymptomatic, HIV-infected patients or in patients with ARC and AIDS. Males are more frequently affected than females, with variations in incidence in different geographic areas (i.e. high in New York City, low in San Francisco). Black patients are affected more frequently than white. Proteinuria is always present and is usually heavy. The course is usually marked by early and

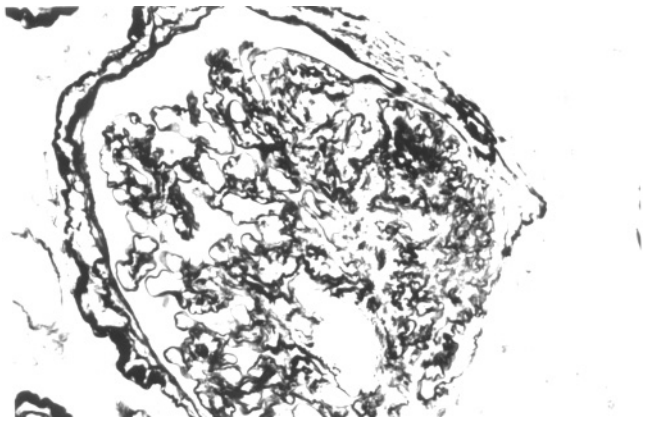

FIG. 27.24. Biopsy specimen from a patient with AIDS nephropathy. The glomerulus is segmentally sclerosed (Gomori's methenamine-silver, × 200).

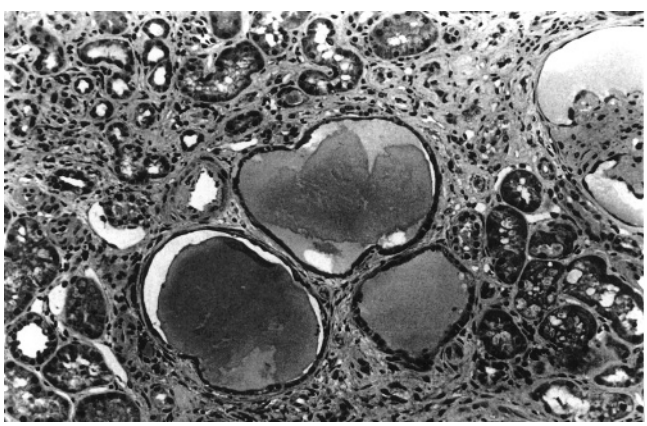

FIG. 27.25. Biopsy specimen from a patient with AIDS nephropathy. The interstitium is brotic and dilated tubules are present (hematoxylin and eosin, × 200).

rapidly progressive azotemia. Mild hypertension is present (379).

Morphologic manifestations of HIV-associated nephropathy are found in glomeruli, tubules, and interstitium (pan-nephropathy). The kidneys are usually enlarged. Progressive changes may be seen in the affected glomeruli. Initially, the epithelial cells become swollen and the underlying capillary loops are wrinkled or collapsed (collapsing variant of focal segmental glomerulosclerosis). Occasional intraluminal foam cells are seen. In the more advanced lesions the capillary loops are completely collapsed, with expansion of the mesangial matrix-basement membrane material (Fig. 27.24). Plasma proteins may accumulate within the capillary loops (hyalinosis). In far advanced lesions more glomeruli are involved and there is complete capillary obliteration.

Microcystic dilation and proteinaceous casts may be found in the tubules. Interstitial Þbrosis and tubular atrophy are seen as the glomerular lesion advances (Fig. 27.25). Interstitial inÞltrates contain lymphocytes, histiocytes, and plasma cells.

Ultrastructural studies demonstrate a diffuse effacement of epithelial foot processes and detachment of cells from the basement membranes. Inclusions of tubuloreticular bodies are almost always present in the vascular endothelium. Cytoplasmic inclusions of parallel stacks and cyclindrical confronting cisternae, also known as test tube and ring-shaped forms, have been described. Immuno-ßuorescence studies demonstrated C3, C1q, and IgM in the sclerotic segments. Granular deposits of these proteins may also be seen in mesangial areas. In situ hybridization techniques have demonstrated proviral HIV DNA in the tubular and glomerular epithelial cells.

PATHOLOGY OF AIDS IN CHILDREN

Since the Þrst published series of AIDS cases in children (380,381), it is estimated that at the end of 1999, 1.2 million children are living with HIV-1 infection and an additional 3.6 million children have died of the disease. In 1999 alone, 570,000 children became infected with HIV, primarily through mother-to-child transmission (382).

There are basic similarities between pediatric and adult AIDS in major clinical features and pathologic lesions. However, pediatric AIDS is different with respect to mode of transmission, frequency and types of certain clinical features, immunologic abnormalities and pathologic lesions. These differences will be highlighted and the pathologic lesions, which occur more frequently or predominantly in children, and perinatal pathology of HIV infection will be described in this section.

Mode of Transmission

There are two major modes of transmission (383): (a) Transplacental/perinatal transmission from the HIV-infected mother (the mother may or may not have symptomatic HIV infection or full blown AIDS) and (b) parenteral transmission through transfusion of infected blood or blood products (e.g. factor VIII). Of the two, the former is by far more common. Transmission can occur prenatally, during delivery, and postnatally via breast milk (384,385). Of these, transmission during delivery presents the greatest risk. The risk estimates of transmission of HIV from infected mothers to their infants vary greatly. Administration of antiretroviral agents during pregnancy and labor and to the infant after birth can decrease transmission rates to as low as 2% (386,387).

Clinical Features and Immunologic Abnormalities

Failure to thrive, fever, lymphadenopathy, respiratory symptoms and signs and neurologic abnormalities are among the most common clinical features (380,381,388). The immunologic features include both T and B cell

abnormalities such as cutaneous anergy, low absolute T helper cell counts, reversed T helper/suppressor cell ratio, lack of response to mitogens and polyclonal hypergammaglobulinemia. With longer survival due to early diagnosis and intensive therapy, cardiomyopathy, renal disease, neoplastic disorders, etc., are seen with increasing frequency.

Pathologic Lesions

All the lesions described in adults occur in children with AIDS. Although these lesions occur with different frequency, lesions of lymphoreticular, central nervous, respiratory and digestive systems, and heart and kidneys have all been reported in pediatric AIDS, as are various infections and malignancies (4,389). The following lesions are seen predominantly or more frequently in children with AIDS: (a) Thymic lesions (primary lesions), (b) Pulmonary lymphoid lesions (associated lesions), (c) Lymphoproliferative disorders, including lesions of mucosa associated lymphoid tissue (MALT), (d) Arteriopathy (lesion of undetermined pathogenesis), and (e) certain types of neoplastic lesions.

The lesions in the thymus and lungs, those related to the lymphoproliferative disorders, arteriopathy, and neoplastic lesions, will be discussed. Similarities, differences and frequency of these lesions in adults with AIDS will also be described.

Lesions of the Thymus

Three types of lesions have been noted in the biopsy and autopsy specimens of the thymus (4,389):

a. Precocious involution is characterized by marked depletion to virtual absence of lymphocytes, loss of corticomedullary differentiation, and microcystic dilation of Hassall's corpuscles (HC) which are present in normal numbers. Hyalinization of the cortex and medulla is present in some cases at autopsy. The location, conÞguration and the blood vessels of the thymus are normal. These features resemble age and stress related involutionary changes but occur prematurely and are out of proportion to the severity of stress. There is marked reduction in weight often to less than 1 g.
b. Dysinvolution (Fig. 27.26) is characterized by all the features noted above except that HC are virtually absent. Rare HC can be demonstrated in an occasional lobule by studying step serial sections of the thymus. These features particularly the virtual absence of HC resemble the dysplastic thymus seen in certain congenital immune deÞciency syndromesÑ hence the term dysinvolution.
c. Thymitis is characterized by one of the following features: Lymphoid follicles with germinal centers in the medulla, focal or diffuse lymphomononuclear or lymphoplasmacytic inÞltrate disrupting the normal architecture of the thymus or multinucleated giant cells in the medulla. Recently another reactive lesion, multilocular thymic cyst has been described in children with AIDS (390,391). This lesion should be considered in the differential diagnosis of a mediastinal mass in a child with AIDS.

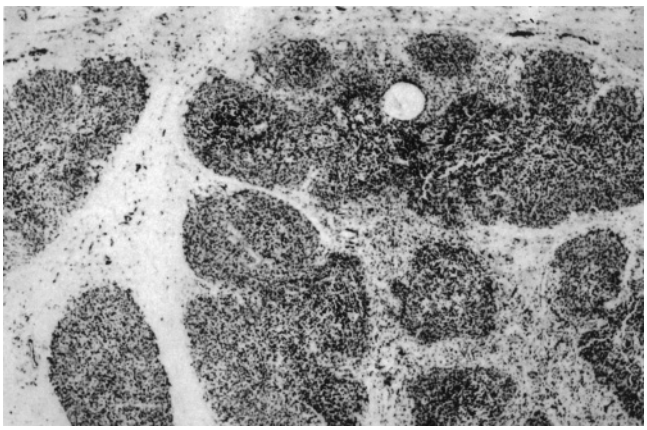

FIG. 27.26. Dysinvolution of thymus. Note loss of corticomedullary differentiation, lymphocytic depletion of both cortex and medulla and markedly reduced number of Hassall's corpuscles—only one of which is present in one of the lobules (hematoxylin and eosin, × 20).

The etiology and precise target cell in thymic injury are not known. However, it is possible that the thymic epithelial cells may be injured by HIV since: (a) HIV has been isolated from a thymic biopsy specimen in one of our cases and from the thymus of a 20-week fetus and (b) Simian immunodeÞciency virus (SIV) has been demonstrated by immunoperoxidase staining in the thymic epithelial cells in rhesus monkeys with an AIDS-like disease complex (4).

It is of interest to note that severe involutionary changes with thymic epithelial injury have been reported in adults with AIDS (392). Thymic enlargement with thymitis characterized by presence of lymphoid follicles with germinal centers and vascularized lymphoid follicles in the medulla resembling those seen in Castleman's disease has been observed in an adult with AIDS (393). Thymic injury in AIDS thus may lead to defective T cell differentiation and maturation and contribute to the immune deÞciency in AIDS both in children and adults.

Lesions of the Lungs

Pulmonary lesions associated with the frequently observed respiratory symptoms and signs in children with AIDS are listed in **Table 27.4**. Lesions related to pulmonary opportunistic and pathogenic bacterial infections are the same as in adults. The pulmonary lymphoid

lesions including the systemic lymphoproliferative disorder are seen much less frequently in adults.

Lymphoid Lesions

These are of two types: Pulmonary lymphoid hyperplasia/lymphoid interstitial pneumonitis complex (PLH/LIP complex) (394,395) and polyclonal polymorphic B cell lymphoproliferative disorder (PBLD). PBLD, a systemic disorder with prominent pulmonary involvement, will be described separately in the next section. PLH is characterized by peribronchiolar lymphoid nodules commonly with germinal centers (Fig. 27.27). The lymphoid nodules are composed of mature and immature lymphoid cells of the germinal center with plasma cells at the periphery. LIP is characterized by diffuse inÞltration of the alveolar septa by mature and immature lymphoid cells, plasmacytoid lymphocytes and plasma cells with occasional Russell bodies (Fig. 27.28). Nodular aggregates of lymphoid cells are seen in some foci. There is no involvement of blood vessels or destruction of bronchi. No viral inclusions are seen. Special stains for fungi, acid-fast bacilli, and *Pneumocystis carinii* are negative. Immunoperoxidase stains for kappa and lambda light chains of immunoglobulins show that the pulmonary lymphoid inÞltrates are polyclonal.

With experience of larger number of cases, the overlap between PLH and LIP was evidenced by (a) the variable degree of alveolar septal inÞltration in cases which could otherwise be labeled as PLH, (b) the presence of peribronchiolar lymphoid nodules in cases which could be labeled as LIP and (c) the gradual transition between (a) and (b) became more evident and of more common occurrence. Therefore, it was recommended that these lesions should be designated as PLH/LIP complex (395).

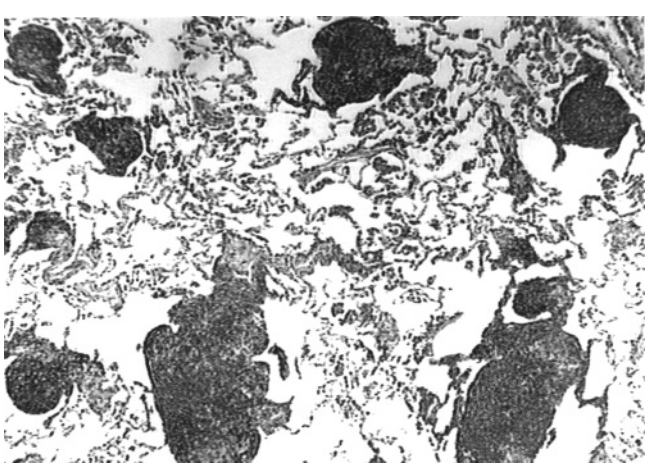

FIG. 27.27. Lung biopsy specimen showing lymphoid nodules around bronchioles. Note slight extension of lymphoid in ltrate into adjacent alveolar septa and germinal centers vaguely disconcernible at this magni cation (PLH/LIP complex) (hematoxylin and eosin, × 10).

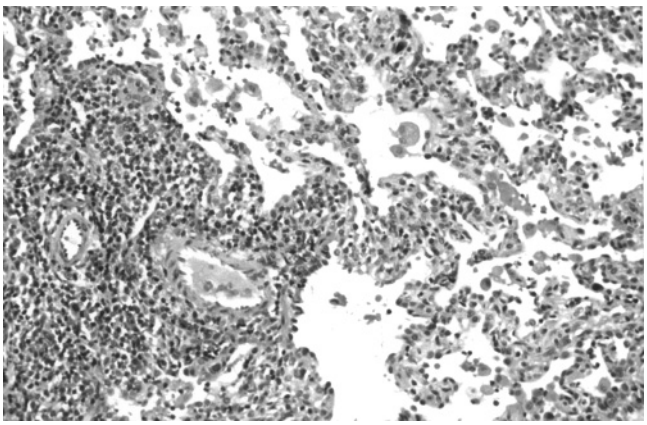

FIG. 27.28. Lung biopsy specimen showing dense in ltration of alveolar septa by lymphoid cells (PLH/LIP complex) (hematoxylin and eosin, × 100).

Besides B cells demonstrated by routine histologic methods and by immunoperoxidase stain for light chains, T cells are also present in the lesions of PLH/LIP complex shown by cell marker studies on bronchoalveolar lavage ßuid or the lung biopsy tissue. T_8 cells predominate in this T cell population.

Although infrequent, PLH/LIP complex has been described in adults with AIDS (57). However, the recent study by Guillon et al. (396) indicates that PLH/LIP complex diagnosed by lymphocytosis in bronchoalveolar lavage ßuid is more common in adults than generally thought. The lesion in adults has been labeled as LIP or lymphocytic alveolitis. Although detailed descriptions and illustrations are lacking, it is apparent from the limited accounts (57,396) that the lesion in adults represents PLH/LIP complex similar to that in children.

Pathogenesis: Although the pathogenesis of pulmonary lymphoid lesions is not yet established with certainty, there is evidence to suggest that both Epstein-Barr virus (EBV) and HIV may be etiologically related (4,397). In view of the concept of mucosa associated lymphoid tissue (MALT) of various organs such as the gastrointestinal tract, respiratory tract, etc. (398), the pulmonary lymphoid lesions can be considered to represent MALT lesions.

Diffuse Alveolar Damage (DAD): DAD is seen in both biopsy and autopsy specimens. The exudative phase is characterized by hyaline membrane formation, interstitial and alveolar edema, mild mixed interstitial inßammatory inÞltrate and focal intra-alveolar hemorrhage (Fig. 27.29). In the proliferative phase there is cuboidal metaplasia of alveolar epithelium, interstitial Þbroblastic proliferation and interstitial edema.

DAD is related to a variety of pathogenetic factors, which include oxygen toxicity, pulmonary infections, sepsis, and shock. One or more of these factors are operative in individual cases. DAD may obscure the infectious nature of the lesion. In adults, DAD related to similar factors is seen. However, in some cases of adult AIDS, features of DAD with proliferative changes in the

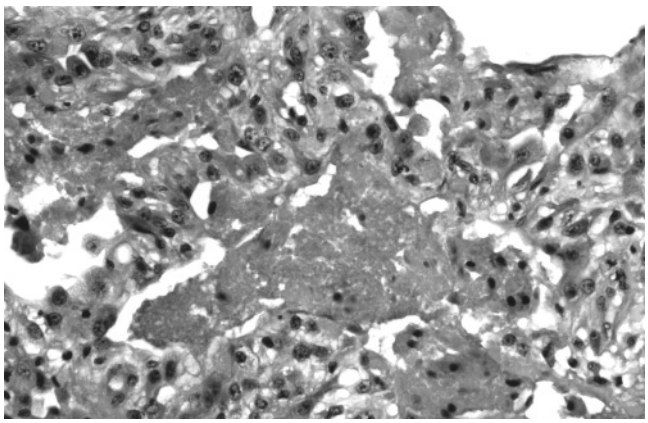

FIG. 27.29. Lung biopsy showing organizing diffuse alveolar damage. Note the hyaline membranes and alveolar foamy exudate (Gomori's methenamine silver stain revealed *Pneumocystis carinii*) (hematoxylin and eosin, × 400).

lung biopsy are present without any demonstrable pathogenetic factors (399).

In addition to these lesions occurring in children and adults, two inßammatory lesions viz. nonspeciÞc interstitial pneumonitis (NIP) (400) and lymphocytic bronchiolitis (LB) (401) have been described in isolated cases of AIDS in adults having respiratory symptoms and signs. In NIP, histologic features are essentially those of a combination of exudative and proliferative phases of DAD and consist of alveolar edema, Þbrin deposition, hyaline membranes, interstitial inßammation and loose and dense interstitial Þbrosis. The lesion is labeled as NIP presumably because the inßammatory component is more prominent (402). In most instances NIP is seen in association with or following other pulmonary lesions such as KS, experimental therapy, drug abuse or PCP and shows prominent Þbroproliferative changes. However, in a minority of the patients no associated factor or other pulmonary lesion is found. In this latter group, NIP is characterized by interstitial lymphoid cell aggregates and the absence of loose or dense interstitial Þbrosis. Hyaline membranes are seen in some cases. NIP with only interstitial lymphocytic inÞltrate without hyaline membranes has also been described in adults with AIDS having no respiratory symptoms or signs. The importance of recognition of NIP is related to similarities between the clinical features of NIP and of pulmonary opportunistic infections particularly PCP.

LB has been reported only in one case (401). It is characterized by an intense inÞltration of the wall of terminal and respiratory bronchioles by lymphocytes and plasma cells. Increased numbers of T_8 suppressor cells were found in the bronchoalveolar lavage specimen.

Both NIP and LB need to be further characterized with a detailed description and illustration of the pathologic features. It is possible that NIP and LB in adults are less severe variants of PLH/LIP complex described above in children.

Lymphoproliferative Disorders

Two types of these disorders occur in children: (1) systemic lymphoproliferative disorder and (2) spectrum of MALT lesions. In four children with AIDS a systemic lymphoproliferative disorder with prominent involvement of lungs and less severe involvement of liver, spleen, kidneys, other extranodal sites and lymph nodes, was seen (4) (Figs. 27.30 and 27.31). There was no involvement of the brain.

Histologically, the cellular inÞltrates in the various organs mentioned above and in skin, skeletal muscle, and salivary glands were polymorphic and consisted of an admixture of lymphocytes, plasma cells, plasmacytoid lymphocytes, and immature lymphoid cells or immunoblasts. The polyclonal nature of the lymphoid inÞltrates was demonstrated by the presence of both kappa and lambda light chain immunoglobulins in parafÞn sections of different organs stained by an immunoperoxidase method. In the kidneys and spleen, vascular invasion by the cellular inÞltrate was noted. It appears that this lymphoproliferative disorder is distinctive and was designated as polyclonal polymorphic B cell lymphoproliferative disorder (PBLD). It is considered to be intermediate between benign and full-ßedged malignant lymphoproliferations. In two cases PBLD appeared to represent a progression of PLH/LIP complex, while in other cases it seemed to arise *de novo*.

A spectrum of lymphoproliferation (follicular hyperplasia of lymph nodes, PLH/LIP complex, lymphoid hyperplasia of G.I. tract, lymphoid follicles in the thymus,

FIG. 27.30. Polyclonal polymorphic B-cell lymphoproliferative disorder (PBLD) involving the kidney. Whole mount of histologic section showing multinodular involvement of cortex with extracapsular extension (hematoxylin and eosin), × 5).

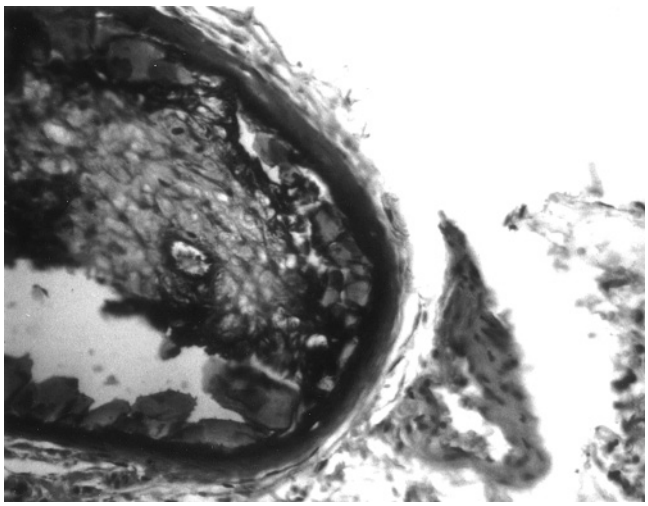

FIG. 27.31. Arteriopathy characterized by intimal brosis with calci cation and brosis of media of an artery. Note the markedly narrowed lumen (hematoxylin and eosin, × 25).

and nodal and extranodal malignant lymphoma) similar to that in children can also be proposed for adults with AIDS. Thus the following lymphoid lesions described in adults may belong to such a spectrum: Follicular hyperplasia of lymph nodes, adenoidal hypertrophy (403), PLH/LIP complex, EBV related lymphoproliferative disorder (404) similar to PBLD in children, and nodal and extranodal malignant lymphoma.

The other major category of lymphoproliferative lesions is the spectrum of MALT lesions (405,406). The spectrum includes: typical and atypical PLH/LIP complex, myoepithelial sialadenitis with or without focal lymphoma, low grade MALT lymphoma of parotoid gland and diffuse large cell lymphoma of parotid gland and of tonsil. MALT lymphomas respond to therapy and follow an indolent clinical course. Both EBV and HIV may be involved in the pathogensis of MALT lesions (407).

There is overlap between the systemic lymphoproliferative disorder and MALT lesions. The concept of MALT has been extended to include tissues and organs such as liver, thymus, genital tract, etc., besides the respiratory and digestive tracts (405). Therefore, it appears that most of the lesions seen in the systemic lymphoproliferative disorder described above belong to the spectrum of MALT lesions. We would like to suggest that since PLH/LIP complex, which is a MALT lesion, is considered one of the criteria for the diagnosis of AIDS in children, that the other reactive MALT lesions described above should also be included as criteria for the diagnosis of pediatric AIDS.

Arteriopathy

The lesion designated as arteriopathy is seen in small and medium sized arteries of different organs (heart, lungs, kidneys, spleen, intestine, brain) (4). It is characterized by intimal Þbrosis, fragmentation of elastic Þbers in the media, Þbrosis and calciÞcation of internal elastic lamina and media with variable luminal narrowing (Fig. 27.32). (Vasculitis seen only in the brain in association with progressive HIV encephalopathy is not considered as part of the arteriopathy described here.) Arterial aneurysms affecting young black patients occurring in atypical sites and with a tendency towards multiplicity have been described (408). In one case, fatal outcome resulted from aneurysms of the right coronary artery with thrombosis (**Fig. 27.33**) and myocardial infarction (4). Aneurysm formation of cerebral arteries has been reported in another case.

The pathogenesis of arteriopathy is not clear. Arteriopathy is therefore included under the category of lesions of undetermined pathogenesis. However, it is possible that repeated bacterial and opportunistic infections secondary to immunodeÞciency might result in increased exposure to endogenous and exogenous elastases. Elastic tissue damage, which is the striking feature of the arterial lesions, may be related to such an increased and repeated exposure.

The arteriopathy seen in children with AIDS appears to be distinctive. Similar arterial lesions have not been reported to occur in adults with AIDS, although we have seen similar ÞbrocalciÞc lesions of medial of small and medium sized arteries of thyroid, mesentery and kidney in a 30 year old male intravenous drug abuser who died of AIDS.

Recently, other types of vascular lesions have been described in children and adults with AIDS. These include: inßammatory lesions (mycotic aneurysms, systemic vasculitis, polyarteritis nodosa like vasculitis, hypersensitivity vasculitis), artherosclerosis, thomboembolic lesions, proliferative lesions (e.g. bacillary angiomatosis) and miscellaneous lesions (zidovudine induced leukocytoclastic vasculitis, etc.) (409).

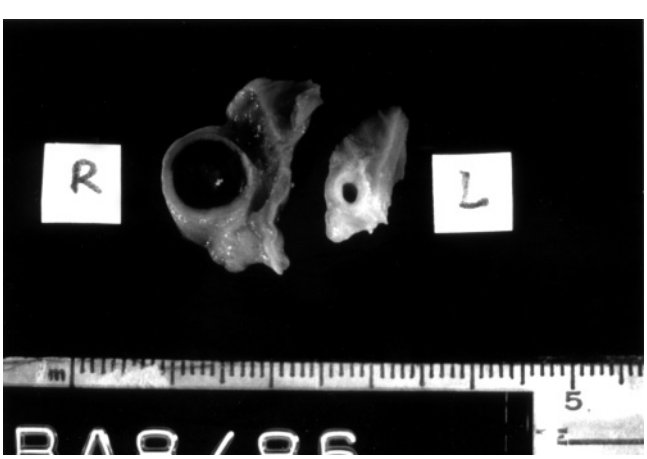

FIG. 27.32. Gross photograph of both coronaries. Note the aneurysmal dilatation with thrombotic occlusion of the lumen of right coronary artery.

PROLIFERATIVE AND NEOPLASTIC DISORDERS

Of the various neoplastic lesions described in association with pediatric AIDS, non-Hodgkin's lymphomas (NHLs) are the most common. There are no basic differences between NHL in adults described above and NHL in children with AIDS (410). Other neoplastic lesions in children include smooth muscle tumors, Kaposi's sarcoma, and leukemias. With the increasing incidence of human papilloma virus (HPV) infection and HIV infection in adolescent girls, it is conceivable that the incidence of HPV related genital lesions will increase in HIV-infected adolescent girls. These lesions, which include condyloma acuminatum with or without various grades of cervical intraepithelial neoplasia (CIN), can progress to invasive cervical carcinoma. Rapid progression of these lesions may occur due to immunosuppression in HIV infection. In fact a case of invasive cervical carcinoma has been reported in a 16 year old HIV-infected girl (411). Pediatricians should be alert to the possibility of HPV-related genital lesions and take steps for early diagnosis and treatment of these lesions so their progression is prevented.

It appears that smooth muscle tumors (SMTs) are the second most common neoplastic disorder in pediatric AIDS. Thirteen cases of SMTs involving GI tract, lungs, soft tissue, skin, and spleen (most of which are leiomyosarcomas and a few leiomomas) have been reported (412). Quantitative PCR done on the fresh frozen samples of the tumor tissue revealed 170 to 455 copies of EBV per 100,000 tumor cells. EBV receptor (CD21) was demonstrated in high concentration in the tumor cells by an immunoperoxidase method. In situ hybridization studies using an EBER probe showed the presence of EBV in tumor cells but not in the adjoining normal cells (413). These findings suggest that the EBV receptor may be upregulated in children with AIDS and EBV may enter the muscle cells and cause their transformation.

PERINATAL PATHOLOGY

Transplacental/perinatal transmission is by far the commonest route of transmission of HIV to children. Several large studies have demonstrated the ability of short-course antenatal/intrapartum prophylaxis with zidovudine or nevirapine to reduce perinatal HIV transmission dramatically (414,415). There are only a few systematic virologic and pathologic studies on placenta, abortuses, and stillborn fetuses from HIV-infected women to document intrauterine transplacental HIV transmission. HIV was isolated from amniotic fluid, thymus, lungs, spleen and brain of a 15- and a 20-week fetus, but pathologic description of the various fetal tissues and placenta was not given (416). Jauniaux, et al. (417) studied 49 placentas, seven fetuses and two stillbirths from Central African and European HIV-infected women with or without fully developed AIDS. No villitis was noted but irrespective of gestational age, the villi were coarse, cellular, hypovascularized and the intervillous spaces were narrow with fibrin deposition and calcification. There was a high incidence (43%) of chorioamnionitis, which was unrelated to HIV infection. The fetuses did not show any histologic lesions in the viscera. The two stillbirths had pneumonia (associated with chorioamnionitis) and nodular peribronchiolar and alveolar septal lymphocytic aggregates in the lungs. Ultrastructural studies in 13 placentas revealed isolated retrovirus-like particles with some morphologic similarities to HIV (100 nM in size, dense central or eccentric core) in the syncytiotrophoblasts, fibroblasts and endothelial cells in villous capillaries and free membranes. Other investigators have demonstrated the presence of HIV RNA and p24 protein in the trophoblasts, Hofbauer cells and endothelium of placentas from HIV-infected women (418,419). Systematic prospective virologic, immunologic, and pathologic studies of placenta, abortuses and stillborn fetuses of HIV-infected mothers with and without full-blown AIDS are needed to confirm the observations outlined above. Such studies on larger number of pregnant women in various stages of HIV infection would also provide data regarding timing of HIV infection of the fetus, the cell types and tissues of the fetus infected by HIV and the relationship of severity of HIV infection in the mother to that in the fetus.

Perinatal transmission, (i.e. transmission shortly before, during, or shortly after the process of birth) is probably far more common than intrauterine transplacental transmission. Findings in the cervical biopsy tissue of four HIV-infected women described by Pomerantz, et al. (420) and isolation of HIV from vaginal and cervical secretions reported by Wofsy, et al. (421) support the concept of perinatal transmission to the neonate (and also of heterosexual transmission of HIV). The findings described by Pomerantz, et al. (420) were as follows: Chronic cervicitis characterized by mononuclear cell infiltration and lymphoid aggregates in the mucosa and/or submucosal, isolation of HIV from cervical biopsy tissue and demonstration of HIV antigens in the monocytes, endothelial cells and lymphocytes in the cervical biopsy.

PATHOLOGY OF AIDS IN AFRICAN PATIENTS

The epidemiology and clinical spectrum of AIDS in Africa differ markedly from that in the United States and western European countries (422,423). Heterosexual activity, a history of prostitution or sexual contact with prostitutes, blood transfusion, the use of unsterilized needles in medical facilities, and vertical transmission from mother to infant are considered the primary modes of HIV transmission in African countries (424). Published histopathologic studies of AIDS in Africa are meager as a result of inadequacies of laboratory facilities in these

developing countries. Limited investigations have shown pathologic lesions similar to those described in western patients. However, the following differences in the frequencies and types of opportunistic infections and other lesions have been noted between these two groups (118,425–428): (1) MTB infections are more common, (2) MAI infections are infrequent, (3) Cryptosporidium and *Isospora belli* are frequently found in endoscopic biopsies, (4) PC pneumonitis is infrequent, (5) a generalized, pruritic, maculopapular dermatosis is seen in about 50% of patients as the initial disease presentation, and (6) growth retardation and chronic diarrhea are more frequently seen in African children with AIDS (118,429).

There are few studies comparing HIV-associated Kaposi's sarcoma and malignant lymphoma in African and western patients.

PREVENTING HIV TRANSMISSION IN THE CLINICAL LABORATORY

Occupational transmission of HIV to health care workers by sharps injuries or mucocutaneous contact is well reported (430–438); however, appropriate use of needle stick prevention devices, especially as part of a comprehensive prevention program, can significantly reduce the incidence of such injuries. Federal law now requires the use of sharps "devices to protect workers and many states have passed similar legislation.

To minimize exposure, microbiologic and other specimens requiring manipulation during processing should be handled under a laminar flow hood to control aerosol dispersion. Mechanical pipetting devices are routinely used and mouth pipetting strictly forbidden. Specimens requiring centrifugation are capped and placed in a centrifuge with a sealed dome. Accidental spillage of a specimen is promptly cleaned with a suitable disinfectant solution (439–442).

All laboratory work areas should be cleaned and sanitized at the end of each shift or at least daily using an appropriate chemical disinfectant such as 0.5% sodium hypochlorite (1:10 dilution of household bleach). Specimens leaking from their containers are discarded if specimen replacement is possible. Otherwise, the outside of the container is appropriately disinfected.

Tissue sent from the operating room for frozen section studies should be placed in leak-proof containers during transport and handled with latex or vinyl gloves to prevent inadvertent skin contamination. During cryostat cutting of specimens, the use of tissue-freezing aerosol is discouraged to prevent aerosolization. The cryostat and all cutting areas exposed to blood or body fluids should be decontaminated at frequent intervals with an appropriate germicide. Face shields or a facemask and goggles should be routinely worn during the gross examination and cutting of surgical specimens.

For health care workers, who are occupationally exposed to HIV, the U.S. Public Health Service issued guidelines for prevention and chemoprophylaxis in 1998. The exposure site should be washed with soap and water as soon as possible after exposure and then cleaned with sterile saline or disinfectant solution. Thorough irrigation should occur if the exposed area is mucus membrane. For post high-risk exposure, antiretroviral chemoprophylaxis should be offered (443). Agents used for post-exposure prophylaxis have had serious adverse effects, such as bone marrow suppression, peripheral neuropathy, nephrolithiasis, and hepatocellular injury. However, there are no reports of long-term sequela.

Autopsy Procedures

Currently, there is no documentation of an autopsy assistant or pathologist having contracted AIDS or seroconverting as a result of performing an autopsy on a patient with AIDS (444,445).

The autopsy recommendations of the National Committee of Clinical Laboratory Standards are for total-body barrier protection with water repellent protective clothing. This includes a hood that covers the hair, face shield, or safety goggles with mask to cover the nose and mouth, as well as water-protective boots, double surgical gloves, and, in certain instances, heavy overgloves (446). Aerosols generated by an oscillating electric saw (Stryker) can be minimized by the use of a plastic bag over the head and neck of the corpse and shielding the spray by keeping wet towels immediately adjacent to the blade. A circulating assistant outside the work area should be designated to perform tasks such as answering phones and pages and obtaining supplies. Some institutions, including ours, have elected to perform autopsies in special "isolation" rooms.

Following the autopsy, the closed body is washed with a detergent solution and then with dilute sodium hypochlorite, rinsed with water, and placed into a body bag. Tables, instruments, and floors are washed with a detergent to remove dried blood and fluids, and then decontaminated with a dilute solution of household bleach or other effective germicides. The prosector and assistant should discard all disposable materials into a specially marked bag for incineration. Reusable materials are cleaned and disinfected or autoclaved. Adequate washing or shower facilities should be available in the vicinity of the autopsy suite.

Recommendations for preventing nosocomially acquired HIV infection are designed to minimize the risk of mucosal or parenteral exposure to potentially infectious materials. This can be accomplished through the establishment of departmental protocols developed from known scientific, clinical, and epidemiologic information about HIV and its associated diseases. Special attention to inservice training of laboratory workers on the hazards posed by all specimens should diminish the risk of acquiring HIV in the clinical laboratory setting.

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Chapter 28

Neuropathology of AIDS

Umberto De Girolami, Leroy R. Sharer, Dana Gabuzda and Ana Sotrel

HIV-1 causes neurologic dysfunction in as many as 20Ð30% of infected adults and children at some stage of their illness and can be isolated from the brain and cerebrospinal ßuid (CSF) of patients with neurologic syndromes (1Ð3). The spectrum of neurologic disorders includes HIV-1-associated dementia (HAD) and related cognitive and motor disorders (termed minor cognitive/motor disorder), vacuolar myelopathy, and peripheral neuromuscular disorders (1Ð5). The etiologic basis of these clinical syndromes is principally attributable to the direct and indirect effects of HIV-1 on the nervous system, to opportunistic infections, or neoplasms. It is well known that multiple etiologic agents may cause neurologic disease in any one affected individual.

The incidence of these disorders has declined remarkably since the introduction of combination highly active antiretroviral therapy (HAART) (6Ð11). However, neurologic disability continues to be an important complication of AIDS, since most current antiretroviral drugs have relatively poor central nervous system (CNS) penetration and the CNS is a reservoir for long-term viral persistence. In response to this challenge, through intensive research over the past decade, the mechanisms of injury of the nervous system are slowly beginning to be understood (3,12Ð19).

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This review will discuss the range of neuropathologic abnormalities found in adults and children with AIDS.

NEUROPATHOLOGY OF AIDS

Post-mortem neuropathologic studies have demonstrated abnormalities in up to 80% of AIDS cases (see reviews (20Ð26)), though in the authors' recent personal experience and in that of published series (27), the percentage of cases in the various diagnostic categories listed below seems to be changing when the decades 1980Ð1990 and 1990Ð2000 are compared.

The neuropathologic abnormalities in adults can be subdivided into Þve categories: (1) direct and indirect effects of HIV-1 on the central nervous system (CNS)Ñ including HIV-1 encephalitis and vacuolar myelopathy; (2) opportunistic infections; (3) neoplasms, including primary CNS lymphoma and metastatic Kaposi sarcoma; (4) neuromuscular disorders; (5) AIDS-related illnesses in children and other less common conditions.

Direct and Indirect Effects of HIV-1 on the Central Nervous System in Adults

It was recognized early in the course of the study of AIDS, that some patients developed a unique encephalitic syndrome. The neurologic syndrome is characterized by a variably-progressive, subacutely-evolving dementia (including principally slowness of thought, loss of retentive memory, apathy and language disturbances) and is associated with motor dysfunction (incoordination of limbs, ataxia of gait, and eye movement abnormalities) and sphincteric disturbances (28,29). This syndrome has been termed *HIV-associated AIDS dementia complex* (ADC) (30).

Over a Þfteen year span, the possibility that HIV-1 could infect CNS cells was supported by isolating the virus from the cerebrospinal ßuid (CSF) of patients early in the

course of their disease and by identifying it in the neural tissues of patients with (and without) ADC, utilizing a variety of virological and molecular methods (31–34). Meanwhile, numerous autopsy studies of patients with AIDS, including thorough examinations of the CNS, were conducted in this country and in Europe (4,20,22,35–46). These post-mortem studies established the existence of a new form of viral encephalitis with the now generally accepted designation "*HIV-encephalitis*". Review of this large body of work indicates that the neuropathologic aspects of HIV encephalitis in adult patients differ considerably depending on the stage of evolution of the disease. These neuropathologic, and to some extent neurologic, stages can be grouped into three phases of the illness*: acute HIV-1 encephalitis, subacute HIV-1 encephalitis and late manifestations of HIV-1 encephalitis*.

Acute HIV-1 Encephalitis

Within one week of seroconversion patients may develop symptoms of meningitis and encephalopathy lasting several days or weeks. In these mildly symptomatic patients and in HIV-1 positive, neurologically asymptomatic people, antibodies to HIV-1 are detectable in the cerebrospinal fluid (CSF), and/or virus can be isolated from the CSF (31,47–49). Furthermore, in this population, there is radiologic imaging and clinical laboratory evidence of CNS injury in the acute stages of the disease (50–52). Individuals who have come to post-mortem examination at the time of this early phase of HIV-1 invasion, or shortly thereafter, having died of unrelated causes (e.g. accidental death, suicide, homicide) have been found to have mild to moderate meningeal lymphocytosis, focal cerebral white matter myelin damage, perivascular gliosis, and chronic inflammatory lesions in and around small blood vessels, principally in the white matter (23,24,32,53–56). Immunohistochemical studies with antibodies directed against the p24 HIV-1 viral antigen and PCR studies to detect HIV-1 DNA in paraffin-embedded human brain tissue have demonstrated the presence of the virus in HIV-positive asymptomatic cases (57).

Subacute/Late Manifestations of HIV-1 Encephalitis

The most complete post-mortem studies of patients dying of AIDS months or years after the onset of the disease, as reported from this country and Europe in the first decade of the study of the disease, described the histopathology of a unique viral encephalitis (4,20,22, 35–45). This newly recognized illness was called "*subacute encephalitis*" by Nielsen and coworkers (37), and the term retains validity today inasmuch as it emphasizes the cellular response of the inflammatory reaction in this phase of the disease. Subsequently, with further virological understanding of the etiologic basis of the illness, the term "*HIV-1 encephalitis*" became accepted in a "Consensus Report", 1991. For purposes of description, we like to indicate the stage of evolution of the illness, thereby integrating the original and the newer terminology.

In the majority of cases of acute or subacute HIV-1 encephalitis *macroscopic* external examination of the brain shows that the meninges are clear and that there is no evidence of cortical atrophy. Brain weight is also normal (20,22). Progressive loss of cerebral volume in AIDS patients has been reported in studies using magnetic resonance imaging methods (52). On imaging studies, sectioning of the formalin-fixed brain, or whole brain sections, in a minority of cases, there is ventricular dilatation and widening of the Sylvian fissures and adjacent cortical sulci (Fig. 28.1a and b). The cortical mantle is of normal thickness as established by quantitative measurements (58). Posterior fossa structures and the spinal cord are not remarkable.

By far the most careful neuropathologic *microscopic* studies of patients with AIDS have been carried out in individuals who have died within months or years of the onset of the illness. The full range of abnormalities which characterize subacute HIV-1 encephalitis has been defined in these cases. This distinctive spectrum of microscopic findings which has been found to affect both the white matter and gray matter will be described in detail.

The Inflammatory Lesion in the White and Gray Matter (Fig. 28.2a–c)

The earliest neuropathologic studies described the *microglial nodule* as a cardinal feature of subacute HIV-1 encephalitis. These are aggregates of nuclei of elongated or bean-shaped microglial cells that cluster around regions of tissue disruption (sometimes with foci of necrosis). The surrounding tissue shows reactive astrocytosis and slight pallor of myelin-staining. The nodules can occur diffusely throughout the brain, though the extent and density of lesions varies considerably, apparently irrespective of the clinical severity of neurologic manifestations. In the experience of Petito and co-workers (39), Kure and collaborators (59), and in our observations (22), microglial nodules occur most often in the subcortical white matter, though they also be seen in the diencephalon/basal ganglia and brainstem/cerebellum, but are ordinarily less common in the cerebral cortex.

Infiltrates of *macrophages,* characterized by abundant foamy (sometimes pigment-laden) cytoplasm, are often found in association with the microglial nodules, or they may occur in sizable collections perivascularly, especially in the white matter of the cerebral hemispheres and cerebellum. Diffuse microglial proliferation has also been noted, particularly in the late stages of the disease (see below).

Thirdly, an important component of the inflammatory lesion are the *multinucleated giant cells*. These are

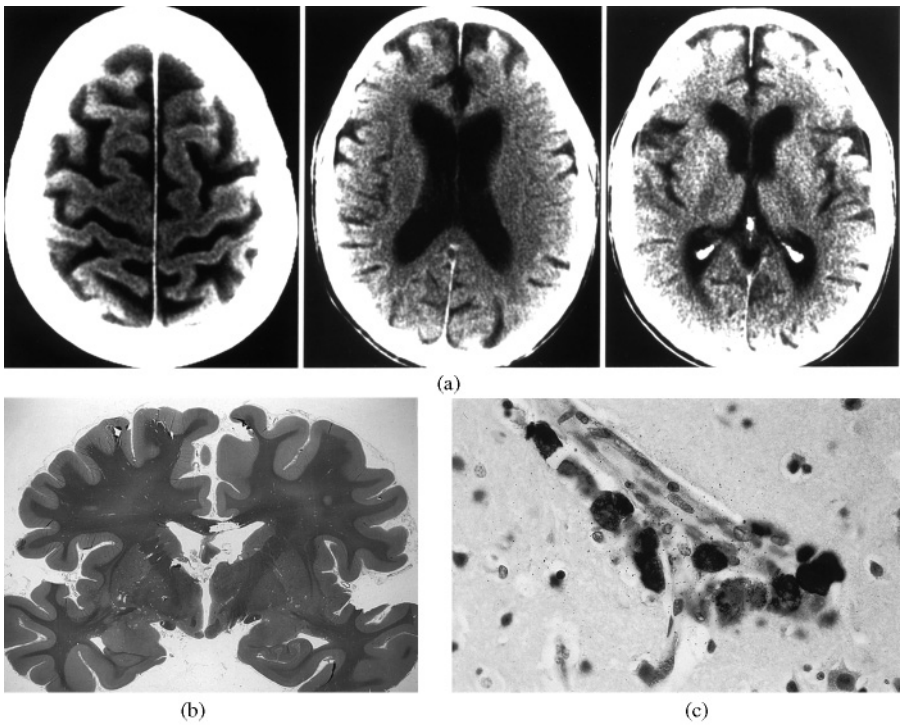


FIG. 28.1. HIV-1 encephalitis. (a) CT scan showing ventricular dilitation and sulcal widening in a 61 year old man with clinically progressive dementia and pathologically-proven HIV encephalopathy. (b) Whole brain section of paraf n-embedded brain at level of mammillary body showing some pallor of myelin staining in the centrum semiovale, mild ventricular dilatation, and widening of sulci (LFB stain for myelin).

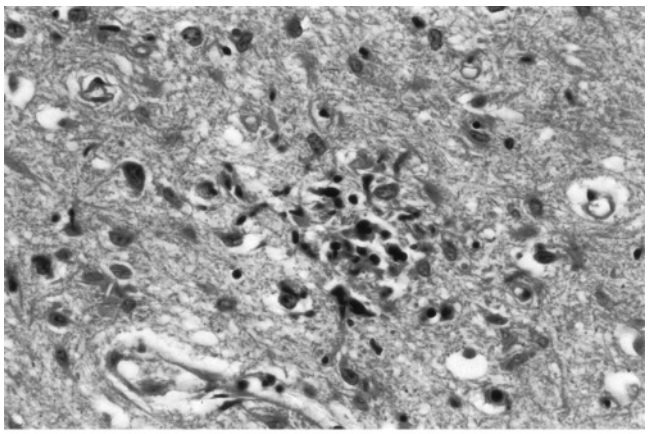
macrophage-derived cells with several nuclei either haphazardly-arranged or aggregated within a brightly eosinophilic, irregularly-shaped cytoplasm. They can occur admixed with other cells in microglial nodules or appear in isolated clusters in the parenchyma and perivascular spaces. They tend to occur in the same topographic distribution as the microglial nodules and macrophages. Multinucleated cells have been demonstrated to result from fusion of macrophages and have the same immunohistochemical properties as macrophages. The diagnostic importance of the multinucleated giant cells was Þrst emphasized by one of us (60), and other investigators have interpreted their presence as an essential component of the histopathologic picture (23,61,62). It is now apparent that their occurrence is characteristic of subacute HIV-1 encephalitis, as they are not often seen at either extreme of the illness (22,23,46).

Many studies, utilizing electron microscopy, anti-HIV-1 antibody immunocytochemistry, *in situ* hybridization, and polymerase chain reaction methods, have conclusively demonstrated the presence of HIV-1 in tissue sections of brain and spinal cord (20,25,63Ð72). HIV-1 has been found principally within the macrophages, multinucleated giant cells and microglial cells that characterize the lesion of subacute HIV-1 encephalitis as described above. These affected cells have been shown to be CD4-positive, which HIV uses as a binding site (73,74). Although there had been early speculation that the microglial nodules and multinucleated giant cells might be the result of non-HIV-related opportunistic viral infections, there seems to be no evidence of immunoreactivity to HSV, CMV and papovavirus antigens within microglial nodules containing HIV-positive mononuclear cells when these agents are searched for with immunohistochemical and electron microscopic methods (64,66,74,75).

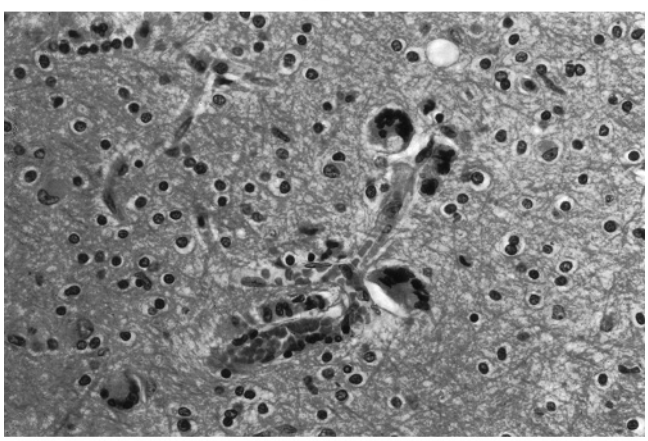
Until very recently, in spite of an assiduous search, there has been no incontrovertible evidence of HIV-1 infection of neurons or oligodendrocytes in human tissue (77,78). The work of Torres-Mu–oz and collaborators has demonstrated HIV-1 gene sequences in neurons in the CA3, CA4 and CA1 regions of the hippocampus utilizing laser capture microdissection methods on autopsy tissue; these investigators postulate non-productive latent infection of neurons (78). The implications of this interesting new Þnding in the pathogenesis of AIDS-associated dementia will be discussed below. Furthermore, recent evidence also suggests limited and nonproductive infection of astrocytes (57,72,79Ð81) particularly in the early stages of the disease. Endothelial cell infection by HIV-1 will be discussed below.

Lesions of the Microcirculation

The second striking abnormality seen in subacute HIV-1 encephalitis is evident in the intracerebral capillaries and venules (microcirculation). As mentioned above, an important manifestation of *acute* HIV-1 encephalitis consists of foci of perivascular and intravascular inßammation (24,56); recent evidence indicates HIV-1 infection of endothelial cells in this early phase of asymptomatic involvement of the CNS (57). In the *subacute* phase of the illness the inßammatory component subsides and the microcirculatory changes consist of thickening of the wall



(a)



(b)

FIG. 28.2. Subacute HIV-1 encephalitis. (a). Microglial nodule. Note cluster of elongated irregular nuclei (H&E). (b) Multinucleated giant cell. Note aggregate of nuclei next to swath of eosinophilic cytoplasm (H&E).

of the blood vessels, increased cellularity, with enlargement and pleomorphism of endothelial cells (82). These vascular abnormalities are usually associated with prominent perivascular aggregates of HIV-1-positive monocytes and multinucleated cells (Fig. 28.3). Microcirculatory abnormalities are most commonly observed in the white matter of the centrum semiovale and in posterior fossa structures. A particularly interesting aspect of both the acute and subacute vascular lesions is that they are sometimes related to microinfarcts. The microinfarcts are characterized by small regions of white matter necrosis and prominent axonal swelling with little inßammatory reaction. The brain microinfarcts are histologically identical to those seen in the nerve Þber layer of the retina, recognized clinically as cotton wool spots (Fig. 28.4a and b). Cotton wool spots are an important and characteristic funduscopic abnormality in patients with AIDS and have an established relationship with the retinal microcirculation (83).

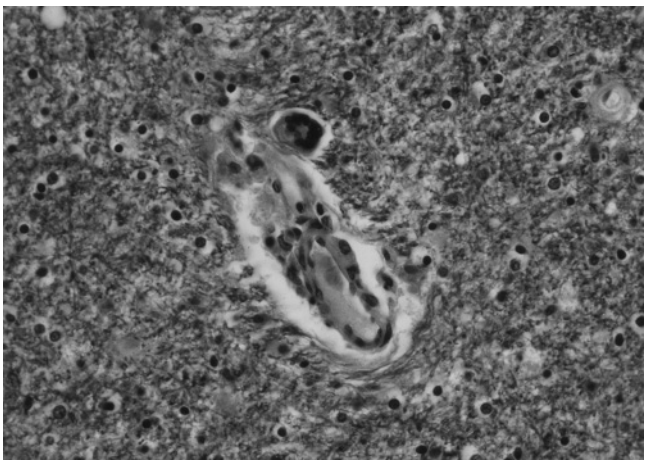
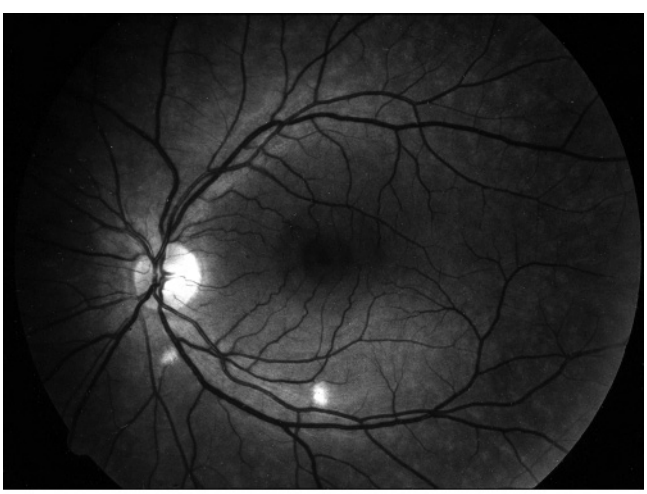
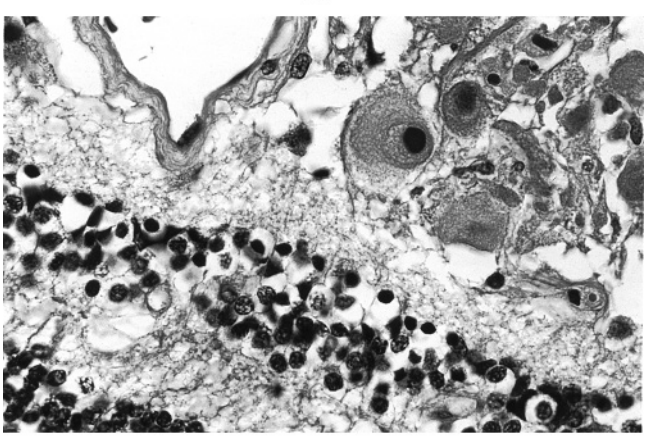


FIG. 28.3. Thickening of blood vessel in the white matter and perivascular multinucleated giant cell (LFB stain for myelin).



(a)



(b)

FIG. 28.4. Cotton-wool spots. (a) Funduscopic image of uffy super cial exudates. (b) Microscopic section through lesion in nerve ber layer, next to vessel, showing multiple concentric cores within axonal swelling (H&E).

The pathogenesis of these lesions of the microcirculation could well be related to infection of cerebral endothelial cells by HIV-1 as reported by some investigators (57,66,72); there are also reports demonstrating infection of retinal endothelial cells (84,85). These abnormalities of the microcirculation were at Þrst postulated to give rise to altered vascular permeability (82). Leakage of ßuid and plasma proteins around the perivascular extracellular spaces was subsequently demonstrated by Rhodes (86), Petito et al. (87), Power et al. (88), and Dallasta et al. (89). There has been no systematic neuropathologic study of the topographic distribution of these sites of increased vascular permeability over the course of the disease, although neuroradiologic studies indicate the basal ganglia have increased early enhancement suggesting disruption of the BBB in these regions over other areas of the brain (90).

The sequence of events that leads to penetration of HIV-1 into the human brain are unknown (13,15,16,21,91). At the time of initial viremia it is speculated that the endothelial cells of cerebral microcirculation may well become transiently infected; indeed there is now evidence of infection of the brain endothelial cells of HIV-1-positive asymptomatic individuals who died of unrelated causes (57). The considerable numbers of circulating, latently-HIV-infected monocytes (77) may then adhere to the damaged endothelial cells and perivascular astrocytes and be transported across the blood-brain barrier (BBB) (92,93).

The potential injurious effect of this BBB breakdown on the surrounding white and gray matter is discussed below.

Demyelinating Lesions

One of the truly remarkable aspects of the disease, which was especially evident to us upon examination of whole brain sections embedded in celloidin or in paraÞn and stained with conventional myelin stains, is the presence of a multifocal, poorly-circumscribed or diffuse faint pallor of myelin staining which involves primarily the centrum semiovale but also less often affects the white matter of the cerebellum and the brain stem. At high magniÞcation the typical white matter lesions consist of poorly deÞned regions of tissue rarefaction wherein there is a moderate reduction in the number of myelinated Þbers, scattered macrophages/microglial cells, few reactive astrocytes, relative sparing of axons, and virtual absence of any other inßammatory cell. Microglial nodules and/or multinucleated giant cells are sparse or absent within these lesions. Less severe forms of this process (perhaps early lesions) are characterized by focal, often angiocentric regions of myelin loss often around microcirculatory abnormalities as described above. Transitions between the focal and more diffuse white matter lesions are frequent. Very severe examples (late lesions) show extensive destruction of myelinated Þbers, conspicuous axonal damage with axonal spheroids in the centrum semiovale, and secondary degeneration. In patients with long-standing disease, on macroscopic examination, the white matter of the centrum semiovale appears unusually Þrm (20,94). Giometto et al. (95) and Raja and co-authors (96) have documented axonal injury within areas of demyelination using an antibody to b-amyloid precursor protein. This leukoencephalopathy has been studied in detail by several workers (38,39,62,82,88,96Ð98).

The pathogenesis of these white matter abnormalities is uncertain. Some investigators (40,99) have noted alterations in the number and size of oligodendrocytic nuclei within areas of myelin pallor, while others have not (100). Thus far, conclusive evidence of HIV infection of oligodendroglial cells in human tissue has been lacking.

Alterations in BBB permeability as discussed above could cause accumulation of edema ßuid within the extracellular space as well as allow various circulating macromolecules to enter the cerebral parenchyma which could cause injury to myelinated Þbers.

Other as yet hypothetical causes for the white matter degeneration include the destruction of myelin and axons by soluble substances elaborated by HIV-infected monocytes or autoimmune-associated demyelination. In experimental models, HIV-1-infected monocytes are believed to migrate through the BBB (101Ð103), and recent work by Dallasta and co-workers has provided structural evidence that BBB tight-junction proteins are affected in HIV-1 encephalitis. Viral replication within the transformed macrophages might then follow, with a release of virions and subsequently, infection of other cells which express CD4 receptors on their surface (i.e. macrophages and microglial cells) through the binding of the HIV envelope glycoprotein gp120 to the CD4 receptor. Cytokines released by the infected cells might then diffuse into the extracellular spaces of the white matter and cause damage to myelin and axons.

Neuronal injury and apoptosis

A particularly elusive and troublesome issue in the understating of the effects of HIV-1 on the CNS has been the issue of neuronal injury. This has been an especially critical issue to resolve because, as mentioned above, one of the cardinal clinical neurologic manifestations of the disease is dementia, a neurologic abnormality ordinarily associated with structural, physiological and biochemical abnormalities of neurons.

It is now believed by most workers in this Þeld that the neuropathologic substrate of ADC is *subacute HIV-1 encephalitis* as described above (104,105). However, as mentioned above, light microscopic evidence of neuronal injury is not a prominent feature of the illness. To address this issue, workers have postulated that indeed there is neuronal damage in HIV-1 encephalitis, but that the injury requires specialized techniques to demonstrate it morphologically and that perhaps the nature of the injury is a

physiologic/molecular dysfunction of the neuron, rather than an overt destruction of the cell.

There is considerable evidence that the brain serves as a reservoir of large quantities of proviral unintegrated DNA which apparently is predominantly localized to selective regions, including basal ganglia and hippocampus (34,106). While some quantitative studies suggest that brain viral burden correlates with the severity of ADC (25,107), others reach opposite conclusion (33). We have already reviewed above which CNS cells seem to be primarily infected with the virus. What then might the mechanisms of HIV-1 neuronal injury?

A number of workers have suggested that the neuronal dysfunction is due to indirect neurotoxic effects. Cytokines, including tumor necrosis factor and other soluble factors or proteoloytic enzymes, liberated by the inßammatory cells (largely macrophages and microglial cells) which permeate the brain in subacute HIV-1 encephalitis, have been shown to be toxic to both neurons and their processes and to glia (108Ð112) (see also review (16)). The work of Glass et al. (113) has shown that the severity of the ADC in the patients analyzed correlated best with postmortem brain tissue density of macrophages and microglial cells, as identiÞed by immunohistochemical methods, rather than with the viral burden within the infected macrophages and microglial cells. This fact has led to the speculation that control of systemic HIV replication might limit the development of the dementia (114). According to the *in vitro* studies of Lipton (115,116), another mechanism of impairment of neuronal function involves the HIV-1 envelope glycoprotein gp120, or a fragment of this protein, which was shown to be toxic to rodent neurons by inducing an inßux of intraneuronal free calcium. This toxicity was later shown to be mediated through the participation of macrophage/microglial cell-derived factors which acted via NMDA receptors (117,118). It was also demonstrated that neurons poor in calcium-binding proteins (parvalbumin and calbindin), located in the cerebral cortex, are relatively vulnerable to injury, whereas those rich in these proteins (basal ganglia and hippocampus) are unaffected (119,120). Further evidence of the participation of infected inßammatory cells in mediating neuronal toxicity comes from the experimental study of Tardieu and collaborators (121) who have shown neuronal cell injury only after adhesion between HIV-infected monocytes and neurons. Cell-cell interactions have also been shown to exist between HIV-infected macrophages and astrocytes (13,79,93). Finally, Dallasta and collaborators have reported that the disruption in the BBB which occurs in the brains of patients with HIV-1 encephalitis and ADC is associated with HIV-infected macrophages (89), again suggesting that it is the infected macrophage which is the key culprit in causing neuronal injury.

The relationship between these neurotoxic factors and the development of neuronal and glial injury currently under active investigation (see reviews (15,46,93,122, 123)). Morphologic evidence of neuronal injury, possibly caused by the release of macrophage-derived neurotoxic factors or other mechanisms, has been presented by several laboratories in an attempt to explain the clinical syndrome of ADC. This evidence rests on the demonstration of quantitative neuronal loss in the cerebral cortex, reduction in cell size, and/or dendritic injury in cortical neurons (119,124Ð130). On the other hand, in a prospective study of six patients with HIV-associated dementia compared with six controls (non-demented, non-AIDS) there was no statistically signiÞcant difference in the number of cortical neurons (Brodmann areas 4, 9, 40) in the two groups (58). Other methods of determining cell death utilizing the TUNEL technique to detect apoptosis have documented both neuronal and astrocytic injury in HIV-encephalitis, though careful quantitation of this phenomenon correlated with morphometric cell-counting has not yet been accomplished (81,131Ð135).

Still, in spite of all the cited studies, a plausible pathogenesis for the ADC has remained elusive. It remains a complete mystery how the putative neurotoxic substances could be acting on neurons of the cerebral cortex and elsewhere when systematic neuropathologic examinations in a number of laboratories have shown relatively few microglial nodules, multinucleated giant cells and macrophages/microglia in patients with clinical evidence of severe dementia. Quantitative assessments of neuronal injury or death have not uncovered damage of such magnitude that would easily explain the clinical syndrome. The abnormalities of the microcirculation described above are rare in the cortex. Possibly, the white matter injury caused by a breakdown of the BBB might be giving rise to a sub-cortical dementia, though often enough the neuropathologic changes do not appear to be so severe to afford a reasonable explanation for the dementia in most cases.

Vacuolar Myelopathy and Vacuolar Encephalopathy (Fig. 28.5a and b)

In early 1985 two separate groups of investigators (5,136) described the clinical and pathologic aspects of a previously unrecognized disorder of the spinal cord in patients with AIDS, occurring late in the course of their disease. The disease, named vacuolar myelopathy by Petito and collaborators, was characterized neuropathologically by multiple 10Ð100 μm vacuoles within the white matter of the posterior and lateral columns of the lower thoracic cord. They also stressed the importance of Þnding foamy macrophages in association with these vacuolar lesions so as to be certain to rule out artifactual changes. In the twenty cases recorded, the severity of the lesions was graded (I-mild, II-moderate, III-severe) and transition forms between the prototypes were frequent. In subsequent large studies (39,76,137Ð140), the disorder has been estimated to be demonstrable at post-mortem in

20–30% of unselected patients with AIDS in the Western hemisphere; less often in Europe. It has also been noted that, although extensive reactive gliosis is rare, microglial nodules and multinucleated giant cells may be seen in the cord away from the zones of vacuolar myelopathy. Ultrastructural studies indicate both axonal and myelin injury (141,142).

The etiology/pathogenesis of vacuolar myelopathy is unknown (143). The early reports pointed out the striking resemblance of the cord lesions to those of Vitamin B12 deficiency (subacute combined degeneration) but this etiology was negated by appropriate serum assays (5). The pathogenetic role of HIV-1 in vacuolar myelopathy has been controversial. Ho et al. (31) were able to isolate the virus from CSF and post-mortem spinal cord tissue in a patient with clinical manifestations indicative of a myelopathy. Budka et al. (144), Maier et al. (145), and Eilbott et al. (146) have demonstrated by immunohistochemistry and in situ hybridization that the macrophages and multinucleated giant cells in the vicinity of the vacuoles are HIV positive suggesting that the cause of vacuolar myelopathy is HIV. On the other hand strong evidence has been adduced to demonstrate just the opposite viewpoint. Kamin and Petito (147) reported 12 cases of vacuolar myelopathy in immunosuppressed patients without AIDS. Rosenblum et al. (148) demonstrated, in a comprehensive study combining immunohistochemistry (p24), in situ hybridization (DNA), and HIV isolation, that the presence of the virus correlated not with the presence of vacuolar myelopathy but with an inflammatory myelitis which was in every way similar to subacute HIV-1 encephalitis as described above. Grafe and Wiley (138) also showed a complete lack of association between immunocytologic localization of HIV antigens and vacuolar myelopathy. Shepherd and colleagues (140) studied the spinal cords of 90 patients with AIDS and found no evidence that proximity of productive HIV-1 (as assessed by quantitative PCR) infection is implicated in the pathogenesis of vacuolar myelopathy. Our own experience with a series of cases from Paris and Boston agrees with these findings (76). Similarly, the reports of neuropathologic observations of the spinal cords of children with AIDS again dispute the assertion that vacuolar myelopathy is caused by HIV-1 (149,150). Finally, there has been some interest in the role of s-adenosyl methionine deficiency or malutilization in the pathogenesis of vacuolar myelopathy which has resulted in clinical trials (151,152). Similar lesions have been described in the cerebrum (multifocal vacuolar leukoencephalopathy) (153). When they occur in the basis pontis, (multifocal pontine leukoencephalopathy) (154) they can to a large extent be necrotizing and demonstrate focal calcification.

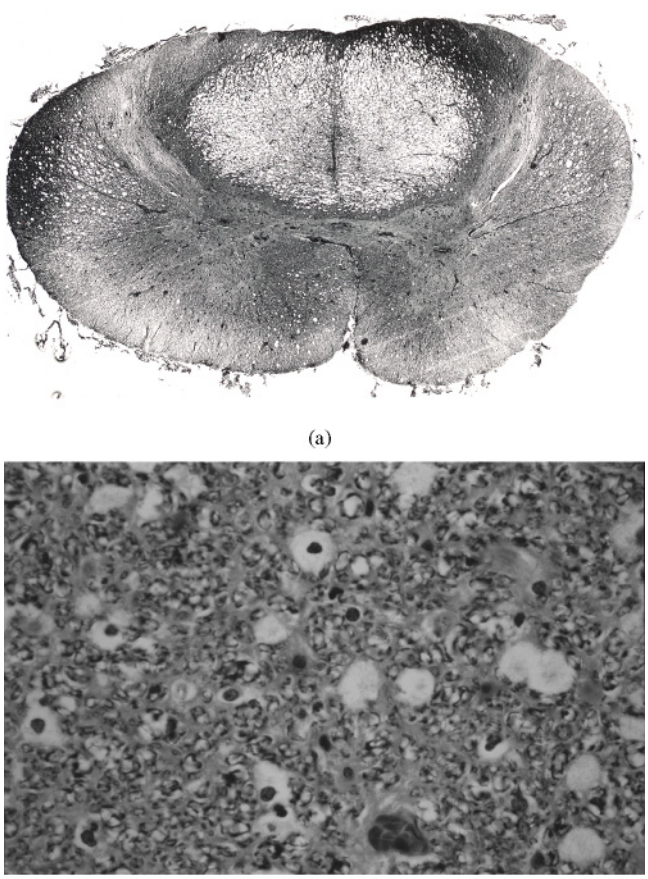


FIG. 28.5. Vacuolar myelopathy of AIDS. (a) Transverse section through high lumbar cord. Note extensive vacuolization of myelin in posterior columns and lateral corticospinal tracts (LFB stain for myelin). (b) High magnification view of posterior column abnormality showing numerous macrophages (LFB stain for myelin).

It can now be concluded that AIDS-associated vacuolar myelopathy is probably not related directly to HIV-1 infection of the spinal cord. Available evidence suggests that it may be the result of a yet unidentified indirect effect, possibly a myelinolytic cytokine (155). The report by Goudreau et al. (156), in which transgenic animals expressing HIV genome in oligodendroglia were found to develop vacuolar changes in the white matter of the spinal cord lends credence to the speculation there is an indirect role for HIV in the pathogenesis of vacuolar myelopathy.

The spinal cord has also been reported to be the site of "degenerative" changes to the corticospinal tracts or the posterior columns. Such changes have been attributed to secondary Wallerian degeneration consequent to proximal injury in either the pyramidal system or the dorsal root ganglia (97,157).

OPPORTUNISTIC INFECTIONS

In the early days of the AIDS epidemic in the United States and Western Europe, opportunistic infections (OIs) of the central nervous system (CNS) were a major cause of morbidity and mortality. Since that time, there has been improved recognition and medical management of these conditions. In addition, the incidence of many of CNS OIs

has declined in people who have been treated with highly active antiretroviral therapy (HAART). Several parameters affect the occurrence of various OIÕ in the CNS of patients with AIDS, including:

1. The severity of immune deÞciency.
2. The age of the patient (adult, child).
3. The geographical location where the patient resides.
4. The effectiveness of control of HIV-1 infection in a particular patient.

Much information about CNS OIs is derived from older data, from the 1980s and early 1990s, prior to the availability of HAART, which became the standard of care in the U.S. from 1996 onwards. However, CNS OIs continue to occur in people living in countries like the U.S., for several reasons:

1. Patient compliance with complex antiretroviral treatment regimens may be poor.
2. People with limited access to medical care may have unrecognized, and hence untreated, HIV-1 infection.
3. The development of resistance mutations of HIV-1 can lessen the effectiveness of antiretroviral therapy.

In countries where HAART and adequate prophylaxis of OIs are largely unavailable, the incidence of CNS OIs is startlingly similar to what was observed in the pre-HAART era in the U.S., as has for example been reported from India 158).

The common CNS OIs in AIDS will be described below, beginning with viruses, and continuing through parasites, bacteria, and fungi. It should be recalled that patients with AIDS may have more than one type of CNS pathology, and more than one OI of the CNS (159).

Viral Opportunistic Infections

Cytomegalovirus (Fig. 28.6aÐd)

In patients who are either untreated or inadequately treated with antiretroviral agents, cytomegalovirus (CMV) is the most common cause of OI in the CNS, according to published autopsy studies from the U.S. (39). Despite this high incidence, the diagnosis of CMV encephalitis during life is difÞcult to make, since this entity has few clinically distinguishing characteristics. This is not surprising, given the pathology of this disorder, in which there are widely scattered glial-microglial nodules, usually with no large lesions that would be apparent with radiographic imaging techniques. A pathological variant, seen in up to 10% of cases (160), exhibits involvement of the ependymal surfaces of the ventricles termed ÒventriculofugalÓ (66). This periventricular pattern is reminiscent of the congenital form of CMV that occurs in newborn infants. On microscopical examination of both the glial-microglial nodule form and the periventricular form, one Þnds typical, enlarged cells containing large, dark, intranuclear inclusions that are virtually pathognomonic of CMV. On immunohistochemical examination, using an antibody that recognizes CMV antigen, both the intranuclear inclusion and the cytoplasm are positive.

Clinically, patients with CMV encephalitis often manifest a non-speciÞc, subacute encephalopathy that is difÞcult to distinguish from either HIV-associated dementia or a metabolic encephalopathy. The clinical diagnosis can be assisted by the Þnding of speciÞc CMV nucleic acid sequences in cerebrospinal ßuid (CSF), as determined by polymerase chain reaction (PCR) (161). The CSF examination is otherwise not speciÞc, except in those relatively rare cases of CMV lumbrosacral polyradiculitis, in which polymorphonuclear leukocytes may be seen, in the absence of bacteria or fungi (162).

CMV is the most frequent OI of the CNS in children with AIDS (163). HIV-1-infected children have a higher incidence of developing non-congenital CMV infection than do non-HIV-1-infected children, and CMV infection is a risk factor for poor outcome in young children with AIDS (164). Young HIV-1-infected children who are co-infected with CMV have a higher incidence of neurological deterioration, including impaired brain growth and progressive motor deÞcits (164), probably due to synergistic deleterious effects of CMV and HIV-1 on the brain and spinal cord.

Progressive Multifocal Leukoencephalopathy (PML) (Fig. 28.7aÐd)

PML is an opportunistic viral infection of the CNS that is caused by the papova virus JC (165). In the earliest reports of this disease, prior to its identiÞcation as a viral OI, PML was noted to be associated with either immune deÞciency or immune suppression (166). Thus it was not surprising that this disease should be reported in patients with AIDS (167). JC virus has particular tropism for oligodendrocytes, the cells that produce and support CNS myelin; this accounts for the main clinical and pathological features of PML, which is characterized by multiple white matter lesions. There is some tropism for astrocytes as well (168), which can result in enlarged, bizarre astrocytes in late stages of the lesions, a histopathological change that can be mistaken for astrocytoma.

JC virus has high seroprevalence in adults, with seroconversion usually occurring during late childhood (169). For this reason, PML is uncommon in children with AIDS (170), although it can be expected to occur in adolescents. There is evidence from *in vitro* studies that HIV-1 infection can exacerbate JC virus infection, since the HIV-1 gene product *tat* can transactivate the JC virus promoter (171).

Grossly, the lesions of PML are highly characteristic, with scattered, patchy, granular zones of loss of white matter, with relative sparing of gray matter. On microscopical examination, evolving lesions of PML contain enlarged oligodendroglial nuclei that contain dark intranuclear inclusions that Þll the nucleus, although in some instances they resemble Cowdry type A inclusions, with a space between the inclusion and the thickened nuclear membrane. These inclusions can be shown to harbor papova virus antigen by immunocytochemistry and characteristic papova virus particles by electron microscopy (169), as well as speciÞc JC virus nucleic acid sequences by in situ hybridization (168). More longstanding lesions, which may extend focally into gray matter, are associated with loss of myelin and axis cylinders, with few or no intranuclear inclusions, since the oligodendrocytes have largely been destroyed. These late lesions contain bizarre astrocytes, and they may be associated with gross atrophy of both white and gray matter structures.

The occurrence of PML in people with AIDS who are not on adequate antiretroviral therapy is associated with a poor prognosis (172). Since the introduction of HAART, the ÒnaturalÓ history of PML in these patients has changed, with improved survival and quality of life (173). However, the incidence of PML in patients on HAART has declined less than that of other OIs of the CNS. PML can be reliably diagnosed by Þnding characteristic, multiple white matter lesions on MRI scans, combined with detection of speciÞc JC virus sequences in CSF by PCR, thereby obviating the need for brain biopsy in most instances (174,175). The Þrst reported randomized treatment protocol for PML found that cytosine arabinoside was ineffective for this

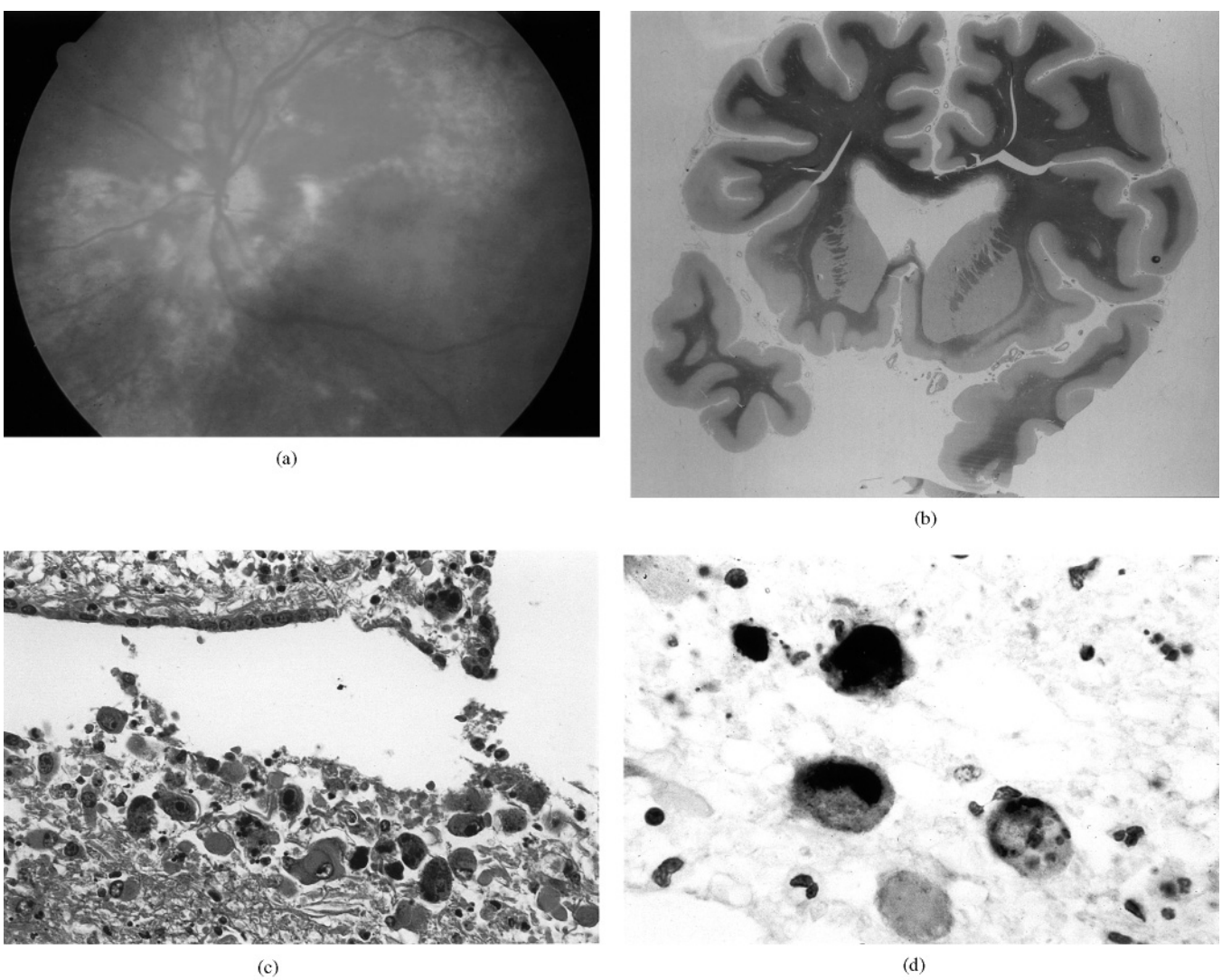


FIG. 28.6. Cytomegalovirus. (a) Funduscopic view of hemorrhagic necrosis of retina due to CMC infection. (b) Coronal section of brain showing disrupted periventricular zone with congestion due to CMV infection (LFB stain for myelin). (c) Periventricular zone showing enlarged, cytomegalic cells contain typical, diagnostic intranuclear inclusions of CMV (H&E). (d) Immunoperoxidase stain for CMV antigen showing intracytoplasmic and intranuclear inclusions in periventricular infected cells.

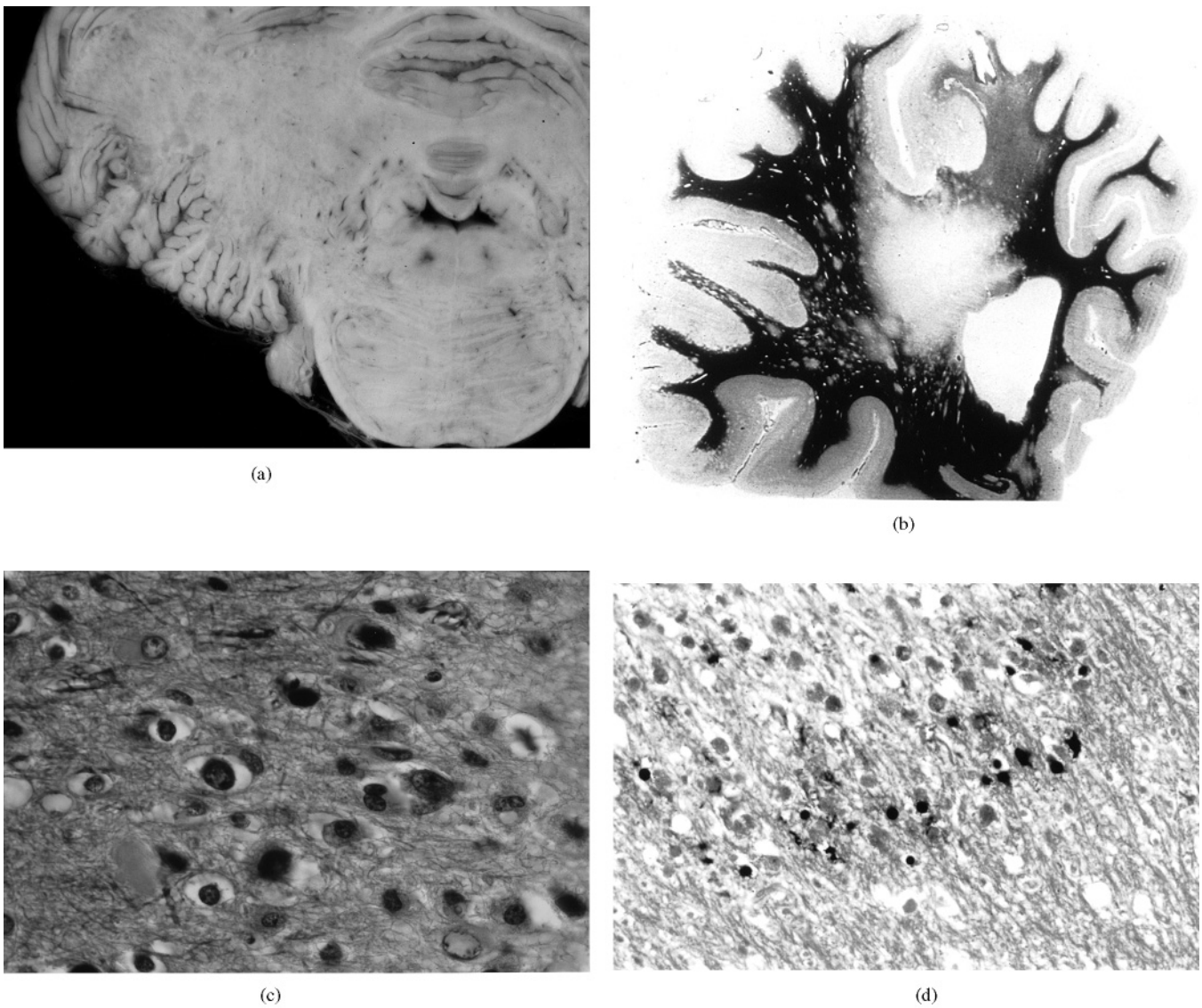


FIG. 28.7. Progressive multifocal leukoencephalopathy. (a) Macroscopic view of horizontal section of cerebellum and pons showing yellow discoloration of white matter in irregular granular patches. (b) Coronal section of occipital lobe in patient with AIDS and PML. Note innumerable foci of irregular, poorly-circumscribed myelin destruction. Heidenhain-Woelke stain for myelin. (c) Gliosis and enlarged, polyomavirus-infected oligodendrocytes (LFB stain for myelin). (d) Immunoperoxidase reaction to demonstrate polyoma virus within the enlarged oligodendrocytes (brown reaction product). The section has also been stain for myelin (LFB) and shows pallor of staining in the center of the lesion.

disorder (172), and no other therapy has as yet proved to be useful. One study has suggested that cidofovir may improve survival in people with AIDS and PML who are on HAART (176), but another European study has not supported these Þndings (177).

Herpes Virus Infection

The most common opportunistic herpes virus infection of the CNS is that due to varicella-zoster virus (VZV). Diagnosis of this virus infection can be difÞcult, since it has protean manifestations. However, the combination of skin lesions and spinal cord involvement should raise suspicion of VZV (178,179). Diagnosis during life is most reliably accomplished by identifying speciÞc VZV nucleic acid sequences in CSF by PCR. Patients with AIDS may have necrotizing myelitis, meningoencephalitis, brain and spinal cord infarcts, or giant cell arteritis (178,180). Pathologically, the diagnosis can be facilitated by Þnding eosinophilic Cowdry type A intranuclear inclusions, which however may be difÞcult to locate and which are identical to those of herpes simplex virus (HSV). Immunocytochemistry and in situ hybridization are necessary for conÞrmation of VZV infection in tissue, in most cases.

A small number of cases of HSV infection, mainly HSV-1, has been diagnosed by pathologists in autopsies of people with HIV-1 infection and AIDS. It is not clear if

this infection occurs more frequently in this patient population than it does in the general population, since HSV encephalitis is uncommon in both. Such cases in patients with AIDS may be atypical, with absence of both CSF pleocytosis and of typical imaging studies of temporal lobe involvement. Pathologically, there may be a necrotizing encephalitis without typical inßammatory features, rendering the clinical diagnosis difÞcult (181,182) From a pathological point of view, HSV encephalitis is characterized by eosinophilic Cowdry type A intranuclear inclusions, which are positive for HSV antigens on immunocytochemistry. However, by current standards, this technique cannot differentiate HSV-1, which has been reported to cause brain infection in patients with AIDS, from HSV-2, which has been reported to cause myelitis; such a distinction needs to be made by either PCR or in situ hybridization (183).

Parasitic Infections

Toxoplasmosis (Fig. 28.8aÐc)

Encephalitis or brain ÒabscessesÓ due to *Toxoplasma gondii* are common in people who have low CD4 counts and high HIV-1 viral loads. In the U.S., toxoplasmosis has classically been the most common cause of a mass or space-occupying lesion in the brains of people with AIDS. CNS toxoplasmosis in people with AIDS is more common in people who live in France and Germany than in people who live in the United States or the United Kingdom (184). It is also a common occurrence in people with AIDS in sub-Saharan Africa, South America, and India (158,185,186).

Pathologically, the diagnosis of toxoplasmosis can be suspected in lesions with abundant necrosis, with a variable inßammatory response, including polymorphs and perivascular lymphocytic cuffs that mimic the angiocentric pattern of primary brain lymphoma (PBL) (187). Diagnostic accuracy is enhanced by the use of immunocytochemistry, since organisms, particularly scattered, free tachyzoites, can be difÞcult to distinguish from cellular debris (188). The Þnding of encysted bradyzoites along with free tachyzoites on routine hematoxylin and eosin-stained sections is highly suggestive of toxoplasmosis, but this appearance is less sensitive for the diagnosis than is immunocytochemistry.

In the mid-1980s, clinicians in the U.S. developed an algorithm for patients with CNS mass lesions, with a recommendation for treatment for 10 days to two weeks using antibiotics directed against *T. gondii* (189). If the lesions regressed during the period of therapy, a presumptive diagnosis of toxoplasmosis was made. If, however, the lesions progressed either clinically or radiographically, the patient would require other diagnostic modalities, including brain biopsy, for diagnosis. The most common alternate cause of CNS mass lesion in patients with AIDS is primary brain lymphoma (PBL), which clinically and pathologically can be confused with toxoplasmosis. Since resistance of *T. gondii* to antibiotics has apparently not developed, treatment failure would rule

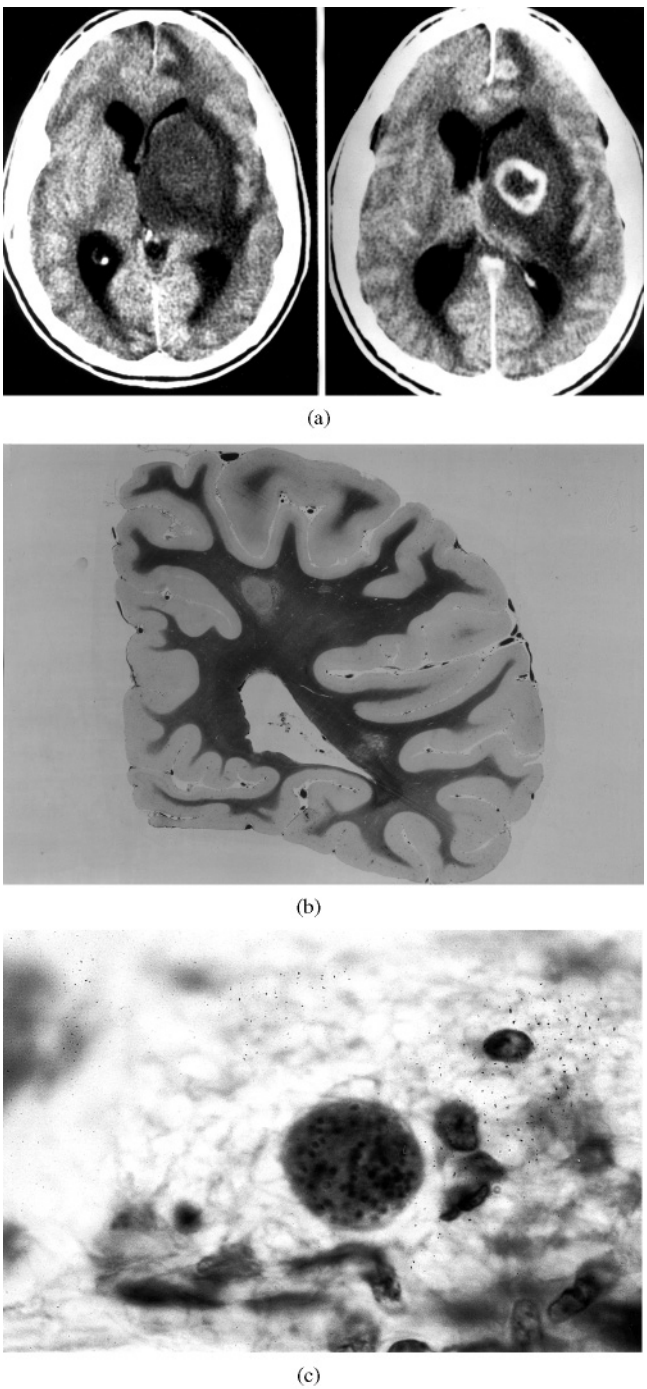


FIG. 28.8. Toxoplasmosis. (a) Computed tomography scan (CT scan) before (left) and after (right) contrast enhancement in a 32 year old patient with AIDS and toxoplasmosis. Note hypodense space-occupying mass with edema and contrast-enhancement. (b) Whole-brain coronal section through occipital lobe showing multiple discrete lesions (Loyez stain for myelin). (c) Bradyzoite of *Toxoplasma gondii* (H&E).

out toxoplasmosis, in most instances. As is the case with HAART, it is important to assess whether the patient has been compliant with anti-toxoplasmosis therapy or not. This was vividly illustrated recently for one of us (LRS), when a brain biopsy on a patient who was alleged to have been treated for toxoplasmosis for two weeks had readily demonstrable *T. gondii* organisms. In retrospect, it was recognized that this patient had indeed not been compliant with his medication and was therefore inadequately treated.

In the U.S., clinicians continue to see patients who have HIV-1 infection that has not been previously diagnosed and who present with CNS mass lesions. The Þnding of *T. gondii* organisms on biopsy of a brain lesion should prompt the physician to evaluate the patient for HIV-1 infection, which in most jurisdictions requires the patientÕs consent. One of us (LRS, unpublished) recently encountered a patient with recurrent hemangioblastoma of the cerebellum who developed new, multiple, rapidly progressing cerebral lesions, after treatment with corticosteroids for edema associated with the recurrent tumor. Biopsy of one of the new lesions revealed toxoplasmosis with numerous free tachyzoites, as seen on immunocytochemistry using an anti-Toxoplasma antibody. This patient was not known to have either HIV-1 infection or a risk factor for acquisition of this virus, but post-biopsy studies demonstrated that he was indeed infected. He succumbed before adequate anti-*Toxoplasma* therapy could be initiated.

In contrast to the situation in adults with AIDS, CNS toxoplasmosis is distinctly unusual in children in the U.S. In our experience, the most common cause of CNS mass lesions in children with AIDS is PBL. For this reason, we have recommended that a child with AIDS who develops a CNS mass lesion should be considered to have PBL until proven otherwise, and this will usually require brain biopsy (190). Children with AIDS who reside in or come from developing countries, on the other hand, may develop CNS toxoplasmosis, as for example was recently reported in a clinical study from Brazil (191).

The decline in incidence of OIÕs in patients treated with HAART has extended to CNS toxoplasmosis. A recent report (192) has suggested that the incidence of PBL may becoming more frequent in these people, leading to the recommendation of study of brain mass lesions with thallium single-photon emission computed tomography (SPECT) and CSF PCR examination for speciÞc Epstein-Barr virus (EBV) nucleic acid sequences, both of which can be helpful in the clinical diagnosis of PBL. Brain biopsy is not without signiÞcant risk in this patient population (1998).

Other Parasitic Infections

A few parasitic infections can resemble CNS toxoplasmosis, on histological examination. Among these is *Trypanosoma cruzii*, or Chagas disease, which can cause brain infection in people with AIDS who either live in or come from endemic areas. Brain involvement with this organism can be seen in acute infection (194) as well as in reactivated infection (195) In addition, microsporidia have also been reported in the CNS in people with AIDS (198), and these organisms also bear some resemblance to the free tachyzoites of *T. gondii*. Immunocytochemical differentiation of these organisms from *T. gondii* is essential, particularly in biopsy tissue.

Bacterial Infections

Bacteria are uncommon causes of CNS OIs in patients with AIDS. Of increasing importance is *Streptococcus pneumoniae*, which has been reported to cause acute leptomeningitis (197). Gram-positive cocci have been reported in brain abscesses (197), and this is an especially important complication in people who self-administer drugs of abuse by the intravenous route, in whom there may be concomitant endocarditis. Although infrequent, *Nocardia* sp. should be considered in the differential diagnosis, when biopsy reveals an acute brain abscess in a patient with AIDS (Sharer, unpublished). A diagnosis of *Nocardia* in biopsy tissue may be facilitated by Gram stain and methenamine silver stain for fungi, which may reveal organisms before they are reported by the microbiology laboratory.

By contrast with bacteria, mycobacterial infections are relatively frequent in the CNS of people with AIDS, especially leptomeningitis due to *Mycobacterium tuberculosis*. Other types of infection with this organism can be seen, including tuberculoma and tuberculous abscess (198). CNS tuberculosis can be expected in regions of the world where both *M. tuberculosis* and HIV-1 infection are common, such as sub-Saharan Africa, as well as in certain populations in the U.S., for example in incarcerated individuals (197). Tuberculous leptomeningitis in AIDS has a poor prognosis (198), and it may also be complicated by vasculopathy (199). Treatment is often unsuccessful, even with strains of the organism that are sensitive to anti-tuberculous therapy.

Mycobacterium avium/intracellulare, or *M. avium* complex (MAC), is a common OI in people with AIDS. However, clinically important CNS involvement with atypical mycobacteria is rare (200) (Fig. 28.9).

Fungal Infections

Cryptococcus (Fig. 28.10a and b)

Cryptococcal meningitis, due to the yeast *Cryptococcus neoformans*, the most common opportunistic CNS fungal infection in general, is also the most common CNS fungal infection in patients with AIDS (187) in whom it may be

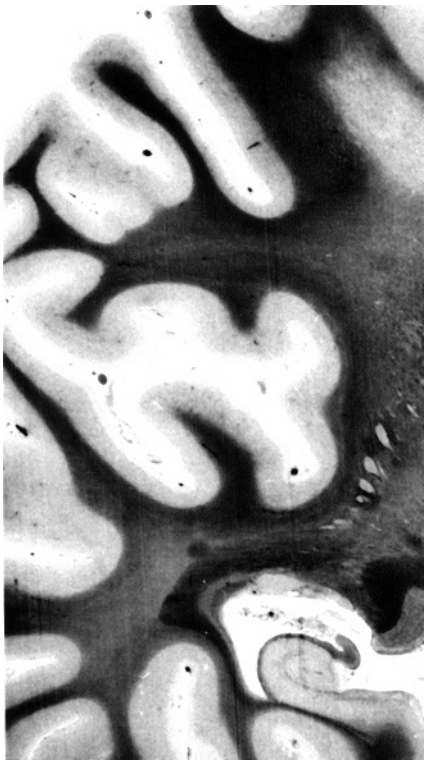


FIG. 28.9. Mycobacterium avium intracellulare. Note small patch of myelin destruction in centrum semiovale (Loyez stain for myelin).

the presenting manifestation of both HIV-1 infection and AIDS. This organism can be successfully treated with oral ßuconazole, if the diagnosis is made before the lesions become too advanced. The use of HAART has rendered the occurrence of this infection less common than it formerly was, with autopsy studies from the mid-1980s showing an incidence of cryptococcal meningitis in up to 6% of cases (39). HAART has also made the management of the disease much easier.

In AIDS, cryptococcal meningitis typically has minimal inßammation in the CSF compartment, although this can vary, from no inßammatory cells at all to well-developed granulomas (189). The organisms also have a propensity to grow in the Virchow-Robin spaces about blood vessels, particularly in the basal ganglia, where large numbers of them can form gelatinous masses that are readily seen on imaging (201) and that can be appreciated grossly at autopsy. For the most part, the organisms do not invade through the pia into the brain parenchyma, and there is usually minimal astrocytic reaction around the masses of organisms surrounding the vessels. However, on rare occasion the organisms can invade the brain, where they can cause hemorrhage and necrosis (Sharer, unpublished).

Cryptococcal meningitis is unusual in children, as are most OIs of the CNS, probably due to low exposure of children to opportunistic and other pathogens, with this exposure increasing with time and therefore with age. Although cases have been reported in children (202,203), we have seen no cases of cryptococcal meningitis at postmortem in our own autopsy series (163), which now numbers 80 cases.

Other Fungal Infections

Aspergillus sp. infections can occur in people who have disseminated aspergillosis, including people with AIDS. The lesions in brain are frequently hemorrhagic, because of the propensity for the fungus to destroy blood vessel walls. Patients with AIDS are no more likely to have

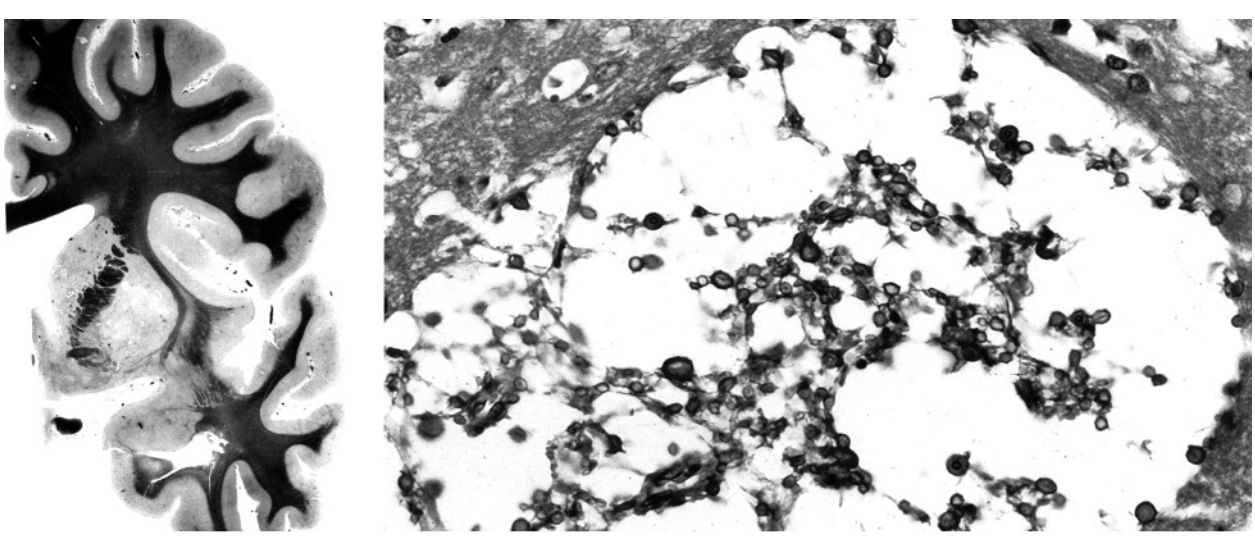


FIG. 28.10. Cryptococcosis. (a) Coronal section of brain showing soap-bubble lesions in the basal ganglia representing massive accumulations of organisms around lenticulostriate blood vessels (Heidenhain/Wolke stain for myelin). (b) Microscopic section of A showing well-circumscribed lesion containing organisms and little surrounding in ammatory response (Mucicarmine stain).

dissemination of *Aspergillus* infection to the CNS than are other immunocompromised patients with disseminated disease (204).

CNS OIs have been reported with *Histoplasma capsulatum* and *Coccidioides immitis* (205), either in areas where these infections are endemic, or in people who have lived in those areas. Clinically both of these fungal infections in patients with AIDS can be confused with infection due to *M. tuberculosis*. On pathological examination of tissue specimens, *C. immitis* can be readily identified, due to the large size (up to 100 μm or more) and characteristic morphology of the spherules (206). Special stains for fungi, such as PAS and Gomori methenamine sliver, are useful for identification of both of these agents, with small forms, 2–4 μm in diameter, often intracellular, for *H. capsulatum* (207).

NEUROMUSCULAR DISEASES

Neuropathies (Fig. 28.11a–c)

Patients with AIDS are susceptible to a wide range of cranial and peripheral nerve disturbances (4,62,208–214) see also reviews (210–212,215–218). The most commonly reported clinical syndromes include: (1) acute and chronic inflammatory demyelinating polyneuropathy (AIDP, CIDP); (2) distal painful sensory polyneuropathy; (3) polyradiculopathy; (4) autonomic neuropathy; and (5) mononeuritis multiplex. A sensory "ataxic" neuropathy due to ganglioneuronitis has also been described (157,219). The histopathologic findings observed in most of these cases include segmental demyelination, axonal degeneration, and epi- and endoneurial mononuclear cell inflammation. The etiology of these AIDS-associated neuropathies is undoubtedly multifactorial and often obscure. HIV has been cultured from the nerve in some cases of AIDS-associated neuropathy (31,64,212); however, in a series of 24 cases the virus could not be demonstrated by immunohistochemistry within Schwann cells or elsewhere in the nerve (139). In one patient reported by Bailey et al. (210), electron microscopy demonstrated retrovirus-like particles within peripheral nerve axoplasm. Necrotizing arteritis has been reported in some cases of AIDS-associated neuropathy (220). In the two patients described by Gherardi et al. (220) HIV replication was demonstrated by in situ hybridization

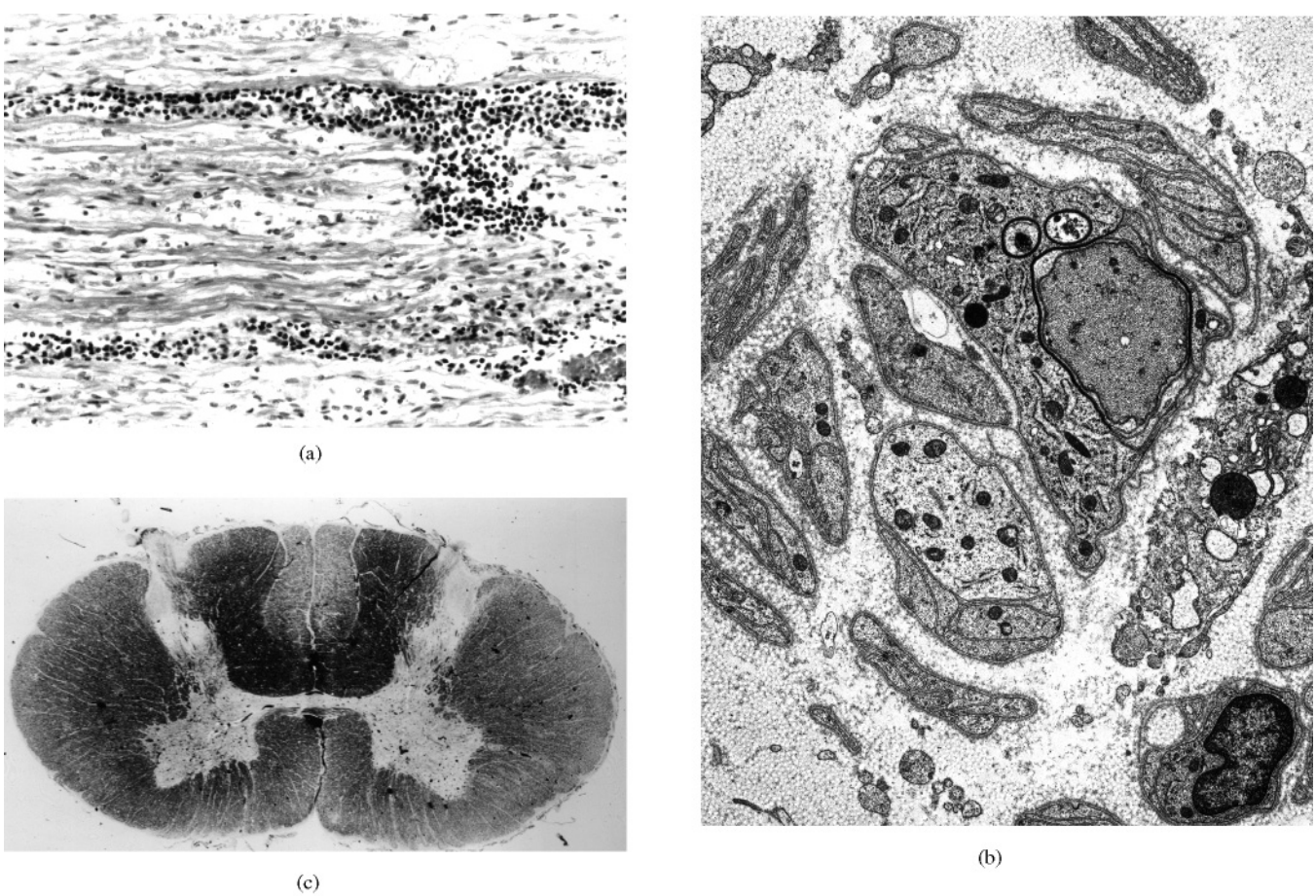


FIG. 28.11. Nerve. Demyelinating polyneuropathy. Note chronic inflammatory cells (a) in longitudinal section of nerve (H&E stain) and ultrastructural image of thinly myelinated fiber (b). (c) Transverse section of cervical spinal cord showing posterior column degeneration in fasciculus gracilis secondary to injure of dorsal root ganglion cells. (Woelke for myelin).

within mononuclear cells inÞltrating the vessels. Other infectious agents may cause a neuropathy in some HIV-infected individuals. In the patients reported by Bishopric et al. (221), Eidelberg et al. (222), and Behar et al. (223) a Guillain-BarrŽ-like syndrome was related to CMV. Lanska et al. (224) described a HIV-positive patient with syphilitic polyradiculopathy. Other possible causes for AIDS-related neuropathies include immune-mediated attack against peripheral nerve components triggered by HIV or other viruses, or by the immunodeÞciency state itself; various toxic, metabolic, and nutritional factors, and inÞltration of nerves and/or roots by neoplasm (e.g. lymphoma).

Myopathies (Fig. 28.12a and b)

Inßammatory myopathy has been the most frequently described skeletal muscle disorder in patients with AIDS or ARC (215,218,225Ð236). The disease is characterized by the subacute onset of proximal weakness, sometimes pain, and elevated serum creatine kinase. The histologic Þndings in these cases have included muscle Þber necrosis and phagocytosis, T-cell lymphocytosis and interstitial inÞltration with HIV-positive macrophages, and in a few cases cytoplasmic bodies and nemaline rods (215,229, 231). There have been no reports of direct viral invasion of skeletal muscle cells. An inßammatory myopathy has also been described in simian AIDS (237). Wiley et al. (238) reported a patient with polymyositis who was seropositive for both HTLV-I and HIV. Using in situ hybridization and immunocytochemistry, they observed direct infection of muscle Þbers by HTLV-I but no evidence of HIV in muscle or inßammatory cells.

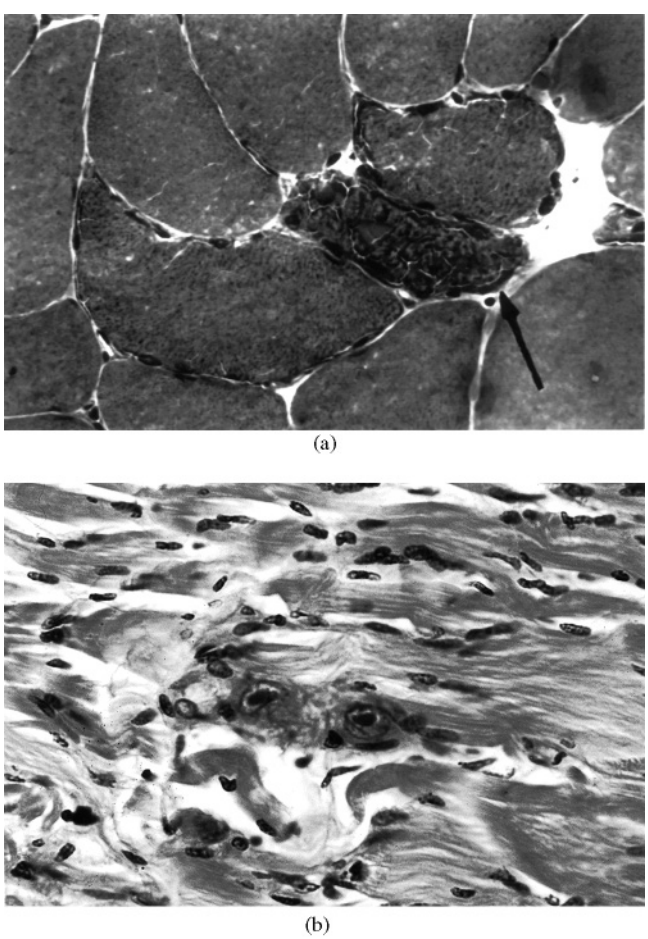


FIG. 28.12. Muscle. (a) Ragged-red ber. (modi ed Gomori trichrome stain on frozen muscle). (b) CMV involving skeletal muscle (H&E stain).

The pathogenesis of the inßammatory myopathy is not clear. The hypothesis that skeletal muscle cells are a primary target of HIV has not been borne out by tissue culture, ultrastructural or viral localization studies. An alternate possibility is that there is an attack on skeletal muscle cells by immune effector cells or that an opportunistic infectious agent alters muscle membranes rendering them the target of an autoimmune reaction akin to idiopathic polymyositis. In a case of HIV-associated myopathy which we have studied, we suggested that the virus might localize to the vascular bed of skeletal muscle, whether or not skeletal muscle is itself a target cell (239). The hypothesis was based on the presence of structural abnormalities of the microcirculation, immunocytochemical localization of the virus within monocytes around vessel walls, and evidence of altered vascular permeability (extravasation of red blood cells, iron pigment-laden macrophages). Such regional distribution of viruses has been proposed as one possible mechanism of viral tropism.

There have also been several reports of an acute toxic myopathy with myoglobinuria developing in AIDS patients treated with zidovudine (AZT) (235,240,241). These cases seem to illustrate a different pathologic process from the inßammatory myopathy described above. In several subsequent series (241,242), muscle biopsies demonstrated the presence of numerous Òragged redÓ Þbers (Þbers containing abnormal mitochondria with paracrystalline inclusions) which suggested the presence of a toxic mitochondrial myopathy. Some patients with AIDS may develop proximal muscle weakness with normal creatine kinase, with severe type II Þber atrophy seen on muscle biopsy (215,243). This condition may be related to poor nutrition, rapid weight loss, prolonged bed rest, or remote effect of malignancy. Instances of infectious myositis due to CMV and toxoplasmosis are also on record (242).

AIDS-RELATED ILLNESSES IN CHILDREN AND OTHER LESS COMMON CONDITIONS (Fig. 28.13)

The neuropathologic abnormalities encountered in children with AIDS, although somewhat different from those in adults, will be brießy reviewed. Up to the advent of intensive antiretroviral treatment of HIV-positive expectant mothers, neurologic disease was reported to be

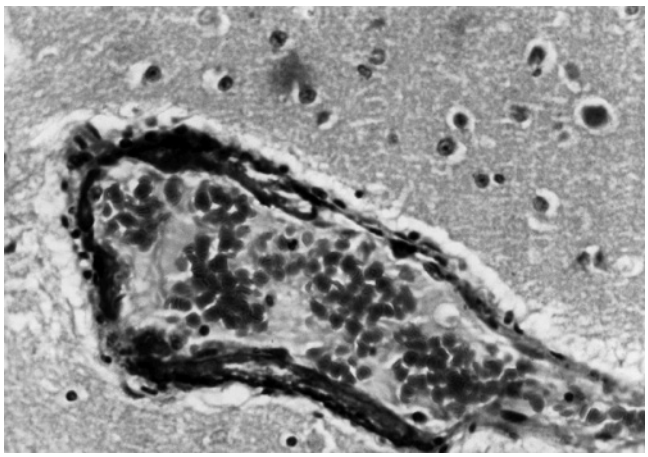


FIG. 28.13. Pediatric HIV-1 encephalitis. Note plate-like mineralization in large vessel of basal ganglion (H&E).

common in children with AIDS (244–246). Clinical manifestations of neurologic dysfunction are evident by the first years of life and include microcephaly with mental retardation and motor developmental delay with long tract signs. The presence of progressive HIV encephalopathy in children with untreated HIV-1 infection indicates a poor prognosis for survival, especially for younger children.

The neuropathologic features of AIDS in children have been discussed in a few comprehensive reports and reviews (21,38,247–256). The most frequently reported macroscopic abnormalities are calcific deposits within the basal ganglia and deep cerebral white matter, micrencephaly and diffuse firmness of the supratentorial white matter. These findings are demonstrable with radiologic studies (246,257). Histopathologically, the characteristic findings include lesions of the medium-sized and small blood vessels of the basal ganglia and subcortical white matter characterized by mineralization of the vessel wall and narrowing of its lumen without associated inflammatory changes of any great severity. Calcific deposits may also be found scattered throughout the white matter and in association with destructive lesions. Many patients have also had histopathological changes of HIV-1 encephalitis, similar to those seen in adults, including characteristic multinucleated giant cells. The severity of the encephalitis in children is often greater than seen in adults, with more numerous lesions and more widespread involvement of the CNS (163). Immunocytochemical and ultrastructural studies of children with HIV encephalitis have also demonstrated the same abnormalities as noted in adults (256,258). Studies have suggested amelioration of some of the lesions of HIV-1 encephalitis by antiretroviral therapy, particularly with zidovudine, with reduction in the percentage of cases having multinucleated giant cells. This change in pathology was not noted for calcifications, however. There have been no detailed quantitative studies of neuronal injury, but neuronal apoptosis has been recorded (259). Also remarkable in children has been the extent of white matter damage in the cerebral hemispheres and cerebellum, which often show extensive pallor of myelin staining, reactive astrocytosis, and evidence of axonal injury.

As mentioned above, CNS OIs are less frequent in the brains of children with AIDS than in the brains of adults (163). The most obvious explanation for this phenomenon is that children have less time to become exposed to the common opportunistic pathogens than do adults. This information should direct the management of children with AIDS, particularly those with expansile lesions in the brain, since, in the United States, these are much more likely to be due to primary brain lymphoma than to toxoplasmosis (190). Vacuolar myelopathy is also encountered in the spinal cords of children with AIDS much less often than it is in adults, for reasons that are not apparent (163).

The introduction of HAART has affected children as well as adults with AIDS. The Pediatric AIDS Clinical Trial Group has recently reported, in PACTG 219, reduction of mortality in children, from 5.3% in 1996, to 0.7% in 1999, following the introduction of HAART. Although the effect of HAART on neurological function was not addressed in this report, other recent studies have noted improved growth and immune function in treated children. Indeed, complete cessation of HIV-1 replication can be achieved by combination therapy given during the first three months of life, an effect that can be expected to cut dramatically the incidence of progressive HIV-1 encephalopathy in children.

Lesions of large blood vessels (macrocirculation) are known to occur in patients with AIDS; these may or may not be associated with HIV encephalitis and include instances of embolic cerebral infarction, hemorrhages at various sites of the gray and white matter, intimal thickening with narrowing of the vascular lumen, and vasculitis (4,260–265).

Rare instances of fulminating multiple sclerosis-like leukoencephalopathy occurring at almost any stage of AIDS have been described in several clinicopathologic studies. The neuropathology of these lesions closely approximates acute disseminated encephalomyelitis or multiple sclerosis-like syndromes (266–269). In this context, some cases showing plaque-like demyelinating lesions have been found to be due to opportunistic infection with herpes viruses (270–273).

NEOPLASMS

Patients with AIDS have a high risk of developing systemic and CNS malignancies, but this trend appears to be subsiding. A recent collaborative study (276, and the National Cancer Institute/AIDS-Cancer Match Registry) that compared the records of 98,336 AIDS patients to that of 1,125,098 non-AIDS cancer patients, revealed that 7% of AIDS patients had Kaposi's sarcoma (KS). This represents 310-fold increase in relative risk (RR) of

developing KS compared to non-AIDS patients; 2% (113-fold) had non-Hodgkin lymphoma (NHL). Moreover, 712 AIDS patients (1%), had other types of cancer, at the incidence rate that was on an average 2-fold higher than that of the general population. These included: angiosarcoma (37-fold); anal and cervical human papilloma virus (HPV)-related cancers (277) (32-fold); Hodgkin disease (8-fold); malignant astrocytic CNS tumors 3.5-fold. The most noteworthy Þgures appeared recently in the National Center for Health Statistics report on cancer death rate associated with HIV-infection in USA (278). Their analysis of death certiÞcates from 1990Ð1995, of AIDS patients aged 25Ð44 years, revealed 22,275 deaths caused by a combined HIV-infection and cancer; 55% had KS, 38% had NHL and about 2% had both.

Kaposi Sarcoma (KS) (Fig. 28.14)

KS-associated with AIDS is an aggressive polyclonal, angioproliferative, usually multifocal disorder, caused by a persistent infection of the endothelial progenitor cells by human herpes virus-8 (HHV-8) also known as KS associated herpes virus (KSHV). The virus induces uncontrollable proliferation of infected cells and neoangiogenesis (277). The incidence of KS has decreased since the introduction of HAART (278). Between 1988 and 1998, KS was diagnosed as an AIDS-deÞning illness, in 9.6% of all Western European AIDS patients; however, there was a signiÞcant decline from 13% in 1988 to 6% in 1998 (275). Likewise, the International Collaboration on HIV and Cancer (ICHC), including 23 cohort studies in developed countries, reported a decline in the incidence rate of KS from 15.2% in 1992 to 4.9% in 1999 (ICHC, 2000). In the CNS KS has been exceptionally rare and invariably as a consequence of metastases from systemic sites (279,280).

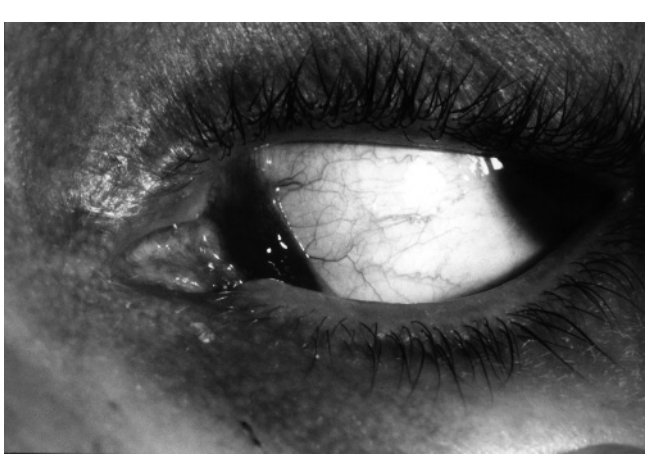


FIG. 28.14. Kaposi sarcoma. Note eshy lesion of conjunctiva.

Lymphoproliferative Disorders (LPD) and AIDS

There are two relatively rare forms of LPD that occur in the patients with AIDS, both causally related to KSHV-HHV-8. One is the primary effusion lymphoma (PEL), also known as a ßuid-Þlled body cavities (peritoneal, pleural, pericardial)-based, large cell type B-lymphoma; the other is a plasmacytic variant of multicentric Castleman disease (MCD) (280,281). Far more commonly, patients with AIDS develop LPD that are pathogenetically linked to Epstein-Barr virus (EBV). This category includes a relatively rare, mostly systemic, aggressive variant of H, and NHL.

AIDS-associated NHL (75Ð95% are extranodal) are classiÞed as: (a) systemic, diffuse Burkitt type lymphoma (BL); (b) systemic, diffuse large cell (DLCL) and LC-immunoblastic lymphoma subtypes; (c) primary CNS lymphoma (PCNSL) (282). According to one study, NHL have increased in all European countries from an average of 3.6% in 1994 to 5.3% in 1998 (283). However, there has been an overall decline in the worldwide incidence rates of NHL from 6.2 prior, to 3.6 following the introduction of HAART (284). The pathogenesis of AIDS-related systemic NHL is yet to be fully understood. Postulated pathogenetic mechanisms include virally-mediated oncogenesis, cytokine dysregulation, and oncogene activation or loss of tumor suppressor genes (285). For the most part, these are phenotypically B-cell tumors that are biologically and genetically heterogeneous with a variable rate of EBV infection (range from 30% in BL to 80% in DLCL) (286).

Nervous System Lymphoma-Associated with AIDS

A 1994 Southeast England study of trends in the incidence of PCNSL, independent of age, gender, or HIV status (287), demonstrated a nine-fold increase in PCNSL over the preceding Þve years. This did not seem to be related to AIDS epidemic, although HIV status was known for only 39 of 210 PCNL patients, of whom eight were HIV-positive. Likewise, another 1995 epidemiological study of AIDS-related CNSL (288), demonstrated an increase in frequency of CNSL over the previous decade; 40% of AIDS patients with systemic NHL also had secondary leptomeningeal tumorous involvement, and 7.6% of autopsied AIDS patients had highly malignant B-cell CNSL.

Secondary meningeal lymphoma, also known as lymphomatous meningitis (LM), most commonly represents a complication of an immunoblastic, or large cell subtype of a B-cell systemic NHL. According to one study (289), detectable EBV infection of the systemic NHL is predictive of the secondary CNS involvement; presence of EBV-DNA in CSF often precedes clinical and neuroradiological signs of a CNS spread. Furthermore, about 7% of PCNSL-s develop CSF-borne dissemination of tumor cells

resulting in LM. PCNSL in non-AIDS patients develop leptomeningeal and subependymal dissemination in 42% of cases at presentation and in 41% at the time of recurrence (290), indicating that the incidence of LM secondary to AIDS-related PCNSL, may be underestimated. CSF obtained through lumbar puncture from all patients with LM contains diagnostic tumor cells (291). For an exhaustive discussion of the clinical presentation (frequently reßecting involvement of cranial nerves), imaging, modes of treatment and outcome of LM see (291). Large B-cell type lymphoma has rarely been reported to originate primarily in the peripheral nerves (292).

Primary CNS Lymphoma (PCNSL) (Fig. 28.15aÐe)

AIDS-associated NHL almost always presents as an extranodal disorder manifesting as a PCNSL in 23% to 25% of all NHL cases (293Ð295). Some studies report no statistically-signiÞcant decline in the incidence of PCNSL as a result of extensive use of HAART (296); others have detected an unequivocal decrease in PCNSL between 1995Ð1999 (297).

A recent Multicenter AIDS Cohort prospective study (MACS) of the natural history of HIV infection among homosexual men in Baltimore, Pittsburgh, Chicago and Los Angeles (298), recorded the most dramatic and statistically signiÞcant decrease in the mean incidence rate (no./1,000 person-years) of AIDS-associated PCNSLs. This went from 4.3, during the 1993Ð1995 predominant employment of combination therapy without protease inhibitors, to 0.4 in the 1996Ð1998 of HAART time (298).

Although pathogenesis of PCNSLs in AIDS may in part be related to presently undeÞned genetic alterations, EBV is considered to be the major etiologic factor. This is based on the virtually omnipresent infection of PCNSLs by EBV, and an expression of EBV-encoded transforming latent membrane protein antigen LMP-1 by about 50% of cases (286). Whereas still speculative, most believe that PCNSL in AIDS arises from EBV-infected and subsequently immortalized B-cells that enter CNS and continue to grow unchecked because of T-cell deÞciency and limited natural killer-cell activity at such an ÒimmunoprivilegedÓ site (293,308).

Presenting neurological signs and symptoms reßect PCNSL propensity for supratentorial location and multicentricity. Involvement of eyes occurs in 5Ð20% of cases (299), whereas spinal cord lesions are rare. Signs of increased intracranial pressure and focal neurological deÞcits are most common. This may include: progressive headache, altered mental status, hemiparesis, aphasia, memory impairment and partial epilepsy. In PCNSLs with meningeal or periventricular dissemination, headache, cognitive decline and cranial nerve palsies may be predominant signs (291,300,301).

Diagnosis of PCNSLs associated with AIDS is usually made by a series of tests that may involve:

a. Neuroradiologic studies (CT&MRI) that detect a solitary lesion in 29% of cases, or multicentric tumors in 50Ð70% of patients (300,301). The most common anatomical sites of PCNSL-s include basal ganglia and frontal lobes, followed by parietal, temporal and occipital lobes. Corpus callosum and periventricular white matter are affected in about 20% of cases; both supra and infratentorial lesions are seen in about 18% of cases; isolated cerebellar or brainstem lesions are very rare; MRI detects more lesions than CT (291). By CT most lesions are of low density; with contrast more than 80% show either rim or irregular peripheral enhancement (IPE); likewise, most contrast-enhanced T1-weighted MRI images show either ring or IPE. Multifocal tumor tissue necrosis is the pathologic correlate of heterogeneous, patchy enhancement; peripherally enhancing lesions, pathohistologically have an extensively necrotic center, surrounded by an abundance of newly-formed vessels cuffed by viable tumor cells. Normal CT images of an affected brain most likely correspond to a diffusely inÞltrative, perivascular, inßammatory-like pattern of tumor growth. Structural imaging tools, CT and MRI, cannot reliably separate PCNSL from a potentially treatable, infectious mass-like lesion, such as toxoplasmosis. Functional imaging tools, such as FDG-PET (302,303), or SPECT (304,305), appear to be more reliable in differentiating actively proliferating mass lesions, such as PCNSLs, from largely necrotic indolent-infectious lesions caused by toxoplasma or other organisms. This is based on a signiÞcantly higher FDG uptake by the metabolically active tumor cells; similarly, radioactive tracers, such as thalium-201 (or technetium-MIBI) used in SPECT studies, become actively incorporated only into living cells by utilizing their Na/K/ATP-ase transmembranous system.
b. Examination of the CSF usually reveals an increased protein, a normal or decreased glucose level and pleocytosis in about 50% of cases. The reported percentage of PCNSL-s cases with diagnostic cytology of the tumor cells, differs widely from one study to the next ranging from 10Ð30% (291,300,301). It has been repeatedly demonstrated (306,309), that EBV-DNA ampliÞcation in CSF is positive in about 85% of all biopsy/autopsy-proven PCNSLs. Most importantly, a combination of a positive CSF-EBV-DNA with a hyperactive SPECT-detectable CNS lesion, is considered diagnostic of PCNSL at 100% level of speciÞcity (305); these diagnostic procedures may eliminate the need for a more invasive brain biopsy.
c. Stereotactic brain biopsy, although invasive, is considered to be Òsafe and effectiveÓ, but less invasive, yet highly speciÞc diagnostic tools (305,313), may presently have better Ònet survival advantage.Ó

Pathohistologically, PCNSLs, that share the same cell of origin with their nodal and extranodal counterparts, are classiÞed according to the working formulation for systemic NHLs (301,306,307). Nonetheless, some argue that such a classiÞcation Òis not reliable and clinically not relevant for PCNSLÓ (308). Rather, on the basis of their biological behavior, frequently atypical morphology, and Òlocation in an organ devoid of lymphatics, PCNSLs

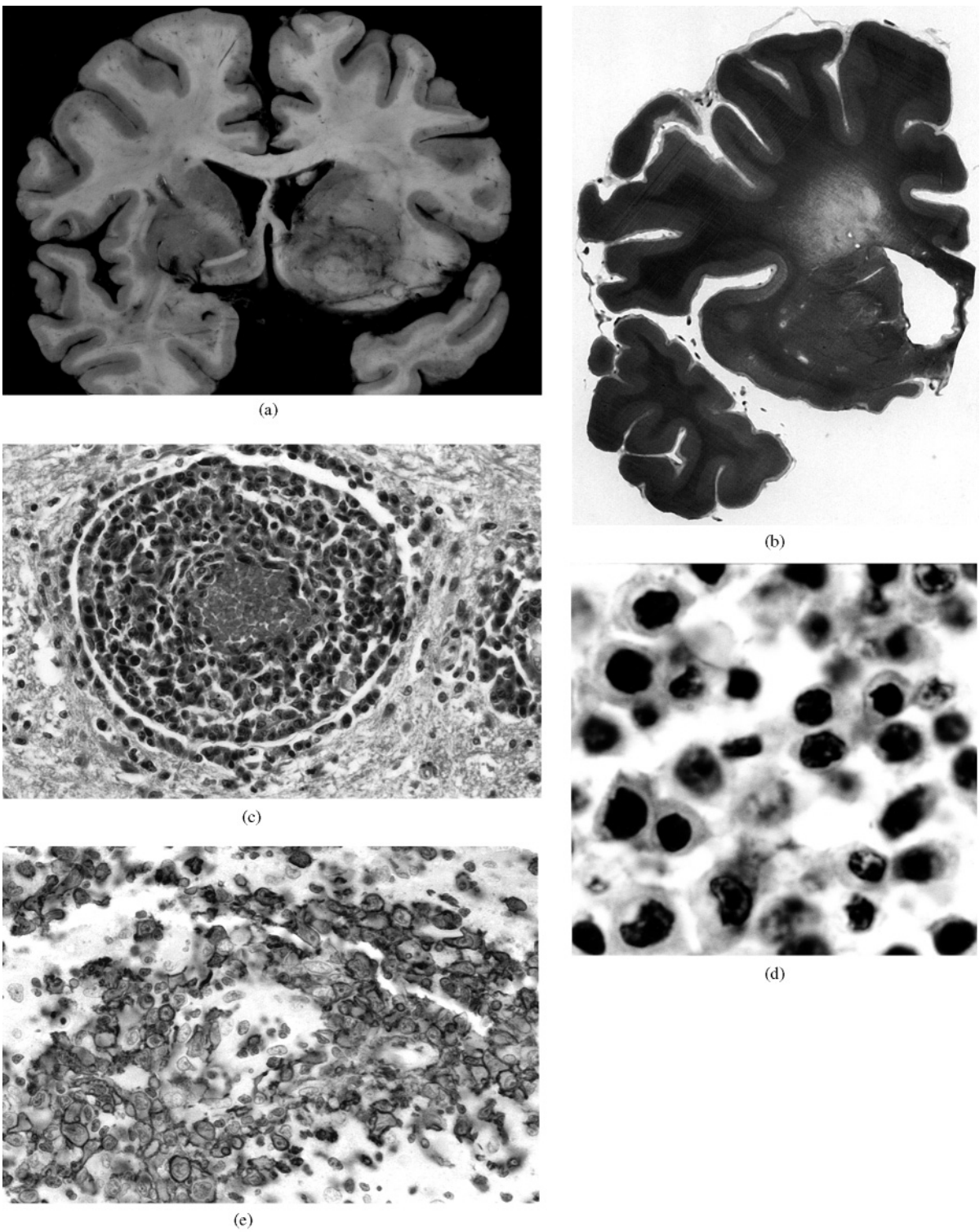


FIG. 28.15. Lymphoma. (a) Coronal section of formalin- xed brain at level of anterior commisure showing discrete whitish lesion in basal forebrain. (b) Whole brain section through frontal lobe showing destruction of tissue and in ltrating lesion in centrum semiovale. (c) Microscopic appearance of lesion characterized by perivascular collections of tumor cells (c) and pleomorphic nuclei (d) (H&E stain); the tumor is a B-cell lymphoma (e) (immunoperoxidase stain for B-cells).

constitute a disease entity unto its ownÓ (307), that in many instances may not be readily classiÞable (301,308). In about 98% of cases PCNL tumor cells express B-cell antigens (CD20); only 2% are T-cell lymphomas, and all other subtypes are exceptionally rare (308). Taken together, most studies of PCNSLs associated with AIDS, have reported a preponderance of the more aggressive large-cell immunoblastic and large non-cleaved-cell subtypes of NHL (280,295,309Ð312). Some are composites of mixed large-cell subtypes, as well as mixed large and small cleaved cell variants, or only the latter (301). Our own experience in patients with AIDS parallels that of others in that PCNSLs are usually highly malignant, morphologically polymorphous and extensively necrotic; routine histopathological work-up almost always demonstrates positive reticulin staining. Treatment of PCNSLs involves one or, more frequently, a combination of the following modalities: whole brain irradiation, chemotherapy, HAART and interleukin-2 (313). With some exceptions, the overall prognosis is still poor.

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INFECTION CONTROL

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Chapter 29

Infection Control Considerations to Prevent HIV Transmission in Healthcare Settings

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Transmission of human immunodeÞciency virus (HIV) from patients to healthcare personnel (HCP), from HCP to patients, and from one patient to another can occur in healthcare settings. Similar to other bloodborne pathogens, HIV is more likely to be transmitted from patients to HCP than from HCP to patients. Needle punctures or other similar percutaneous injuries inßicted by contaminated sharp instruments account for most cases of occupational HIV infection. Transmission from an infected healthcare provider to a patient is unusual, but possible, e.g. when an infected provider is injured by a sharp object, which then recontacts a patientÕs tissues. Transmission between patients is usually attributable to breaches in infection control practices such as reuse of contaminated equipment or injection of contaminated materials. Fortunately, all these modes of transmission are rare and can be prevented. In this chapter, we discuss the epidemiology and prevention of HIV transmission in healthcare settings.

OCCUPATIONAL HIV TRANSMISSION

Occupational exposures to blood and certain other body substances (e.g. cerebrospinal ßuid, amniotic ßuid) pose a risk of HIV transmission. Important determinants of the risk of occupational HIV transmission are the prevalence of infection in the patient population, the likelihood of acquiring infection after a single blood contact from an infected patient, and the nature and frequency of blood contact. These factors have been assessed by seroprevalence studies, prospective studies of HCP after occupational exposure to HIV, and observational studies of occupational contact with blood and other body ßuids.

In prospective studies of HCP, the average risk of HIV transmission after a percutaneous exposure to HIV-infected blood has been estimated to be approximately 0.3% (95% conÞdence interval (CI) = 0.2%Ð0.5%) and after a mucous membrane exposure, approximately 0.09% (95% CI = 0.006%Ð0.5%) (1,2).Although episodes of HIV transmission after nonintact skin exposure have been documented (3), the average risk for transmission by this route has not been precisely quantiÞed but is estimated to be less than the risk for mucous membrane exposures (4). The risk of transmission after intact skin exposures has not been quantiÞed precisely, but is believed to be even smaller; no cases of HIV transmission after exposure to intact skin have been documented (3). The risk of transmission after exposure to ßuids or tissues other than HIV-infected blood also has not been quantiÞed.

As of June 2001, the Centers for Disease Control and Prevention (CDC) had received reports of 57 U.S. HCP with documented HIV seroconversion temporally associated with an occupational HIV exposure (5 and CDC unpublished data). In addition, the CDC has received reports of 137 HCP (one worker was reclassiÞed) with possible occupationally acquired HIV infections; each of these workers reported that his or her infection was occupationally acquired and no other risk for HIV infection was identiÞed, but transmission of infection after a speciÞc exposure was not documented. Of the 57 documented episodes, 49 involved exposure to HIV-infected blood; one to a visibly bloody body ßuid; four to unspeciÞed ßuids; and three to concentrated virus in a laboratory. Forty-eight HCP had percutaneous exposures, Þve mucocutaneous, and two both percutaneous and mucocutaneous; the route for two exposures is uncertain (5).

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The 51 percutaneous exposures involved a hollow-bore needle (45), broken glass vial (two), scalpel (two), or an unknown sharp object (two).

Factors associated with transmission risk have been evaluated in a retrospective case-control study of percutaneous exposure to HIV among HCP (6). The risk for HIV infection was increased with exposure to a larger quantity of blood from the source patient as indicated by a device visibly contaminated with the patientÕs blood, a procedure that involved a needle placed directly in a vein or artery, or a deep injury. The risk also was increased for exposure to blood from source patients with terminal illness, possibly reßecting the higher titer of HIV in blood late in the course of acquired immunodeÞciency syndrome (AIDS) or other factors, such as the presence of syncytia-inducing strains of HIV. A laboratory study that demonstrated that more blood is transferred by deeper injuries and hollow-bore needles lends further support for the observed variation in risk related to blood quantity (7).

HEALTHCARE PROVIDER-TO-PATIENT HIV TRANSMISSION

Since the onset of the AIDS epidemic, only three episodes of HIV transmission from an infected healthcare provider to patients have been reported: one in the U.S. (1990) and two in France (1997) (8Ð11). The episode in the U.S. involved a cluster of six patients whose HIV infections were linked epidemiologically and genetically to a dentist with AIDS (8). Although the investigation indicated that HIV transmission occurred during ofÞce visits for dental care, and was most likely from dentist to patient rather than from patient to patient, the precise event(s) resulting in transmission could not be determined.

The second episode of HIV transmission from an infected healthcare provider involved an orthopedic surgeon in France whose HIV transmission to one patient was conÞrmed through DNA sequence analysis of viral isolates obtained from the surgeon and the patient (9). The surgeon in this case most likely became infected by an occupational injury sustained during a surgical procedure in 1983. However, the surgeon was not aware of his infection until AIDS was diagnosed in 1994. A retrospective (Òlook-back)Óinvestigation of 3,004 patients who had undergone at least one invasive procedure performed by the infected surgeon since 1983 was initiated. One patient, who had a negative HIV antibody test before undergoing the Þrst of three procedures performed by the index surgeon, was found to be infected with HIV when she underwent preoperative testing before a third procedure. Although the precise mechanism of transmission is unknown, the duration of the procedure (10 hours), procedure-related opportunities for percutaneous injury to the healthcare provider, and possible high viral titer in the surgeon are hypothesized as contributing factors. No breaches in recommended infection control practice were identiÞed.

The third report of transmission of HIV from a nurse to single patient in France, conÞrmed by phylogenetic analyses, was published in 2000 (10). The nurse was co-infected with HCV; however, the patient was found to be infected with HIV only. Nursing duties involved monitoring the patient post-operatively and giving two subcutaneous injections. No circumstances that could have contributed to HIV transmission were identiÞed. A lookback investigation of more that 2,300 patients who may have been exposed to the nurse found no other case of nurse-to-patient HIV transmission (11).

Data from retrospective investigations of HCP infected with HIV for the purpose of assessing the risk for HCP-to-patient transmission did not detect any transmission among 22,759 patients treated by 53 infected HCP (12). Despite the limitations of retrospective studies, these data are consistent with previous assessments that the risk for HIV transmission from infected HCP is extremely low.

PATIENT-TO-PATIENT HIV TRANSMISSION

Episodes of HIV transmission from one patient to another have been reported in hospitals and out-patient healthcare settings in the U.S. and other countries; the majority have involved breaches in protocol, improper infection control practices, and/or inadequate disinfection procedures. Reuse of blood-contaminated hypodermic needles was linked to HIV transmission to at least 41 hospitalized children in the Soviet Union and may be responsible for as many as 400 HIV infections in hospitalized children in Libya that occurred in 1998 (13,14). Similar opportunities for such transmission have been reported during immunizations in the U.S. (15).

In three separate incidents (two in the U.S. and one in the Netherlands), patients were inadvertently injected with blood from an HIV-infected patient during nuclear medicine procedures (16). These procedures involved withdrawal of the patientÕs blood, labeling with a radioactive isotope, and then reinjection of the blood. In these reported instances, system errors in identiÞcation of patients and/or materials to be injected contributed to exposure.

In Australia, Þve patients who underwent minor out-patient surgical procedures performed on the same day by an HIV-negative surgeon were subsequently found to be HIV positive (17). Four of these patients had no identiÞable source of infection; the Þfth patient had known risk factors for HIV, was subsequently found to be HIV positive, and is the probable source of infection. Although the mechanism of transmission was not identiÞed, contamination of multi-dose medication vials was hypothesized as a possible vehicle for transmission in this outbreak.

Transmission of hepatitis B and C viruses is well documented in the hemodialysis setting; rarely, HIV transmission has been associated with this procedure (18).

No case of patient-to-patient transmission of HIV during dialysis has been reported in the U.S. However, HIV transmission to at least nine patients in a hemodialysis center in Colombia has been reported and was attributed to inadequate disinfection and reuse of contaminated vascular access devices; similar cases have been reported from Argentina and Egypt (19–21).

There are few reports where HIV transmission from patient-to-patient was suspected but not confirmed. One report from the U.S. involved an infant with AIDS whose source of infection was unknown but was possibly related to exposure to another HIV-infected patient during medical care (22). In Denmark, a child was reported to have acquired HIV during a hospital stay that overlapped 15 days with an HIV-positive child; genotypes of the two viruses were similar (23). Although no mechanism of transmission was identified in this case, a needle disposal box was accessible in the infected child's room. It was hypothesized that an unnoticed needlestick occurred to the second child during an unobserved visit to the source patient's room.

In most cases, these transmissions could have been avoided through adherence to standard infection control practices. These include aseptic technique, cleaning and disinfecting or sterilizing equipment between patients, safe injection practices, and appropriate handling of single-use or single-patient-use devices and equipment.

Epidemiology of Blood Exposures in Healthcare Personnel

Epidemiological data on blood exposure are important for targeting and prioritizing prevention efforts. Aggregate data from more than 50 hospitals participating in the CDC National Surveillance System for Healthcare Workers (NaSH) show that the majority (82%) of reported blood exposures are the result of a percutaneous injury (e.g. needlestick or other sharp object injury) followed by exposures to mucous membranes (14%), non-intact skin (3%), and human bites (1%) (CDC unpublished data).

NaSH data on percutaneous injury demonstrate that a few types of devices are associated with the majority (74%) of injuries, although a large variety of sharps injure HCP (CDC unpublished data). Among these devices are disposable syringes, which account for 29% of injuries, suture needles (17%), winged-steel needles (12%), scalpel blades (7%), intravenous (IV) catheter stylets (6%), and phlebotomy needles (3%). Many of these devices are hollow-bore needles that have been used in a vein or artery that may contain residual blood.

Injuries can occur at multiple points in the course of handling a needle or other sharp device. NaSH data show overall that 39% of injuries occur during use on the patient, 41% occur after use and before disposal, and 16% occur during or after disposal (CDC unpublished data). Table 29.1 lists possible injury mechanisms during each of these time periods and demonstrates the multiple factors that contribute to injuries.

TABLE 29.1. *Common mechanisms of percutaneous injury to healthcare personnel*

While manipulating patient or needle/sharp
Patient moved and jarred device
During needle insertion, manipulation, or withdrawal from a patient or intravenous line
Passing or transferring equipment during use
Handling equipment or specimens
Handling equipment on a tray or stand
Transferring specimens into a container
Recapping
Disassembling device or equipment
During clean-up
In transit to disposal
Collision with other person or sharp device
Disposal-related
Placed sharp in container
Injured by sharp sticking out of container
Over- lled or punctured sharps container
Sharp in unusual location
Found in trash, linen/laundry
Left on tray/table
Left in bed/mattress
Placed in pocket/clothing

Mucocutaneous exposures generally reflect either noncompliance with recommendations for using protective barriers, failure of protective equipment to provide an adequate barrier, or unanticipated circumstances in which the HCW was unable to prepare for the patient interaction. Of the mucocutaneous exposures reported to NaSH, 68% were to the eyes (CDC unpublished data). This is most likely due in part to a reporting bias, since an eye exposure is dramatically apparent to a healthcare worker and is therefore more likely to be reported (24). In 89% of eye exposures, the healthcare worker wore no protective eye wear (e.g. goggles, face shield). That exposures occurred in some instances despite the use of protective eye wear suggests a problem with either the selection or the design of the protective device in use.

Reported nonintact skin blood contacts often involve the hands, arms, and/or face/head and frequently include exposure of a mucous membrane. The distribution of these exposures suggests that in addition to better eye protection, more consistent protection of the hands and arms is needed, including overlap between gloves and the cuffs of cover garments to eliminate the problem of "wrist gap" (24,25).

Prevention Strategies

The strategies for preventing transmission of other pathogens to patients in healthcare settings also prevent transmission of bloodborne viruses to patients. These

include handwashing, using aseptic technique, wearing gloves and other barriers when contact with blood and other body ßuids is anticipated, and cleaning, disinfecting, and sterilizing patient care equipment, instruments, and other devices in accordance with recommendations issued by various authorities (26Ð30).

Healthcare personnel must be educated and trained in the modes of disease transmission and the basic principles and practices of infection control to protect patients during provision of healthcare. Written policies and procedures help guide correct practice and prevent system errors. However, many situations with the potential for disease transmission are not covered by written guidelines and procedures. In these situations, HCP who understand and are able to apply the fundamentals of prevention in a variety of circumstances are best equipped to protect their patients and themselves.

UNIVERSAL AND STANDARD PRECAUTIONS

In 1987, CDC issued recommendations for Òuniversal precautionsÓ based on the concept that all blood and certain body ßuids should be treated as infectious, since it is not possible to know who may be carrying a bloodborne virus (26). Accordingly, CDC recommended that all HCP anticipate the extent of blood contact that is likely, based on the speciÞc type of patient interaction, and use preventive practices. These include careful handling and disposal of sharp devices, positioning patients and using other procedural techniques to avoid or limit blood splashes and using appropriate barriers (e.g. gloves, gowns, eye protection) to provide optimal protection of skin and mucous membrane.

Universal precautions was initially conceived as a strategy for HCP protection from bloodborne virus exposure. However, its relevance to other aspects of patient care was soon recognized and, in 1996, universal precautions was incorporated as a key component of a more comprehensive prevention strategy, designated as standard precautions (27). Standard precautions integrates and expands the elements of universal precautions into a standard of care designed to protect both patients and HCP from pathogens that may be spread by blood, or by enteric and contact routes of transmission.

PREVENTION OF OCCUPATIONAL EXPOSURES

To prevent bloodborne virus transmission to HCP, attention must focus on preventing blood exposure, in particular, percutaneous injuries. This can be accomplished by approaching all blood as potentially infectious and using a combination of strategies, i.e. engineering controls (e.g. safety devices), work practice controls, and personal protective equipment to prevent injury and direct contact with blood (31).

Several strategies can be used to prevent percutaneous injuries to HCP. These include:

- minimizing needle use (e.g. using alternate routes for medication delivery and specimen collection where appropriate and safe for patient care, consolidating specimen collection and implementing needle-free intravenous delivery systems);
- using devices with engineered sharps injury prevention features (e.g. safety IV catheters, hypodermic needles/syringes, and blood collection equipment);
- ensuring safe sharps disposal (e.g. puncture-resistant sharps containers available at the point of use and systems for removing containers before they become overÞlled); and
- promoting injury prevention work practices (e.g. not recapping needles or, if recapping is necessary, using a one-handed scoop technique; keeping the hands behind the sharp; maintaining awareness of the presence of an exposed sharp).

The importance of implementing such measures has been the focus of regulatory and legislative activity since 1991, when OSHA Þrst issued its Bloodborne Pathogens Standard to protect HCP from such exposures (32). The initial focus of this standard was the use of barriers to prevent blood contact. However, as the scientiÞc understanding of bloodborne pathogen transmission from sharp devices increased, attention turned to making safer devices in order to prevent percutaneous injuries; state and federal legislation and regulations now require the use of safety devices to prevent needlesticks.

Hand Protection

Hands are the primary site of cutaneous blood exposure. Gloves serve the dual purpose of protecting patients from pathogens that may be on the hands of HCP and also protecting providersÕhands in situations where blood and body ßuid contact can be anticipated (26,27). The type and number of gloves used should be determined by the procedure being performed.

For most clinical situations that require nonsterile gloves, a single pair of good quality medical gloves that Þt comfortably will provide ample protection. Questions have arisen regarding differences in barrier properties between latex, vinyl, and, more recently, nitrile gloves. Data comparing latex and vinyl gloves have consistently shown that latex is superior to vinyl in distension leak tests (33Ð35) and that sterile surgeonsÕgloves are superior to nonsterile gloves (36). However, differences in failure rates among gloves from different manufacturers were apparent in these studies, suggesting that a good quality vinyl glove sometimes will provide better barrier protection than a poorer quality latex glove. In recent years, concerns about latex allergies have inßuenced glove selection. As a result, gloves made of nitrile, neoprene, and various polymers are now being manufactured for clinical use. One in-use study of barrier performance has found nitrile gloves comparable to latex (37).

Body, Face and Eye Protection

The use of barriers to protect the eyes, face, and other exposed areas of skin during procedures when blood contamination can be reasonably anticipated is recommended (26,27). The type of equipment needed should be appropriate to the procedure being performed (38). Eye protection minimally requires the use of goggles, as neither contact lenses nor glasses provide sufficient protection. Face shields often are used when more extensive splashing might occur. Cover garments protect the arms, torso, and legs in situations where blood contact is likely. The effectiveness of garment barriers depends on their design and the level of liquid resistance of the material of which they are made. Garments should have a high neck and long sleeves and should close in the back or have a well-sealed front closure. The conventional "lab coat" should not be considered a protective garment for preventing blood and body fluid exposures since its front opening, V-neck, and loose cuffs do not provide sufficient coverage of body areas that might be exposed. In addition, the cotton-blend fabric of the lab coat and the traditional reusable cloth or disposable isolation gown generally lack the protective qualities necessary for preventing penetration of blood or body fluids.

PREVENTION OF HIV TRANSMISSION TO PATIENTS

Episodes of patient-to-patient transmission of bloodborne viruses, including HIV, serve as sentinel events for the detection of breaches in infection control practice and are reminders that such lapses have important public health implications. Such transmissions occur indirectly through lack of adherence to infection control by HCP and to date have not involved direct contact between two or more patients/persons. Almost all of the transmissions reported are preventable through adherence to recommended practices for infection control (Table 29.2).

SPECIAL SETTINGS: OPERATING ROOMS

Among NaSH hospitals, the operating room (OR) is a common location where sharps injuries occur, accounting for 27% of reported percutaneous injuries; 39% of OR injuries are reported by residents (24%) and surgeons (15%) (CDC unpublished data). However, this most likely underestimates the true incidence of injuries among surgeons; surveys performed by NaSH hospitals between 1996 and 1998 found that surgeons reported only 25% of their exposures, compared to other physicians and nurses who respectively reported 45% and 48% of their exposures (39).

The epidemiology of sharps injuries in the OR differs from that in other hospital locations. Observational studies of operative procedures have recorded some type of blood exposure in 7–50% of cases; in 2–15% of cases, the event is a percutaneous injury, usually from a suture needle (40–44). Aggregate data from reported injuries among OR staff in nine hospitals also reflect the importance of suture needles, which in this study accounted for 43% of the injuries in the OR (45). Most suture needle injuries occur when palpating a needle tip being drawn through tissue, loading or unloading a needle holder, and/or passing a

TABLE 29.2. *Measures to prevent patient-to-patient HIV transmission*

Safe injection practices
use only single-use disposable needles/syringes
use single-dose vials when available and cost-effective
use aseptic technique when handling injection equipment
prevent contamination of multidose vials; never reenter a vial with needle/syringe used on one patient if that vial will be used to withdraw medication for a subsequent patient
prepare medications in clean areas, physically separated from areas where blood and other body fluids are handled
Gloves and hand hygiene
wear gloves for procedures where blood contact is reasonably anticipated
change gloves between patients
wash hands if visibly soiled with blood
perform hand hygiene (washing hands or using a waterless hand hygiene agent) after changing gloves
Environmental practices
use barriers as appropriate to protect equipment that may become blood contaminated
avoid touching clean equipment and surfaces with blood contaminated hands or gloves
follow recommended procedures for cleaning, disinfection, or sterilization of reusable equipment and instruments and blood-contaminated surfaces
maintain a physical separation between "clean" and "contaminated" equipment and supplies
Other practices
assign equipment with potential for blood contamination (e.g. reusable spring-loaded lancet devices) to individual patients
evaluate new devices and equipment for cross-contamination potential; establish procedures to ensure proper between-patient handling

suture needle to someone else (43–45). Other injuries unique to this environment involve wires, scalpels, and bone spurs. These injuries have additional implications as they may result in recontact with the patient's tissue and patient exposure to a healthcare provider's blood (44).

Observational studies in surgical and obstetrical settings have found that 71–92% of blood contacts are mucocutaneous (40–46). In these studies, most of the observed blood contacts involved intact skin and therefore theoretically did not pose a risk for bloodborne virus transmission. In contrast, of the mucocutaneous exposures reported to surveillance systems, 80–83% involve a mucous membrane or nonintact skin and therefore might pose a risk for bloodborne virus transmission (38, CDC unpublished data).

Prevention of sharps injuries during surgery requires a comprehensive approach that includes eliminating the unnecessary use of needles and other sharp instruments and using safer devices and preventive work practices. The least invasive surgical approach that will achieve the desired patient outcome is preferable. For example, fiberoptic techniques usually pose a lower risk of injury and blood exposure than do more invasive surgical approaches. Likewise, when patient safety permits, alternatives to needles and other sharps (e.g. use of adhesive tape, staples, or tissue glue rather than sutures, or use of blunt electrocautery rather than scalpels) should be used (47,48).

The protective effect of wearing two pair of gloves in reducing percutaneous injury and blood contamination during surgery has been reported (40,51–56). When perforation rates are compared, rates for outer double gloves and single gloves are remarkably similar; however, inner double glove perforation rates are much lower. In these studies, wearing double gloves reduced inner glove perforation between 55% and 84%. In addition, double gloves were shown to reduce blood contamination of surgeons' hands (43,57).

Despite concerns about comfort, sensitivity, and dexterity (58,59), the majority of surgeons who wear double gloves find them acceptable and do not report that tactile sense is significantly impaired (60). While double gloving appears to have important implications for preventing blood contact and has been shown to be acceptable for use during many types of surgical procedures, the impact of this intervention on disease transmission has not been determined.

SPECIAL SETTING: DIALYSIS

When AIDS was described in 1981 and its epidemiology was subsequently determined to be similar to that of hepatitis B, concern increased for transmission of HIV in the dialysis settings. However, as the AIDS/HIV epidemic progressed, it became evident that HIV was less efficiently transmitted than HBV, presumably because of the substantial difference in the amount of infectious virus circulating in the blood of infected individuals. Thus, standard infection control strategies using basic barrier precautions could be used to prevent transmission of HIV from patients to staff members and from patient to patient in the dialysis setting (61,62). From 1985 to 1997, the percentage of centers that reported providing dialysis for patients with HIV infection increased from 11% to 39%. The percentage of patients receiving dialysis who were known to be infected with HIV increased from 0.3% in 1985 to 1.3% in 1997 (63). An HIV serosurvey among hemodialysis patients at 28 dialysis centers found that none of 254 HIV-seronegative patients became HIV positive during a one-year follow-up period (64).

Routine testing of dialysis patients or staff members for anti-HIV for is not necessary for infection control. Patients with HIV infection can be treated with either hemodialysis or peritoneal dialysis and they need not be isolated from other patients, either in separate rooms or by using dedicated machines. The type of dialysis treatment should be based on the needs of the patient and not on the presence or absence of HIV infection. The infection control precautions recommended for dialysis centers are sufficient to prevent HIV transmission between patients. These include the use of both standard precautions and additional measures that are specific for the hemodialysis setting (18,65). These precautions include the routine use of gloves, handwashing, and cleaning and disinfection of external surfaces of dialysis machines and other environmental surfaces. Disinfection and sterilization strategies routinely practiced in dialysis centers are adequate to prevent HIV transmission. In addition, patients who are HIV-positive may participate in dialyzer reuse programs. Because HIV is not transmitted efficiently through occupational exposures, reprocessing dialyzers from HIV-positive patients should not place staff members at increased risk for infection.

PROTECTION OF PATIENTS INFECTED WITH HIV

Patients with HIV vary in the degree to which their immune system is compromised. However, no special measures during any stage of HIV disease are indicated to protect these patients from acquiring other diseases during hospital care (27). The use of standard precautions for all patients and appropriate isolation of patients who have diseases that pose a risk for transmission should protect HIV-infected patients from acquisition of institutionally acquired pathogens from other patients and environments.

MANAGEMENT OF HEALTHCARE PERSONNEL INFECTED WITH HIV

HCP infected with HIV should follow the same precautions to prevent exposure to transmissible agents

recommended for all other personnel (27). There are no recommendations for modifying the work environment to protect these workers from exposure. However, as with anyone who may be immunocompromised, referral to personnel health professionals who can provide individual counseling on the worker's job-related infection risks is advised (66).

The risk of HCP-to-patient transmission of HIV is extremely low. Guidelines for the management of HCP infected with HIV and other bloodborne viruses have been published (67,68). The focus of these recommendations is on HCP who perform a subset of highly invasive procedures where a percutaneous injury to the provider could result in patient exposure to the healthcare provider's blood. Healthcare organizations are advised to develop comprehensive occupational health programs to manage impaired workers, including evaluation of personnel fitness for duty based on competence, ability to perform routine duties, and compliance with established guidelines and procedures. Restriction of an infected provider is rarely indicated. Recommended strategies to prevent blood exposure during surgery, especially percutaneous injuries, should be emphasized.

SUMMARY

The prevention of HIV transmission in healthcare settings requires the use of strategies to prevent blood exposures. All blood should be considered potentially infectious and appropriate barriers and personal protective equipment should be used to prevent blood contact between patients and HCP. Percutaneous injuries from contaminated needles and other sharp devices pose a particular risk for HIV transmission to HCP; during certain invasive procedures, such injuries can also result in blood exposure to both a HCP and patient. The prevention of percutaneous injuries is therefore a high priority. In recent years, devices with engineered sharps injury prevention features have been developed to reduce percutaneous injuries. These devices, in combination with the elimination of unnecessary needle use, use of safe work practices, and organizational efforts to promote a culture of safety are necessary to reduce percutaneous injuries effectively during the provision of healthcare.

Strategies to protect patients rely on the basic principles and practices of infection control, including standard precautions; aseptic technique; handwashing or hand hygiene; use of gloves and other barriers to prevent blood contact; and the cleaning and disinfection or sterilization of equipment and environmental surfaces. Using care to prevent patient-to-patient blood contact when handling injection equipment and multidose vials is particularly important.

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Chapter 30

HIV Era Occupational Exposures and Risks

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The initial recognition in 1981 of the acquired immunodeÞciency syndrome (AIDS) epidemic was based on the recognition by clinicians and the conÞrmation by epidemiologists of the unusual occurrence of a few cases of Kaposiõ sarcoma and *Pneumocystis carinii* pneumonia (1Ð6). An infectious etiology was supported by cases of unusual infections in intravenous drug abusers, children of drug abusers, and blood transfusion recipients that were judged likely to be related to the illnesses occurring in homosexual men (7Ð10).The Þrst occupational guidelines were formulated on the premise that the agent might be similar enough to the hepatitis B virus (HBV), known to be a highly infectious bloodborne pathogen that was also common among homosexual men, that guidelines based on our knowledge of HBV epidemiology would be sufÞcient for interim protection. In 1982 (just a year after the Þrst report of AIDS), and again in 1983, guidelines were formulated and widely disseminated (11Ð13).

U.S Public Health Service (USPHS) guidelines have since been revised many times, most recently (as of this writing) on June 29, 2001 (14). These guidelines highlight the potential risk of parenteral exposure to blood and other body ßuids that might contain HBV, hepatitis C virus (HCV) or the human immunodeÞciency virus (HIV). These major bloodborne pathogens are transmitted sexually as well. The risks of percutaneous acquisition for these three agents vary by more than 100-fold (Table 30.1). The initial section of this chapter focuses on HIV, with the other major bloodborne pathogens of concern for the modern health care worker covered in the latter portion.

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TABLE 30.1. *Comparative transmission risks for HIV, HBV and HCV, from a needlestick with blood contaminated with that agent*

Agent	Percutaneous Exposure Transmission Risk (Range)	References
HIV	0.3% (95% CI 0.2–0.5%)	(14–16)
HCV	1.8% (range 0–7%)	(14,17–19)
HBV, HBsAg + , HBeAg negative	Serologic: 23–37% Clinical hepatitis: 1–6%	(14,20,21)
HBV, HBsAg + , HBeAg positive	Serologic: 37–62% Clinical hepatitis: 22–31%	(14,20,21)

CI—Con dence interval.

THE HIV ERA

Building upon the isolation and culturing of HIV type 1 (HIV-1) in 1983Ð1984 (22Ð24), reliable serological screening for HIV became available in 1985 (25Ð27).The HIV antibody test also helped to establish a convincing etiologic connection between HIV and AIDS (27,28).

These discoveries subsequently led to the realization that the magnitude of the epidemic was far greater than had been imagined, with the early numbers of AIDS cases dwarfed by the prevalence of (number of persons infected with) HIV (25). This meant that in the United States transmission of HIV had silently occurred during the 1970s with continuing, extensive spread in the ensuing decades. Thus, in the early years of the epidemic healthcare and laboratory workers were unknowingly in routine contact with the blood or body ßuids of a great many more patients infected with HIV than those recognized to have AIDS.

During the early 1980s, few workers took precautions against blood-borne agents, with a somewhat cavalier

attitude reinforced by the reassurance that no worker had (yet) become infected with AIDS/HIV as a result of an occupational exposure and an under-appreciation of the risks from the hepatitis viruses. At that time, the standard infection control precautions were disease specific and aimed at prevention of exposure of staff and other patients with regard to a limited set of diagnosed or suspected diseases. There were some fearful workers who heavily garbed to protect themselves from AIDS patients. These efforts were misplaced as well, representing both an over-reaction with AIDS patients (excessive, unnecessary precautions) and an under-reaction (as no protection at all was used with the even greater number of patients who were HIV-infected but had not been identified as having AIDS).

It became increasingly evident that most persons with bloodborne pathogens were, in fact, not visibly "sick" and thus not recognized to be infected and infectious. (This recognition underlies the development of universal and standard precautions.) Needlestick injuries in the medical setting occurred frequently (29) but were infrequently reported or documented. Sharps injuries continued to occur (30) after the risks of sharps exposure had been documented (21) and despite the implementation of standard precautions, giving the impetus for safer devices and safer work practices.

DIAGNOSIS OF HIV INFECTION

The mainstay of HIV diagnosis is testing for antibodies ("seropositivity") (confer Chapter **00**, "Laboratory **Diagnosis** of Retroviral Infection," for a more complete discussion), connoting chronic, persistent infection (as opposed to protection). In the "window" period that exists before antibody becomes detectable but after exposure and infection with HIV, other tests such as p24 antigen (p24Ag, with and without immune complex disassociation), nucleic acid amplification tests (NAT) and viral load measures, or immunologic tests may be positive (31–35). These tests have some specific applications in various sections of the "window" period, discussed in detail in that portion of Chapter **00** (the LAB chapter). These additional tests are not routinely used in the clinical evaluation of persons with an exposure to HIV, in part related to the costs involved, the low pre-test probability of HIV infection leading to a limited positive predictive value), questions concerning the specificity of the tests, and the lack of standards.

Available data have been consistent with the picture painted by HIV antibody testing alone (36–38). Antigen and nucleic acid amplification assays, on the average, detect HIV only a few days before standard antibody tests (31), are expensive, and results are usually not immediately available. Some have not been licensed as screening tools in the clinical setting. In contrast, tests for screening for HIV antibody are commonly available, a confirmed result can be obtained shortly afterwards, and rapid screening tests were licensed in 2002 (39,40).

In a person with possible acute retroviral seroconversion syndrome, for whom the pre-test probability of true positivity rises, there is justification for use of alternative tests, since an unconfirmed positive research assay has an increased likelihood of ultimately being a true positive (see Chapter **00**, "Laboratory **Diagnosis** of Retroviral Infection"). It is believed that most persons who acquire HIV from an exposure will demonstrate antibodies to HIV within six months, although exceptions occur (see Chapter **00**, "Laboratory **Diagnoses** of Retroviral Infection"). The data are sparse with regard to how frequent these longer latency exceptions might be, but anecdotal cases of prolonged seroconversion intervals continue to be reported sporadically, with varying levels of documentation (41–43).

HISTORICAL DISCOVERY OF HIV OCCUPATIONAL RISKS FOR THE WORKER

By early 1985, there were some clues that HIV infection was a chronic disease, with long latency (25,44). The demonstration of a long incubation period between first acquiring HIV and developing AIDS or other severe clinical sequelae involved the use of both prospective epidemiologic data and tests for the causative agent (45). It was, at least theoretically, possible that many healthcare workers might have (unknowingly) become infected with HIV and feel and look perfectly well; the initial studies of occupational risk could conceivably have failed to detect widespread transmission.

It was therefore quite reassuring in January 1985 when two groups found HIV antibody to be absent serologically in a combined total of 273 persons drawn from institutions and laboratories where AIDS and HIV, respectively, were routinely encountered (15,27,46).

The CDC subsequently reported on 40 healthcare workers, representing persons who had specific potential HIV exposures. These exposures included 29 needlestick injuries, five cuts, five mucosal exposures, and five skin exposures (47). No worker was seropositive, with a median follow-up period of eight months. Additionally, the T-cell subsets were normal and HIV clinical sequelae were absent among over 200 workers followed prospectively (47). This reinforced the message that the initial U.S.PHS guidelines for AIDS had been reasonable initial measures. These data, however, still did not exclude the statistical possibility of a substantial public health risk (48), since needlestick transmission rates for HIV exceeding 1% were within the 95% upper confidence bounds given that limited observation base. The transmission by a needlestick injury from an AIDS patient of HBV, *without* transmission of HIV (49), suggested that transmission of HBV was more likely compared to HIV (21). These findings supported the tendency to offer reassurances to a somewhat nervous

work force, but also tended to instill a sense of complacency in many institutions. The medical community largely ignored the limitations of the data.

In late 1984 a nurse in Great Britain became infected with HIV as a consequence of injection of fresh blood from an arterial blood gas syringe in an intensive care unit setting (50). The source patient was known to have AIDS. Healthcare workers could no longer deny that they were at some risk for occupational acquisition of HIV.

Needlestick transmission was next documented in early 1985 in a cross-sectional study of high risk hospital workers in the United States (21), conducted before the commercial availability of any HIV tests. One (2.4%) of 42 persons who had reported parenteral exposure to patient(s) with AIDS was HIV seropositive, with no other risk identified in a prospective questionnaire that had been completed prior to initial phlebotomy nor after a thorough investigation, which included assessment of her seronegative heterosexual spouse. Two other seropositive healthcare workers with nosocomial parenteral exposure but no other risk factors were simultaneously reported (21), further cementing the reality of risk.

First positive reports not infrequently overestimate the magnitude of risk, as negative data from other studies do not enter the denominator. It is not surprising that current estimates of risk (see Table 30.1) are significantly lower than the 2.4% transmission risk above. The actual rate remains high from a public health viewpoint. Several other large studies as of that time had not yet observed any unexplained HIV infection or seroconversions among exposed workers (27,51,52). Numerous authorities thus chose to continue to interpret incorrectly the risk to workers as minimal, awaiting future reports. Given the clear evidence of other types of parenteral transmission from other settings, such viewpoints may have reflected more a political assessment than a balanced scientific assessment.

In retrospect, it is clear that most summary analyses systematically tended to underestimate risk, the flaw in part reflecting methodological biases. For example, health care workers with non-occupational risks were excluded from the numerator (i.e. from the cases of occupational transmission), on the assumption that the other risks were the responsible factor(s) (or, at best, that such factors were not easily excluded). However, workers with these risk factors were nevertheless still included in the denominator (reflecting all those considered to be at risk), artifactually reducing the risk estimates. Indeed, only a single review (15) addressed (and adjusted for) this type of methodological bias.

Documentation of HIV transmission to healthcare and laboratory workers by multiple investigators around the world has since occurred (15,53–65), leading to a broader acknowledgement that infectious risks in the workplace deserve attention. This apparent change in viewpoint by some infection control experts led some physicians, particularly surgeons at high risk of percutaneous injury, to feel they had been misled and even betrayed by earlier advice and reassurances. A few retreated from active clinical practice because of their concerns, and some attracted national publicity in doing so.

The American Medical Association, through its insurance agency, by the late 1980s had developed and promulgated an insurance policy for HIV negative physicians who were concerned about the risk of contracting HIV infection and its consequences. The cost of the insurance policy is fairly high compared to the low risk of occupational acquisition for most workers. However, this insurance coverage was apparently not limited to workplace risks, which account for very few of the incident HIV infections among workers. Thus, the worker is insured against possible loss of income irrespective of the cause of infection. Furthermore, the issue of demonstrating mode of acquisition, which might become the subject of litigation and dispute, is removed from consideration in obtaining benefits. There is reason for concern about the possibility of work restrictions for healthcare workers in the event of HIV infection, as discussed below. Thus, a professional who is physically well enough to work (and thus ineligible for standard disability policy benefits) might nevertheless be suffering loss of income and incurring substantial medical expenses. This "HIV insurance" is probably most useful, and on a cost-benefit basis practical, for those with high sexual risk and for workers in high HIV prevalence areas who suffer many parenteral injuries.

IS THERE A RISK OF TRANSMISSION TO THE PATIENT?

Given the theoretical possibility that HIV might be transmitted to a patient and recurrent inquiries to the New Jersey Board of Medical Examiners for advice, a multidisciplinary conference was convened in 1990 to explore what the risks to patients might be (66). Interest was subsequently heightened by the CDC investigation of the practice of a dentist in Florida (67), in which purported HIV transmission from a health care provider to multiple patients gained notoriety. This initially led to a public outcry for further regulation. This reflected an environment in which only absolutely zero risk in the medical care setting was to be tolerated.

As we struggle to protect patients and healthcare providers and to render appropriate care despite limited resources, compromises shall occur that may have long-standing impact on the delivery of healthcare services. As the quantitative risks and costs continue to be weighed by the medical community, public policy is likely to continue to evolve on this issue. It is unclear how lawyers, politicians and others will ultimately weigh such data.

Developing Institutional Policies

This chapter discusses the magnitude of risks to workers and to patients in perspective, and outlines key medical issues involved in the development of institutional policies. Periodic policy review at individual institutions by a multidisciplinary committee will be useful. Members of the committee might include an epidemiologist, an occupational health professional knowledgeable about nosocomial risks, an infectious diseases expert knowledgeable about HIV and its treatment, an infection control professional, administrative and legal staff representatives, a bioethicist and a patient advocate.

HIV OCCUPATIONAL RISK TO THE WORKER

Anecdotal case reports are important in describing modes of transmission, but these cannot be used to calculate the magnitude of risk from those speciÞc types of exposure. HIV transmission to healthcare workers has been documented from several types of HIV exposure: parenteral injury from contaminated sharps (particularly hollow bore needles) and other cuts, mucosal splashes, and skin contact. Even persons rendering home health care are at some risk (68,69). Despite common perceptions to the contrary, transmission of HIV has occurred with needlestick injuries involving only limited blood infusion or relatively superÞcial injury. However, one study has reported Þnding evidence of a dose-response with sharps injury exposures (70)Ñ greater exposure to HIV increasing risk of transmissionÑ consistent with our expectations.

The reported frequency of cutaneous injury with sharp instruments in various surgical procedures is 1.5Ð15%, with an average risk of Þve injuries per 100 procedures (71Ð73). However, injuries from needlesticks may be even more common than suggested by institutional reports (74Ð77). Over 70% of U.S. resident physicians in one study reported at least one needlestick injury (78,79). In a longitudinal study of medical students, the percentage exposed to blood increased from 45 to 65% over the seven-year study period 1990Ð1996 at teaching hospitals afÞliated with the University of California San Francisco School of Medicine (80). The risks outside the Unites States are likely to be at least as great. In a Pakistani study of operating room personnel, 59% reported more than four needlestick injuries per year, 37% reported one to three per year, and only 4.4% reported no needlestick injury in the prior Þve years (81). A British survey of house ofÞcers and senior house ofÞcers in 1996 found that 68% reported at least one needlestick injury (82).

The cumulative number of needlesticks, and the ensuing exposure risk from parenteral agents, can therefore be quite substantial (83Ð86).

Historically, it was also the impression that the majority of injuries were never reported. With increasing attention by OSHA and focused efforts by individual institutions, there are anecdotal reports of improvement. The International Healthcare Worker Safety Center of the University of Virginia, led by Dr. Janine Jagger, estimated that an average of 30 percutaneous and blood and body ßuid exposures occur per 100 occupied hospital beds each year, and that only 39% of all exposure injuries are never reported (87). Other types of occupational exposure are also common in the healthcare environment (88,89). Analysis of where and what causes sharps injuries can help assist in focusing injury prevention programs on speciÞc devices and departments (90).

In a study of percutaneous injuries in 1993Ð1995, nearly 40% of the cases of HIV transmission in the healthcare workplace were associated with phlebotomy procedures (91). The magnitude of the needlestick problem was thus considerable (30). Instruction in universal precautions and clinical procedures is not sufÞcient to prevent exposures to blood (80,92). These data highlight the potential role for modern safety devices and safe work practices (87,93,94). Safety improvements at the phlebotomy service at the Mayo Clinic succeeded in reducing accidental needlestick exposures from 1.5/10,000 venipunctures in 1985 to 0.2/10,000 in 1996 (95,96).

Surgeons reacted beginning in the late 1980s to these risks of HIV with greater attention to the potentials for occupational exposure. Changes in surgical techniques have been recommended (91,94,97Ð100). An example of one of the early, effective safety attempts was the use of blunt suture needles during gynecologic surgical procedures, which reduced the risk of suture-related percutaneous injuries to healthcare workers (94). Many efforts to re-engineer medical products to reduce the risk of injury are ongoing; vascular access ports are a typical example (101). Comprehensive education programs may be needed to introduce successfully and gain acceptance of new devices (102). A cross-sectional study reported a reduced injury rate among surgeons in 1993 as compared to 1988 (103).

Although HIV has been detected in many body ßuids (28,104), strong epidemiologic evidence of HIV transmission has only been demonstrated in association with blood, semen, cervicovaginal secretions and breast milk. There are other potentially infectious material (OPIM). Those OPIM currently deemed to pose a potential risk for HIV include (105):

a. Human body ßuids: any other body ßuid or tissue containing visible blood, cerebrospinal ßuid, synovial ßuid, pleural ßuid, pericardial ßuid, amniotic ßuid, saliva in dental procedures, and all body ßuids in situations where it is difÞcult or impossible to differentiate between body ßuids;
b. any unÞxed tissue or organ (other than intact skin) from a human (living or dead);
c. HIV-containing cell or tissue cultures, organ cultures, and HIV-containing culture medium or solutions; and

d. blood, organs, or other tissues from experimental animals infected with HIV.

The importance, if any, of saliva is unclear. In the dental setting, saliva is invariably contaminated with blood and thus viewed as infectious. Persons with AIDS are also more likely to have bloody saliva, due to hematological disorders (106). In the initial case report that suggested HIV transmission by a human bite (107), the skin was not broken and neither temporal seroconversion information nor viral strain comparison data was available. In a second case report (108), some question remained as one sexual partner (a "one-night stand," who thus might be from a pool of higher risk partners) of the healthcare worker was never tested for HIV. No other cases of transmission by bite were reported (109) leaving transmission by bite uncertain until a well-documented report in 1996 (110). Similarly, one report of female-to-male HIV transmission implicating saliva exposure during fellatio (111) was questioned (112) and the initial report was partially retracted in a follow-up by the authors, but some risk does exist (106,110,113,114). The magnitude of risk from orogenital sex is controversial (115–117). Household transmission has occurred (118,119). In summary, saliva alone has generally not been included among the body fluids posing a high risk of HIV transmission. However, saliva or other body fluids that are contaminated by blood should generally be treated in the same manner as blood itself (120).

The CDC periodically publishes tables summarizing "documented" and "suspected" cases of occupational HIV transmission within the United States. These reports highlight the diversity of jobs where workers have become infected, and some of the non-needlestick transmission routes. The actual total number of transmissions is unknown, but likely greatly exceeds these CDC tabulations. Anecdotal cases of cutaneous and mucocutaneous transmission have been reported (121) and represent acknowledged sources of risk (14).

The risk of HIV transmission is thought to be related to the mode and dose of exposure, but the data to substantiate this are sparse, especially since measures of HIV infectivity and even surrogate measures such as viral load (122) have generally not been utilized in prospective transmission studies. Specimens with high p24 antigen titers or a detectable viral load are believed to have increased infectivity (risk of transmission) (110). Host susceptibility factors to HIV and immunologic defenses remain important areas of investigation (33,38,123–131). It is also possible that some HIV genetic strains may have altered virulence. Superinfection with a genetically different HIV can occur during the course of established HIV-1 infection (128,132). These issues may help explain the lack of a clear relationship between magnitude of blood exposure and risk of transmission to workers. Nevertheless, virtually all transfusion recipients of HIV-positive blood do acquire HIV infection. Thus, at some exposure dose HIV transmission clearly becomes very likely, notwithstanding some specific form of resistance such as absence of a necessary chemokine receptor molecule (124,126).

Within an individual patient infected with HIV, the titer of HIV will vary over the course of infection, so that a given person's infectiousness likely varies, perhaps considerably, over time. In terminal AIDS patients, the HIV viral load often rises—evidenced in early studies by the re-emergence of measurable p24Ag titers. In an epidemiologic study, the CDC found that several clinical surrogate markers of dose exposure, including deep injury, visible blood on a device, a procedure involving a needle in an artery or vein, and terminal illness in a source patient (defined as disease leading to death from AIDS within two months of the exposure) were significant predictors of HIV percutaneous transmission to health care workers (70).

During the "window" before seroconversion, the plasma HIV titers, and hence the potential for infection, may be particularly high (133). This risky situation would be devoid of protection if clinical precautions were taken only for patients with known, documented infection. Some occupational exposures to blood and bodily fluids occur where the HIV status of the source patient is unknown, and many such patients may refuse HIV testing even in the setting where a worker has been exposed (134). These considerations are part of the rationale behind the concepts of "universal precautions" and "standard precautions" (135–137), and are especially important in regions where the incidence of seroconversion remains high. Policies to deal with these situations, which balance the worker's right to a safe workplace against the privacy rights of an individual, are essential for all health care facilities.

The many epidemiologic studies of occupational transmission risk provide numerators for cases of work-related acquisition and denominators for persons-exposed and specific exposures. Within these studies, persons who have had parenteral exposure have the greatest risk: roughly about 0.3% per parenteral exposure (see Table 30.1) (14,138). In other words, the vast majority of exposures do not lead to infection. Among research laboratory workers who handle highly concentrated HIV, the annual risk of HIV acquisition is about 0.3% (58). Three persons working in research laboratories were reported to have acquired HIV infection occupationally (59).

Several studies have sought to quantify level of exposure and other risks (89,139–142). Only one prospective study, by Ippolito et al. in Italy, has so far observed a risk with mucosal membrane or cutaneous HIV exposures (143). The limited number of persons in the denominator still leads to wide statistical confidence limits concerning the specific level of risk (144). A CDC summary cited a risk estimate for a mucosal membrane or cutaneous HIV exposure of 0.09%, with a 95% upper confidence bound of 0.5% while the lower bound was just 0.006% (14). This estimate is apparently based on the

single case noted above by Ippolito (1/158, 0.63%, 95% CI 0.018%–3.47%), with adjustment of the denominator with data from other studies (which were negative). The existence of multiple anecdotal cases confirms that such exposures convey some risk (142). While it can sometimes be difficult to pinpoint the precise mode of HIV acquisition (142), the evolving tools of molecular epidemiology hold some promise for verification (58) as well as elimination of suspected transmission (112). The interpretation of these research tests remains uncertain, given the newness of the techniques and applications, the complexity of HIV and its host interactions, and the very limited data from controls (confer below, and also Chapter **00**, Laboratory Diagnosis). Further complicating the picture is that coinfection by multiple HIV-1 strains can occur *in vivo* (145), and pre-existing infection does not prevent super-infection with another strain (128,132).

STANDARD PRECAUTIONS

The initial CDC recommendations called for blood and body fluid precautions when a patient was known or suspected to be infected with bloodborne pathogens (13). In August 1987 the CDC and OSHA called for "Universal Precautions" (UP) to be consistently used all the time, regardless of the apparent blood-borne infection status of a given patient (146). UP applied to blood, to body fluids that had been implicated in the transmission of blood-borne infections (i.e. semen and vaginal secretions), to body fluids from which the risk of transmission was unknown (i.e. amniotic, cerebrospinal, pericardial, peritoneal, pleural, and synovial fluids), and to any other body fluid visibly contaminated with blood. Feces, nasal secretions, sputum, sweat, tears, urine and vomitus were not included unless they were visibly contaminated with blood. At the same time (1987), a new system of isolation called Body Substance Isolation (BSI) was developed by infection control personnel in Seattle, Washington and San Diego, California, as an alternative to diagnosis-driven isolation precautions. BSI focused on the isolation of all moist body substances with the exception of tears and perspiration (120). BSI and UP shared many similar features and thus became the basis for "Standard Precautions." Standard Precautions reduces the risk of transmission of blood-borne and other pathogens. Standard Precautions applies to all body fluids and body substances with the exception of perspiration. Standard Precautions are used with all patient's body fluids/body substances in all situations where contact is, or can be, reasonably anticipated.

Since it was acknowledged that available medical and laboratory data might not always identify those persons and specimens with blood-borne pathogens, Universal and subsequently Standard Precautions in effect eliminate the "need to know" and provide broader safety and infection risk reduction for patients and healthcare workers. Implementation of Standard Precautions is currently the primary strategy for successful nosocomial infection control (120). Barrier precautions and hand washing are among the mainstays of Standard Precautions (147).

A century ago, surgical gloves were introduced to practice as part of the new antiseptic technique and originally to protect the hands of the surgeon and his assistants from the harmful dermatological effects of powerful antiseptics (e.g. carbolic acid) in use at that time (148). Since then, the wearing of gloves during surgery has been standard practice. Furthermore, the protection value of surgical gloves in preventing cross-infection has stood the test of time.

The estimated prevalence of HIV infection within the U.S., projected from mathematical models, is under one million persons (25,149,150). There is wide geographic variation in HIV seroprevalence. In some hospitals, e.g. public hospitals in San Francisco, New York City or Newark, a very high proportion of patients are infected (151). In such locations a person of unknown HIV status has a high probability of seropositivity, as highlighted by a study of emergency room patients in Baltimore (152). The incidence of new infections (community seroconversion rates) may also be sufficiently high that HIV seronegativity cannot be relied upon to rule out HIV infection. The implementation of "Standard Precautions" for blood, body fluids, and body substances, once controversial in such settings, is now commonly used and well accepted. Nevertheless, many professionals still express the desire to "know" their patient's status.

Universal and Standard Precautions certainly do not have perfect efficacy in removing all risks (140,153–163). Gloves are not expected to prevent needlestick or other penetrating sharps injuries (164–168). However, gloves may exert some protective effect (169). In a study of simulated needlestick injury, glove material reduced the transferred blood volume by 46% to 86% in two models (169). After standard pre-operative hand preparation, glove perforations were found to be of no clinical significance to the patient, but the high incidence of perforations suggested that a main indication for preoperative change of damaged gloves was for protection of surgeons against pathogens transmissible during surgery (170). These data lend further support to the recommendation that gloves be worn whenever sharps are to be handled.

Since gloves can tear (168), and breaks may not even be recognized by the worker, hand washing should be routinely performed whenever gloves are removed (147). The risk of glove perforation during surgery may be higher than generally appreciated (171,172). The quality of disposable (and of sterile) gloves can be variable (173–176). Latex gloves appear preferable to poly-vinylchloride, since the rates of perforation in latex gloves are generally lower than those made from poly-vinylchloride and latex gloves tend to fit better, improving tactile sensation and reducing injury risks (154,165,173,177–180). It has been suggested that increased government

supervision may be needed world-wide in monitoring the quality of surgical gloves (181), beyond that of current FDA approval (182).

Data suggest that the powder used in gloves may increase bacterial environmental contamination. In animal models, corn starch (a material used as a glove powder) promotes wound infection (183). Talc also may elicit tissue toxicity (184,185). As discussed below, concerns related to latex allergy have further led to promotion of powder-free gloves. Infection control teams should support switching from use of powdered to powder free gloves.

Gloves may reduce dexterity and thereby increase the risk of percutaneous injury (186). In one study of dentists, latex glove wear was associated with a 16-fold increase in percutaneous injury with endodontic Þle manipulation compared to when the same group was tested barehanded. Dynamometer tests showed a 36% decrease in light-touch proprioception when the dentists were gloved as compared to scores when they were tested barehanded. In a study of surgeons, statistically signiÞcant differences in moving two-point discrimination for the dominant hand index Þnger were found for no gloves versus double gloves ($p = 0.05$) and single versus double gloves ($p = 0.02$) (187).

The advent of newer gloves which can provide an excellent Þt and improved dexterity compared to products in the past, such as some current nitrile, polyurethane and other polymer gloves, may now offer possible alternatives to latex (180,188Ð190).

During a surgical operation when an overt sharps injury occurs, the aseptic barrier is broken and except under emergency circumstances the injured worker leaves the operating Þeld and returns upon the re-establishment of asepsis. Thus, the primary risk in these circumstances is generally from patient to worker (170). Inapparent barrier breakdown, however, appears to be common (77,191). If a single glove is worn, surveys have found frequent gross contamination of the hands (192). With double gloving, such gross contamination is greatly reduced (192), which led to the suggestion of double gloving for surgeons (193). Some surgeons went further, including a thin cloth glove between the two latex gloves, in an effort to impede minor needlestick injuries. The problem with double gloving can be a loss of dexterity. A study of vascular surgeons recommended against double gloving, Þnding that the loss of dexterity led to an actual increase in glove perforation rates for the operating room surgeon and scrub nurse (194). Similarly, an analysis of the routine dental treatment of HIV-infected patients concluded that there was unlikely any signiÞcant beneÞt from the use of a double-gloving technique, or of a glove perforation detection system (195). Other analyses have favored double gloving, particularly with deep surgical procedures (172,196,197). Further complicating the picture are studies in which methodological problems and biases may exist (193). In summary, the relative merits of double gloving are controversial, so that recommendations will continue to need to be adapted to the particular specialty and speciÞc procedures based upon available evidence.

One study found that over half of needlestick injuries occurred to the index Þnger of the non-dominant hand (83). Thus, the addition of reinforcement to this portion of the glove or the routine use of a thimble-equivalent could dramatically reduce occupational exposures. Various safety devices to guard against needlesticks and sharps injuries have been marketed (198,199). Although these can add signiÞcant costs to the workplace, this is offset by risk reduction, and the unit costs may be reduced with increased use and production efÞciencies of scale. Training in the use of all new devices is essential. The Final Rule from OSHA in 2001 detailed some of the dilemmas and costs (200).

The Needlestick Safety and Prevention Act of 2000 and the 2001 revised Blood-borne Pathogens Standard require health care facilities to maintain a sharps injury log. The log must include, at a minimum, the type and brand of device involved in the exposure incident, the department where the exposure occurred, and an explanation of how it occurred. Among the proprietary systems developed for such purposes is the Exposure Prevention Information Network (EPINet[a]), which was developed by Dr. Janine Jagger and colleagues in 1991 to provide standardized methods for recording and tracking percutaneous injuries and blood and body ßuid contacts (http://hsc.virginia.edu/medcntr/centers/epinet/). The EPINet[a] system consists of a series of report forms and software for entering and analyzing the data from the forms, and has been utilized by a wide variety of institutions. Data from EPINet[a] and other sources have been used to estimate the number of annual percutaneous injuries in the U.S. (201). Although the extent of reduction in needlestick injury rates with initial protective devices had been questioned (202), beneÞts have since been establishedÑ although cost-efÞcacy issues remain (203,204). With increasing production of devices and expected concomitant reduction in unit cost, the cost-beneÞt balance can be expected to improve further.

Healthcare workers are at greatest risk of exposure to blood-borne pathogens when handling contaminated sharps. More than half a million sharps related injuries occur each year, according to OSHA.

The International Healthcare Worker Safety Center of the University of Virginia estimates that an average of 30 percutaneous and blood and body ßuid exposures occur per 100 occupied beds each year. It is estimated that 39% of all exposure injuries are never reported. Annually, this could potentially cause 18 to 35 new cases of HIV infection, about 400 new cases of hepatitis B infection, and 39 to 1,967 new cases of hepatitis C infection. It is commonly accepted, based on evolving studies, that sharps safety devices signiÞcantly reduce the risk of injury during procedures. Studies have demonstrated that safer intravenous systems have reduced needlesticks associated with

piggy-back tubing and intravenous medication administration (87). Needlestick injuries which carry the highest risk of HIV, HBV and HCV transmission are those associated with the hollow-bore needles used in intravenous catheters, phlebotomy, and injecting medications; further evaluation of the efficacy of risk reduction and safety improvement are needed (87). It should be noted that for a few safety devices, because of design flaws, the risk of injuries increased – for example, safety features that are clumsy or difficult (and therefore never activated by the worker). Thus, implementing the use of safer sharps products alone will not eliminate all blood and body fluid exposures. Safer work practices much be reinforced in all work areas.

Safety devices include needleless systems, engineered protective devices for needles and other sharps as well as breakage resistant capillary tubes to decrease exposure. General guidelines for sharps safety rules are as follows: (1) use of a safety needle device or needleless system for withdrawal of body fluids, accessing a vein or artery or administering medications or fluids; (2) use of either a needleless system or a needle with engineered sharps protection for any other procedure requiring needle devices when available; and (3) use of a non-needle sharps with engineered sharps protection when available. When using sharps, always follow effective, safe handling techniques to prevent injury. Bending, breaking, manipulating, shearing or recapping contaminated needles or sharps are prohibited, except in specific procedures when recapping is procedurally required and then only a resheathing recapping device or a one-handed "scoop" method may be used.

Disposable sharps are not to be reused. Contaminated broken glass should always be picked up using a broom and dustpan, forceps or tongs and never ones' hands. Sharps safety includes discarding contaminated sharps immediately or at "point-of-use" in an appropriate, puncture-resistant, color-coded container. NIOSH suggests this risk can be decreased by placing sharps containers within easy reach – slightly below eye level, and not allowing containers to overfill. These safe work practices may reduce needlestick or sharps exposure.

Continued refinements and improvements towards injury reduction, which can protect both patient and worker, include safer work practices, engineering controls, improvement of the design and organization of jobs and tasks, and facilitation and reinforcement of safer behavior by institutions (161). Sharps injuries and fluid splashes will not be eliminated by simply buying and using sharps with engineered safety protection. Many types of injury occur during use and will not be prevented by safety mechanisms. There will also remain unpredictable elements both within the facility and from patients. The system envisioned under the OSHA regulations seeks to minimize the risks by systematic safer work practices and safer devices. All of this needs to be documented and evaluated for efficacy; the safety logs mandated by OSHA will be valuable in ongoing assessments by individual facilities and in research studies.

Latex Sensitivities and Allergies

Exposure to latex and latex products has greatly increased over the last two decades. With the advent of universal precautions, latex glove usage greatly increased. Standard precautions has maintained that practice for bloodborne pathogens, but has further expanded the overall use of gloves to include contact with any body fluid or body substance (except for perspiration). Other exposures to latex products have also significantly increased in the population as a whole over this time period, including the use of condoms for safer sex (205–207).

There has emerged a recognition of latex-related allergic phenomena (208–215). A wide variety of products contain latex: medical supplies, personal protective equipment (PPE), and numerous household objects (214). A recent study by the National Institute for Occupational Safety and Health found no significant difference in work-related symptoms between latex sensitized and non-sensitized workers (216). Environmental concentrations of latex were higher in the work areas of the non-sensitized workers, and higher in the clinical than in the non-clinical areas. Occupational latex glove use was judged not to be a risk factor for latex sensitization (216).

Within health care facilities, powdered latex gloves represent one of the largest sources of latex antigen. Glove powder (cornstarch) can function as a carrier for latex allergens (217,218); antigens may be carried in association with the powder and liberated into the air when gloves are applied or removed (211). Once airborne, the antigen may be inhaled or come into contact with mucous membranes. Powder-free gloves, with reduced latex protein content, are thus now preferable if using latex. Significant reductions in the airborne latex antigen can be accomplished by using low-allergen-containing latex gloves or latex-free gloves (such as polyurethane or nitrile gloves). Low-allergen latex gloves are now comparable in price to high-allergen-containing latex gloves. Cost remains a major concern of institutions when moving to latex-free gloves. Some workers complain of increased sweating when using nitrile gloves, with attendant personal complaints. Also, tactile sensation may be better with latex gloves, and thus may reduce the risk of sharps injuries. Thus, when dealing with possibly infectious fluids or materials, low-allergen latex gloves are often still recommended as the preferred product.

It has been recommended that high-allergen-containing latex gloves be replaced, at the least, in places such as the operating room, to protect both patients and health care workers but primarily to reduce exposure in general and thereby reduce the incidence of latex allergies. These and other efforts, such as frequent cleaning of ventilation filters and vacuum bags (which attract such particles) and

frequent cleaning of areas contaminated with latex (such as upholstery, carpets, ventilation ducts, and plenums) to reduce the amount of allergen-laden dust in the environment should be employed when possible (214).

Education of personnel is important. All workers should avoid using oil-based hand creams when wearing latex gloves, since these can cause glove deterioration and increase latex sensitization. For activities that are not likely to involve contact with infectious materials, such as in food preparation, routine housekeeping, maintenance, and so on, non-latex gloves are preferred in the workplace.

OSHA currently requires that the employer provide PPE in appropriate sizes and accessible locations (219). In addition, "hypoallergenic" gloves, glove liners, powderless gloves, or other similar alternatives must now be readily available and accessible at no cost to those employees who are allergic to the gloves normally provided (219). (The FDA, as of September 30, 1998, prohibited products that contain natural rubber from being labeled as "hypoallergic.") It has been suggested that it may be beneficial for a worker with latex sensitivity to work during the earliest part of the day, before workplace antigen levels begin to rise higher. Workers with true immediate hypersensitivity-type reactions to latex should avoid exposure to latex, and may need to be reassigned to other roles in the health care facility. Workers with latex sensitivities may also already have, or develop, cross-sensitization with other substances (212). Cross-reactivities may lead to impaired specificity in diagnostic testing when trying to determine specific allergic propensities.

Although life-threatening anaphylaxis has occurred in patients invasively exposed to latex (220,221), no deaths attributable to latex exposure have occurred among health care workers. This may in part reflect the significant exposure differences between an invasive procedure and routine occupational exposure with respect to route and dose. A recent review concluded that "increased risk of (latex) sensitization was not clearly associated with the duration of work in health care, the time spent wearing latex gloves, the frequency of exposure, the specific job categories, the use of powdered versus non-powdered latex gloves, the use of latex versus non-latex gloves, or any measurements of ambient exposure to latex proteins. The epidemiologic studies do not support a conclusion that health care workers are at clearly increased risk of latex sensitization and type I allergies compared to other occupations in the United States" (222). Others have argued that workers are at risk (223,224). Further epidemiologic studies, especially prospective studies, are needed that "include measured exposures to latex antigens, that compare health care workers to appropriate referent groups, and that address confounding by atopy, age, sex and race" (222) and avoid methodological biases.

These matters raise issues in terms of workplace regulations, modifications of the environment, and the cost-benefit analyses of universal precautions. Further studies concerning risk reduction, efficacy, and economic assessments also will aid in continuing the development, implementation and assessment of policies.

Compliance and Costs

Universal precautions are expensive, both for materials (such as gloves) as well as the educational effort required. Studies that have assessed efficacy are mixed in their results (77,137,160–162,191,225–233), and the effort may not be cost effective (202,234). Compliance correlates with the perception of risk and extent of knowledge. Adherence to guidelines may be greater when employee compliance is monitored. Institutions may find it useful to work with employee unions to effect meaningful changes that enhance workplace safety.

With the first U.S. documentation of occupational transmission of HIV to a healthcare worker (a woman in a residency program), it was recommended that attention to structured education was important to reduce the risks (21). Other studies have since clearly delineated the significant risks to students and others in training (89,235–243). The need to strengthen our prevention efforts remains (74,162). Enhanced attention to education of those at risk, and in particular those with limited prior experience, is of the utmost importance (21,162).

POST-HIV EXPOSURE PROPHYLAXIS— A HISTORICAL INTRODUCTION

The time period immediately following an occupational exposure is a difficult one for both the exposed worker and his physician or occupational health service. The low statistical risk of acquisition of a deadly infection provides limited comfort. It is helpful to have a comprehensive program in place to deal with the anticipated accidental exposures (53,244,245).

Early on, researchers pondered the potential value of treatment in the event of a significant exposure event. In a multi-institutional prospective study initiated in 1985 by investigators at the National Institutes of Health of workers at high risk for HIV occupational exposure, consideration was given to post-exposure prophylaxis in the very trial design, at a time when all therapies were strictly experimental (58) (and S. H. Weiss, unpublished data).

Around the time of FDA approval of the first antiretroviral agent for treatment of HIV, zidovudine (246), the manufacturer (then the Burroughs-Welcome Company) sponsored a randomized trial offering zidovudine to workers exposed to HIV (247). Given the low risk of seroconversion, it was unlikely that a sufficient sample size could ever have been accrued for this clinical trial to prove efficacy. Furthermore, the ready availability of the

approved drug, worker apprehensions, and the delay in drug access through the trial led few to enroll. The trial was abandoned after very limited enrollment. A decade later, a new effort to enroll exposed workers so as to monitor for possible drug toxicity was undertaken (the HIV Post Exposure Prophylaxis Registry) with much fanfare, but it had no greater success at enrollment, and was abandoned without having accomplished its primary objective (248).

Evidence from animal studies suggesting that zidovudine might be useful (249–251) prompted the USPHS to formulate guidelines concerning post-exposure prophylaxis (252). Zidovudine does have some toxicity in this setting, sometimes severe (253–256). Data, on otherwise healthy workers given a limited course of zidovudine, found a worker whose neutrophil count declined to 500/mm^3 (257), a level at which serious infections might occur. Due to considerable side effects, a very high proportion of workers discontinue zidovudine prophylaxis even in the context of research studies (255). There is also concern about the potential for delayed mutagenicity or carcinogenicity (258). Embryonic toxicity in mice at high zidovudine doses has been demonstrated (259). The initial USPHS judgment was that it could "not make a recommendation for or against the use of zidovudine for this (post-exposure prophylaxis) purpose because of the limitations of current knowledge" (252). Nucleoside analog drugs are known to induce mitochondrial dysfunction. Concerns for toxicity exist for other anti-retroviral drugs as well (258).

In the absence of demonstration of high clinical efficacy in the post-exposure setting, some clinicians have questioned the wisdom of administration. Since some laboratory data suggest that administration of zidovudine within an hour or two may be requisite to achieve a substantial likelihood of prophylactic efficacy (260), mechanisms for rapid decision making and initiation of therapy are important if zidovudine or other anti-retroviral therapy is to be given at all.

A decision analysis study concerning the issue of post-exposure prophylaxis (PEP) for needlestick exposures to HIV concluded that even a very low zidovudine efficacy (of 3–8%) might warrant its use (261). On the other hand, subsequent laboratory data suggest a limitation to its usefulness (260,262,263). Zidovudine benefits do not clearly outweigh the risks after exposure to blood of unknown serologic status, or if there is a delay in starting therapy (261,264–266). Furthermore, multiple documented instances of seroconversion in which prophylactic zidovudine was given, and sometimes initiated extremely rapidly, demonstrate it is not 100% efficacious (141,267–275,275–278).

In December 2000, two instances of life-threatening hepatotoxicity were published concerning health-care workers in Chicago who had been taking nevirapine (NVP) for postexposure prophylaxis after occupational HIV exposure (279,280). In one case, a 43 year old female healthcare worker required liver transplantation after developing fulminant hepatitis and end-stage hepatic failure while taking NVP, zidovudine, and lamivudine as PEP following a needlestick injury. In the second case, a 38 year old male physician was hospitalized with life-threatening fulminant hepatitis while taking NVP, zidovudine, and lamivudine as PEP following a mucous membrane exposure.

Based on these anecdotal case reports, the CDC and FDA reviewed MedWatch reports of serious adverse events in persons taking NVP for postexposure prophylaxis received by FDA to better characterize NVP-associated postexposure prophylaxis toxicity (281). (MedWatch, under the aegis of the FDA, **passively** accrues toxicity data as part of routine post-licensure and marketing surveillance.) Including the two case reports of fulminant hepatitis, FDA had received reports of 22 cases of serious adverse events related to NVP taken for postexposure prophylaxis from March 1997 through September 2000. These 22 events included hepatotoxicity (12), skin reaction (14), and rhabdomyolysis (one); four cases involved both hepatotoxicity and skin reaction, and one case involved both rhabdomyolysis and skin reaction. In the CDC HIV postexposure prophylaxis registry, which collected data on occupational HIV postexposure prophylaxis use from October 1995 through March 1999, six cases of serious adverse events related to postexposure prophylaxis were found retrospectively among 492 registered participants; a severe skin reaction occurred in one of 11 health-care workers taking a regimen that included NVP.

Their analysis indicated that healthy persons taking abbreviated four-week NVP regimens for postexposure prophylaxis were at risk for serious adverse events. Because most occupational HIV exposures do not result in transmission of HIV, the USPHS recommended that clinicians considering prescribing postexposure prophylaxis for exposed persons must balance the risk for HIV transmission represented by the exposure and the exposure source against the potential toxicity of the specific agent(s) used. When postexposure prophylaxis is prescribed, the manufacturer's package insert should be consulted for dosing instructions, possible side effects, and potential drug interactions. In many circumstances, the risks associated with NVP as part of a postexposure prophylaxis regimen outweighed the anticipated benefits (281).

Thus, whether to administer zidovudine or other post-exposure therapy depends upon the individual circumstances, including the nature of the exposure, documentation of the likelihood that the source was infected with HIV and any surrogate measures of infective titer, the risk-taking perceptions of the exposed worker, and the availability of alternative experimental therapies. The issues of pregnancy and contraception are also relevant related to safety issues. Some workers may reasonably decide to "take their chances." Others who have a particularly high risk of seroconversion (e.g. an

inoculation with a substantial amount of blood) might decide to try experimental combination chemotherapy in consultation with an infectious disease expert, rather than zidovudine alone. These issues were addressed in part by provisional guidelines released in 1996 (282), although variations in approach are appropriately being debated, as discussed further below (255,256,283–286).

POST-EXPOSURE PROPHYLAXIS—CURRENT GUIDELINES

In late 1995, the CDC reported in summary form in *MMWR* an analysis of 31 selected healthcare workers who seroconverted to HIV as a consequence of well-documented occupational exposures (278). A full report, extended to 33 seroconverters, was published in 1997 (70). These data on seroconverters include critical details that were collected only retrospectively. These data were then compared with prospectively collected data from 665 (679 in *MMWR*) healthcare workers who had participated in CDC studies of occupational exposure but who were not known to have seroconverted ("controls"). Thus, although case-control in design, the sources and types of bias in the two study groups (seroconverters and "controls") differ.

The results were consistent with an increased risk of HIV transmission from exposure events where high viral load exposure was likely, such as visible contamination of a device with an HIV-infected patient's blood, deep injury, or the patient being terminally ill.

In the univariate analyses (in Cardo's Table 1), there was no evidence of protection against seroconversion by prophylaxis with zidovudine (95% confidence interval for the odds ratio was 0.3 to 1.4, p = not significant). In contrast, in a multivariate model zidovudine prophylaxis was statistically significant in terms of reduction in risk (70). This unusual and surprising biostatistical situation sometimes can arise where statistical confounding exists.

However, this multivariate model included many variables (five) despite only a limited number of outcomes (33 seroconverters). In such situations, the robustness of the multivariate model is highly questionable from a statistical viewpoint. As pointed out by Concato and colleagues in a 1993 general biostatistics critique, "risk estimates may be unreliable if the multivariable data contain too few outcome events relative to the number of independent variables" (287). Multivariate methods of analysis can yield problematic results when methodological guidelines and mathematical assumptions are ignored (288). "In general, the results of models having fewer than 10 outcome events per independent variable are thought to have questionable accuracy and the usual tests of statistical significance may be invalid" (287). This guideline would permit a maximum of only three variables in Cardo's logistic regression model. Simulation work concerning the number of events per variable in logistic regression analysis have confirmed these guidelines (288–293). "The key issue in the overfitting is an ample number of outcome events, not just a large sample size. When numerous variables are included in an attempt to "control" or "adjust" the data, accuracy of results can be threatened by overfitting or by other mechanisms. The number of variables selected for analysis should therefore be parsimonious, based on clinical sensibility and suitable data quality" (287).

Although the report by Cardo et al. has been widely cited as demonstrating that zidovudine post-exposure prophylaxis is 79% efficacious and as a key justification for its routine use, these claims in fact represent a gross overstatement of our current knowledge base. There is no convincing evidence for any protection at all, and the claim for protection resides only in an over-analysis that is questionable statistically. It must be noted that the absence of proof that prophylaxis provides protection does *not* rule out the possibility that there may be some protection. But, all post-exposure prophylaxis policy recommendations are best considered tentative. Thus, as the USPHS guidelines presented in Tables 30.2 and 30.3 presume efficacy of post-exposure prophylaxis, there is adequate reason to consider alternative guidelines.

Given the widespread use of zidovudine and, more recently, other therapies among patients, the issue of primary resistance to zidovudine or to combination chemotherapy regimens is a valid concern (294). Indeed, multi-drug clinical trials have demonstrated increasingly subsets of patients with primary HIV infection in whom underlying zidovudine as well as other anti-retroviral drug resistance exists (295). This phenomenon may even have somewhat limited the ability to detect postexposure prophylaxis protection from zidovudine in the Cardo study, and supports the theoretical basis for using combination therapy in this setting.

In light of the expectation that HIV strains resistant to combination therapy would arise (296), it was predictable that a case would eventually be reported in a prominent journal of HIV transmission to a healthcare worker despite full and prompt recommended "prophylaxis" (14,297,297,298). However, anecdotal case reports do not necessarily undermine the concept. They serve as a reminder of the necessity of continually reassessing guidelines and of the complexity of HIV and of the pandemic. Indeed, genotypic analysis of HIV-1 isolates to identify antiretroviral resistance mutations from source patients involved in health care worker occupational exposures has been proposed (299). In one small study, the majority of HIV-exposed workers who remained virologically and serologically negative demonstrated transient cellular immune responses to HIV-specific peptides in temporal association with their HIV exposures (32,123). As these responses may have been to non-infectious particles, such data do not prove widespread transient infection, but merely demonstrate the mounting of an immunologic response. Others have reported finding autoantibodies against CD4 significantly before sero-conversion (300). A

TABLE 30.2. *The 2001 update of the U.S. Public Health Service recommendations for basic and expanded HIV postexposure prophylaxis (PEP) regimens*

1. BASIC REGIMEN
   - Zidovudine (RETROVIR™; ZDV; AZT) + Lamivudine (EPIVIR™; 3TC); available as COMBIVIR™
   – ZDV: 600 mg per day, in two or three divided doses, and
   – 3TC: 150 mg twice daily.

   Advantages
   – ZDV is associated with decreased risk of HIV transmission in the CDC case-control study of occupational HIV infection.
   – ZDV has been used more than the other drugs for PEP in HCP.
   – Serious toxicity is rare when used for PEP.
   – Side effects are predictable and manageable with antimotility and antiemetic agents.
   – Probably a safe regimen for pregnant HCP.
   – Can be given as a single tablet (COMBIVIR™) twice daily.

   Disadvantages
   – Side effects are common and might result in low adherence.
   – Source patient virus might have resistance to this regimen.
   – Potential for delayed toxicity (oncogenic/teratogenic) is unknown.

2. ALTERNATE BASIC REGIMENS
   - Lamivudine (3TC) + Stavudine (ZERIT™; d4T)
   – 3TC: 150 mg twice daily, and
   – d4T: 40 mg (if body weight is < 60 kg, 30 mg twice daily) twice daily.

   Advantages
   – well tolerated in patients with HIV infection, resulting in good adherence,
   – serious toxicity appears to be rare, and
   – twice daily dosing might improve adherence.

   Disadvantages
   – Source patient virus might be resistant to this regimen.
   – Potential for delayed toxicity (oncogenic/teratogenic) is unknown.
   - Didanosine (VIDEX™, chewable/dispersible buffered tablet; VIDEX™ EC, delayed-release capsule; ddI) + Stavudine (d4T)
   – ddI: 400 mg (if body weight is < 60 kg, 125 mg twice daily) daily, on an empty stomach.
   – d4T: 40 mg (if body weight is < 60 kg, 30 mg twice daily) twice daily.

   Advantages
   – Likely to be effective against HIV strains from source patients who are taking ZDV and 3TC.

   Disadvantages
   – ddI is dif cult to administer and unpalatable.
   – Chewable/dispersible buffered tablet formulation of ddI interferes with absorption of some drugs (e.g. quinolone antibiotics, and indinavir).
   – Serious toxicity (e.g. neuropathy, pancreatitis, or hepatitis) can occur. Fatal and nonfatal pancreatitis has occurred in HIV-positive, treatment-naive patients. Patients taking ddI and d4T should be carefully assessed and closely monitored for pancreatitis, lactic acidosis, and hepatitis.
   – Side effects are common; anticipate diarrhea and low adherence.
   – Potential for delayed toxicity (oncogenic/teratogenic) is unknown.

3. EXPANDED REGIMEN
   Basic regimen plus *one* of the following:
   - Indinavir (CRIXIVAN™; IDV)
   – 800 mg every eight hours, on an empty stomach.

   Advantages
   – Potent HIV inhibitor.

   Disadvantages
   – Serious toxicity (e.g. nephrolithiasis) can occur; must take eight glasses of uid per day.
   – Hyperbilirubinemia common; must avoid this drug during late pregnancy.
   – Requires acid for absorption and cannot be taken simultaneously with ddI in chewable/dispersible buffered tablet formulation (doses must be separated by at least one hour).
   – Concomitant use of astemizole, terfenadine, dihydroergotamine, ergotamine, ergonovine, methylergonovine, rifampin, cisapride, St. John's Wort, lovastatin, simvastatin, pimozide, midazolam, or triazolam is not recommended.
   – Potential for delayed toxicity (oncogenic/teratogenic) is unknown.

TABLE 30.2. *Continued.*

- Nel navir (VIRACEPT™; NFV)
  - 750 mg three times daily, with meals or snack, or
  - 1250 mg twice daily, with meals or snack.

Advantages
- potent HIV inhibitor, and
- twice dosing per day might improve adherence.

Disadvantages
- Concomitant use of astemizole, terfenadine, dihydroergotamine, ergotamine, ergonovine, methylergonovine, rifampin, cisapride, St. John's Wort, lovastatin, simvastatin, pimozide, midazolam, or triazolam is not recommended.
- Might accelerate the clearance of certain drugs, including oral contraceptives (requiring alternative or additional contraceptive measures for women taking these drugs).
- Potential for delayed toxicity (oncogenic/teratogenic) is unknown.

- Efavirenz (SUSTIVA™; EFV)
  - 600 mg daily, at bedtime.

Advantages
- Does not require phosphorylation before activation and might be active earlier than other antiretroviral agents (note: this might be only a theoretical advantage of no clinical bene t.)
- One dose daily might improve adherence.

Disadvantages
- Drug is associated with rash (early onset) that can be severe and might rarely progress to Stevens-Johnson syndrome.
- Differentiating between early drug-associated rash and acute seroconversion can be dif cult and cause extraordinary concern for the exposed person.
- Nervous system side effects (e.g. dizziness, somnolence, insomnia, and/or abnormal dreaming) are common. Severe psychiatric symptoms are possible (dosing before bedtime might minimize these side effects).
- Should not be used during pregnancy because of concerns about teratogenicity.
- Concomitant use of astemizole, cisapride, midazolam, triazolam, ergot derivatives, or St. John's Wort is not recommended because inhibition of the metabolism of these drugs could create the potential for serious and/or life-threatening adverse events (e.g. cardiac arrhythmias, prolonged sedation, or respiratory depression).
- Potential for oncogenic toxicity is unknown.

- Abacavir (ZIAGEN™; ABC); available as TRIZIVIR™, a combination of ZDV, 3TC, and ABC
  - 300 mg twice daily.

Advantages
- potent HIV inhibitor, and
- well tolerated in patients with HIV infection.

Disadvantages
- Severe hypersensitivity reactions can occur, usually within the rst six weeks of treatment.
- Potential for delayed toxicity (oncogenic/teratogenic) is unknown.

4. ANTIRETROVIRAL AGENTS FOR USE AS PEP ONLY WITH EXPERT CONSULTATION
   - Ritonavir (NORVIR™; RTV)

Disadvantages
- dif cult to take (requires dose escalation),
- poor tolerability, and
- many drug interactions.

- Saquinavir (FORTOVASE™, soft-gel formulation; SQV)

Disadvantages
- Bioavailability is relatively poor, even with new formulation.

- Amprenavir (AGENERASE™; AMP)

Disadvantages
- Dosage consists of eight large pills taken twice daily.
- Many drug interactions.

- Delavirdine (RESCRIPTOR™; DLV)

Disadvantages
- Drug is associated with rash (early onset) that can be severe and progress to Stevens-Johnson syndrome.
- Many drug interactions.

TABLE 30.2. *Continued.*

- Lopinavir/Ritonavir (KALETRA™)
  – 400/100 mg twice daily.

Advantages
– potent HIV inhibitor, and
– well tolerated in patients with HIV infection.

Disadvantages
– Concomitant use of ecainide, propafenone, astemizole, terfenadine, dihydroergotamine, ergotamine, ergonovine, methylergonovine, rifampin, cisapride, St. John's Wort, lovastatin, simvastatin, pimozide, midazolam, or triazolam is not recommended because inhibition of the metabolism of these drugs could create the potential for serious and/or life-threatening adverse events (e.g. cardiac arrhythmias, prolonged sedation, or respiratory depression).
– May accelerate the clearance of certain drugs, including oral contraceptives (requiring alternative or additional contraceptive measures for women taking these drugs).
– Potential for delayed toxicity (oncogenic/teratogenic) is unknown.

5. ANTIRETROVIRAL AGENTS GENERALLY NOT RECOMMENDED FOR USE AS PEP
   - Nevirapine (VIRAMUNE™; NVP)
     – 200 mg daily for two weeks, then 200 mg twice daily.

   Disadvantages
   – Associated with severe hepatotoxicity (including at least one case of liver failure requiring liver transplantation in an exposed person taking PEP),
   – Associated with rash (early onset) that can be severe and progress to Stevens-Johnson syndrome,
   – Differentiating between early drug-associated rash and acute seroconversion can be dif cult and cause extraordinary concern for the exposed person, and
   – Concomitant use of St. John's Wort is not recommended because this might result in suboptimal antiretroviral drug concentrations.

speculative possibility is that low level, active replication (infection) does occur in some instances and that cellular immune responses play a role in controlling this initial, early infection Ðto the point of eradication or to inducing latency (with residual, unmeasurable infection). The rare cases of delayed seroconversion might then reßect instances of delayed escape from such control.

If active replication occurs in a sizeable proportion of those who do not seroconvert, the rationale for control by aggressive treatment with multiple anti-retroviral drugs is enhanced; the object of this being to reduce viral load at the earliest possible stage and maximize the chances for immune control. The tradeoff is treating many persons with potentially toxic drugs who do not need them (since most workers do not seroconvert) versus waiting, with close monitoring, until some sign or symptom of HIV infection (either clinical or virologic) occurs before initiating treatment. In the absence of animal studies that demonstrate the efÞcacy of therapy for protection in the post-exposure situation, if not human clinical trials, post-exposure therapy must still be considered experimental.

The CDC developed an interagency working group to explore these issues, culminating in guidelines concerning chemoprophylaxis (282) that tried to characterize degree of risk, and stratify suggested prophylaxis into Òrecommend,ÓÒoffer,Óand Ònot offer.ÓKey aspects to consider in assessing an exposure are summarized in Table 30.4.

In 2001, the USPHS guidelines were further revised (14). The number of drugs to be offered or recommended varies with the circumstances (Table 30.3). In the highest risk scenarios, where the expected risk of seroconversion can be far greater than the overall summary estimate, there is clearly strong theoretical support for intervention with multi-drug therapy. It is likely that the provisional guidelines in Tables 30.2 and 30.3 will continue to be modiÞed periodically, so the sources discussed below should be routinely consulted for updates in conjunction with experts in the Þeld.

Concordant with the above guidelines, some clinics, ofÞces and hospitals stock these drugs for potential immediate use in postexposure prophylaxis and others arrange for emergency prescriptions to be Þlled. It is not widely recognized that the labeling for these drugs in the FDA approved package inserts has not included post-exposure prophylaxis as one of the Òapproved indications.Ó (For example, Merck Òdoes not recommend the use of its products in any manner other than described in the prescribing informationÓ(Personal communication, David Horn, M.D., Merck Director of Medical Services, November 5, 1996).) The joint FDA and USPHS promulgation of postexposure therapy is a notable (and perhaps a sole) exception to standard FDA policies regarding promulgation of off-label uses of prescription pharmaceuticals.

One of the remaining difÞculties is the absence of toxicity and safety information for these regimens among healthy adults. An anonymous registry set up to assess postexposure prophylaxis drug use and toxicity closed in

TABLE 30.3. *Recommended HIV postexposure prophylaxis for percutaneous injuries (from the CDC, 2001 (14))*

	Infection status of source				
Exposure type	HIV-Positive Class 1*	HIV-Positive Class 2*	Source of unknown HIV status†[1]	Unknown source	HIV-Negative
Less severe[1]	Recommend basic 2-drug PEP	Recommend expanded 3-drug PEP	Generally, no PEP warranted; however, consider basic 2-drug PEP** for source with HIV risk factors	Generally, no pEP warranted, however, consider basic 2-drug PEP** in settings where exposure to HIV-infected persons is likely	No PEP warranted
More severe	Recommend expanded 3-drug PEP	Recommend expanded 3-drug PEP	Generally, no PEP warranted; however, consider basic 2-drug PEP** for source with HIV risk factors	Generally, no PEP warranted; however, consider basic 2-drug PEP** in settings where exposure to HIV-infected persons is likely	No PEP warranted

* HIV-Positive, Class 1—asymptomatic HIV infection or known low viral load (e.g. < 1,500 RNA copies/mL). HIV-Positive, Class 2—symptomatic HIV infection, AIDS, acute seroconversion, or known high viral load. If drug resistance is a concern, obtain expert consultation. Initiation or postexposure prophylaxis (PEP) should not be delayed pending expert consultation, and, because expert consultation alone cannot substitute for face-to-face counseling, resources should be available to provide immediate evaluation and follow-up care for all exposures.
? Source of unknown HIV status (e.g. deceased source person with no samples available for HIV testing).
? Unknown source (e.g. needle from a sharps disposal container).
[1] Less severe (e.g. solid needle and super cial injury).
** The designation "consider PEP" indicates that PEP is optional and should be based on an individual decision between the exposed person and the treating clinician.
?? If PEP is offered and taken and the source is later determined to be HIV-negative, PEP should be discontinued.
?? More severe (e.g. large-bore hollow needle, deep puncture, visible blood on device, or needle used in patient's artery or vein).

1999 (248). That registry was largely a failure due to a paucity of participants, which biases results from a potentially skewed sample and provided insuf Þcient statistical power for many types of analyses. Although the CDCÕ9 World Wide Web home page on the Internet was expected to carry updated information so as to broadly disseminate results of this monitoring, this never came to fruition. The CDC site (http://www.cdc.gov) can also be consulted for updates on USPHS guidelines. As with any drug, any unusual or severe toxicity should also be reported to the respective manufacturers as well as the Food and Drug Administration (800-332-1088).

The issue of post-exposure prophylaxis for non-occupational HIV exposure has also been raised, e.g. after a sexual exposure or an inadvertent transfusion of contaminated product. Although the published guidelines speci Þcally refrain from extension of these recommendations to other types of exposure, study and recommendations are warranted.

All exposed persons must be counseled regarding the risks of transmission to others, particularly sexual partners, and the need for follow-up past the seronegative window. For those persons who unfortunately acquire HIV infection, the period just prior to the appearance of antibodies (i.e. their blood test is misleadingly still negative) is likely to be a time of particularly high circulating viral loadÑ conveying a high risk of transmission to others through the conventional HIV transmission routes (122).

RISKS TO OUR PATIENTS?

There have been a limited number of clusters of HBV transmission from dentists and surgeons to patients (301), including very rare instances of recurrent HBV transmission despite attempts to improve the professionalÕ9 infection control techniques. Such risks from professionals to patients were not perceived as a major issue, as no such clusters of HBV transmission were reported from 1987 through 1991.

The 1989 provisional guidelines published by the Occupational Safety and Health Administration (OSHA) are oriented towards the protection of both worker and patient (302). However, injuries remained commonplace (77,303). The cumulative HIV risk to some providers, such as trauma and orthopedic surgeons in regions of high HIV prevalence, may be substantial. In summary, the cumulative career risk of occupational infection with HIV may vary widely based on individual circumstances and the population served (304).

With the widespread publicity concerning the CDC investigation of a dental practice in Florida (67,305,306),

TABLE 30.4. *(form the CDC, 2001 (14))*

Factors to consider in assessing the need for follow-up of occupational exposures

- Type of exposure
  - Percutaneous injury
  - Mucous membrane exposure
  - Nonintect skin exposure
  - Bites resulting in blood exposure to either person invloved
- Type and amount of uid/tissue
  - Blood
  - Fluids containing blood
  - Potentially infectious uid or tissue (semen; vaginal secretions; and cerebrospinal, synovial, pleural, peritoneal, pericardial, and amniotic uids)
  - Direct contact with concentrated virus
- Infectious status of source
  - Presence of HBsAg
  - Presence of HCV antibody
  - Presence of HIV antibody
- Susceptibility of exposed person
  - Hepatitis B vaccine and vaccine response status
  - HBV, HCV, and HIV immune status

Evaluation of occupational exposure sources

Known sources

- Test known sources for HBsAg, anti-HCV, and HIV antibody
  - Direct virus assays for routine screening of source patients are recommended
  - Consider using a rapid HIV-antibody test
  - If the source person is **not** infected with a blood-borne pathogen, baseline testing or further follow-up of the exposed person is **not** necessary
- For sources whose infection status remains unknown (e.g. the source person refuses testing), consider medical diagnoses, clinical symptoms, and history of risk behaviors
- Do not test discarded needles for blood-borne pathogens

Unknown sources

- For unknown sources, evaluate the likelihood of exposure to a source at high risk for infection
- Consider likelihood of blood-borne pathogen infection among patients in the exposure setting

much attention was focused for a while on a related issue: the potential for transmission of HIV from healthcare workers to their patients and the risk of acquisition related to the healthcare environment.

In previous studies, no evidence of risk of AIDS or HIV transmission to patients was found (307Ð311). Several other subsequent investigations failed to reveal any new evidence of transmission risk, and comparative assessments based on the epidemiology of hepatitis B virus have been made.

In the Florida investigation, several patients who had a history of invasive dental procedures were found to be HIV seropositive. Molecular studies suggested the possibility of transmission from the dentist, who died with AIDS, based on the degree of strain similarity. However, limited data on the degree of homology within epidemiologic clusters exist (67). Some studies suggest extremely little variation in such clusters (312Ð314)Ñ raising the possibility that the changes observed by the CDC may be in excess of that associated with immediate (direct or indirect) transmission. Also, the period from Þrst dental visit to AIDS in one person (patient ÒA)Ówas less that two years. This would be an exceptionally short latency period, raising the question of possible earlier acquisition from an unidentiÞed source outside the dental practice (315).

Investigations of the Florida dental practice indicate general (although not absolute) compliance with the CDC recommendations for infection control in dentistry (316), and the CDC has not concluded how transmission might have taken place (315). This is problematic, since the public health implications of transmission to patient(s) via a direct blood-blood transfer versus an indirect route (e.g. faulty decontamination) differ considerably. Nevertheless, revised guidelines for dentistry were issued (317).

Mathematical models of direct transmission suggest a very low risk even with invasive procedures (304,318, 319). When the HIV status of a surgeon is unknown, the overall likelihood of reverse HIV transmission (to the patient) is about one chance in 21 million hours of surgery, with an upper bound 95% risk of one in four million (319). If the surgeon were known to be HIV seropositive, the estimate is one chance in 83,000 hours of surgery. These risks are of about the same magnitude as fatal injury to the patient on the way to the hospital (319). Risks have been similarly estimated for radiologists. If the radiologist is HIV-positive, the risk of transmission to a patient was estimated (by modeling) to be 7.5 per million procedures (95% upper conÞdence interval 15.3 per million) (304). These data would imply limited need to restrict the work of persons infected with HIV (304).

A meeting held by the CDC in February 1991 explored many related issues (320). Should the practices of HIV-infected surgeons and dentists engaged in invasive procedures be restricted? What procedures are ÒinvasiveÓ or Òexposure-prone"? Who, if anyone, and under what conditions, should be tested for HIV? The guidelines from the CDC issued in July 1991 concerning these matters (321) focused on several of these key issues, but left many questions unresolved. Subsequently, some proposals were found to be unworkable and quietly discarded (317,322).

Spurred by public apprehensions, new studies looking back at over 25,000 former patients of healthcare workers known to be HIV seropositive, have been conducted (323). It has even been suggested that in general look-back ventures may not be worth their considerable cost (324) and that they perhaps should only be conducted when there is a clearly identiÞable risk of transmission (324). Multiple additional studies also failed to document any evidence of HIV transmission from workers to any patients (304,325Ð327). These data led some medical groups to reappraise the scientiÞc rationale of the CDC recommendations (320) and to recommend that implementation of policy decisions should await further data or that global restrictions were not warranted (304,323). Other groups seized upon these issues, utilizing them as a surrogate for mobilizing public reaction amid long-standing fears, with the debate becoming charged and politicized.

Revised position papers on the issue of healthcare workers infected with blood-borne pathogens are being developed by organizations such as the Society for Healthcare Epidemiology of America, Inc. (SHEA) (323). The draft 1997 SHEA paper concluded that Òno blanket restriction on the performance of any procedure should be recommended for HIV positive healthcare workersÓ (323).

However, two reports from Europe in 1997 Ð one concerning HIV and one concerning HBV Ðwill undoubtedly lead to further discussion of the issue of possible disease transmission from healthcare professionals to their patients and policy guidelines.

According to press accounts, a French orthopedic surgeon reportedly acquired HIV occupationally in 1983, but did not have his infection diagnosed until 1994 when he developed multiple bouts of unidentiÞed illness (328Ð330). The French Health Ministry subsequently contacted 5,000 persons on whom he had operated since 1983. Of 936 tested, only one patient reportedly tested positive for HIV. The preliminary news reports suggest this female patient had no other risk factors and that analysis of the sequences of viruses from the surgeon and this patient were consistent with probable transmission of HIV from the surgeon to her. Given the complexities of such genetic analyses and the large number of studies conducted, it will be important to critically review any research reports that are published and to ascertain the statistical robustness and the adequacy of the controls utilized. It is conceivable that an apparent relationship might be detected as a chance occurrence given the universe of a large number of (negative) studies, and the issues discussed above concerning the Florida dental case remain pertinent to scientiÞc assessments.

These preliminary data reopened a debate in France concerning whether doctors and/or patients should be tested for HIV before surgery, and the issues of disclosure of HIV status. The French National Council for the Order of Physicians (Conseil National de lÕOrdre des Medcins), which sets standards for the medical profession in France, has considered asking surgeons infected with HIV to voluntarily stop performing some activities, although peer pressures and hospital managers might also inßuence individual responses.

In the United States, some HIV-infected workers concerned about possible loss of employment have not disclosed their HIV status to their employer. Evolving U.S. case law suggests that the anti-discrimination laws may not offer protection to such employees, even though HIV is considered a Òdisability.Ó However, in the event of notiÞcation the terms of employment may change. In short, the issues surrounding both disclosure and non-disclosure remain extremely complicated legally, medically and emotionally, and necessitate expert counsel for both employer and employee.

There have been sporadic, infrequent reports of clustered HBV transmission from surgeons and dentists to patients (331) with instances of abrogation of spread when routine glove use has been implemented (331Ð333). Transmission of HBV is signiÞcantly more likely when the index person is e antigen (HBeAg) positive and HBV also poses a greater risk for transmission than HIV does (21,333Ð335). Since healthcare workers infected with the hepatitis B virus who are ÒeÓ antigen positive (21) who perform certain types of invasive procedures may pose limited but heightened risk to their patients that is insufÞciently ameliorated with the use of gloves, it has been recommended that such workers may need to entirely refrain from such activities (323).

Molecular epidemiologic approaches have been applied to HBV clusters (336). Of interest is a 1997 analysis using molecular approaches that implicates four different surgeons in transmitting HBV to their patients, despite each of the surgeons being HBeAg negative (337). This report raises new concerns about the adequacy of standard precautions and testing procedures.

Hepatitis C virus can be transmitted by percutaneous exposure to blood (338,339). Hepatitis C virus is approximately 10 times more infectious than HIV by percutaneous blood exposure to small volumes of blood (340). (In contrast, HIV is more transmissible than HCV between heterosexual partners and from a mother to her infant (340).) An anesthesiologist who acquired HCV from a patient is believed to have transmitted the infection to another patient, based on molecular analyses of quasi-species sequences and the clinical histories (341). An HCV

outbreak in an ambulatory surgical center has been linked to a technician with chronic HCV who was involved in narcotic tampering (342). An anesthesiologist addicted to opioids was similarly linked to transmitting HCV to over 217 patients in Spain (343).

In summary, further attention to the improvement of workplace safety, which offers bilateral enhancement of protection to both the worker and the patient, remains critical.

OTHER WORKPLACE TRANSMISSION ISSUES

Other Retroviruses

Several studies have examined laboratory workers who work with human T-cell lymphotropic viruses (HTLV-I and HTLV-II), but no evidence of occupationally-related transmission was documented (58,344,345). In a survey of California blood donors (346), one HTLV-II carrier was identified who had no known traditional risk factors for HTLV-II (347) but who was a dentist. Similarly, a Belgian study found a midwife infected with HTLV-I who had no standard risk factors (348). A history of an accidental needlestick or cut was recently linked in a U.S. study of blood donors with their HTLV-I and HTLV-II infections (349). However, no prospectively documented instance of occupational acquisition of HTLV has so far been reported.

In contrast, the CDC reported transmission of the simian immunodeficiency virus (SIV) to two workers related to workplace exposure (350,351). The guidelines for handling these agents, as well as HTLV, are the same as for HIV.

Blood-Borne Pathogens: HBV and HCV

The other possibly infectious materials (OPIM) (see above) that may pose a risk for HBV and HCV include all those noted above for HIV, with the caveat that HBV or HIV, respectively, be substituted in the descriptions of fluids, tissues or cultures that may harbor these agents (105). The early report of transmission of HBV but not HIV after a needlestick injury from an AIDS patient demonstrated the apparent higher infectivity of HBV (Table 30.1) (49). Simultaneous exposures to HIV and HCV leading to transmission have been reported (41), including a case in which there was delayed seroconversion to both HIV and HCV (41).

The hepatitis B vaccination status and the vaccine-response status (if known) of an exposed person should be reviewed (14). A summary of the USPHS guidelines for HBV prophylaxis recommendations for percutaneous or mucosal exposure to blood according to the HBsAg status of the exposure source and the vaccination and vaccine-response status of the exposed person are summarized in Table 30.5. When HBIG is indicated, it should be administered as soon as possible after exposure (preferably within 24 hours). The effectiveness of HBIG when administered >7 days after exposure is unknown. When hepatitis B vaccine is indicated, it should also be administered as soon as possible (preferably within 24 hours) and can be administered simultaneously with HBIG at a separate site (vaccine should always be administered in the deltoid muscle).

The source patient should also be tested for anti-HCV (14). Indeed, some source patients, such as any person with a history of injection drug use after (about) 1970, may be at high risk for HCV and be an as yet undiagnosed carrier. Studies have reported serologically confirmed HCV prevalences in drug users ranging as high as 99% (352).

For the person exposed to an HCV-positive source, the USPHS recommends baseline testing for anti-HCV and ALT activity; and follow-up testing (e.g. at four to six months) for anti-HCV and ALT activity (if earlier diagnosis of HCV infection is desired, testing for HCV RNA may be performed at four to six weeks). All anti-HCV results reported positive by enzyme immunoassay should be confirmed using supplemental anti-HCV testing (e.g. recombinant immunoblot assay (RIBA[a])).

Healthcare professionals who provide care to persons exposed to HCV in the occupational setting should be knowledgeable regarding the risk for HCV infection and appropriate counseling, testing, and medical follow-up. Immunoglobulin and antiviral agents are not recommended for postexposure prophylaxis after exposure to HCV-positive blood. In addition, no guidelines exist for administration of therapy during the acute phase of HCV infection. However, limited data indicate that antiviral therapy might be beneficial when started early in the course of HCV infection. When HCV infection is identified early, the person should be referred for medical management to a specialist knowledgeable in this area.

Other Agents

The immune system impairment that results from infection with HIV leads to an inability to control some infectious organisms, including some pathogens such as *Mycobacterium tuberculosis* that are causing increasing burdens of disease in association with HIV (353–358). This has also led to secondary transmission within urban communities as well as in hospitals, since *M. tuberculosis* (unlike HIV) can be highly infectious through aerosol transmission routes (359). Close-contact spread has been compounded by the crack cocaine epidemic (360), the existence of anergy in HIV-infected persons (leading to delay in diagnosis of new infection with *M. tuberculosis* as well as diagnosis of active tuberculosis) (361–363), and reduced physician prescription of isoniazid prophylaxis (due to hepatotoxicity concerns) during the 1980s

TABLE 30.5. *Recommended postexposure prophylaxis for exposure to hepatitis B virus*

Vaccination and antibory response status of exposed workers*	Treatment		
	Source HBsAG† positive	Source HBsAg† negative	Source unknown or not available for testing
Unvaccinated	HBIG§ × 1 and initiate HB vaccine series¶	Initiate HB vaccine series	Initiate HB vaccine series
Previously vaccinated			
Known responder**	No treatment	No treatment	No treatment
Known nonresponder#	HBIG§ × 1 and initiate revaccination or HBIG × 2	No treatment	If known high risk source, treat as if source were HBsAg positive
Antibody response unknown	Test exposed person for anti-HBs 1. If adequate,** no treatment is necessary 2. If inadequate, administer HBIG × 1 and vaccine booster	No treatment	Test exposed person for anti-HBs 1. If adequate,¶ no treatment is necessary 2. If inadequate,¶ administer vaccine booster and recheck titer in one to two months

* Persons who have previously been infected with HBV are immune to reinfection and do not require postexposure prophylaxis.
† Hepatitis B surface antigen.
§ Hepatitis B immune globulin; dose is 0.06 mL/kg intranuscularly.
¶ Hepatitis B vaccine.
** A repsponder is a person with adequate levels of serum antibody to HBsAg (i.e. anti-HBs □10 mlU/mL).
? A nonresponder is a person with inadequate response to the vaccination (i.e. serum anti-HBs < 10 mlU/mL).
? The option of giving one dose of HBIG and reinitiating the vaccine series is preferred for nonresponders who have not completed a second 3-dose vaccine series. For persons who previously completed a second vaccine series but failed to reposnd, two doses of HBIG are preferred.
? Antibody to HBsAg.

(364Ð366). In addition, multiply-drug resistant strains of *M. tuberculosis* have been described, particularly in HIV seropositive prisoners and intravenous drug users (367,368). A death of a healthcare worker (who had an underlying malignancy and worked in close contact with infected prisoners) attributed to occupational acquisition of multiply-drug resistant tuberculosis highlighted the risks (368).

These issues have important ramiÞcations regarding implementation of respiratory precautions, as well as the design and adequacy of ventilation systems in many types of medical institutions (369). Some buildings are clearly inadequate in terms of the availability of private rooms with appropriate ventilation, and the cost would be great to correct these deÞciencies. Initial OSHA regulations, and in particular those related to respirator use (including type of device and Þt) (370), were revised in response to public comment and vigorous actions by multiple medical groups (371Ð373). Occupational risks in the healthcare setting due to airborne organisms have been reviewed (374). Practices concerning the use of masks and other respiratory personal protective equipment have been evolving (375,376).

Infections by classic pyogenic pathogens also occur in HIV-infected persons (377,378), including pneumococcal infections (379). Reports of antibiotic resistant strains in some common bacteria (380) suggest a possible, future problem arising regarding nosocomial spread of such agents either to other patients or to workers, as the epidemic progresses and new resistance patterns emerge. The possibility of nosocomial spread of *Pneumocystis carinii* has been raised (381Ð384). However, since this agent has not yet been cultured *in vitro*, the genetic (and possible antibiotic resistance) variability has been difÞcult to study and the question difÞcult to address and resolve.

Nosocomial outbreaks of other highly infectious agents, such as measles (385), have also been described. Among laboratory workers and animal care handlers, the speciÞc agents with which they work and to which they might be exposed further broadens the spectrum of occupational risks (58,59,350,386Ð388). A high clinical index of suspicion and adherence to infection control guidelines can be expected to minimize these problems, and re-assessment of the biosafety level of precaution may sometimes be necessary (388).

Other occupationally acquired infections transmitted by blood-borne routes (e.g. hemorrhagic fever viruses such as Ebola virus (389)), organisms spread through the fecal-oral route (salmonella species, hepatitis A virus), and organisms spread by direct contact (herpes simplex virus, *Sarcoptes scabiei*) have been reviewed elsewhere (390). Violation of at least one of three basic infection control principles was noted to be associated with most instances of transmission: hand washing, vaccination of the workers,

or the prompt placement of infectious patients into appropriate isolation (390).

In the aftermath of the terrorism events of September 11, 2001 and the anthrax cases thereafter, there are intensiÞed concerns about bioterrorist threats. Healthcare and laboratory workers would be in the forefront of the Þrst responders to such agents, so new issues continue to ariseÑ such as vaccination status of workers and the persistence of protection for agents such as smallpox, as well as plans for decontamination and surveillance (391Ð399). It will be important for public health professionals, epidemiologists, infection control and infectious disease experts to be integral in the development and implementation of plans, with dissemination of this information at the local level, to maximize our ability to cope if there is an ÒeventÓ while minimizing the substantial risks to workers (399).

SUMMARY

The perception of degree of risk can vary markedly from actual risk. About 5% of the cases of AIDS and HIV infection in the U.S. have occurred in healthcare workers, a percentage that has remained stable over time. Nearly all of these infections are related to lifestyle factors, not occupational risk. The primary occupational risk for HIV is related to percutaneous exposures. The risk to patients appears to be very much smaller, but has received even more publicity. Apprehension exists concerning the future framework of our medical care delivery system, and who will care for whom (400,401). The sensitive handling of legitimate fears and the balancing of conßicting risks will continue to be a challenging task in the decades ahead.

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VII

TREATMENT AND PREVENTION

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Chapter 31

Antiretroviral Chemotherapy

Robert T. Schooley

The past seven years have witnessed an unprecedented reduction in morbidity and mortality due to HIV-1 in areas of the world in which antiretroviral chemotherapy has been routinely available (1). During this period an ongoing discovery and development effort has resulted in the introduction of additional antiretroviral agents to the point that over 15 are now available and several more are on the verge of approval. These agents have made it increasingly possible to apply a set of principles of antiretroviral chemotherapy that were derived from a series of clinical trials and translational research efforts undertaken in the preceding Þve years. This chapter outlines a current approach to antiretroviral chemotherapy and provides insight into the directions likely to be followed by the Þeld over the next several years.

ANTIRETROVIRAL CHEMOTHERAPEUTIC AGENTS

The rational development of antiretroviral chemotherapy compounds is based on the development of agents that are directed at aspects of the viral replicative cycle that are not shared by the host. In the case of HIV-1, additional complexity is added by the wide variety of cell types in which the virus replicates, artifacts that are introduced by the *in vitro* cultivation of the virus in continuous cell lines and the highly error-prone process of reverse transcription. The high rate of errors introduced into the replicative process results in major strain diversity at the population level and in a propensity in individual patients for the emergence of strains with reduced susceptibility to antiretroviral agents following prolonged exposure to antiviral drugs unless near complete suppression of viral replication is achieved with combination chemotherapy. Finally, HIV-1 poses unique problems of drug delivery, both in terms of general pharmacokinetic principles, which must take into account the need for the continuous maintenance of therapeutic levels of drug, the need for penetration of the central nervous system, and the need to be active in each of the intracellular sites in which the virus replicates.

The initial step in replication of HIV-1 involves its use of the CD4 molecule as its major ligand for interaction of the viral envelope of glycoprotein, gp120, with susceptible cells. This high-afÞnity interaction, which accounts for much of the selectivity of the virus for cells of the CD4 surface phenotype, led to initial efforts directed at interfering with gp120-CD4 binding with soluble forms of the extracellular domain of the CD4 molecule (2). These efforts failed because it had not yet been recognized that CD4 binding was just the Þrst in a series of interactions between the viral envelope and the cell that greatly increased the afÞnity of the virus for the cell (3,4). The interactions that follow CD4 binding include binding with one of two chemokine receptors, CCR5 or CXCR4 (5Ð7). Each viral particle has a tropism for one or both of these ligands that is determined by a short region in the viral envelope and that determines, in part, the tropism of the virus for lymphocytes, monocytes or both types of cells. The viral quasispecies that generally emerges during primary infection is primarily of the CCR5 or monocyte-preferring lineage. As the disease progresses, the virus evolves to the CXCR4-binding subtype in an increasing fraction of patients. CXCR4-binding virus tends to grow preferentially in activated CD4 cells and grows at a more rapid rate *in vitro* than CCR5-binding virus. Emergence of virus of the CXCR4-binding phenotype often signals a more rapid CD4 cell decline and is associated with a more rapid clinical course (8). Recently inhibitors of viral envelope binding to chemokine receptors have been developed, and proof of their activity *in vivo* has been demonstrated (9,10). Each chemokine receptor binding inhibitor is speciÞc for its own viral quasispecies class, i.e. inhibitors of envelope binding to the CCR5 receptor block the growth of CCR5-utilizing virus and those that inhibit

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binding to the CXCR4 receptor inhibit replication of CXCR4-utilizing virus.

After chemokine receptor binding, the viral envelope undergoes a change in conformation and the hydrophobic gp41 domain of the envelope fuses with the cell membrane allowing viral entry into the cell. This step, too, has been shown to be amenable to inhibition with fusion inhibitors that mimic one of the strands of the heptad formed in the process of conformational reorganization of the viral envelope that precedes fusion.

After gaining entry into a susceptible cell, the virus is confronted with the problem of converting its genetic information, which is contained in two identical strands of single-stranded RNA, into double-stranded DNA. This conversion requires reverse transcription of the viral genomic RNA in the cellular cytoplasm by an RNA-dependent DNA polymerase known as reverse transcriptase. This enzyme has served as a major target for antiretroviral drug development. Inhibition of reverse transcription is the mechanism of action for all the currently approved nucleoside analogues or nucleotides (zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (D4T), lamivudine (3TC), abacavir and tenofovir), as well as for the class of allosteric inhibitors known as non- nucleoside reverse transcriptase inhibitors (NNRTIs).

The double-stranded DNA that results from reverse transcription may exist in a free form within the cytoplasm of the cell, or it may be integrated into host cell DNA. Cellular activation favors integration, which is catalyzed by another viral enzyme termed integrase. Systems were developed to screen for inhibitors of integrase activity several years ago, but only recently have compounds emerged from these screening attempts into later stages of drug development (11,12). After integration, the virus may remain within the host cell in latent form for long periods of time. Viral transcription is controlled by the long terminal repeat (LTR) sequence of the virus. Activation of the viral LTR sequence is, like integration, favored by cellular activation. HIV-1 has evolved a complex regulatory strategy to control its replication. This regulatory process includes several HIV-1 gene products that interact to control splicing and intracellular traffcking of viral RNA. Tat is an 86-kd protein that binds to a short segment of viral messenger RNAs. Binding of these segments by tat protein enhances the effciency of viral replication by several thousand-fold. Rev is another regulatory gene of HIV-1 that plays a critical role in determining the success of the virus in production of its structural genes, especially the viral envelope. Rev encodes a protein that binds to messenger RNA encoding the viral envelope and that is required for efficient translation of the viral envelope mRNA. Several other viral genes including nef, vif, vpu, and vpr with regulatory properties or properties affecting effciency of cell-to-cell transmission of HIV have also been identified. No prototypic drugs have yet been developed that inhibit the action of these regulatory proteins. At this point several investigative groups are attempting to delineate in more detail the function of these genes using molecular biologic approaches.

Translation of the viral gag-pol messenger RNA results in a large fusion polyprotein that must be cleaved into separate gag and pol proteins. This cleavage is mediated by a viral proteinase, which is located within the polyprotein near the gag-pol junction. This proteinase activity, which cleaves the large gag-pol fusion polyprotein into the polymerase component and into four separate gag polypeptides, is essential for the production of viral structural proteins that are capable of maturation into infectious viral particles. Compounds that are capable of inhibiting the viral proteinase with a high degree of selectivity were introduced into clinical use in the mid-1990s and essentially ushered in the current era of antiretroviral chemotherapy.

Thus many potential steps in the viral replicative cycle have been identified that might serve as excellent candidates for the rational development of effective antiretroviral agents. This chapter focuses primarily on approaches that have progressed to clinical trials and practice, namely, inhibition of binding or entry, reverse transcription and viral protease activity.

Inhibitors of Reverse Transcription

Initial success in the development of antiretroviral drugs came from agents directed at inhibiting reverse transcription. The prototype drug in this class, zidovudine (AZT, Retrovir), made its debut as an antineoplastic agent in the 1960s and was resurrected as an antiretroviral drug in 1985. Zidovudine and the other nucleoside analogues currently in clinical use serve as competitive inhibitors of reverse transcription. In each case these nucleoside analogues are taken up by cells susceptible to HIV-1 infection and phosphorylated by kinases of the host cell to triphosphate derivatives of the parent compound. These nucleotides are incorporated by the reverse transcriptase enzyme as the viral RNA template is used to construct complementary DNA. Tenofovir is an exception in this class in that it is administered as a nucleotide and does not require intracellular phosphorylation. Incorporation of these competitive inhibitors prevents further elongation of the DNA and terminates reverse transcription. Because such agents serve primarily to protect susceptible cells from initial infection with HIV, these agents have no effect on previously infected cells.

Zidovudine

Zidovudine was initially found to have antiretroviral activity against murine retroviruses in the 1960s. Zidovudine inhibits HIV replication in cell lines at concentrations in the range of 0.1 μM. Broder and his colleagues initiated

a small phase I escalating dose tolerance trial of zidovudine in individuals with advanced HIV infection in 1985 (13). Subsequent large scale blinded, placebo-controlled randomized trials demonstrated the clinical efficacy of zidovudine in individuals with moderate to advanced HIV-1 infection (14–16). This drug remains in widespread clinical use nearly 20 years after its initial introduction. The major dose-limiting toxicity of AZT was bone marrow toxicity but the use of the drug in lower doses than initially studied in combination with other antiretroviral agents to more fully suppress viral replication has greatly reduced this side effect. Zidovudine is also associated with several subjective complaints. In the initial randomized placebo-controlled trial of zidovudine, anorexia, mild to moderate headaches and insomnia were reported significantly more frequently by zidovudine recipients than by those receiving placebo. In practice, anorexia and headache are the subjective complaints that most frequently trouble patients to the extent that discontinuation of the drug is contemplated. In most cases these symptoms subside despite continuation of the drug over the first two to three weeks of therapy. In situations in which anorexia, nausea, or headache are particularly troublesome, symptomatic relief may be offered with antiemetics, aspirin, or nonsteroidal antiinflammatory agents.

ddC (Zalcitabine)

Zalcitabine (ddC) is another nucleoside analogue with selective antiretroviral activity that was developed shortly after zidovudine. The agent is roughly ten-fold more active against HIV-1 *in vitro* than is zidovudine but a narrow therapeutic index imposed by neurotoxicity and the development of several more potent and better-tolerated drugs in this class has rendered ddC primarily a drug of historical interest (17).

ddI (Didanosine)

Didanosine (ddI) followed zidovudine and ddC into clinical trials in 1988 (18,19). ddI has a serum half-life of 30 to 90 min, but the intracellular half-life of the active metabolite of ddI, dideoxyadenosine triphosphate, allows q24 hourly dosing. Pancreatitis and neuropathy are the major toxicities of ddI that require discontinuation of therapy. The initially unpalatable buffered form of the drug has recently been replaced with an enteric-coated formulation that has resulted in a major improvement in its tolerability. The drug exhibits antiviral activity *in vivo* of roughly the same magnitude (0.6–0.7 $\log_{10}$) as that of zidovudine in previously untreated individuals.

D4T (Stavudine)

D4T (Stavudine) exhibits antiretroviral activity *in vitro* of a magnitude that is similar to either zidovudine or ddI and is less cytotoxic to bone marrow progenitor cells *in vitro* than is zidovudine. From the subjective standpoint, the drug is extremely well tolerated by most individuals. A recently developed extended release formulation has made daily dosing possible. The major toxicities that have been reported with D4T are peripheral neuropathy and hepatitis, both of which occur in 15% to 20% of individuals. Each of these toxicitites may be exacerbated by the simultaneous administration of ddI and both are generally reversible if recognized early. Of particular concern, however, these two agents appear to be the most prone of the nucleoside analogs to cause lactic acidosis and hepatic failure (20–24). The presentation of this syndrome may be subtle and the results of missing the diagnosis may be serious. Patients on nucleoside analogs, especially those taking either D4T or ddI should be followed carefully for symptoms of lactic acidosis that may include lethargy, fatigue, anorexia, weight loss and exercise intolerance. Patients exhibiting any of these symptoms should undergo serum lactic acid monitoring, and the drugs must be discontinued if symptomatic lactic acidosis is noted. The apparent propensity of these two agents to be associated with the increasingly appreciated syndrome of lipodystrophy has also tempered the enthusiasm for their use in the past two years. The use of D4T in combination with AZT is discouraged in that D4T utilizes the same intracellular kinases as zidovudine for phosphorylation and, thus, antagonizes the antiviral activity of zidovudine (25).

3TC, Lamivudine (Epivir)

3TC is a nucleoside with potent antiretroviral activity ($\sim$1.5 $\log_{10}$) that is extremely well tolerated. The major difficulty with 3TC lies in the rapid rate at which viral isolates with reduced 3TC susceptibility arise both *in vitro* and *in vivo* if the drug is not used in combinations that suppress viral RNA below the limits of detectability in the serum (26,27). The mutation induced by 3TC at position 184 of the reverse transcriptase sensitizes the virus to AZT and tenofovir (28,29). This finding, coupled with the excellent tolerability of the drug, has resulted in widespread use of the drug in combination regimens, especially those containing zidovudine or tenofovir that allow one to take advantage of this resistance interaction.

Abacavir (Ziagen)

Abacavir is almost an ideal antiretroviral agent. Antiviral potency in untreated patients is in the range of 1.5 $\log_{10}$; the emergence of high-level resistance requires selection of two or more mutations; and the drug is well tolerated by most patients (30). The only significant problem with the drug is the occurrence of a hypersensitivity reaction in 3–5% of individuals in whom the

drug is used (31). The hypersensitivity reaction usually manifests itself in the Þrst three weeks of drug exposure and is characterized by a multisystem syndrome that may include fever, rash, gastrointestinal and/or pulmonary symptoms. If the drug is continued, the symptoms generally progress and may become severe. In general, these symptoms resolve quickly without residua if the drug is discontinued promptly at the time the syndrome is recognized. If, however, the drug is discontinued and the symptoms are allowed to resolve, a rechallenge with the drug may result in an accelerated recurrence of syndrome that can be fatal. The mechanism of the syndrome is not clear but an association with a speciÞc HLA type (B57) has recently been demonstrated in two small studies involving primarily Caucasians in approximately two-thirds of cases (32,33).

Tenofovir (Viread)

Tenofovir is a nucleotide reverse transcriptase inhibitor and, as such, does not require intracellular phosphorylation to form the active drug moiety (34). It has several ideal properties including potency (~1.25–1.5 $\log_{10}$ of plasmaviral RNA suppression in untreated individuals), convenience (once daily dosing), a relatively unique resistance proÞle and excellent tolerability (35). Tenofovir was initially approved by the FDA on the basis of its action in "salvage" situations in which its addition to failing drug regimens added approximately a half $\log_{10}$ of viral suppression in heavily pre-treated individuals (35–37). In these studies that demonstrated durable activity of up to 48 weeks duration, minimal toxicity was observed and little drug resistance developed, even in patients with incompletely suppressed viral replication. There is a certain degree of cross resistance between tenofovir and thymidine-based nucleoside analogs but the cross resistance is not complete and, as noted earlier, the presence of an M184V mutation induced by exposure to 3TC actually sensitizes the virus to tenofovir (38–40). Recently the drug has been studied in a large trial of previously untreated individuals and found to be as effective as D4T in reducing viral replication in a three-drug regimen including 3TC and efavirenz in addition to either tenofovir or D4T (41). In contrast to D4T, however, tenofovir did not elevate levels of cholesterol or triglycerides above the changes caused by the background drugs in the regimen. This study has Þrmly established tenofovir as an agent that is attractive for use in previously untreated individuals in addition to being useful in patients with multidrug resistant virus.

Nonnucleoside-Based Reverse Transcriptase Inhibitors

Several investigative groups have independently discovered a series of nonnucleoside compounds that exhibit potent inhibitory activity against the HIV-1, but not the HIV-2, reverse transcriptase enzyme. The agents are collectively known as nonnucleoside reverse transcriptase inhibitors (NNRTIs). These compounds are superÞcially quite dissimilar in terms of structure but exert antiretroviral activity by the same allosteric mechanism of action. Each group of agents is extremely selective for HIV-1. The agents exhibit activity against strains of HIV-1 that show reduced susceptibility to nucleoside analog reverse transcriptase inhibitors. NNRTIs exhibit synergistic antiretroviral activity with nucleoside analogue antiretroviral agents. The major difÞculty with the use of these agents resides in the rapid emergence of resistance both *in vitro* and *in vivo* if they are employed in incompletely suppressive regimens. The excellent pharmacokinetic proÞle, relatively good tolerability and inexpensive cost of manufacture of this class of drugs has made them extremely important in the management of HIV-1 infection.

Nevirapine (Viramune)

Nevirapine was the Þrst member of this class to enter clinical trials but the failure to appreciate the need to use it in fully suppressive regimens greatly delayed demonstration of its clinical utility. With the realization that antiviral activity can be prolonged indeÞnitely if used in completely suppressive combination regimens, nevirapine has become an important antiretroviral agent in worldwide use (42). Because of its tolerability and its relatively low cost of manufacture, nevirapine has become an important component of regimens designed to reduce maternofetal transmission of HIV-1 (43). The half-life of the drug allows once daily dosing; *in vivo* antiviral activity is in the range of 1.5 $\log_{10}$ of plasma viral RNA suppression. The major toxicities of the drug include rash that may result in drug discontinuation in as many as 15% of patients and a syndrome of fever and hepatitis that can be fatal if not recognized and managed by drug discontinuation.

Delavirdine

Delavirdine is slightly less potent than nevirapine *in vivo* and shares many of the same toxicities. It has been shown to be active both as a single agent and in the context of combination regimens. The initial formulation of this agent imposed a high pill burden on patients, but a new formulation has signiÞcantly improved the convenience of the drug. Because of the potency and convenience issues, delavirdine has not enjoyed as much commercial success as nevirapine but one advantage that it does exhibit is that it more consistently enhances serum levels of HIV-1 proteinase inhibitors than does nevirapine.

Efavirenz (Sustiva, Stokrin)

Efavirenz has become the dominant agent in this class. It has an *in vivo* potency of 1.5–2.0 $\log_{10}$ and has an extremely prolonged serum half-life that easily supports once daily dosing. As with other members of the class, efavirenz administration may be associated with a rash. The major unique toxicity of efavirenz is its propensity to cause CNS toxicity manifest by somnolence and/or by vivid dreams that can interrupt sleep. This toxicity is dose limiting in up to 15% of patients and is directly related to serum drug levels. Most patients prefer to take the drug at bedtime to minimize the effects of somnolence on their daily pattern of living. Combination regimens consisting of efavirenz and two nucleoside analogs such as zidovudine, D4T or tenofovir and 3TC can be expected to reduce HIV-1 RNA levels to below the limit of detection in more than 80% of previously untreated patients (44).

HIV-1 PROTEASE (PROTEINASE) INHIBITORS

As noted earlier, the HIV-1 gag-pol fusion polyprotein is cleaved by a viral proteinase (protease) that is contained within the gag-pol polyprotein. The viral proteinase enzyme has been expressed in *Escherichi coli* and found to be a dimeric aspartic protease. The deÞnition of structure-activity relationships for the viral protease has greatly enhanced insights into mechanisms of action of this enzyme, and into directed approaches to the development of inhibitors. The introduction of HIV-1 protease inhibitors into clinical trials represented the initial phase of the current revolution in antiretroviral chemotherapy.

Saquinavir (Invirase or Fortovase)

Saquinavir was the Þrst member of this class to enter clinical development. The initial formulation of saquinavir as a hard gel capsule (Invirase) was less than optimal in that it had limited bioavailability because of high Þrst pass metabolism. This formulation exhibits *in vitro* activity that is only slightly greater than zidovudine (45). Despite this relatively limited activity, a clinical endpoint trial demonstrated clear clinical beneÞt of this compound (45a), thus establishing the clinical utility of this class of drugs. The hard gel formulation of saquinavir was so limited in its bioavailability that a soft gel formulation (Fortovase) was developed (46). This formulation was much more bioavailable and exhibited antiviral activity *in vivo* in the range of slightly less than 1.5 $\log_{10}$, but its use has been largely supplanted by the use of the ritonavir/saquinavir combination that provides much more certainty with respect to drug levels and a lower pill burden (47). When combined with ritonavir, the hard gel formulation is preferable to fortovase.

Ritonavir (Norvir)

Ritonavir was the Þrst truly potent protease inhibitor to enter clinical practice and formed the basis of many of the initial studies that demonstrated the extremely high daily production rates of HIV-1 (48). The dose (600 mg twice daily) required to achieve its full antiviral activity *in vivo* ($\sim$1.5 $\log_{10}$) is only marginally palatable, and many patients are unable to tolerate this dose for an appreciable length of time because of gastrointestinal side effects. The major use of ritonavir at this point is as a pharmacologic enhancer in which it is used to retard the Þrst pass metabolism of other protease inhibitors, thereby providing longer dosing intervals, reducing food restrictions and raising achievable trough serum concentrations (47). Even in this clinical application, there is a trade-off between the pharmacological enhancing activity of ritonavir and its side effects with respect to tolerability and its propensity to raise serum levels of cholesterol and triglycerides.

Indinavir (Crixivan)

Indinavir is a potent protease inhibitor with *in vivo* activity in the range of 1.5 $\log_{10}$ (49,50). The drug is better tolerated by most patients than is ritonavir but its short half-life and signiÞcant food interactions greatly decrease the convenience of the agent when used as it was initially developed at a dose of 800 mg every eight hours. This shortcoming is largely overcome when indinavir is pharmacologically enhanced with ritonavir that allows twice daily dosing and removes the requirement that the drug be given on an empty stomach. This greatly improves the convenience of indinavir but does not eliminate the other three limitations of the drugÑ the propensity to cause renal stones, gastrointestinal disturbance and abnormalities with nails and other epidermal tissues. Despite these side effects, the combination of ritonavir and indinavir remains a widely used combinationÑ especially in salvage situations in which the addition of ritonavir greatly enhances trough serum levels (51). Although there have been a number of pharmacologically oriented studies to determine the optimal dosing combination of ritonavir and indinavir, there remains some controversy as to the best strategy. Many experts advocate the use of 100 mg of ritonavir and 800 mg of indinavir twice daily when the combination is used in individuals in which resistance testing does not indicate that indinavir resistance is present and the use of a 200 mg ritonavir dose in the presence of reduced susceptibility to indinavir.

NelÞnavir (Viracept)

Until recently nelÞnavir was the most widely prescribed drug in this class (52,53). The drug is generally well tolerated and can be administered twice daily (at 1,250 mg

bid). Major side effects include gastrointestinal intolerance and elevation of cholesterol and triglycerides. Although the *in vivo* antiviral activity is approximately 1.5 $\log_{10}$, it performs slightly less well in head to head comparisons with lopinavir/ritonavir or efavirenz when durability of viral suppression is compared in an intention to treat analysis (54). Combined dosing with ritonavir is not a viable strategy because of the two-way interactions between nelfnavir and ritonavir; thus, it is the only current protease inhibitor that is usually dosed without ritonavir enhancement. Despite these limitations, many clinicians and patients continue to use the drug because of its resistance profile in which the mutation (D30N) that is usually initially induced has few implications for other protease inhibitors, thus providing an opportunity for the use of other protease inhibitors after nelfnavir failure (55).

Amprenavir (Agenerase)

Amprenavir is a well-tolerated agent that, like nelfnavir, has a resistance profile that overlaps less extensively with other agents in the class (56–59). Because the drug is heavily protein bound, initial hopes that it would exhibit potency *in vivo* superior to other first generation protease inhibitors were not realized (57). The high lipid solubility of the compound has required that it be formulated in a large capsule in which 150 mg of amprenavir is emulsified with vitamin E to enhance bioavailability. This has resulted in the need to administer the drug in a very inconvenient dosing regimen of eight capsules twice dailyÑ a regimen that few patients are willing to tolerate. Amprenavir is, however, pharmacologically enhanced by ritonavir and it is now usually administered at a dose of 900 mg in combination with 100 or 200 mg of ritonavir twice daily. Amprenavir, like atazanavir, appears to be less likely than other currently available protease inhibitors to induce lipid abnormalities. Another unique property of amprenavir is the fact that the N88S mutation that can be induced by several other protease inhibitors renders the virus hypersusceptible to amprenavir (60). There are indications that these variants are particularly likely to respond to amprenavir therapy *in vivo* (61). A more conveniently dosed phosphonate formulation of the drug (now termed Ò908Ó) is in late stages of clinical development and will likely replace amprenavir over the next 12 months.

Ritonavir/Lopinavir (Kaletra)

Lopinavir is structurally similar to ritonavir but has limited bioavailability when administered by itself. In combination with ritonavir, however, it achieves much higher and more sustained serum levels and exhibits potency that is probably slightly greater than other currently used protease inhibitors. Because of the dependence upon ritonavir for bioavailability, it is co-formulated with ritonavir in a single capsule as a fixed dose combination and marketed as Kaletra. This agent is currently the most widely used member of the protease class and has been very well studied both as initial therapy and in salvage situations. The major toxicities of the drug are gastrointestinal intolerance and the induction of hypertriglyceridemia and hypercholesterolemia (62,63). It has been demonstrated to be an extremely potent protease inhibitor and significantly outperformed nelfnavir in a recently published head-to-head comparison in antiretroviral naïve patients (54).

Atazanavir

Atazanivir is a generally well-tolerated, once-daily protease inhibitor that appears less likely than currently available agents in this class to elevate cholesterol and triglycerides (64). As in the case of amprenavir, atazanavir has a resistance profile that overlaps less extensively with other agents in this class (65). The major unique toxicity of atazanavir is its propensity to cause a GilbertsÕlike hyperbilirubinemia. This toxicity is related to serum drug levels and is reversible with dose reduction or discontinuation of therapy. The ultimate niche for this drug has not yet been fully delineated but a recently completed trial comparing it to efavirenz in initial therapy (65a) and its Òlipid sparingÓ profile suggests it will be more widely used than initially assumed.

ENTRY INHIBITORS

Over the past several years a much better understanding of the molecular interactions between the virus and host cell surface proteins has been developed. Although initial hopes that interfering with the binding of the viral envelope to the CD4 molecule could lead to effective antiretroviral therapy were not realized (3), interference with subsequent steps of the viral cellular entry sequence has demonstrated significant promise (3,4). Since the experience with recombinant soluble CD4, it has been learned that the viral envelope binds to a second set of cellular proteins termed chemokine receptors before undergoing a structural change that facilitates fusion of one of its hydrophobic domains to the cell membrane (66). Interference with either of these steps has been demonstrated to inhibit viral replication *in vitro* and *in vivo* and FDA approval of the first entry inhibitor appears likely within the next 24–36 months (67).

Chemokine Receptor Antagonists

As outlined in more detail above, following binding of the viral envelope to the CD4 molecule on the surface of

the cell both the CD4 molecule and the viral envelope undergo secondary structural changes that allow binding of another portion of the viral envelope to one of two chemokine receptors (CXCR4 or CCR5) (66). These receptors are present in different amounts on monocytes and activated lymphocytes with the CCR5 molecule being most heavily expressed by monocytes and the CXCR4 molecule being most heavily expressed on activated lymphocytes. Which of the two chemokine receptors is most efficiently bound by a given viral particle is determined by a short sequence of amino acids within the third hypervariable loop of the gp120 viral envelope glycoprotein. In early disease, virus of the CCR5 binding type usually predominates; later in disease a more rapidly growing and more pathogenic quasispecies of CXCR4-preferring viral particles often emerges (68–70). Specific inhibitors for each of these types of virus have been developed and have been studied in clinical trials.

Inhibitors of binding of HIV-1 to the CCR5 receptor have advanced the most in clinical trials because several companies have developed agents that are orally bioavailable (67). The proof of concept that blocking this interaction between HIV-1 and the CCR5 molecule inhibits viral replication *in vivo* has been demonstrated in Phase I/II trials of a compound (Schering ÒCCÓ) that appears to reduce viral replication by over 1.0 $\log_{10}$ (71). Clinical development of the compound was delayed for a period while concerns about its propensity to cause QT segment prolongation were evaluated. Resumption of development of this compound was allowed by the FDA when the QT segment prolongations were found to be related to peak levels of compound in the serum and a twice daily dosing schedule was substituted for daily dosing. CXCR4 antagonism has also been shown to reduce HIV-1 replication *in vitro* and *in vivo*, but the reduction is limited to viral quasispecies of the X4 phenotype (72). Development of X4 antagonists has been further limited by the fact that the only compounds studied to date have not been orally bioavailable and have required intravenous administration.

Fusion Inhibitors

Following chemokine receptor binding the gp41 component of the viral envelope changes configuration and aligns a six-stranded segment of amino acids that fuse with the cellular membraneÑ thereby allowing viral entry into the cell. This process can be inhibited by mimics of these amino acid segments that interfere with this hexamerization. A thirty-six amino acid protein that has been given the name of ÒT20Ó or enfuvirtide has been shown to reduce viral replication by up to 1.5 $\log_{10}$ *in vivo* in a series of Phase I/II studies. Recently reported Phase II/III trials conducted in the U.S. and in Europe have confirmed these findings and have set the stage for FDA approval of this compound early 2003 (73,74). T20 is a clear demonstration that this approach is viable clinically but is greatly limited as a drug both by its high cost of manufacture and by the requirement that it be administered parenterally because of its lack of oral bioavailability. It will likely prove to be a critical agent for patients in salvage situations with limited therapeutic options but the cost and inconvenience of administration will greatly limit its clinical utility.

Integrase Inhibitors

Following reverse transcription, the DNA copy of the HIV-1 RNA must be integrated into the host cell DNA. This process is facilitated by a viral encoded integrase that enters the cell with the viral particle. Inhibitors of this enzyme have only recently entered clinical trials, and it is premature to speculate about the potential clinical utility of this approach since *in vivo* activity against HIV-1 has not yet been demonstrated (12,75).

PRINCIPLES OF ANTIRETROVIRAL CHEMOTHERAPY

The principles of antiretroviral chemotherapy have become increasingly well delineated. The ability to deliver antiretroviral drugs in accordance with these principles has steadily improved as a wider variety of agents have appeared. This rapid development of new agents is both enormously exciting and desperately needed. The pace of this evolution, however, poses a significant challenge to physicians in that antiretroviral chemotherapeutic agents are often widely available at significantly earlier stages of development than in the case of drugs used in other illnesses. This, coupled with the need for a deep understanding of the pathogenesis of HIV-1 infection, requires that clinicians stay abreast of a wide body of data, and necessitates therapeutic decisions that are based on fewer data from formal clinical trials than in the case of more traditional infectious diseases. Given the complexity of the disease process and the wide variety of objective and subjective factors that must be considered in therapeutic decision-making, a rigid algorithm for making therapeutic decisions that is valid in all situations cannot be recommended in HIV medicine. Nonetheless, from the available data, a general pathogenesis-based approach to antiviral chemotherapy has been developed that provides a reasonable framework from which to base therapeutic decisions in individual patients.

These principles are based on the premise that HIV-1 replication is the major driving force in the deterioration of the immune response that ultimately leads to most HIV-1 associated morbidity and mortality. HIV-1 replicates at an extremely high rate with the production of over a billion viral particles daily in the infected host (76–78) (Fig. 31.1). Approximately 99% of viral replication occurs in activated CD4 cells, thus accounting for the preferential

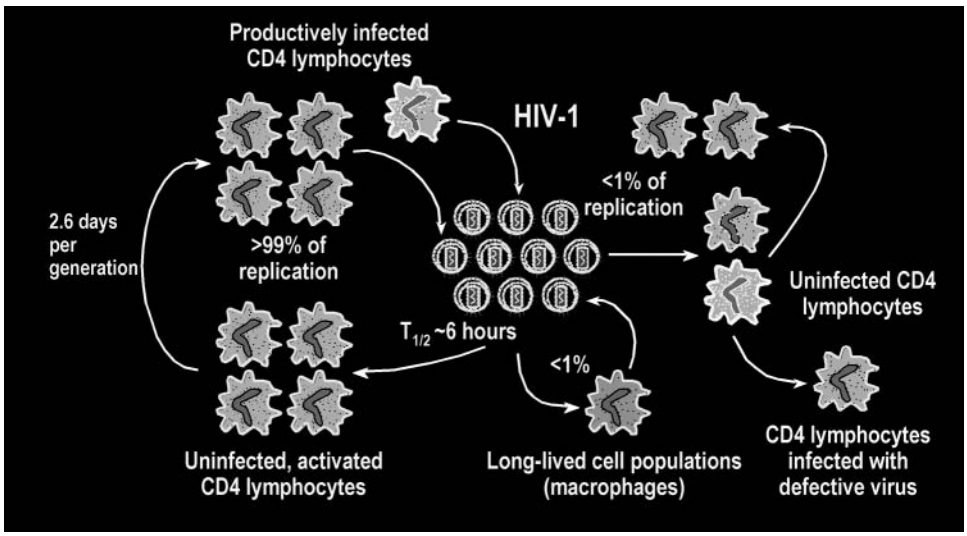

FIG. 31.1. Immunologic damage is mediated by viral replication.

depletion of this subset of immune cells. The remaining 1% of viral replication occurs in resting CD4 cells and in cells of the macrophage/monocyte lineage. Viral replication in this immunologic compartment is less important from the standpoint of inducing immunologic dysfunction but it is a critical component of the ability of the virus to retain a foothold within the host even when viral replication is nearly totally inhibited in the activated CD4 cell compartment. The rate of development of immunologic dysfunction is directly related to the rate of viral replication. Partial or complete inhibition of HIV-1 replication facilitates immune reconstitution. Because reverse transcription is error prone, the selection of drug resistant mutants is rapid if virus is allowed to replicate in the presence of incompletely effective antiretroviral drugs. With this set of principles in mind, a rational approach to antiretroviral therapy can be developed that must be adapted to Þt the individual patient.

Starting Therapy

The optimal time to initiate therapy must be determined by weighing the degree and rate of development of immunologic dysfunction, potential complications of the speciÞc drugs to be used and the willingness of the patient to initiate therapy. This determination is a complex one that varies from patient to patient and has changed over time as information has been developed about the pathogenesis of the disease and as new agents have been developed. Over the past several years, increased concerns about potential long-term toxicities of the agents utilized to treat the disease coupled with the realization that immunologic dysfunction is highly reversible in many patients even when therapy is delayed until late in the disease process have resulted in a tendency for many practitioners and patients to delay the initiation of therapy until CD4 cell counts have declined to lower levels than were tolerated in the past. Since it is also apparent that the plasma viral RNA level is mainly germane from the standpoint of the rate at which immunologic dysfunction develops and that the correlation between plasma HIV-1 RNA levels and the likelihood of viral suppression is relatively limited, most clinicians rely primarily on the CD4 cell count to determine when to recommend the initiation of therapy. At this writing most clinicians recommend starting therapy in the range of 350 CD4 cells/mm^3. This downward trend in the CD4 cell count at which therapy is recommended will likely to reverse with the advent of better tolerated and more convenient drug regimens and with the realizations that the lipodystrophic Þndings initially ascribed to antiretroviral therapy (especially protease inhibitors) are both drug and disease related and that the extent to which these complications occur is highly inßuenced by the CD4 cell count nadir. Patients with lower CD4 cell counts at the time of therapy initiation are much more likely to manifest these complications.

Selection of the Initial Drug Regimen

As in the case of when to initiate therapy, the speciÞc regimen with which to start therapy is one that must be individualized. Once started the goals of therapy are to reduce viral replication to a level at which HIV-1 RNA cannot be detected in the plasma with the most sensitive available assays (e.g. 20Ð50 copies/ml) with the least degree of toxicity and inconvenience for the patient. The ultimate success of a given antiviral regimen requires that it be potent, well-tolerated, convenient and that it require the virus to make multiple genetic changes to replicate successfully in its presence. At this writing there are several regimens that will accomplish this in a signiÞcant majority of patients.

Most current initial regimens include at least two nucleoside analogs to provide a high genetic barrier for

TABLE 31.1. *Options for initial therapy*

Nucleoside backbone	
Preferred regimens	*Comments*
Zidovudine and lamivudine (AZT/3TC)	Extensive clinical experience; available in co-formulated tablet; generally well tolerated.
Tenofovir and lamivudine (Tenofovir/3TC)	More limited clinical experience; highly ef cacious; well tolerated, low toxicity.
Alternative regimens	
Stavudine and 3TC	Extensive clinical experience; well tolerated symptomatically but D4T component contributes to neurotoxicity and hyperlipidemia.
Stavudine and didanosine	Extensive clinical experience; relatively dif cult resistance barrier; D4T and ddI interact to cause neurotoxiticy.
Abacavir/3TC	Limited clinical experience but theoretically a reasonable nucleoside combination.
Combinations that should not be used	
Stavudine and zidovudine	Antiviral antagonism
Potent "Third" Drug	
Preferred agents	
Efavirenz	Extensive clinical experience; highly ef cacious when combined with either of the preferred nucleoside backbone combinations; CNS toxicity limits use in 5–15% of patients.
Abacavir	Available in a tablet co-formulated zidovudine and 3TC allowing a compact one-pill, twice daily regimen; likely less ef cacious at HIV-1 RNA levels > 100,000 copies/ml.
Alternative agents	
Nevirapine	Acceptable alternative to efavirenz when CNS toxicity or baseline psychiatric abnormalities make use problematic; potential for hepatotoxicity should be monitored closely.
Nel navir	Extensive clinical experience but longer-term toxicities associated with protease inhibitors make PI's less attractive in treatment naïve patients.
Atazanavir	Recently completed clinical trials support its ef cacy and indicate less toxicity related to lipid abnormalities.
Lopinavir/ritonavir Indinavir/ritonavir Saquinavir/ritonavir Amprenavir/ritonavir	Potent regimens but side effects of ritonavir-forti ed protease inhibitor based regimens make these regimens more attractive in more experienced patients.

resistance and at least one additional agent (a "third" drug) to maximize potency (Table 31.1). Although many nucleoside combinations are possible, clinicians are increasingly using combinations of zidovudine and 3TC as their nucleoside backbone although the combination of tenofovir and 3TC also has been shown to have signi cant merit (41). Two recently completed studies (ACTG 384 and Gilead 903) have demonstrated the utility of these regimens (79,80). Based on currently available information, the combination of zidovudine and 3TC or tenofovir and 3TC should be offered to most patients as the initial nucleoside options. With the accumulating evidence that D4T is more likely than AZT to be associated with lipodystrophy, the previously heavily used nucleoside backbones of D4T and 3TC or D4T and ddI should be preserved for those who experience toxicities or are unable to tolerate one of the primary regimens.

Efavirenz is the most frequently used "third" drug at this writing but it is not the ideal agent for all patients and exibility in the selection of this component of the regimen is critical. Efavirenz has the advantage of once-daily dosing and is now available in a 600 mg tablet allowing this dose to be given as a single pill. When combined with AZT and 3TC (which can be administered in the xed dose formulation of Combivir), a highly potent regimen can be administered that requires the patient to

take only one pill in the morning and two at night. Especially in patients with lower baseline plasma HIV-1 RNA levels, abacavir is a very attractive third drug as well because of its tolerability and its compact dosing proÞle. With the development of Trizavir (a co-formulation of abacavir, AZT and 3TC), a daily regimen that consists of only one pill twice daily is now available. Although Þrst-line use of protease inhibitors as the ÒthirdÓ drug is also a very viable initial strategy, concerns about the longer-term effects of this class of drugs on cholesterol, triglycerides and body habitus have greatly reduced the tendency of clinicians and patients to use these agents in the initial regimen.

Although many arguments can be made about which regimen is the ÒbestÓ initial regimen, it is clear that no single regimen is best for all patients and that in the individual patient, one of the most important considerations is ßexibility. At this point, it should be expected that the initially selected regimen will drive HIV-1 RNA below the limit of detection in over 80% of patients. It is critical to communicate to patients the most likely side effects of the regimen chosen and to indicate to them that it is equally critical that they communicate to you any difÞculties they encounter with this regimen. If patients are made aware that the side effects of a speciÞc regimen may be difÞcult to predict in a given patient and that it is almost always possible to make an adjustment to the regimen, it is relatively easy to assure them that a regimen that they will tolerate with minimal toxicity can be developed in the vast majority of patients.

When to Switch Therapy

Once therapy is started the goal should remain to suppress viral replication to the point that HIV-1 RNA is not detectable in plasma with as little toxicity to the patient as possible. The two main reasons for changing therapy are a failure of the regimen to achieve or to maintain control of viral replication (virologic failure) or the occurrence of unacceptable toxicity. Virologic failure is best deÞned as the conÞrmed presence of detectable levels of HIV-1 RNA in plasma. Whether or not one should change therapy immediately when viral RNA is detected in plasma depends both on the reasons for failure and upon the therapeutic options remaining. In general, the Þrst detection of HIV-1 RNA in the plasma should trigger a return visit to the clinic and a discussion as to whether the patient had been adherent to the prescribed regimen during the period of time just prior to the phlebotomy. If a lapse of adherence is detected, a discussion should ensue as to whether the lack of adherence is due to either subjective side effects of the regimen or to the fact that the regimen is unduly complicated. If either of these factors can be addressed, this would be the time to do so. If the patient is adhering to the regimen and if viral RNA is detected on a repeat determination, it is usually prudent to change the drug regimen to one that will fully suppress viral replication although patients often do well clinically and immunologically for a prolonged period of time despite measurable amounts of virus in plasma. The main reason for changing therapy when possible is that if the regimen is not changed, selective pressure will inevitably lead to an increasing amount of drug resistanceÑ further limiting eventual treatment options.

There are clearly circumstances during which there are no treatment options likely to restore full control of viral replication. Under these circumstances, the physician must weigh the ongoing clinical and immunological beneÞt of partial suppression of viral replication against the damage done by broadened resistance to any remaining antiviral agents. It is clear from a number of contemporary studies as well as from studies conducted prior to the availability of potent combination regimens that any measurable reduction in viral replication from baseline reduces morbidity and mortality over the short to intermediate term. It is also clear that the problem of drug resistance worsens with ongoing viral replication in the presence of partially effective antiretroviral drugs. Although there is no speciÞc point at which antiretroviral therapy must be changed in all patients, one of the more common errors made by treating physicians is to stay with a failing regimen because the patient is immunologically and clinically stable at the expense of downstream treatment options. The physician should tolerate much less viral replication when there are many remaining treatment options than in the case of patients who already have multidrug resistant virus and, thus, have less likelihood of achieving virologic control with changes in the treatment regimen.

What Drugs to Change the Regimen to in the Case of Regimen Failure

In the past it was fashionable to speculate about a magic sequence of drug regimens that would be most efÞcacious for the majority of patients. It has become apparent that the selection of regimens after the failure of one or more treatment regimens is even more complex than the selection of the initial regimen. With each regimen failure, considerations related to drug susceptibility become increasingly important. The prior dictum that regimen failure mandates the change of as many of the drugs in the prior regimen as possible has given way to the concept that drug resistance testing is the key component in choice of subsequent regimens. This change in thinking has arisen with the realization that drugs in treatment regimens fail sequentially rather than simultaneously and with the wider availability and appreciation of the role of resistance testing. Although generalizations in this regard are dangerous, the sequence with which drugs fail follows a general pattern that drugs with the most potency and the least number of mutations required to develop high-level

TABLE 31.2. *Comparison of attributes of genotypic and phenotypic testing methodologies*

Genotypic testing
Advantages
Turn-around time ~ 1 week
Generally less expensive
Disadvantages
Interpretation dif cult except in experienced hands
Not quantitative
Not predictive of phenotypic susceptibility in some viral isolates—especially those with multiple mutations
Phenotypic Testing
Advantages
Quantitative
Interpretation more straightforward
Not affected in unpredictable ways by complex genetic interactions
Disadvantages
Turnaround time 14–21 days
More expensive than genotypic testing in most settings

resistance are the most vulnerable when regimens initially fail. This would include Òone mutationÓdrugs such as 3TC and the NNRTIs as compared to protease inhibitors and drugs such as abacavir. If a regimen is continued after resistance to these drugs has developed, it is highly likely that the stepwise selection of broader resistance to other agents in the regimen will follow.

A full understanding of resistance testing is a required component of contemporary management of antiretroviral chemotherapy. Resistance testing should be undertaken each time a regimen fails, and in situations in which the transmission of drug resistant virus is common (> 5% of isolates), when therapy is initiated for the Þrst time (81). Viral characterization as it relates to drug susceptibility may be undertaken by either genotypic or phenotypic techniques (Table 31.2). Genotypic approaches rely on associations between previously deÞned mutations in speciÞc locations and drug susceptibility; phenotypic testing actually measures drug susceptibility of the virus in question to available agents. Genotypic testing is usually less expensive and usually has a more rapid turnaround time than phenotypic testing but it does not provide quantitative data and it is increasingly limited with the evolution of the more complex viruses that emerge after exposure to sequential drug regimens. Although genotypic testing is adequate in patients with limited drug exposure, this approach becomes increasingly limited in its predictive capabilities in patients in whom it matters the mostÑ namely in those with fewer treatment options. Under ideal circumstances, physicians would use both approaches in all patients because the information provided by the two techniques is complementary. Considerations of cost often require the physician to choose one approach or the other in a speciÞc circumstance. In general, it is acceptable to use genotypic testing in patients with relatively limited treatment experience and to increasingly use phenotypic testing as patients have more treatment experience.

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Chapter 32

Discovery and Development of New HIV Medicines

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The success of current HIV chemotherapy and the research that supported it has created both opportunities and hurdles for the development of new medicines to combat this infection. The opportunities range from novel molecular targets to avenues for overcoming drug resistance or undesired side effects of current drugs. However, because of the many drug approvals (16 to date) and the emergence of generic versions of existing drugs in the near future, a new drugÕ attributes must be striking to play a signiÞcant role in standard care and be commercially viable. Because many patients will proceed through multiple regimens during their course of treatment, and there is widespread acceptance of the drugs in the initial lines of therapy, many new drugs may be limited to later treatment regimens. This chapter will discuss speciÞc opportunities and hurdles in the development of new antiretroviral agents in the context of the broader difÞculty of developing any new drug.

The chapter will focus on the discovery and development of small organic molecules for treatment of HIV infection. DNA or RNA-based therapies have been and will continue to be examined in clinical trials (1,2), and the 36mer peptide T20 (3) most likely will be FDA approved. However, the preference for small molecules is supported by the molecular weight range for compounds approved to date for HIV therapyÑbetween approximately 300 for nucleoside analogs and 700Ð800 for protease inhibitors, with non-nucleoside reverse transcriptase inhibitors falling in between. In addition, among the approved HIV drugs, those with higher molecular weights usually have poorer pharmacokinetic and oral absorption properties, a common observation within drug development in general (4). Some of the desired properties of a new drug and various approaches to predict achieving the product proÞle are listed in Table 32.1.

The drug discovery and development process begins with a hypothesis and ends with approval to sell a drug for speciÞc indications. For organizational purposes, the entire process is sometimes divided into three broad research efforts: generation of a lead molecule, preclinical development of that lead, and clinical development of the drug candidate. Even though the possibility exists for a *fast track* approval by the USFDA (which can reduce the clinical process by one to two years), the entire process usually takes at least ten years. For example, the viral entry inhibitor peptide T20 was Þrst described in the scientiÞc literature in 1994 (5), and even with a fast track designation, the soonest that it will be approved will be 2003. Furthermore, new drugs targeted against HIV integrase will take even longer due to the difÞculty of generating leads for this particular target. Integrase was cloned and expressed in 1990 (6), and the Þrst inhibitors described in 1993 (7). However, the Þrst molecules with cellular antiviral activity proven to be due to inhibition of integrase (8,9) have only just recently entered Phase I trials. Thus, at best, the process will take at least 15 years for integrase. Figure 32.1 provides a graphic look at the magnitude of the effort from screening to launch of a new drug. Because of the length and expense of this overall process, pharmaceutical companies spend considerable time evaluating particular targets before any work commences. The speciÞcs of this exercise will be reviewed in this chapter.

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TABLE 32.1. *Predicting the desired properties*

Property	Preclinical Predictors
Effective	Biochemical assays, cell-based assays, animal models
Safe	Selectivity assays, receptor panel screening, toxicology studies
Convenient (small oral pill, QD)	Solubility, cell permeability, *in vitro* metabolic stability, pharmacokinetics in animals
Financially viable	Differentiated from competition
	Low dose, simple synthesis

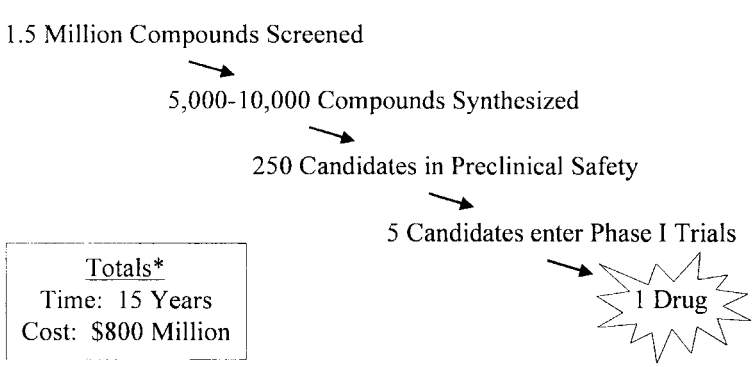
TARGET TRACTABILITY AND VALIDATION

To decrease the attrition rate of drug discovery projects, targets are analyzed prior to commencing research for their tractability (the chances of developing a desired modulator of that target) and validity (the certainty that the target plays an essential role in the disease in question). Such analyses do not give an absolute score of attractiveness, but can lead to a relative ranking of many targets. We will highlight the various components considered in these exercises, and then describe the tractability and validity of the proteins encoded in the HIV genome as an example.

In judging tractability, the most important question is what is known about the proposed binding site for small molecule modulators. This understanding can range from very high with a binding site of a well studied class of enzymes with many structural classes of modulators already identiÞed (e.g. the ATP pocket of protein kinases (10,11)) to non-existent for a protein-protein interaction where no structural information is available. In general, enzymes or receptors that bind small biomolecules as substrates or ligands are more tractable than enzymes or receptors that bind other macromolecules. The least tractable targets are macromolecular interactions of relatively large surfaces. However, if this interaction is dominated by a relatively small number of residues (e.g. the RGD pocket of integrins (12)), small drug-like molecules could mimic those residues. In addition, there are examples of small molecules modulating macromolecular interactions through noncompetitive mechanisms (e.g. modulators of G protein-coupled receptors that bind small proteins/large peptides). The existence of such noncompetitive binding sites cannot be predicted *a priori* and may be identiÞed through random screening. Obviously, such targets are given relatively low tractability scores.

Aside from knowledge of the potential small molecule pocket, each of the speciÞc steps that make up the drug discovery process can be accessed for the likelihood of success. The following questions and answers enumerate some of the major steps.

1. If necessary, can the molecular target be cloned, expressed, and puriÞed? Possible difÞculties are the size of the protein, and post-translational modiÞcations, membrane-bound nature of the protein, and overexpression of protein being toxic to cells. Solutions may include truncations or mutations, and thus the biological relevance of the altered construct must be considered.
2. If random screening is required, can a biologically relevant high throughput assay be developed? It is relatively easy to envision and develop an enzyme or binding assay that can be used to screen hundreds of thousands of compounds in a few weeks. However, there are many examples of difÞculties in establishing biological relevance (for example, wrong ÒformÓ of the enzyme, missing componentsÑ either macromolecular or small molecule, incorrect concentrations relative to *in vivo* environment). Alternatively, cell-based assays can sometimes be considered. However, such assays often produce relatively high response rates or biological activity with unclear mechanism.
3. If structure-base design is desired, is the target obtainable in high quantity and purity, and can crystals



*Source: PhRMA

FIG. 32.1. Finding new drugs: an exercise in beating the odds.

of both apo and higher order complexes be grown? This is hard to predict, with answers empirically determined. Obviously, large proteins of over 100 kD in size and membrane-bound proteins are usually problematic.

4. Can small molecule starting points be identiÞed? (See discussion above on small molecule pockets.)
5. Can secondary assays be developed to progress starting points into clinical candidates? Minimally, a cell-based assay is required to test whether compounds that are active *in vitro* are also active in a cell-based assay, especially if the target is intracellular. If the target is not fully validated (see below), such assays can also provide further evidence for disease association.
6. Can chemical starting points be progressed to clinical candidates? Many hurdles need to be overcome with speciÞc goals often conßicting with one another: increase in potency and selectivity, appropriate oral bioavailability, favorable *in vivo* pharmacokinetics, and metabolism proÞles, no or low *in vivo* toxicity. Usually, these issues do not weigh heavily in determining initial target tractability. However, knowledge may be available to give conÞdence or cause concerns.
7. Have biological patents of the target or compound patents describing modulators of the target been issued? Though not a scientiÞc issue, it is critical in starting a drug discovery program within a pharmaceutical company that research directions are not blocked by issued patents.
8. How easy or difÞcult will it be to monitor modulation of the proposed target in clinical trials? Again, it is not critical to know the details of the answer at the time of target evaluation. However, the earlier details are known, the sooner preclinical experiments can be devised to support clinical trials. It should be stressed that clinical trials of antiviral agents to treat HIV infection have a major advantage in the relative ease of assessing efÞcacyÑ the reduction of viral load in plasma samples.

Separate from target tractability is the evaluation of target validation. Target validation rests on the hypothesis that the target plays a critical if not essential role in the given disease. If true, modulation of the target will improve the disease state. Compared to complicated metabolic diseases such as type 2 diabetes, validation of microbial infection targets is relatively straightforward if the microbe is well characterized and a cell culture system is established. This is certainly true of HIV. Through any number of genetic or biochemical techniques, selective disruption of speciÞc HIV genes or gene products can and have demonstrated that particular gene products are essential to the viral lifecycle. Genetic techniques include deletion or mutation of the gene in question, or blocking transcription or translation of the genetic information into protein via antisense, RNAi, or ribozyme nucleic acids introduced transiently into the infected cell. Alternatively, protein reagents such as antibodies or dominant negative forms of a protein can block the target proteinÕs function.

Although biological reagents can probe for the microbeÕs dependency on a particular protein, there can be a subtle difference between using such tools and knowing for certain that a small molecule can clinically modulate the target. With some biological tools, the target is not made, which results in two subtle differences compared to small molecule modulation. (1) The protein in question may have an unknown secondary function that could also impact the lifecycle. (2) The microbeÕs dependency on the target could be absolute and inhibition of the lifecycle only obtained by 100% alteration of the targeted function. Because of intracellular concentrations of target, substrates, ligands, or inhibitors, a small molecule may not achieve complete modulation. Usually, biological validation does predict clinical success of a small molecule. However, the highest degree of target validation is *clinical* data demonstrating efÞcacy with a small molecule modulator.

Finally, target validation has a second component in addition to conÞrmation of efÞcacy, that is determination of whether speciÞc modulation of the target would induce toxicity. With microbial targets, unless there is a highly homologous host counterpart, target-based toxicity is usually not a concern. (It should be emphasized that this issue is not the same as compound-based toxicity that is always a concern until proven otherwise.) However, lifecycles of microbes often depend on host proteins. Because a host protein may be demonstrated to be essential and also be viewed as tractable, host proteins such as the chemokine receptors have become attractive targets for new HIV therapies. However, such proteins may also play critical roles in normal human physiology, and thus modulation may lead to target-based toxicity.

Using the above descriptions, the proteins encoded by the HIV genome have been ranked in terms of their *relative* attractiveness (Table 32.2). This analysis should not be viewed as a deÞnitive description of the likelihood of developing drugs targeted against each protein, but rather as a vehicle for exemplifying the issues outlined above. This exercise will not include descriptions of the structure and function of the HIV proteins; recent reviews can be referred to for such details (13,14).

Reverse transcriptase and protease are clearly in the top bracket when evaluating HIV targets. The three small molecule pockets for nucleoside reverse transcriptase inhibitors (NRTIs) (15), nonnucleoside reverse transcriptase inhibitors (NNRTIs) (16) and protease inhibitors (PIs) (17) within these two proteins are well characterized, and mutant proteins that lead to drug-resistant virus are now targets for second-generation drugs. Both enzymes have the highest validation as several inhibitor series for both have been approved based on robust efÞcacy in suppressing viral replication in patients. All components of the lead generation process mentioned above have been in place for years. However, even proven targets such as RT

TABLE 32.2. *Relative ranking of HIV proteins as potential targets for development of small molecule antiviral therapy*

Target	Likelihood of Small Molecule Drug	Target Tractability			Target Validation	
		Precedence	Relevant Assay	Structural Data	Biological	Small Molecule
RT	Very High	approved drugs	yes	pockets well de ned	High	approved drugs
Protease	Very High	approved drugs	yes	pocket well de ned	High	approved drugs
Integrase	High	clinical trials ongoing	yes	apo protein	High	*in vitro* resistance
Rev/RRE	Medium	little (aminoglycosides)	probably relevant	pocket w/in RRE	High	none
Tat/TAR	Medium	little (aminoglycosides)	probably relevant	pocket w/in TAR	High	none
RNase H	Medium	little (no antivirals)	possibly relevant	unbound to duplex	High	none
gp41	Med/Low	large peptide	possibly relevant	not encouraging	High	T20 approval expected
gp120	Low	none	possibly relevant	not encouraging	High	CCR5 inhibitors; sol CD4
Gag	Low	none	no	not encouraging	High	PIs; Zn ejectors; cyclosporin
Nef	Low	none	unknown relevance	unknown relevance	LTNP	none
Vpu	Low	none	no	none	accessory	amantadine (by analogy)
Vif	Very Low	none	no	none	accessory	none
Vpr	Very Low	none	no	none	accessory	none

and protease contain inherent weaknesses. NRTIs (and their anabolized phosporylated forms) are close analogs of many naturally occurring metabolites (i.e. nucleosides and nucleotides), and thus may interfere in many critical cellular pathways. The pocket that NNRTIs bind is near to but separate from the active site of RT. Amino acid residues that are critical in binding the inhibitors are not as critical to the normal function of the enzyme compared to active site residues, and therefore resistance arises more rapidly for this drug class. The broad pocket that binds the protein substrates of HIV protease dictates that relatively large small molecules are required for sufficient potency. This in turn has led to relatively poor oral bioavailability and difficulties in formulation. However, these issues are more challenges than obstacles, and RT and protease remain the most attractive molecular targets for new HIV drug development.

HIV integrase resides alone between the targets successfully attacked and all other HIV proteins. This ranking is due to the strength of both tractability and validation for integrase, whereas the other targets are all relatively weak in their perceived tractability. Research on integrase has been ongoing for nearly as long of RT and protease, with most of the components for lead generation in place by the early 1990s. Integrase catalyzes two reactions (reviewed in 18)Ñ hydrolysis of terminal dinucleotides at both 3′-ends of the viral DNA (3′-processing) and insertion of both new 3′-hydroxyls into host cell DNA (strand transfer). Early assays were developed similar to most enzymatic assaysÑ integrase was added *last* in its free or apo form. Inhibitors found using such assays were inactive against the form of the enzyme isolated from virally infected cells (bound in the preintegration complex, or PIC) (19) and were inactive in cellular antiviral assays. Because PICs contain the viral DNA tightly bound to integrase (in addition to other viral proteins), assays were subsequently developed (20) where the oligo substrate that mimics the viral DNA was prebound to integrase. The strand transfer reaction was then initiated by addition of the oligo substrate that mimics host DNA. Such assays identified 4-aryl-2,4-diketobutanoic acids that inhibit integrase within the PIC and HIV replication in cell assays (21). Additionally, the mechanism of antiviral activity has been demonstrated to be inhibition of integrase (21,22), and structure-activity relationship studies have demonstrated a tight correlation between enzyme and cellular potencies (23). Thus, there is little doubt that integrase is fully validated, awaiting clinical data as final proof.

The other four proteins listed under medium attractiveness all have strong biological validation, but concerns exist surrounding the likelihood of finding small molecule modulators. The transmembrane gp41 protein has the highest validationÑ it is the target of the fusion inhibitor peptide T20 which has potent antiviral activity in clinical trials (24). However, there are serious concerns in finding small molecules that could circumvent the difficulties of delivering a large peptide. T20 is derived from gp41 itself and blocks the formation of the fusogenic state of gp41 by competing with the analogous stretch of peptide within gp41. When T20 (a 36mer) is truncated by more than six amino acids, its potency decreases by greater than 1,000-fold (25). Thus, it is difficult to imagine how a small molecule could mimic this peptide. Consistent with this concern is that the crystal structure of the proposed fusogenic conformation (26) shows no obvious pocket in which a small molecule could bind and effectively block the formation of that state. Assays have been developed to monitor the formation of the fusogenic conformation (27,28), but no identification of small molecule inhibitors has been reported.

The other three targets in this section (RNase H, Rev, and Tat) all have strong biological data supporting HIVÕs essential dependence on their function. However, no small molecule modulator has been developed against these targets which has antiviral activity. Thus, they lack definitive validation. The tractability of RNase H is similar to that of integrase prior to the key findings that led to changes in assay format for integrase. Like integrase, RNase H binds nucleic acid as its substrate in a relatively shallow groove (29). In addition, initial RNase H assays were simple RNase assays where cleavage of RNA within RNA/DNA duplexes was monitored. Inhibitors were identified but had no observable antiviral activity in cells and were often toxic most likely due to nonspecific inhibition of RNases. Recently, more sophisticated assays have been described (30) that appear to better recapitulate RNase HÕs function *in vivo* (31). However, no selective HIV RNase H inhibitors have been reported to date.

The final targets within this section are the two essential viral protein/RNA interactionsÑ Rev/RRE and Tat/TAR. The Tat/TAR interaction is required for robust transcription of the integrated viral DNA into viral mRNA. The Rev/RRE interaction is the essential switch between the translation of early and late viral proteins. Because of the many similarities between these targets, comments will be focused on Rev/RRE. This interaction has been demonstrated by use of a variety of different biological reagents (32,33), and has also been validated by natural occurrences of low viral infectivity in association with suppression of Rev function (34,35). Concerns regarding this target mainly reside with its tractability. The only small molecule inhibitor of this interaction with antiviral activity has been neomycin B (36), an aminoglycoside that binds in the major groove of a stem loop of RRE. However, no mechanistic studies were reported to demonstrate that inhibition of viral replication was due to inhibition of Rev/RRE. The antiviral IC_{50} value was in the hundreds of micromoles (36), and neomycin B binds to structured host RNAs at much lower concentrations (37). Although the NMR structure of a critical helical peptide from the Rev protein bound to the stem loop RRE pocket was solved in 1996 (38), no Rev/RRE inhibitor has been reported through a structure-based approach. Alternatively, a high throughput screen of Rev/RRE did identify modulators

(39). However, no antiviral activity was observed, leading again to the question of whether the *in vitro* assay accurately reproduced the *in vivo* interaction. For example, the concentrations of target inside the nucleus of the infected cell may be much higher than the nanomolar concentrations of Rev and RRE used in the screen, leading to too sensitive of a system. Alternatively, unknown components could be missing in the binding assays. To circumvent such issues, cell-based assays where expression of a reporter gene is dependent on Rev/RRE have been developed (40), although no screens using such assays have been reported.

There are enough concerns regarding the remaining seven HIV proteins listed in Table 32.2 to make drug discovery research extremely risky. Whereas there is little doubt that functional modulation of gp120 or the gag proteins would lead to clinical efficacy, there are significant reservations surrounding validation of the last four proteins listed in Table 32.2 (Nef, Vpu, Vif, and Vpr). Compounding these concerns are the low tractability of these four targets. Within this analysis, ranking is still relative and Nef could be considered more tractable than Vif or Vpr. Most likely Nef's function is tied to its binding of cellular SH3 domains (41), although what aspect of Nef's function is due to binding what particular SH3 domain is not clear. There is even less clarity as to the interactions that drive the functions of Vif and Vpr. Because these proteins are not essential to viral replication, there are no cell assays to use in the progression of lead molecules into clinical candidates. Additionally, how modulators of these accessory proteins would be used and their effects monitored in clinical trials is questionable.

Finally, the structural HIV proteins gp120 and gag (matrix, capsid, and nucleocapsid) fall into the low priority ranking due solely to their low tractability. Each protein's function results from complicated macromolecular interactions. For example, gp120 interacts with three proteins—CD4, a chemokine receptor (either CCR5 or CXCR4), and gp41. The limited structural understanding of its interactions with CD4 and the chemokine receptors (42) does not suggest that small molecule pockets exist. Although binding assays have been developed to measure the interaction of gp120 and CD4 (43), only recently have small molecule antivirals been identified (43a). Furthermore, there are no published reports of a possibly more relevant ternary complex assay. Matrix, capsid, and nucleocapsid have equal if not greater tractability concerns. For example, the interaction that appears to be critical for capsid function is capsid dimerization where the structure is dependent on a large subunit surface (44). Such a molecular target is simply not conducive for drug discovery.

DRUG DISCOVERY

Once a target is selected for a drug discovery effort, the first step is to find a starting point for the drug discovery and optimization process. Two useful, yet quite different approaches are broad screening and structure-based drug design.

Broad screening is not unlike playing the lottery, in the sense that discovering an appropriate starting point, often referred to as a "hit," involves an element of luck. Assuming that finding a hit is a random event, the more compounds you screen, the better your chances are of finding a good hit. This provides scientists with an incentive to seek access to very large collections—the equivalent of buying multiple lottery tickets rather than just one. Broad screening, however, is not a completely random exercise since compound libraries contain bias. Most large compound collections are owned by pharmaceutical companies who have accumulated them over a number of years, and the primary source is former projects within the company. Since the number of structural analogs required to find a single drug candidate is usually well over 5,000, a single drug discovery project may contribute a significant number of high quality specimens to corporate collection over the life of a project. Since many of these compounds are likely to be closely related, however, the structural diversity contributed to the collection may be surprisingly low. This, consequently, is the source of the collection bias. This can be an advantage if a company is attempting to start working with a target that is structurally similar to the target of an existing research project. For example, in the early days of HIV protease research, a number of companies with renin inhibitor research programs were able to easily find hits. Since renin and HIV protease are quite similar, collections of renin inhibitors were fertile hunting ground for the HIV scientists. Of course, the disadvantage of this bias is that it can add an additional hurdle to companies attempting to start work in a novel target area.

Most companies attempt to compensate for bias in their compound libraries by supplementing from outside sources. This may be done in a deliberate fashion, by analyzing the library for gaps in its chemical diversity and making or purchasing structures that fill those gaps, or it may be done in a more opportunistic fashion, by simply buying compounds that become available from commercial or academic sources.

The compound collection is not the only necessary component for a broad screening strategy. One must also develop an assay that is amenable to high throughput screening. Much of this screening is now automated and done on 384-well plates. The automated approaches obviously will have some constraints on assay conditions, but do offer the advantage of evaluating hundreds of thousand of compounds in the space of a few days. As mentioned previously, it is vital that the *in vitro* screening assay recapitulate the *in vivo* situation as closely as possible.

Once a data set has been generated by a high throughput screen, the next step is hit selection. A high throughput screen typically generates a percent activity (usually

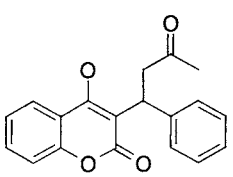
inhibition) of target at a single concentration of compound. The Þrst step is usually to conÞrm the activity of the most promising compounds by generating titration curves. Once the activity is conÞrmed, structures for optimization will be chosen based on both activity in the assay and chemical tractability. It is of particular importance to gain a sense of structure activity relationships (SAR). Medicinal chemists will look for patterns of structural changes, which correlate with varying degrees of target inhibition, to gain conÞdence that the compound is modulating the assay via the intended mechanism of action.

An example of broad screening used to generate a novel lead can be found in the HIV protease discovery process. As mentioned above, peptidomimetic renin inhibitors were expected to generate HIV protease hits, and as will be discussed in more detail later, those hits generated some excellent drugs. In this situation, however, a broad screen found the coumarin derivative warfarin (**x**). Warfarin was a known anti-coagulant, but scientists at Upjohn were able to separate the antiviral activity from the anti-coagulant activity during optimization of the structure, and this research effort produced tipranavir. It is not coincidental that tipranavir has a unique resistance proÞleÑ starting from a different hit than the other protease inhibitors gave it an advantage in this respect (Fig. 32.2) (45,46).

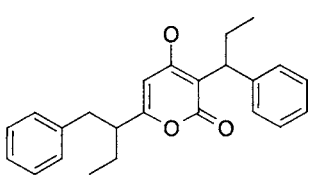
The second approach for identifying a hit is structure-based drug design. This approach is much more elegant than the broad screening approach, but limitations in our knowledge of target make it harder to implement. In this approach, knowledge of the structure of a particular target is used to design effective inhibitors. Some of the best examples are found in the HIV protease literature. As discussed, structural features of the protease were known to be similar to renin, a familiar target to many in the pharmaceutical industry. For example, scientists at Merck screened their renin inhibitor collection to Þnd a starting point for the design of HIV protease inhibitors. Through a series of structural modiÞcations, which were guided by molecular modeling and X-ray crystal structures of the inhibited enzyme complex, they were able to produce indinavir, one of the HIV protease inhibitors which helped achieve prolonged viral load suppression through combination therapy in the early 1990s (Fig. 32.3) (47,48).

It is important to note that broad screening and structure based drug design approaches are by no means exclusive. Using them together can be an extremely powerful approach. Note in Fig. 32.2 that the coumarin structure warfarin underwent signiÞcant structural alteration on its way to tipranavir. Scientists working on this project got many clues about improving the binding afÞnity of the compounds by studying X-ray crystal structures of warfarin and subsequent analogs in the HIV program bound to the HIV protease.

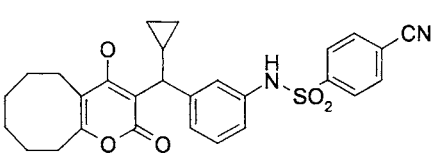
Once a hit has been identiÞed, either through broad screening or design work, the next step is to optimize the structure to contain all of the desired properties and none of the undesired properties. This is very much an iterative process: medicinal chemists make novel compounds



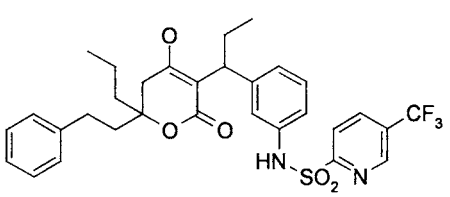
1, Warfarin



2, PNU-96988

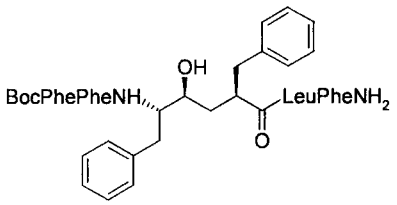


3, PNU-103017

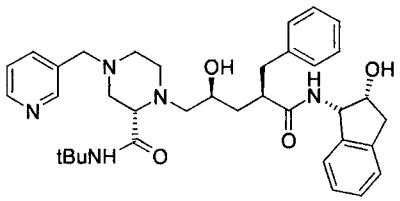


4, Tipranavir

FIG. 32.2. Compound **1**, the anticoagulant warfarin was identi ed through broad screening against HIV protease. Warfarin had minimal activity; the IC_{50} value against HIV protease was ca. 30 μM, and there was no sign of activity in cell culture. Optimization of this screening hit originally focused on increasing inhibition of the HIV protease and decreasing anti-coagulant activity, both of which were achieved with **2**, the rst generation clinical candidate from this series (ED_{50} in HIV-1_{IIIB} infected MT4 cells was 3 μM). Additional optimization resulted in the preparation of more potent compounds, including the second generation clinical candidate **3** (ED_{50} = 1.5 μM) and tipranavir (ED_{50} = 0.03 μM), which is currently in Phase III clinical trials. Early data indicate that tipranavir has very promising clinical ef cacy against viral strains resistant to current approved HIV protease inhibitors (45).



5, L-364,505



6, Indinavir

FIG. 32.3. Peptide derivative **5** was identi ed through screening of a renin inhibitor collection. Compound **5** was quite potent against HIV-1 protease (IC_{50} = 1 nM) and showed some selectivity over renin (IC_{50} = 73 nM), but it had activity against HIV in cell culture only at high micromolar concentrations (47). Extensive modi cation, guided by molecular modeling and X-ray crystal structures, led to the discovery of indinavir, an extremely potent HIV protease inhibitor (IC_{50} = 0.52 nM against HIV-1 protease; no inhibition of human proteases, including renin; and complete inhibition of HIV-1 $_{IIIB}$ spread in cell culture at 50 nM) (48).

which are designed to have the desired properties, these compounds are evaluated in biological assays, then the chemists use these results to guide structural modiÞcations and a new set of analogs are made. This exercise generally takes at least a couple of years, in part, because a number of properties must be optimized. In addition to potency, the scientists on the team need to design compounds that have favorable physical properties (e.g. solubility), good pharmacokinetics, excellent selectivity, and a structure amenable to efÞcient synthesis to keep cost of goods low (Fig. 32.4).

Generally, the Þrst aspect of a lead structure that is optimized is the *in vitro* potency in the primary assay. One of the challenges of this part of the optimization effort is knowing when the compoundÕs potency is sufÞcient for clinical efÞcacy. The clinical efÞcacy will, of course, depend on both the intrinsic potency (which is ideally measured by the primary assay) and the exposure levels achieved *in vivo*. Recent examples in the chemokine receptor Þeld illustrate this concept. Schering has developed a CCR5 antagonist, SCH-C, with excellent activity in assays measuring RANTES binding (Ki = 2 nM) and in antiviral assays (mean IC50 = 2 nM against range of M-tropic viral isolates in PBMC cells). In rats, SCH-C had a half life of Þve hours and an oral bioavailability of 63% (49). Recently, clinical studies showed that this preclinical proÞle translated into promising viral load reductions, even at a dose of 50 mg of SCH-C twice a day. At this dose, 10/12 subjects had at least a 0.5 log drop in viral load over the study period of 10 days (50). In contrast, AMD-3100, which is also a nM inhibitor of HIV replication *in vitro* (EC50 = 4 nM against T-tropic virus), performed much poorer in a clinical setting. In a dose-escalating study of 40 patients, only one patient achieved a viral load drop of more than one log (51). There are at least two major differences between these two studies. First, the pharmacokinetic proÞles of the two compounds are quite different, although dosing of AMD-3100 intravenously would overcome some of the exposure differential. The second difference is in the targets. Although both CCR5 and CXCR4 are both chemokine receptors which are necessary for HIV entry, the CCR5- and CXCR4-tropic viruses exhibit differences in both prevalence and pathogenicity.

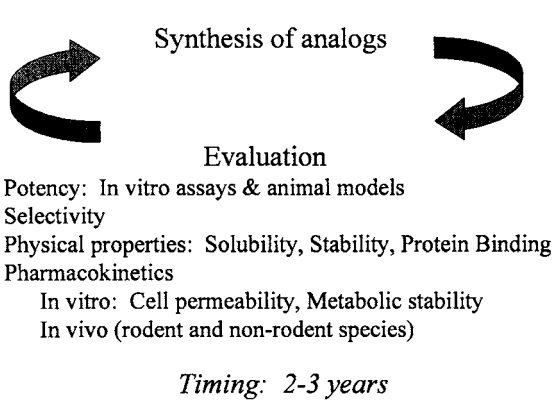
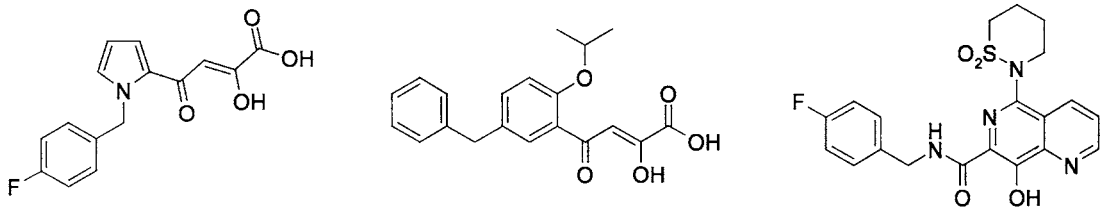


FIG. 32.4. Lead optimization.

As mentioned above, potency is not the only criterion considered in the optimization stage. One elegant example of optimization to improve metabolic stability is found in MerckÕs HIV integrase inhibitors. As mentioned in the earlier section, Merck identiÞed 4-aryl-2,4-diketobutanoic acids in early screening, and they were able to signiÞcantly improve the antiviral activity of this series by modifying the aryl portion of the molecule (23). Although the *in vitro* potency of the modiÞed compound was over 100-fold better than the initial lead compound, the modiÞed agent still had metabolic liabilities that restricted its utility as a drug. Replacement of the acid with an isostere, followed by replacement of the resulting 1,3-diketone with a 1,6-naphtyridine produced compounds such as L-870,810, which has both potency and metabolic stability (Fig. 32.5). This compound also demonstrates good pharmacokinetics in three species (52).

Optimization culminates in selection of a candidate, but there is still signiÞcant work to do before that candidate can enter clinical trials. The focus at this stage is on preclinical developability and safety.



7, L-731,988 **8** **9**, L-870,810

FIG. 32.5. The original integrase inhibitor lead **7** (IC_{95} = 9.6 μM in HIV-1 $_{IIIB}$ infected MT4 cells), was modi ed to improve potency, resulting in compound **8** (IC_{95} = 0.10 μM). Further modi cation of this structural template led to the discovery of clinical candidate **9**, which has both improved potency (IC_{95} = 0.019 μM) and metabolic stability, relative to earlier structures in this class (52).

Developability refers to characteristics of a new drug that are necessary for efficient manufacture and *in vivo* delivery. At this point, stable crystal forms of the drug candidate will be identified and the synthetic route will be optimized for efficient manufacturing. Stability of the drug candidate will be evaluated, and formulation work will begin in earnest.

Preclinical safety work also begins in earnest once a candidate is selected. The potential for mechanism-based toxicities were addressed at target selection stage, but compound-based toxicities are difficult to predict and remain a potential issue until proven otherwise. Before beginning animal studies, scientists will use any available *in vitro* screening methods, such as broad panels of receptors, to look for potential difficulties. Of course, toxicity studies in at least two species are required before clinical trials can begin.

For some classes of compounds or targets, a potential safety liability has been identified, and scientists can attempt to screen for this at the preclinical safety stage, or even earlier during compound optimization. For example, HIV nucleoside reverse transcriptase inhibitors have been associated with symptoms of mitochondrial toxicity, such as peripheral neuropathy, in the clinic. The mitochondria employ a γ-polymerase, and *in vitro* studies have shown differential γ-polymerase inhibition by different nucleoside triphosphates (53). Correlating the *in vitro* profile with clinical side effects over time can be an important clue for designing safer drugs. Since mitochondrial toxicity is both quite serious and difficult to detect in animal models, such information can be quite valuable.

Toxicity and side effects are not a black and white issue. The level of undesired effects a drug candidate can have will depend on several factors, including the severity of the adverse events relative to the severity of the disease being treated and the ability to control adverse events with other drugs. The success of pharmaceutical scientists in controlling HIV infection with HAART has allowed current drug discovery efforts to focus more on both the long term toxicities associated with chronic HAART and the convenience of drug regimens that can promote adherence. For example, although atazanavir, a protease inhibitor in late stage development, does not have significantly better *in vitro* potency than other available protease inhibitors, its once a day dosing and lack of effect on patient lipid profiles are creating significant enthusiasm (54).

CLINICAL DEVELOPMENT

The final step in the drug development process is clinical evaluation. This will involve an initial safety study (Phase I), usually in healthy volunteers, a small-scale efficacy study (Phase II), and large registration trials to evaluate both safety and efficacy in a broad population (Phase III). In HIV infection, the endpoints of these studies are usually based on viral load, but it is important to note that this is a surrogate marker. Registration of the early HIV drugs was based on clinical endpoints, such as progression to an AIDS-defining illness or death. Although viral load surrogate markers are widely used and readily accepted by the regulatory agencies, clinical endpoints are still the gold standard and may be appropriate in some cases. A recent example where viral load measures may not have told the whole story was the AMD3100 study cited above. Although only one patient reached the goal of a □1 log drop in viral load, a closer look at the data indicated a dose dependent shifting of viral populations from X4/R5 dual-tropic to R5-tropic. In other words, the X4-tropic population was reduced below the limits of detection (55). Since X4-tropic disease has been associated with increased viral pathogenesis, there may still be some benefit to a drug with this profile. Of course, a clinical endpoint trial would be required to prove any such benefit.

Given all of the potential issues cited above, it should not come as a surprise that despite the best efforts of scientists to predict clinical success, only approximately 20% of drugs which enter Phase I trials make it to registration. We have already noted the challenges of predicting clinical efficacy and safety based on pre-clinical data, especially for novel targets and new chemical classes. For these reasons, it is routine for large pharmaceutical companies to bring forward multiple drug

candidates. For example, we have already noted that for tipranavir there had been two earlier generation protease inhibitors which entered the clinic. Even though initial efficacy studies with SCH-C are very promising, Schering-Plough has begun development of a second generation compound, SCH-D (56). Although it may be somewhat discouraging to need multiple attempts to get a good drug to patients, the initial failures often yield valuable information for design and development of the drug candidate which is ultimately successful.

Following a successful phase I study where safety and tolerability and drug exposure levels are determined, Phase II studies are designed to provide evidence of efficacy. Early efficacy studies of novel agents face many challenges. ÒProof of ConceptÓstudies are relatively small and begin to provide data as to whether the compound is likely to meet a desired product profile. These studies may not be adequate for regulatory submission but provide data to reduce some of the risks inherent in committing to a full-scale clinical development program. Time and resource pressures usually make it imperative that Proof of Concept is achieved as quickly as possible. It is particularly important to decide in what patient population a new agent is most likely to show efficacy. This population is not necessarily the one that is the ultimate target population based on medical need and commercial viability. For example, it may be appropriate and expedient for early Phase II studies to be conducted in anti-retroviral na•ve subjects who have progressed to a stage where initiation of therapy is recommended based on current guidelines. This may not be the ultimate target population if, for example, the drug is intended to treat drug-resistant strains of HIV. The advantage may be an opportunity to enroll volunteers and complete the trial more rapidly but the tradeoff is the risk that efficacy in the trial population may not translate to efficacy in a more difficult-to-treat population chosen for the Phase III study. Recruitment for these studies may also be a challenge as patients who are on effective regimens are often less likely to be willing to switch to a regimen with a novel drug in early development where the Proof of Concept has not been met. These patients may be more willing to participate in Phase III studies where there exists some evidence of efficacy. At the other extreme are salvage patients who have few therapy options remaining. For many new agents this is the target population and Proof of Concept studies are conducted in this population straight away. The most straightforward way to demonstrate antiviral activity of a novel agent is to test it as a single agent for a short period of time. The duration of treatment in monotherapy is typically from a few days to one or two weeks. Based on what is known about current HIV drugs, monotherapy will almost certainly be sub-optimum. Thus, if monotherapy is used, it should be limited to short duration especially if there is a low genetic barrier to drug resistance for the target and resistance is expected to occur rapidly. Furthermore, it is important to consider whether such resistant virus is likely to be cross-resistant to other members of the class (if the novel agent is a member of a current target class). Another consideration is what therapy is appropriate for the subjects following the short duration testing with the novel agent. Here again, one is confronted with several options. If the new agent is shown to be effective in this short duration monotherapy study, one could add two or more current drugs to constitute a regimen and continue the trial along with an appropriate comparator arm without the novel agent. The toxicology studies for the novel agent must be of sufficient duration to match the duration of human exposure in order to continue such a trial. Often in early development such toxicology is not available and following short exposure to the novel agent, subjects may switch to a standard first line or other appropriate regimen depending on their treatment history. Additional, longer term clinical studies are designed based on the data from the short duration studies, and they may be initiated when sufficient toxicology coverage is available. The timing of preclinical and clinical study results is quite important to ensure rapid and uninterrupted development of the new agent.

While monotherapy may a straightforward way to demonstrate activity of a drug in an early Proof of Concept study, it is not the standard of care and novel drugs must be tested in combination with other drugs. Indeed, in the vast majority of Phase II studies a novel agent is tested in combination with one, two or three other agents. Preclinical studies can give some guidance on the suitability of certain combinations, especially if antagonism is suspected. Regulatory agencies often request *in vitro* combination studies of the new agent with approved agents.

For the larger, longer term Phase III studies it is very important to conduct the appropriate studies to ensure that the desired indication for the new drug is achieved. Given the large number of approved anti-retroviral drugs and the massive number of possible combinations, it can be a daunting task to design a well-controlled study to determine the efficacy of a new drug in a combination regimen. Close interaction between the sponsor and the regulatory agency is essential as this phase of development requires significant investment in time and resources and volunteer subject commitment. Following regulatory approval of a drug, the greatly expanded use outside of the carefully controlled clinical trial setting often brings more information about safety. Continued monitoring by drug company sponsors in Phase IV is important in capturing and interpreting this information. Furthermore, as long term use of drugs continues, long term side effects are revealed. For examples, mitochondrial toxicity mentioned previously and lipodystrophy syndrome have emerged as serious side effects of HAART (also discussed elsewhere in this volume (Chapter 00). A better understanding of the etiology of the metabolic complications of HAART could lead to development of preclinical models and assay

systems for next generation drug discovery and development. For example, PI inhibition of adipocyte differentiation (57,58), NRTI effects on mitochondrial function and the associated lactic acidosis (59) and CCR5 inhibitor QTc prolongation (60), provide some tools and benchmarks to apply to progressing new compounds.

CONCLUSIONS

There are indeed many challenges to drug discovery and development as outlined in this chapter. However, it is likely that the coming years will see new safe and effective drugs to treat HIV-infected patients. Viral gene products will remain the best drug targets and integrase is likely to be the most important new target for drug development. Although after integrase, additional viral targets will represent large challenges. There is a shift in the perception of acceptability of host cellular targets and the chemokine co-receptors are expected to constitute another area likely to be successful. Drug resistance and side effects to currently approved agents will continue to drive discovery efforts to Þnd improved drugs to the well-validated targets. Improvements in preclinical assessments to reduce attrition of drug candidates is a constant goal of the pharmaceutical industry and this will be especially important for HIV infection, as this area becomes even more crowded with approved drugs and the hurdles to initiate new programs gets higher and higher.

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Chapter 33

Toxicities of Antiretroviral Therapy

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Recognition of HIV as the pathogen behind the AIDS epidemic in the 1980s led to an intensive effort within medical, research and pharmaceutical establishments to develop therapies that could delay progression of disease and possibly eradicate the infection. Due to the signiÞcant morbidity and mortality associated with AIDS, it was imperative to move potentially life-saving therapies quickly from the laboratory to the clinic. For example, it took only two years from the initial recognition of zidovudineÕs (AZT) antiretroviral effect *in vitro* for the drug to receive FDA approval for general clinical use in March 1987 (1). The development of nucleoside reverse transcriptase inhibitors (NRTI) delayed progression and improved mortality in many cases, but it was not until the introduction of protease inhibitors (PI), and the recognition that eradication of HIV was not a realistic treatment outcome (2), that HIV-infection, in developed countries, was widely regarded as a chronic disease requiring long-term management strategies.

Many phase II and III trials of new antiretroviral medications identiÞed potential immediate and short-term side effects of the drugs. Many side effects were avoidable with appropriate patient monitoring and alteration of dose, or were of a severity where continued treatment was thought to be beneÞcial compared to the dangers from untreated disease (3). In many cases, pre-licensing data regarding the medium to long-term side effects of speciÞc drugs or drug combinations was not readily available. Consequently, within several years of the use of combination highly active antiretroviral treatment (HAART) involving PIs and NRTIs, the emergence of metabolic abnormalities, chießy related to lipid metabolism, and changes in body shape were described (4,5). These have since been grouped together under the umbrella of HIV-associated lipodystrophy (HIVLD). Of all the side effects of antiretrovirals, this condition has drawn most attention due to its impact on the general appearance of affected patients, as well as the possibility of increased cardiovascular disease associated with the metabolic abnormalities (6).

As treated HIV-infected patients live longer, grow older, and cope with more co-morbidities, such as concurrent chronic viral hepatitis infection, so the spectrum of toxicities is evolving. In addition to HIVLD, concerns have arisen with regard to osteoporosis and hepatotoxicity associated with long-term antiretroviral treatment. This chapter will aim to address issues of toxicity, concentrating on HIVLD. In addition to prevalence of speciÞc side effects and risk factors associated with their development, possible pathogenic mechanisms behind the abnormalities will be discussed. The text will also provide an insight into current and future management strategies to cope with these problems.

HIV-ASSOCIATED LIPODYSTROPHY

Description

Lipodystrophies have been described as far back as the late 19th century (7), although prior to the recognition of HIVLD, lipodystrophy was conÞned to relatively rare congenital and acquired disorders (8). HIVLD is characterised by the development of morphological and metabolic abnormalities associated with the use of antiretroviral medications (ARV). Morphological abnormalities revolve around adipose (fat) tissue, hence the term Ôlipodystrophy.Õ Wasting of subcutaneous adipose tissue

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from the peripheries (4), and accumulation of adipose tissue in the intra-abdominal space, the dorsocervical regions and the breasts are hallmarks of the condition (4,5) (Fig. 33.1) although discreet lipomata have also been described (9). Metabolic abnormalities include hyperlipidemia, insulin resistance, diabetes mellitus and lactic acidemia (4,10).

These morphological and metabolic abnormalities share similarities with previously described congenital and acquired lipodystrophies (8) (Table 33.1). Mechanisms underlying these conditions have been used as models in the investigation of the pathogenesis of HIVLD (11). What is emerging is that the aetiology of HIVLD is complex, with genetic, environmental and drug factors all playing a signiÞcant role.

When a cohort of anti-retroviral na•ve patients was studied after starting their Þrst antiretroviral regimen, an initial increase in body fat in general was observed, associated with an increase in lean mass, increases in CD4 count and decreases in viral load (12). This may represent recovery from the chronic active viral state and the associated catabolic metabolism. After six months of treatment, a selective loss of limb fat tissue occurred, which likely reßects the lipoatrophy described in HIVLD.

Risk Factors

Many complex medical disorders have multifactorial aetiology, involving genetic factors, which predispose an individual to a certain condition, environmental factors, which alter the severity of or susceptibility to the condition, and triggers, which bring the condition to the fore. This is true for diseases such as diabetes (13) and Crohnõ disease (14) and evidence exists to support such a model for HIVLD.

The HIVLD phenotype exhibits marked heterogeneity with some individuals experiencing predominantly peripheral lipoatrophy rather than central adiposity, while others develop metabolic abnormalities before any morphological changes become apparent. This may reßect a combination of environmental and genetic factors contributing to the condition. Certain genetic polymorphisms in fat related genes may result in variations in the severity of

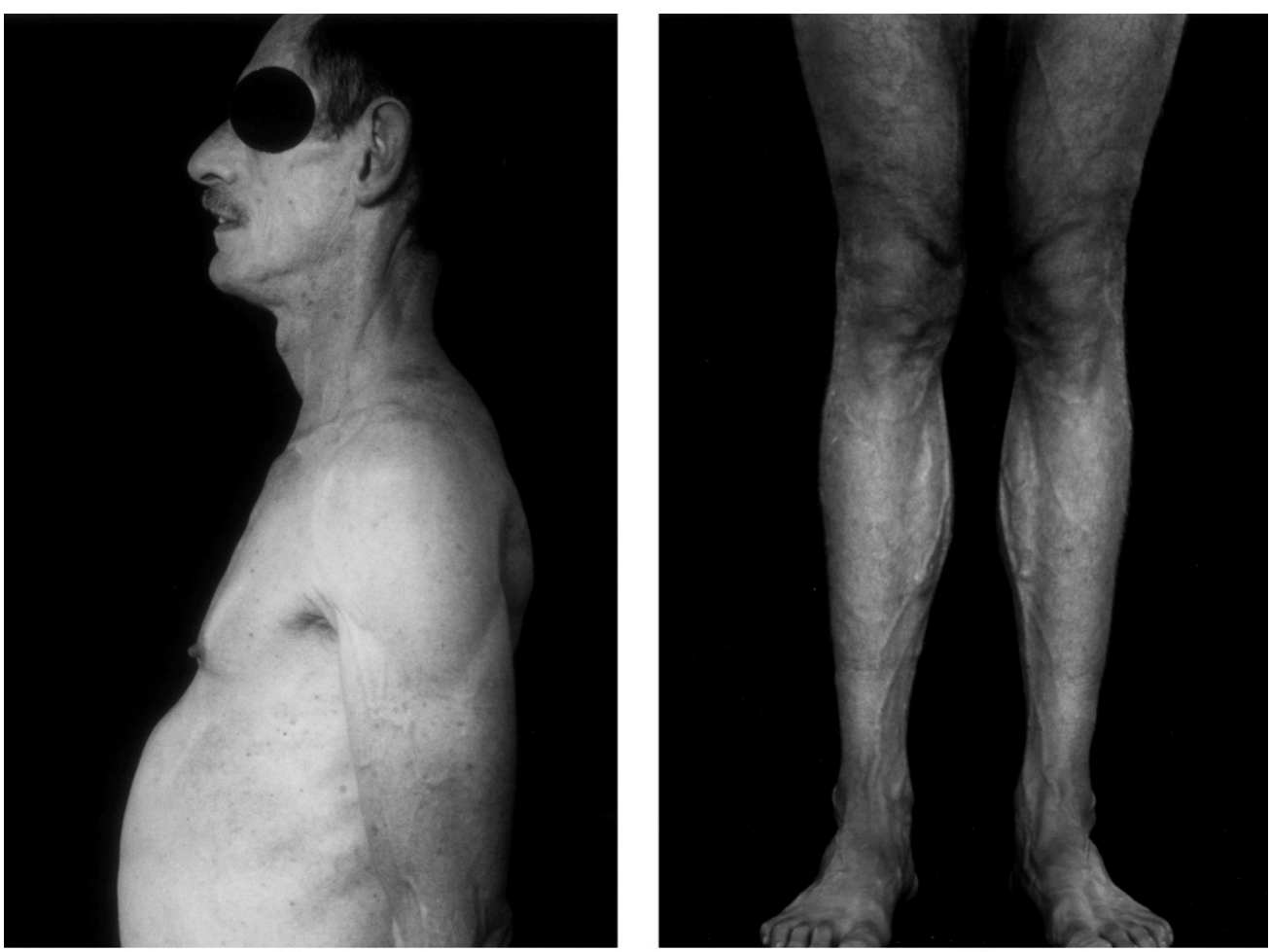

FIG. 33.1. Morphological features of lipodystrophy. (a) Facial and upper limb lipoatrophy with accumulation of central abdominal fat leading to a ‘pot belly’ appearance. (b) Lower limb lipoatrophy resulting in prominence of super cial veins.

TABLE 33.1. *Comparison of HIVLD with other conditions in which lipodystrophy is a feature*

		Defect	SAT loss	VAT gain	buffalo hump	↑ serum lipids	insulin resistance	lactic acidemia	immune related
Acquired	**HIVLD**	Unclear	+	+	+	+	+	+	?
	Generalised Lawrence Syndrome	? Autoimmune	+	ns	ns	+	+	ns	+
	Partial Barraquer-Simons syndrome	C3 nephritic factor mediated adipocyte lysis	+	–	–	rare	rare	ns	+
Congenital	**Generalised** Congenital Generalised Lipodystrophy(CGL)	Gene defect at 9q34	+	–	–	+	+	ns	–
	Partial Familial Partial Lipodystrophy (FPLD)	missense mutation in LMNA gene (1q21–22)	+	–	+	+	+	ns	–
Other	**MSL I**	Mitochondrial DNA (Mt DNA) mutations	–	–	+	–	–	ns	–

"+" = feature of disease. "–" = not a feature of condition. "ns" = not speci ed as feature of condition. ↑ = increase. ↓ = decrease. (8,32). SAT = subcutaneous adipose tissue; VAT = visceral adipose tissue.

HIVLD (15,16) with additive and protective effects described.

Numerous cross sectional cohorts and one prospective cohort have been analysed to determine the effect of multiple environmental factors, factors such as age and sex, and triggers such as antiretroviral treatment on the presence of HIVLD (10,17–24) (Table 33.2). Major associations include prior or continuing use of PIs and/or NRTIs, and increasing age. Minor associations include male sex, AIDS diagnosis and greater CD4 and HIV RNA response to treatment. A signiÞcant role for non-nucleoside reverse transcriptase inhibitors (NNRTIs) was not apparent. The use of combinations including NRTIs and PIs seems to have a synergistic effect with regard to the severity of HIVLD (25).

Pathogenesis

There are complex interactions between adipose tissue, serum lipids, lipids in cells other than fat cells, such as muscle and liver, and insulin resistance. Balanced interactions, under hormonal control, are required for the normal handling of fats within the body (Fig. 33.2) (26). This delicate homeostasis can be upset by numerous factors, including both genetic factors, such as those seen in familial hyperlipidemia and congenital lipodystrophies, and environmental factors, such as diet or drugs.

Lipoatrophy

Lipoatrophy of subcutaneous adipose tissue (SAT) was among the Þrst recognised manifestations of HIVLD and often proves most distressing to patients, explaining much of the apprehension many patients in the developed world feel about starting treatment. Three factors inßuence the mass of SAT. These are differentiation of preadipocytes to mature adipocytes, adipocyte death, either by apoptosis or necrosis, and adipocyte size. Evidence exists supporting a role for each of these factors in the lipoatrophy seen in HIVLD.

$$\text{SAT mass} \propto \frac{\chi[\text{rate of differentiation}].\gamma[\text{adipocyte size}]}{\kappa[\text{rate of adipocyte death}]}$$

Adipocyte Differentiation

Differentiation of adipocytes takes place in two stages. An initial cell mitotic phase (normally two cell divisions from day 0 to day 2) is followed by a differentiation phase (day 2 to day 6–8). This process is regulated by intracellular transcription factors such as the CCAAT/enhancer-binding proteins (C/EBPα & C/EBPβ), sterol regulatory element-binding protein-1 (SREBP-1) and peroxisome proliferator-activated receptor gamma (PPARτ) (27). Changes in levels of these factors during differentiation are illustrated in Fig. 33.3. *In vitro*, protease inhibitors have been shown to decrease the intracellular levels of these transcription factors, resulting in abnormal differentiation of the pre-adipocytes to mature adipocytes (27,28). Similar changes in expression have also been reported *in vivo* (29).

SREBP-1 is thought to be a particularly important factor in the regulation of fat differentiation and metabolism (26). It resides in the cytoplasm before migrating to the nucleus where it stimulates expression of genes involved in lipogenesis and adipogenesis. There are three stages at which its action can be affectedÑ at the transcription phase, limiting the amount of immature SREBP-1 produced, in the cytoplasm where immature SREBP-1 is cleaved to produce an mature fragment that passes into the nucleus, and at the nuclear membrane where the ability of the mature molecule to cross the membrane into the nucleus can be affected (30). Protease inhibitors have been shown *in vitro* to result in accumulation of SREBP-1 outside the nucleus of adipocytes (28), presumably affecting nuclear localisation and subsequent function. Whether they do this by inhibiting the proteases involved in cleavage of immature SREBP-1 to mature SREBP-1, or whether they affect the structure of the nuclear membrane, thereby affecting transport of SREBP-1into the nucleus, remains to be resolved. Interestingly, abnormalities in lamins, structural proteins within nuclear membranes, have been identiÞed in a congenital lipodystrophy called familial partial lipodystrophy FPLD (31,32). This form of lipodystrophy shares many features with HIVLD (Table 33.1).

Additional research has suggested a possible genetic contribution to the function of SREBP-1. Recent identiÞcation of a single nucleotide polymorphism (3Õ22C/G) in SREBP-1c (16) and its association with antiretroviral induced hyperlipidaemia support a scenario where certain individuals may have molecular defects which under normal circumstances do not cause signiÞcant disadvantage, but which place them at higher risk of developing morphological and metabolic abnormalities when combined with added insults to adipose tissue function from antiretroviral therapy. Further population based research in HIV-infected, treated individuals is required to verify such mechanisms.

Adipocyte Death

Increased apoptosisÑ or programmed cell deathÑ has been sought in fat biopsies from individuals with HIVLD (33) and in adipocyte cultures exposed to antiretrovirals (27). The terminal deoxynucleotidyl transferase dUTP-digoxigenin nick end labelling (TUNEL) assay was used to demonstrate apoptosis. In adipocyte cultures, increased TUNEL reactivity was detected after as little as 48 hours

TABLE 33.2. *Factors associated with presence of HIVLD*

Cohort	N	↑Age	Sex	PI now	PI duration	NRTI now	NRTI duration	NNRTI	ARV duration	CD4 count	HIV RNA	AIDS diagnosis	↑Lactate	↑Lipids	Insulin Resistance
Aquitaine (17)	581	LA	LA ♂	ns	+	ns	+	ns		–	–	+	ns	+	+
Australia (18)	1,348	+	LA	+	+	+	+	–		–	+	+	–	+	+
HOPS (19)	1,077	+	–	IDV	IDV	d4T	d4T	–		Nadir	+	+	ns	+	ns
Italian (20)	2,250	–	–	+	+	+	+	–		–	+	–	+	ns	ns
Sydney (10)	221	–	ns	+	+	+	+	–		–	–	–	+	+	+
Spanish (21)	494	+	♀	–	–	–	–	–	+	+	+	–	ns	+	–
German (22)	115	+	♂	LH	ns	+	ns	ns	+	–	+	ns	ns	LH	ns
French (23)	685	+	–	+	+	d4T	ns	–		–	+	+	ns	+	ns
Swiss (24)	1,480	–	ns	+	+	+	+	–		ns	–	–	+	+	ns

" + " = positive correlation with development of HIVLD. "–" = negative correlation with development of HIVLD. ns = not studied. D4T = stavudine. IDV = indinavir. NRTI = nucleoside reverse transcriptase inhibitor. NNRTI = non-nucleoside reverse transcriptase inhibitor. PI = protease inhibitor. LA = association with development of predominant lipoatrophy. LH = association with the development of predominant lipohypertrophy. PI/NRTI "now" = subject on PI/NRTI containing regimen at time of study. PI/NRTI "duration" = total length of exposure to stated class of drug. HOPS = HIV Outpatients Study. ↑ = increase. ↓ = decrease. ♂ = male. ♀ = female.

exposure to PIs (27), while in fat biopsies, mild to moderately increased TUNEL reactivity was seen (33). In addition to apoptosis, other causes of cell death such as necrosis can also result in increased TUNEL reactivity (34). Other speciÞc markers of apoptosis such as procaspase 9 cleavage and DNA laddering were not detected in the cell culture models (27). Therefore, although evidence exists for increased apoptosis in HIVLD, it is by no means conclusive. Nevertheless, evidence exists for increased cell death in HIVLD, whether by apoptosis or necrosis.

Tumour necrosis factor alpha (TNF-α), a molecule released by numerous types of cells within the body, particularly in response to stress, has been implicated in the pathogenesis of HIVLD especially in relation to adipocyte death. It is hypothesised that increased levels, which could be produced in response to HIV infection, could contribute to lipolysis (35,36). Excess TNF-α is known to lead to changes in body composition, as seen in the generalised wasting seen in other aggressive conditions such as metastatic disease (37). However, when plasma TNFα levels were examined in a cross sectional cohort of HIV-infected individuals, neither PI use or lipodystrophy were associated with signiÞcant differences in TNFα concentration (38).

Adipocyte Size

Size of adipocytes is largely determined by the size of the intracellular lipid reservoir. Intracellular lipid reservoirs are depleted in cells exposed to PIs (27,28). Cross sectional analysis of subcutaneous fat from individuals with HIVLD has show marked heterogeneity in the size of adipocytes (33). Obviously the accumulation of lipids within adipocytes will depend on adipocyte function. In addition to the PI effects on transcription factors such SREBP-1 and PPARγ, much interest has been directed at the effect of NRTIs on mitochondria within fat cells.

Mitochondria are essential for the normal physiological function of cells, including the production of acetyl coenzyme A (acetyl CoA) from glucose. Production of acetyl CoA is vital to normal cellular functionsÑ it is the main substrate for the citric acid cycles and the electron transport chain reactions that provide a cell with almost all of its energy requirements (39). Excess acetyl CoA can be stored for later energy use as lipid within a cell and is an important substrate for lipogenesis within adipocytes (26). Mitochondrial dysfunction, resulting in decreased acetyl-CoA, could starve the cell of energy and also prevent an adipocyte from accumulating fat within the cell. Without this energy source, the cell is forced to metabolise

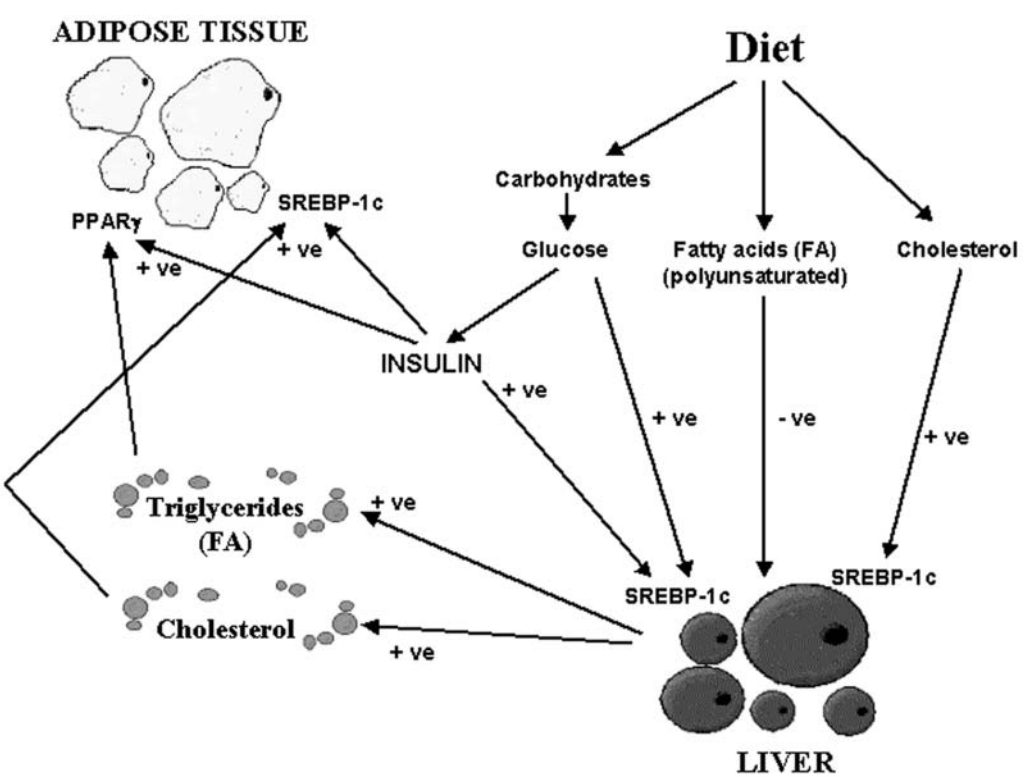

FIG. 33.2. Handling of dietary lipids. Dietary cholesterol and glucose stimulate lipogenesis (production of lipids) in liver. Glucose does this directly, by acting as a substrate for lipogenesis, and indirectly via insulin action. Polyunsaturated fatty acids inhibit lipogenesis. Both serum and intracellular lipid levels and insulin action also in uence lipogenesis in adipose cells. SREBP-1 (sterol regulatory element-binding protein-1) and PPARγ (peroxisome proliferator-activated receptor gamma) are thought to be two important intracellular molecules through which various factors exert their actions (26).

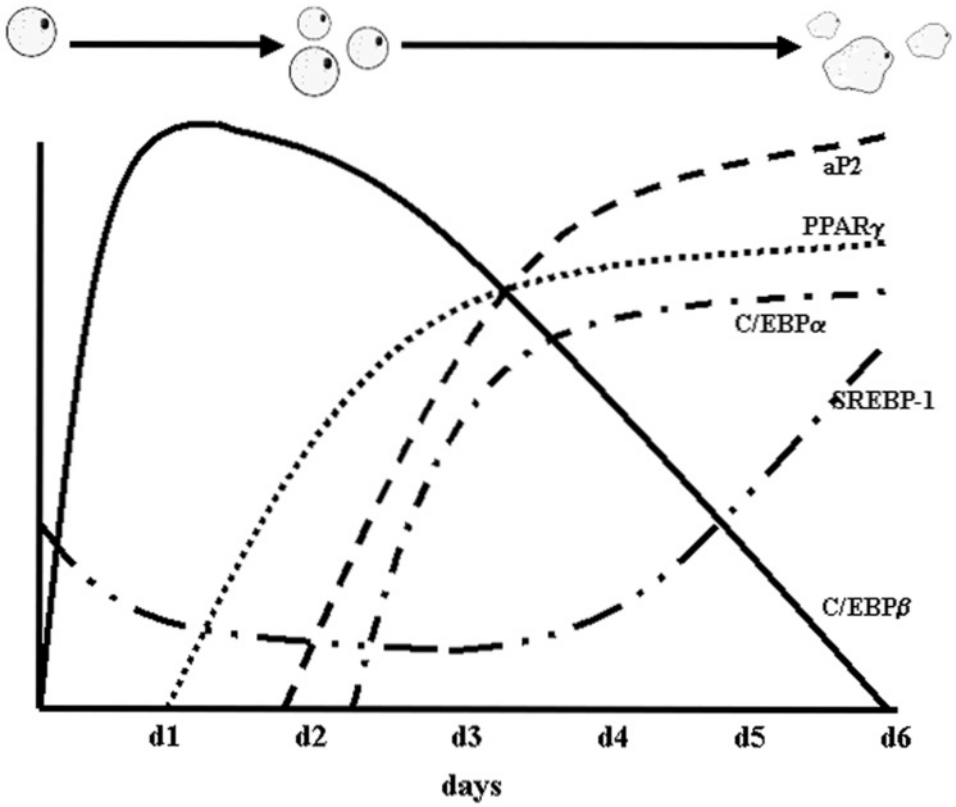

FIG. 33.3. Regulation of adipocyte differentiation. Slow decline in levels of C/EBPβ towards the end of the cell division phase correlate to increasing levels of C/EBPα and PPARγ. Levels of SREBP-1 drop initially but increase towards the end of the differentiation phase. As levels of PPARγ and C/EBPα increase, so do levels of the aP2 protein, the expression of which is regulated by these two transcription factors. C/EBPα and C/EBPβ = CCAAT/enhancer-binding proteins alpha and beta, SREBP-1 = sterol regulatory element-binding protein-1, PPARγ = peroxisome proliferator-activated receptor gamma (27).

pyruvate to lactate, a much less efficient energy creating process (39).

NRTIs exert their effect by incorporating themselves into nucleic acid during gene transcription resulting in the inhibition of polymerases, such as viral reverse transcriptase, thereby causing the termination of transcription. NRTIs may adversely affect mitochondrial function in adipocytes by inhibiting the function of DNA polymerase γ, the major DNA polymerase active in mitochondria, which is susceptible to such inhibition (40). This would almost certainly affect the normal function of adipose tissue, which has a high concentration of mitochondria. Evidence exists to support a role for NRTI induced mitochondrial toxicity in HIVLD. Some individuals develop HIVLD with exposure to NRTIs alone (10). These cases are often characterized by high serum lactate levels suggestive of mitochondrial toxicity. In addition, subcutaneous fat from individuals with HIVLD has revealed decreased levels of mitochondrial DNA (41). This toxicity could result in smaller size of adipocytes.

Fat Accumulation

Accumulation of visceral fat (VAT) and lipomatosis are features of HIVLD (5,10,42). The mechanism whereby VAT accumulates is poorly understood. It has been suggested that, in addition to genetic predisposition (15), shunting of dietary fats towards the visceral compartment due to increased production of lipids (which will be discussed later) and relative loss of the subcutaneous fat compartment may explain the gains seen (42). Visceral fat is significantly different metabolically from SAT, therefore exposure to antiretrovirals may not have as great an effect on its function (43). Prospective data have shown that gain in central fat occurs relatively early into treatment, at the same time when individuals also gain limb fat and lean mass, but is maintained over the period where limb fat mass is lost (12). This could reflect a general improvement in nutrition. A combination of nutritional improvements, decreased energy requirements resulting from treated HIV infection, and shunting of serum lipids away from depleted subcutaneous stores to the visceral compartment could go a considerable way to explaining the persistent increases in VAT seen in HIVLD.

Insulin Resistance

Insulin resistance has been described in both HIV associated and non-HIV associated lipodystrophies (4,8).

Insulin resistance has been related to the fat content within tissues such as muscle, and liver, and has also been associated with abdominal obesity (44Ð46). In HIVLD, its pathogenesis is complex and involves actions by antiretrovirals on several organs (Fig. 33.4).

Insulin, acting through the insulin receptor (IR), activates an intracellular messenger cascade, resulting in the redistribution of an intracellular glucose transport molecule (GLUT4) to the cell surface to facilitate glucose uptake by the cell. GLUT4 is the principal molecule involved in glucose uptake from plasma by adipose and muscle cells in response to insulin (47). Abnormalities in GLUT4 expression affect insulin resistance. These effects may not be tissue speciÞc. Experimental models have shown that decreases in GLUT4 expression in a speciÞc organ results in insulin resistance in other organs with normal GLUT4 expression (48). Abnormalities in the function of GLUT-4 and decreased expression of the IR as a result of PI exposure have both been proposed as possible additional mechanisms for the insulin resistance seen in HIVLD (28,47), although the exact nature of the abnormalities remains unclear.

The etiology of insulin resistance in HIVLD is obviously complex, involving interactions with insulin action and fat metabolism, and a probable role for molecules synthesized in one tissue having an effect on the insulin sensitivity of other tissues (49). One such molecule, the hormone leptin, is secreted by adipocytes. In congenital lipodystrophies, where there is a lack of SAT, levels of leptin are low and are associated with severe insulin resistance (8). In animal models of lipodystrophy, leptin infusions improved insulin sensitivity (50). In contrast high levels of leptin are seen in cases of obesity related insulin resistance (51). In one HIV study, signiÞcantly lower levels of leptin were seen in patients on PIs and correlated with total body fat (34), suggesting that low leptin concentrations may be a consequence rather than a cause of HIVLD. Therefore, a lack of leptin under such circumstances, may contribute to insulin resistance. In obesity, adipose tissue produces excess leptin in an unsuccessful attempt to overcome insulin resistance. A possible explanation is that intracellular lipid concentrations have a dominant effect on insulin resistance, with attempted compensation by increased leptin levels unable to overcome the effect. In lipoatrophic patients, however, leptin replacement may have a beneÞcial effect on insulin resistance (52).

Hyperlipidemia

Accumulation of excess lipids in the serum is thought to be, in part, the result of increase in production of lipids, primarily by the liver (53), and a decrease in the removal and storage of circulating lipids by depleted adipose tissue stores. Loss of SAT affects the bodyÕs ability to adequately store excess circulating lipids. The amount of residual functioning adipose tissue and its capacity to store lipids will vary between individuals, explaining the heterogeneity of lipid proÞles seen in HIVLD.

In vitro work has shown that hepatoma cells exposed to PIs produce excess apolipoprotein B (ApoB) lipoproteins (54). This is thought to be due to a combination of PIs causing inhibition of the proteasome, preventing the normal breakdown of ApoB molecules within the cell, and hypersecretion of these molecules following stimulation from increased intracellular levels of fatty acids (54). This

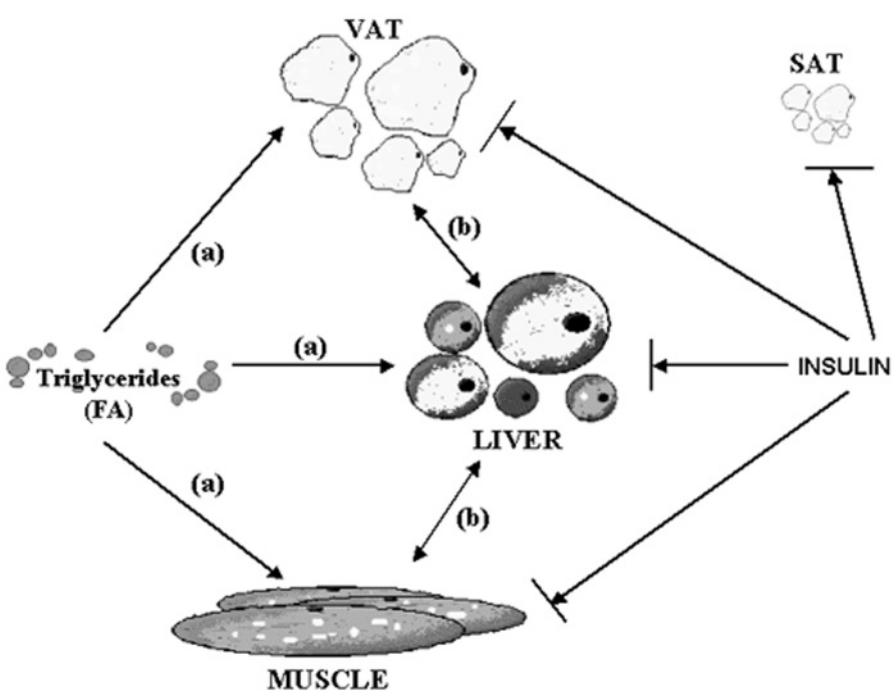

FIG. 33.4. Tissue lipid accumulation and insulin resistance. In HIVLD, as in congenital lipodystrophies, lipid accumulates in tissues such as muscle, liver and visceral fat (a). Lipid accumulation within these tissues is associated with increased insulin resistance (IR). Decreased expression of the insulin receptor gene and abnormalities in the function of GLUT-4 have both been shown with exposure to protease inhibitors (PIs). This could contribute to insulin resistance. Abnormalities of GLUT-4 in one tissue can also lead to insulin resistance in other tissues (b) suggesting a role for other molecules in the regulation of GLUT-4 and insulin resistance (8,42,44,45,47, 48).

combination of events could explain the observation of increased hepatic lipid production in HIVLD (53), resulting in hyperlipidemia and contributing to the insulin resistance seen in HIVLD.

In HIV negative populations, increase in VAT is associated with increased insulin resistance (44). In HIVLD, abdominal fat accumulation correlated with increased intramyocellular lipid levels, which in turn was associated with insulin resistance (42). In addition, increased VAT in those with HIVLD has also been associated with reduced bone mineral density (55).

Lactic acidemia

Associations between the increased frequency of elevated lactate levels seen in treated HIV-infected individuals and the development and severity of HIVLD have been described. Higher serum lactate concentrations have been observed in antiretroviral treated HIV-infected subjects (24). Its pathogenesis is thought to be further reßection of mitochondrial dysfunction. A subgroup of treated individuals with lactic acidemia was seen to suffer from accelerated lipodystrophy, which was of greater severity when compared to those with normal serum lactate concentrations (10). This may either reßect a more severe mitochondrial disturbance in these individuals, or a separate pathogenic effect of lactic acid on adipose metabolism. Lactic acidosis, referring to symptomatic lactic acidemia, will be discussed in more detail later in this chapter.

Complications

Of principal concern is that the metabolic abnormalities seen in those treated with antiretrovirals will result in premature cardiovascular disease (CVD) over the long term. Hyperlipidemia, hyperinsulinemia and central obesity are associated with increased risk of CVD in settings other than HIV infection (56,57). In addition, factors such as increased oxidative stress and cytokine production associated with HIV infection can independently contribute to accelerated atherosclerosis (58). Various techniques have been used to determine an individualÕs risk of cardiovascular disease. Changes in measurements of endothelial function, scans to detect asymptomatic vascular plaques and measurements of increased intimal thickness of blood vessels are thought to correlate well with cardiovascular risk (59).

Although there have been reports of premature cardiovascular disease in HIV-infected individuals on treatment, at present these episodes are associated more with classical cardiovascular risk factors, such as smoking, hypertension and positive family history, than the use of antiretrovirals (60). Risk of cardiovascular disease is usually expressed as a percentage increased risk of a clinical cardiovascular event (such as myocardial infarction) over a 10-year period (61). Subsequently, current increased risk developing from the use of antiretroviral medications may not become clinically apparent for a number of years to come.

Changes in vascular function, such as increased vessel intima thickness and abnormal endothelial function, predate clinical cardiovascular events and therefore may give a more accurate, early indication of the effects of antiretrovirals on cardiovascular risk (59). Abnormalities in endothelial function have been detected in individuals taking PI-containing antiretroviral regimens (62). In addition, in a group of HIV-infected subjects, elevations in systolic and diastolic blood pressure, contributors to cardiovascular risk, were also shown to be more prevalent in those with HIVLD, correlating with increased abdominal girth (63). Several abnormalities may combine to result in signiÞcant increased risk in individuals with HIVLD. This risk will be magniÞed in those with non-HIV speciÞc risk factors such as smoking and a positive family history. There are currently several large observational cohort studies underway to monitor HIV-infected populations to determine if these risks do translate to increased cardiovascular events.

Treatment

Numerous interventions have been investigated as possible treatment options for speciÞc metabolic abnormalities associated with HIVLD (64Ð76), with varying degrees of success. The major interventions and their outcomes are listed in Table 33.3. Overall, many of the interventions, considered effective at rectifying metabolic abnormalities in the HIV-uninfected population, are much less effective in those with HIVLD.

There are several possible explanations. First, in the presence of continuing insult from antiretrovirals, these interventions may not be as potent as in the settings in which they are normally used. Secondly, some of the interventions may beneÞt some aspects of the syndrome while making other features worse. Examples include recombinant human growth hormone, which can reverse visceral fat accumulation but which may exacerbate lipoatrophy and insulin resistance (72), or metformin, which at high dose can help with insulin resistance but can result in further lipoatrophy (68). On the other hand, thiazolidinediones, which act as PPARγ agonists, are the subject of intense investigation, as a side effect of their use in the general population was gain in peripheral fat in addition to its antidiabetic effect (74). In the setting of HIVLD, this would result in an overall beneÞt. Lastly, many of the interventional drugs are metabolised by the same metabolic pathways as antiretrovirals, resulting in altered serum levels of drugs, such as that seen with the use of some lipid lowering drugs from the statin class.

A treatment strategy that has received much attention is that of changing regimens or ÔswitchingÕ away from drugs

TABLE 33.3. *Interventions used in the treatment of HIVLD*

	Lipodystrophy		Metabolic complications						
Intervention	Peripheral fat	Central fat	Trigs	Cholesterol	IR	Lactate	Risk	current use	Q
Diet & exercise	↓	↓	No Δ	↓LDL ↓HDL	↓	No Δ likely	↑ lipoatrophy	VAT accumulation	III* (64)
Switch PI to NNRTI/abacavir	No Δ or ↓	↓	↓	↓ LDL ↑HDL	No Δ or ↓	No Δ likely	Drug reactions Virologic failure	Hyperlipidaemia VAT accumulation	I* (65)
Switch PI to NNRTI	Apoptosis	No data	No Δ	No Δ	↓	No data	Virologic failure	Research	II * (66)
Switch NRTI	↑	No Δ	↓	No Δ	Unclear	No data	Initial further loss of fat	Research	II * (67)
Metformin	↓	↓	No Δ	No Δ	↓	No Δ	↑ lipoatrophy	Diabetes VAT accumulation	I * (68)
Thiazolidinediones	May ↓	May ↓	↓	↓ HDL ↑ LDL	Improves	No Δ likely	P450 interaction Hepatitis	Research	II ψ (69)
Fibrates	No Δ likely	No Δ likely	↓	No Δ	No Δ	No Δ likely	None	Hypertriglyceridaemia	I * (70)
Statins	No Δ likely	No Δ likely	↓	↓	No data	No Δ likely	Adverse event P450 interaction	Hypercholesterolaemia	II * (71)
Growth hormone	↓	↓	No Δ	No Δ	May ↑	No Δ likely	Lipoatrophy Hyperglycaemia	Research Limited by side effects	III * (72)
Plastic surgery	Improved facial appearance	Transient ↓ buffalo hump	No Δ	No Δ	No Δ	No Δ likely	Surgery	Unknown Recurrence likely	II * (73)

Q = quality of evidence. I = evidence from at least one RCT. II = evidence from at least one randomised trial, dramatic results from uncontrolled trial, or cohort or case control trial. III = evidence from respected authorities or expert committee reports. * = evidence from trials involving HIV-infected subjects. ψ = evidence from trials of HIV-uninfected subjects. Adapted from revised ISDA rating system (76). ↑ = increase. ↓ = decrease. HDL = high density lipoprotein. LDL = low density lipoprotein. Δ = change. NRTI = nucleoside reverse transcriptase inhibitor. PI = protease inhibitor. NNRTI = non-nucleoside reverse transcriptase inhibitor. P450 = hepatic cytochrome P450 enzyme system.

suspected of contributing to HIVLD to ones with more metabolically friendly proÞles. Numerous Ôswitch studiesÕ have been conducted. Most involved in switching from a PI based regimen to a non-PI based regimen (65Ð67,75). The results have varied greatly with some improvement in metabolic abnormalities, but little change or an exacerbation of body composition changes (75).

Augmentation of diet and exercise certainly has an important role to play in the general management of HIVLD. It is a role that is often overlooked in the search for an effective pharmacological treatment. With concerns regarding increased cardiovascular disease risk, cessation of smoking, and maintaining regular exercise are essential in that they counterbalance independent risk factors for CVD, and it is possible to modify these risks even in the setting of HIV infection and ongoing ARV treatment.

Summary

With considerable research into the etiology and pathogenesis of HIVLD continuing, the mechanisms behind the syndrome are starting to unravel. The overall picture is a complex one, with multiple insults from various medications resulting in the phenotype observed. By increasing our understanding of fat metabolism, this research has also helped us to realise the important role of adipose tissue function, not only in the setting of HIV infection, with essential roles for fat in many of the bodyÕs functions such as lipid and glucose homeostasis. The complex nature of HIVLD is reßected in the difÞculties experienced and the relative lack of success seen in treatment strategies for the condition. The eventual effect of these abnormalities on long-term incidence of CVD remains to be ascertained.

OTHER TOXICITIES

Lactic Acidemia

As discussed in the previous section (HIVLDÑ lactic acidemia), use of nucleoside reverse transcriptase inhibitors (NRTIs), particularly the synthetic thymidine analogues, has been associated with increases in serum lactate concentrations (24). The underlying mechanism is thought to be NRTI inhibition of the enzyme DNA polymerase gamma, the principal polymerase active in mitochondria (11,24). Evidence to support this includes *in vivo* studies demonstrating decreased mitochondrial DNA content of adipocytes in patients treated with NRTIs (41), thought to represent decreased functioning capacity of the organelles within these cells. Decreased mitochondrial function leads to disruption of intracellular oxidative capacity, leaving the cell unable to meet its energy needs adequately through aerobic metabolism alone. Under such circumstances, cells tend to revert to anaerobic metabolism, the end product of which is lactate, which is usually cleared from the circulation by the liver.

On rare occasions, build-up of serum lactate can become symptomatic (lactic acidosis). This may occur as a result of increased lactate production resulting from mitochondrial dysfunction. Once metabolic decompensation occurs with lactic acidosis, multiple end-organ damage follows rapidly, resulting in a high incidence of mortality (77).

The symptoms of lactic acidosis are non-speciÞc. Occasionally, the condition is preceded by a period of fatigue, weight loss and rapidly progressive peripheral lipoatrophy (10,77). Those presenting with overt acidosis may complain of symptoms such as nausea, vomiting, abdominal distension, tachypnea and occasionally myopathy or neuropathy, developing anywhere from days to several weeks prior to presentation.

Diagnosis is based on demonstration of high lactate levels, anion gap metabolic acidosis, and relevant clinical symptomatology in an individual being treated with NRTIs. Although often deranged, liver function tests may be deceptively normal, as the predominant histopathological lesion appears to be hepatic steatosis rather than hepatitis (77). Management involves the immediate cessation of antiretoviral medications, along with treatment of the metabolic acidosis. Despite intensive treatment and early detection, mortality remains high, quoted at 42% in one study (77).

The use of intermittent measurements of serum lactate as a screening tool remains controversial. The incidence of stable, chronic hyperlactatemia is relatively high (> 8%) in patients treated with antiretrovirals (24) but the development of lactic acidosis is rare (3.9Ð14.5 cases/1,000 patient years) (78,79). However, serum lactate helps identify a subset of individuals who may be at risk of severe lipoatrophy, osteopenia, peripheral neuropathy and insulin resistance (10,24,80), allowing for identiÞcation and closer monitoring of these conditions. Monitoring of lactic concentrations should also be performed in situations where there is a higher risk of lactic acidosis, such as during pregnancy or during concurrent treatment with ribavirin.

Abnormalities of Bone

Osteoporosis, osteopenia, avascular necrosis (osteonecrosis) and pathological fractures have all been reported in adult males and females, and children treated with antiretrovirals (81Ð88). With the exception of avascular necrosis, these abnormalities result from abnormalities in bone metabolism. Avascular necrosis is better explained by abnormalities in vascular function (for example secondary to hyperlipidemia).

Osteopenia

Cross sectional cohorts report a prevalence of osteopenia between 20Ð50% (80Ð82). Decreases in BMD have

been described in antiretroviral-experienced adults (82) and children (85) compared to those who were antiretroviral naïve. Hormonal, HIV and antiretroviral-related factors have all been implicated (80,81,83,84). The underlying defect results in uncoupling of the equilibrium normally existing between bone formation by osteoblasts and bone resorption by osteoclasts (83,84) with increases in markers of bone resorption detected in several studies (84,85). The nature of the underlying defect(s) is unclear but a multifactorial etiology is likely. Currently, principal areas of interest are effects of protease inhibitors, effect of nucleoside reverse transcriptase inhibitors, effects of changes in body composition on bone metabolism and the effect of pre-morbid body composition (eg wasting).

HIV-infected individuals taking protease inhibitor containing regimens may be at higher risk of osteopenia and osteoporosis (81,82). However, one study has demonstrated stable bone mineral density over time in individuals taking protease inhibitor-containing regimens (82).

Mitochondrial toxicity, induced by NRTI, can also lead to loss of bone mineral density. Osteopenia could occur as a by-product of lactic acidemia, resulting from NRTI use. Chronic metabolic acidosis, like that seen with lactic acidemia results in mobilization of calcium from bone, used to buffer high serum acid prior to its excretion by the kidneys. Lactic acidemia has been shown to be an independent risk factor for the presence of osteopenia in HIV-infected, antiretroviral-treated individuals (80). The duration and magnitude of lactic acidemia is thought to play a major role in loss of bone mineral density.

The association between HIV associated lipodystrophy (HIVLD) and reduced bone mineral density is unclear at present. Although reduced BMD has been demonstrated in groups of adults with HIV wasting and those on treatment (82,89), one cross-sectional study found no relationship between body composition changes and osteopenia (80). A smaller cross-sectional study revealed a significant association between increases in central abdominal fat and reduced bone mineral density (55). In this study, no other component of treatment or body composition had a similar association.

Case reports have described the use of bisphosphonates for the treatment of antiretroviral-induced osteopenia or osteoporosis, although randomized controlled trials of their efficacy and safety in this situation have not been published, and concerns regarding possible drug interactions with antiretrovirals persist (90).

Avascular Necrosis (Osteonecrosis)

Bilateral avascular necrosis of the femoral head has been described with increasing frequency in antiretroviral treated adults and children (86,87), many of whom had evidence of lipodystrophy and insulin resistance. Diabetes, insulin resistance and hypertriglyceridemia, all recognized side effects of antiretroviral treatment, are recognized risk factors for avascular necrosis in the general population, although similar risk factors in the HIV-infected population have yet to be identified.

Hepatotoxicity

Many factors contribute to liver dysfunction in the setting of HIV-infection. All antiretroviral medications have the potential for hepatotoxicity. In addition, HIV associated factors that are not drug-related such as opportunistic infections (especially CMV and MAC) can contribute to liver dysfunction, and co-infection with hepatitis viruses such as hepatitis B (HBV) and hepatitis C (HCV) also play an ever greater role in hepatotoxicity. Hepatotoxicity is becoming increasingly more important in the management of HIV infection as it results in considerable morbidity and mortality. It is, in itself, a large topic and therefore this chapter will deal only with antiretroviral related hepatotoxicity.

Antiretroviral related hepatotoxicity can be classified as early (occurring within 1 to 6 weeks of starting therapy) or late (after six weeks) (Table 33.4). Early hepatotoxicity usually occurs either as a result of hypersensitivity to a drug, or as a component of immune recovery (sometimes referred to as immune reconstitution). Hypersensitivity hepatotoxicity, has been described with the use of all PI and NNRTI drugs, with much interest directed against the NNRTI, nevirapine. Use of nevirapine results in hepatitis in approximately 6% of cases irrespective of CD4 count (91) and has rarely ($<1\%$) resulted in fulminant hepatitis in both HIV negative and HIV positive individuals (92,93). Incidence of hepatotoxicity was increased in subjects co-infected with HCV, in those with higher baseline transaminases, and in those with longer exposure to antiretrovirals. The condition usually resolves with discontinuation of the drug, but can rarely be fatal if not recognised early.

Immune recovery against infections such as HBV, HCV, CMV and *Mycobacterium avium complex* (MAC), especially in those with lower CD4 counts pre-treatment, can give rise to an acute and often self-limiting hepatitis, thought to be secondary to immune mediated injury. This hepatitis can be more severe in those with HBV or HCV co-infection but is usually self-limiting (94).

Late hepatotoxicity is usually independent of immune status but is associated with long-term exposure to antiretroviral medications. In such individuals, co-infection with either HBV or HCV can result in increased severity of toxicity. Drugs implicated include the thymidine analogue NRTIs didanosine and stavudine, the NNRTI nevirapine, and certain protease inhibitors. The predominant lesions are steatosis and cirrhosis rather than hepatitis (95–97). NRTI-induced mitochondrial toxicity may contribute to hepatic steatosis, apoptosis and fibrosis, while PI-induced hyperlipidaemia and insulin resistance can also result in steatosis, as seen in cases of congenital

TABLE 33.4. *Liver dysfunction associated with antiretroviral therapy*

	Lactic acidemia	Hyper-sensitivity	Isolated hepatitis	Immune reactivation	HBV reactivation
Antiretroviral (class)	NRTIs	NNRTIs, Abacavir	NNRTIs and PIs	All Classes	None
Speci c ARVs	stavudine-didanosine	nevirapine	ritonavir, nevirapine	any	lamivudine
Onset[a]	late	early	early/late	early	early
Risk Factors (possible in italics)	*NRTI duration* *Pregnancy* *HCV treatment (ribavirin)*	*Female*	HCV/HBV co-infection ↑ ALT pre-therapy	CD4 + < 100 cells/μL pre-ARVs, *MAC bacteremia*	HBV DNA + pre-lamivudine or n*t*RTI
Clinical Features					
Fever	No	Common	Occasional	Yes	Yes
Jaundice	No	Occasional	Occasional	Common	Common
Dyspnea	Common	No	No	No	No
Rash	No	Common	No	No	No
Fulminant Hepatic Failure*	Occasional	1%–2%	Unknown	Relatively common	Relatively common
Chronic Liver Failure	Rare	Not described	2° to HCV/HBV	Not described	Not described
Laboratory Features					
ALT > 10 x ULN	rare	common	common	common	common
lactate > 2 mmol/L	Yes	no	no	no	no
Therapy	cease nRTIs if lactate > 5 mmol/L or symptomatic	cease NNRTI if symptomatic	cease drugs and treat HBV/HCV	cease ARVs until underlying OI treated	restart HBV therapy (usually lamivudine)
Prognosis	mortality 80% if lactate > 10 mmol/L	good	good	good if OI treated effectively	risk of lamivudine resistance

Abbreviations: ALT = alanine aminotransferase. ARV = antiretroviral. HBV = hepatitis B virus infection. HCV = hepatitis C virus infection. MAC = *Mycobacterium avium* complex. NNRTI = nonnucleoside reverse transcriptase inhibitor. NRTI = nucleoside reverse transcriptase inhibitor. n*t*RTI = nucleotide reverse transcriptase inhibitor. OI = opportunistic infection. PI = protease inhibitor. ULN = upper limit of normal. * Features of acute liver failure are peripheral edema, ascites, encephalopathy, hypoalbuminemia, and prolonged clotting times. [a] early onset is de ned as within six weeks of starting the medication.

lipodystrophy, in which severe insulin resistance and hepatic steatosis co-exist (8). Although toxicity is often reversible, or slower to progress upon switching regimens to those with less potential for hepatotoxicity, liver failure and death have been reported (95,96), in some cases several years after drug cessation (95).

Co-infection with HIV and HCV is increasing worldwide, with a reported 25% of those infected with HIV also co-infected with HCV. In this setting, drug associated hepatotoxicity often occurs with increased frequency, with an estimated 21-fold increased incidence of cirrhosis in patients with HIV/HCV co-infection. HCV is a treatable condition and eradication of the infection is achievable in many cases using newer combination regimens of ribavirin (a nucleoside analogue) and pegylated interferon (98). Treatment of HCV concurrently with the use of antiretrovirals has raised concerns with respect to side effects, which could limit the effectiveness of either the HCV or HIV treatments. For that reason, many now consider delaying the introduction of antiretrovirals, providing the patientÕs CD4 count is compatible, until treatment and eradication of HCV infection is complete.

In those co-infected with HBV, antiretroviral treatment with drugs active against HBV, such as lamivudine, can provide added beneÞt. Rebound hepatitis has been reported in several cases where lamivudine was withdrawn for reasons related to failure of HIV therapy (99).

Management of hepatotoxicity in the setting of HIV-infection should include vaccination against viral hepatitis, preventive strategies to avoid exposure to HCV, treatment of concurrent viral hepatitis, careful monitoring of liver function tests while receiving antiretroviral therapy, and cessation of suspected agents in cases of derangement of liver function.

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Chapter 34

HIV Drug Susceptibility Testing

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Resistance of clinical HIV isolates to the Þrst antiretroviral drug, the nucleoside analogue azidothymidine (zidovudine, ZDV), was identiÞed in 1989 soon after its introduction into clinical trials (1Ð3). These studies utilized standard methods of virus isolation and *in vitro* assays for susceptibility based on measurement of inhibition of viral replication by varying concentrations of drug. The following year, the genotypic basis for ZDV resistance was deÞned (4) and opened the Þeld of HIV drug susceptibility testing based on viral genetic sequence determination.

Since that time, the introduction of each new antiretroviral agent has been quickly followed by the recognition of resistance. HIV drug resistance is both a cause and an effect of incomplete viral suppression, but it is not the only impediment to effective, long-term antiretroviral therapy (discussed in Chapter **00**). A number of techniques have been developed to diagnose and study HIV drug resistance, each with particular strengths and weaknesses, but the testing strategies that may be most clinically effective still need to be determined. Based on current data: circumstances that merit drug resistance testing include patients who need to switch therapy due to drug failure, patients with primary HIV infection, patients with established infection from regions where the prevalence of primary resistance is known to be high who are about to initiate therapy and pregnant patients starting long term treatment or perinatal prophylaxis. (5,6).

The apparent ease with which HIV evades both pharmacologic inhibition and immune control through sequence change is explained by the massive scale of HIV replication (7Ð9) and by the high mutational rates characteristic of replication of RNA viruses, which lack proof-reading mechanisms (10). These circumstances result in the random generation on a daily basis of viral variants bearing every single mutation (and many double mutations) along the 9kb HIV genome (11,12). Therefore, the genetically complex population (termed quasispecies) at any given time has a high likelihood of containing viral variants that harbor mutations that can contribute to resistance. Incomplete suppression of viral replication in the presence of antiviral drug(s) results in the selective outgrowth of these variants with reduced susceptibility. For some drugs, single mutations can render mutants fully resistant (13,14), while for other drugs and for combinations of drugs the evolution of high level drug resistance requires iterative events during successive viral generations that result in the accumulation of multiple mutations (15,16). Genetic recombination among viral quasispecies may facilitate the emergence of multi-drug resistant virus (17Ð19).

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OVERVIEW OF RESISTANCE TESTING

Most clinical resistance testing is now performed on HIV RNA in plasma (or serum). This has the advantage of sampling contemporaneous virus because the HIV population in plasma turns over rapidly (8,9). Additionally, the typically large concentrations of free virus in plasma from untreated patients or from those failing treatment with high level drug resistance increases the likelihood that the sampling will be more representative and less susceptible to sampling artifact (8,20,21). Alternatively, peripheral blood mononuclear cells (PBMC) can be used for virus isolation as well as direct molecular studies. However, this viral compartment turns over relatively slowly and may not provide an accurate picture of the most recent evolutionary events (22,23). Drug resistance can be detected in cell free virion RNA weeks and even months before their appearance in PBMC DNA (8,24Ð27). In addition, direct molecular assays targeting HIV DNA in PBMC risk over sampling the relatively large numbers of Òreplication defectiveÓ genomes known to exist in PBMC (28,29), which may not accurately reßect the replicating virus pool.

Two categories of resistance testing are available. Phenotypic assays determine drug susceptibility by directly measuring viral growth rates or biochemical activity of the viral molecular target in the presence of varying concentrations of a particular inhibitor. Genotypic assays assess resistance based on the absence or presence of mutations or combinations of mutations known to confer reduced susceptibility to a particular drug or class of drugs.

Some considerations and caveats are generic to all resistance testing methods. Plasma collected for resistance testing should be stored at -70¡C to minimize degradation of viral RNA prior to processing. Methods that include polymerase chain reaction (PCR) amplification are very susceptible to contamination because of the exquisite sensitivity of these amplification procedures. Where these methods are used, active measures to prevent and survey for cross contamination should be employed. This includes separate dedicated areas for specimen processing, PCR set up and post PCR processing, the inclusion of negative controls with all assays, and adjunctive measures to limit aerosol formation during processing such as positive displacement or plugged pipet tips. Additionally, when sequences are generated, each new sequence should be compared to known laboratory strains and previously generated sequences for unexpected similarities or differences.

Another generic issue is the timing of resistance testing. For patients who undergo resistance testing because of a failing drug regimen, sampling should ideally be performed while the patient is still on treatment or as soon after stopping therapy as possible. Most drug resistance mutations result in selective advantage for the virus only in the presence of drug and stopping treatment will result in their replacement by wildtype, drug susceptible virus. However, this does not indicate that all traces of the drug resistant variants will have been eliminated. On the contrary, resumption of therapy results in the rapid re-emergence of drug resistant virus that lingers in latently infected cells (30). This highlights another potential weakness in drug resistance testing as resistance may have developed in response to treatment received in the remote past and is unlikely to be detected with standard assays. An exception may be certain "reversion" mutations that are seen when patients have previously had resistance to ZDV (31). Because of this, drug selection should not be based solely on the most recent drug resistance data but should take into account treatment history and prior resistance test data when available.

GENOTYPIC ASSAYS

Cycle Sequencing

The most broadly available resistance testing strategy is based on sequencing the viral genes that are targeted by individual inhibitor drugs. The utility of genotypic testing is dependent on the accuracy and completeness of existing inventories of resistance associated mutations (32,33). Such inventories are constantly updated, but many of the key (major) and secondary resistance associated mutations are well described for most of the available antiretroviral drugs. The identification of individual mutations as possibly conferring drug resistance is made when mutations reproducibly arise during the use of a drug *in vivo* or when virus is passaged in the presence of the drug *in vitro*. Establishing the relationship of mutations or groups of mutations conclusively to drug resistance depends on the demonstration that the viral isolate has a reduced susceptibility to the drug in phenotype assays *in vitro* and that site directed mutation(s) can confer reduced susceptibility.

Genetic sequencing for resistance testing begins with isolation of RNA from cell free virus in plasma which is used to generate complementary DNA (cDNA) by *in vitro* reverse transcription (RT) or by isolation of total DNA from cells, which contain viral DNA (see earlier discussion of comparative relevance of RNA and DNA sequences). Next, the target sequence is amplified by polymerase chain reaction (PCR), a repetitive procedure of annealing, extension and denaturation, which approximately doubles the amount of the sequence target with every cycle. Both the RT and PCR steps require the presence of primers which have high homology to the targeted genes. The amplified products are separated from unincorporated primers and deoxynucleotides, and can be cloned to generate individual clonal sequences that can be individually sequenced or, more typically, the entire mixture of products can be sequenced as a "population".

The most common sequencing methods are based on the Sanger chain termination strategy (34). Denatured amplicons are annealed to sequencing primers in the presence of one or more DNA dependent DNA polymerases and both unlabelled dNTP and individual 2′, 3′-dideoxy, chain terminating nucleotides. The iterative steps of annealing, extention and denaturation results in generation of chain terminated products of varying lengths in process known as "cycle sequencing." The use of fluorescently ("dye") labeled dideoxy chain terminators (or in some cases of dye labeled primers) and automated genetic analyzers has greatly simplified this method of nucleotide sequencing (Fig. 34.1). The labeled single stranded DNA fragments are separated by electrophoresis and the individual fragments detected as they migrate past a laser source. The raw data from "automated sequencing" are interpreted by base calling software. A chromatogram consisting of a series of peaks representing the pattern of individual fluorescently labeled fragments is generated (Fig. 34.2a). This approach is the basis of both commercial genotyping kits (TrueGene[a] , Bayer Diagnotics; Viroseq[a] , Celera Diagnostics) as well as many "in-house" genotypic assays.

Because HIV exists *in vivo* as a complex genetic mix, more than one base may be present at each position when

bulk sequencing is performed. This is observed as multiple peaks at a single location on the chromatogram (Fig. 34.2b and 34.2c). The threshold for identifying a base position as mutant is set with the base-calling software of the sequencing system (typically >20–30%). Most sequencing protocols involve sequencing both strands of DNA and comparing these results for consistency. In many sequencing laboratories, some manual inspection and editing is also done. In order to screen for sequences that may be the result of contamination by exogenous sequences during PCR amplification, many laboratories perform additional quality assurance procedures by comparing every newly

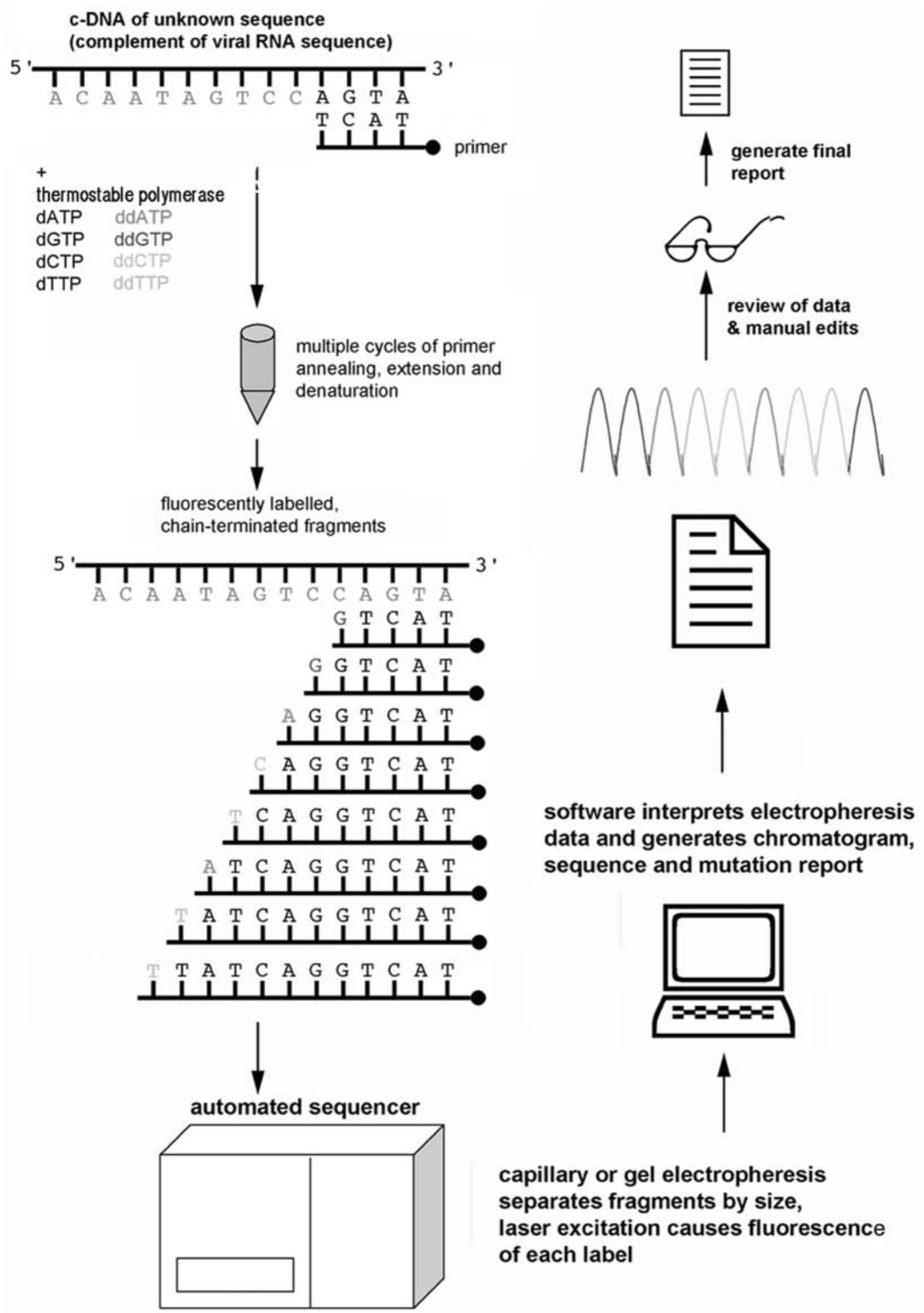

FIG. 34.1.

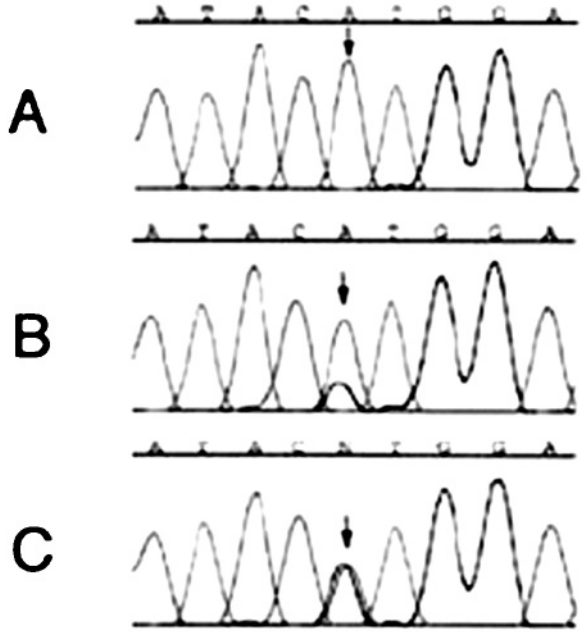

FIG. 34.2.

generated sample sequence with laboratory strain HIV sequences and sequences generated from other patient samples by phylogenetic reconstruction and BLAST searching against sequence databases (35,36). The sample sequence is then compared with a reference Òwild-typeÓ sequence to generate a summary of mutations which can be annotated with information on whether the position is associated with resistance to one or more drugs. (Fig. 34.3).

Hybridization Sequencing: GeneChip

Sequencing the entire length of HIV protease and the relevant 5′ portion of the HIV RT can be performed by large scale hybridization to gene arrays. In one such approach, phosphoramidite chemistry and lithography are used to Þx arrays of probe oligonucleotides based on wildtype and mutant HIV pol sequences to a silicon wafer (37Ð39). These oligonucleotides include various permutations of wildtype and mutant codons together with polymorphisms in the ßanking sequences. The arrays are designed with many redundant probes for each position interrogated. RT PCR is performed to generate complementary DNA (c-DNA) templates, and the inclusion of RNA polymerase promoter sequences in the ampliÞcation primers permits the generation of complementary RNA (c-RNA). These labeled c-RNAs are partially fragmented to generate short oligomers of varying lengths and hybridized to the ÒGeneChipÓ(39). These arrays contain many more probes than can be tested using conventional southern blotting or even the line probe approach. In general, the agreement of this method of resistance testing with cycle sequencing approaches is high (>96% concordance); however, even this method may not be able to detect novel (previously unrecognized) resistance mutations or clusters of mutations and sequence regions with extensive polymorphisms. An example of this limitation are the recently identiÞed insertion mutants between codon 69 and 70 (40,41).

Oligonucleotide Hybridization

An alternatives to long range sequencing are hybridization based assays that inspect only select positions known to confer drug resistance. These assays detect the presence or absence of mutations in the viral genome by the use of individual oligonucleotides that are either complementary to the wildtype (drug sensitive) or the mutant (drug resistant) sequence. The oligonucleotides are used to directly probe for the presence of mutant or wildtype sequences by hybridization under stringent conditions (temperature and salt conditions that prevent annealing unless the probe and template are a perfect match) or to prime ligation or extention reactions that are dependent on annealing of the oligonucleotide to the viral target ÒtemplateÓ Hybridization assays function best to detect well characterized and predictable mutation patterns, but they may yield an indeterminate result when there are ßanking sequence polymorphisms around the interrogated site which affect probe or primer binding. These assays are generally easier to perform with less instrumentation than required for the long range sequencing methods, but they are not designed for discovery of new resistance associated mutations.

Line Probe Assay (LiPA)

The line probe assay (LiPA, Innogenetics) uses oligonucleotide probes that are Þxed onto membrane strips (42). Biotinylated primers are used in RT-PCR ampliÞcation on RNA extracted from plasma and the ampliÞed products are allowed to anneal to the probe strips, washed and treated with streptavidin-alkaline phosphatase conjugate and nitroblue tetrazolium substrate to detect hybridization. Like other hybridization based assays, the applicability of the system depends on well deÞned resistance mutations hence only known resistance mutations can be detected. In addition, binding of the ampliÞed products to the probe strips is also affected by sequences ßanking the codon of interest therefore highly polymorphic regions can be problematic (43). Where high-throughput examination of one or a few resistance codons in well-conserved regions is the principal goal, the time savings with LiPA is appealing. An important feature of the LiPA system is its relative independence from sophisticated instrumentation following the PCR step. Thus, this system may have advantages when a fully equipped molecular diagnostic laboratory is unavailable. The LiPA also appears capable

FOR INVESTIGATIONAL USE ONLY. THE PERFORMANCE CHARACTERISTICS OF THIS ASSAY HAVE NOT BEEN ESTABLISHED.

Applied Biosystems

Antiretroviral Drug Resistance Report [1,2,3]

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Patient: L724-01-23474CSF
D.O.B.: May 8, 2002
Ordering Physician:
Accession Number:
Date Drawn: May 8, 2002
Date Reported: May 8, 2002
Laboratory Technician:

Signature:_____

Nucleoside Reverse Transcriptase Inhibitors

Evidence of Resistance		
High*	Epivir® (lamivudine, 3TC)	E44D V118I **M184V**
High	HIVID® (zalcitabine, ddC)	**M184V**
High	Retrovir® (zidovudine, AZT)	M41L D67N **T215Y** L210W [M184V]
Poss**	Videx® (didanosine, ddI)	M184V
High**	Zerit® (stavudine, d4T)	M41L D67N T215Y L210W
Poss**	Ziagen® (abacavir, ABC)	**M41L D67N** **L210W** M184V **T215Y**
None	Multi-NRTI(a)	
None	Multi-NRTI(b)[4]	**M41L D67N** **T215Y** **L210W**

* WARNING, for at least one mutation shown for this drug, detection by this test has not been verified.
** WARNING, the utility of at least one mutation shown for this drug in the resistance interpretation has not been verified.
*** Both warnings above apply.
(SEE FOOTNOTE 3 FOR MUTATION SPECIFIC INFORMATION.)

FOR INVESTIGATIONAL USE ONLY. THE PERFORMANCE CHARACTERISTICS OF THIS ASSAY HAVE NOT BEEN ESTABLISHED.

Nonnucleoside Reverse Transcriptase Inhibitors

Evidence of Resistance		
High*	Rescriptor® (delavirdine, DLV)	**K103N** G190A **V181C**
High*	Sustiva™ (efavirenz, EFV)	V108I **K103N** **G190A** V181C
High*	Viramune® (nevirapine, NVP)	V108I **K103N** **G190A** **V181C**

Protease Inhibitors

Evidence of Resistance		
Poss***	Agenerase™ (amprenavir, APV)	L10I V32I M46I I54V
High*	Crixivan® (indinavir, IDV)	**L10I** **V32I** M46I I54V **A71V** V82A L90M
High*	Fortovase™ (saquinavir, SQV)	L10I I54V M46I/L A71V V82A L90M
Poss***	Kaletra™ (lopinavir, ABT378/r)	**L10I** **I54V** **F53L** **M46I/L** **L63P** **A71V** **V82A** **L90M**
High*	Norvir™ (ritonavir, RTV)	**L10I** **V32I** M46I **M46L** I54V **A71V** V82A L90M
High*	Viracept® (nelfinavir, NFV)	L10I I54V M46I/L A71V V82A **L90M**

* WARNING, for at least one mutation shown for this drug, detection by this test has not been verified.
** WARNING, the utility of at least one mutation shown for this drug in the resistance interpretation has not been verified.
*** Both warnings above apply.
(SEE FOOTNOTE 3 FOR MUTATION SPECIFIC INFORMATION.)

FIG. 34.3.

of detecting minor resistant mutant populations that make up as little as 5–10% of the total population. In addition to HIV resistance testing, it has been adapted for hepatitis B resistance testing and hepatitis C genotyping (44,45).

Primer Extension and Primer Ligase Assays

The ability of primers, that match either wild-type or resistant sequence, to anneal and prime a PCR amplification reaction has also been used for mutation detection either with conventional detection of product following electrophoresis (46,47) or for use in real-time PCR assays (48,49). These latter assays can be exquisitely sensitive and can be used to detect minority populations $< 0.1\%$. However, each assay is directed at a single codon and not all positions and mutational patterns may be amenable to this approach because of polymorphisms flanking the positions of interest. These assays are currently limited to research applications.

Other assays have been described that use primers whose 3′ ends terminate at the site of the position to be studied. This is the basis of both the Point-mutation Assay (50) and the Oligo-ligase assay (51). In the former, RT-PCR generated template are tested in duplicate polymerase extension assays with either a wild-type or mutant primer and labeled dNTPs. The incorporation of label (originally described with radioactive labeling) in each reaction is proportional to the match between templates to each primer. Minority populations as low as 10% can be detected with this assay but only assays for NRTIs have been described. In the Oligo-ligase assay, mutant is distinguished from wildtype sequence by the ability of respective primers that are differentially modified to anneal to template and become ligated to a third common oligonucleotide (51,52). This system has been used to study NRTI, NNRTI and PI resistance. Sensitivity for detecting minority species approaches 5% which is important because a resistant variant may exist as a minority species, especially when certain drug pressure is not present, but then become the major variant when this drug pressure is reinstated.

Interpretation of Resistance Data

Mutations associated with HIV drug resistance have been classified as primary or secondary. Typical characteristics of primary resistance associated mutations include their ability to alter susceptibility of the virus to the antiviral compound, their early appearance and their relative specificity for one or a class of drugs. Secondary mutations typically accumulate in virus that has already developed primary mutations. By definition, secondary mutations confer little or no reduction in drug susceptibility. However, secondary mutations can increase the level of resistance of virus that already possess primary resistance associated mutations and can also restore or augment the replication capacity of such virus. Secondary mutations that have this latter effect are also called compensatory mutations. The identification of a mutation as important for drug resistance is usually supported by one or more lines of evidence. First, before drugs enter human trials, cell culture passage of virus in the presence of escalating concentrations of drugs can select for mutations *in vitro* that can be detected by sequencing. Virus isolates can then be tested in growth assays (as described in IV. phenotypic assays) for their drug susceptibility in comparison with the parental virus. Further confirmation that particular mutations confer the resistance phenotype can be obtained by creating the individual or groups of mutations in molecular viral clone and testing these "site directed mutants." Second, virus can be recovered from patients on a particular drug and these viruses can be similarly tested. The resistance mutations selected *in vivo* do not always correlate with resistance patterns selected *in vitro*. Finally, some mutations may be consistently observed in the setting of treatment failure with or without the simultaneous development of known drug resistance conferring mutations. The secondary role that such mutations play in enhancing resistance or in restoring replication fitness can be established in some but not all cases.

While a comprehensive inventory of all primary and secondary RAM is beyond the scope of this chapter, a summary of important resistance associated mutations is provided in Figs. 34.4a–c and a discussion of mechanisms of resistance relating to some key mutations concludes this chapter. Comprehensive, updated databases describing known drug resistance genotypic patterns are accessible on the Web at http://resdb.lanl.gov/Resist_DB and http://hivdb.stanford.edu. Additional information can be obtained through the International AIDS Society (http://www.ias.se/).

The standard nomenclature to describe mutations is to use the single letter abbreviation for the wild-type or reference strain amino acid, according to the International Union of Pure and Applied Chemistry (IUPAC) followed by the location of the codon in question followed by the single letter amino acid abbreviation of the new amino acid of the mutant. For example, K103N represents a mutation at the 103rd codon of the RT gene from a Lysine (K) to an Asparagine (N). It is commonly associated with high-grade resistance to NNRTIs (53).

PHENOTYPIC ASSAYS

Inhibition of Virus Replication in PBMC and Cell Lines

A consensus PBMC assay has been described that assesses the phenotypic drug susceptibility of clinical isolates of HIV to AZT by determining the drug concentration necessary to inhibit virus replication *in vitro* (54). The

assay uses virus isolated from co-cultivation of stimulated patient PBMC with sero-negative donor PBMC. The titered virus is then used to infect seronegative donor cells in the presence of varying concentrations of drug. The concentration of drug that reduces the amount of viral p24 production is called the inhibitory concentration 50 or IC_{50}. The fold change in drug concentration for the sample virus to a ÒwildtypeÓ drug sensitive control virus provides a measure of susceptibility. Adaptations of this assay have been used to assess the susceptibility to other antiretroviral drugs as well (55–60). Because of the requirement for virus isolation and titration of the viral stock prior to performance of the actual drug susceptibility tests, this approach is too time consuming and labor intensive for routine clinical monitoring. Furthermore, there are concerns that the process of virus isolation itself can alter the makeup of the viral population and that virus recovered from PBMC may overly represent archival viral forms and

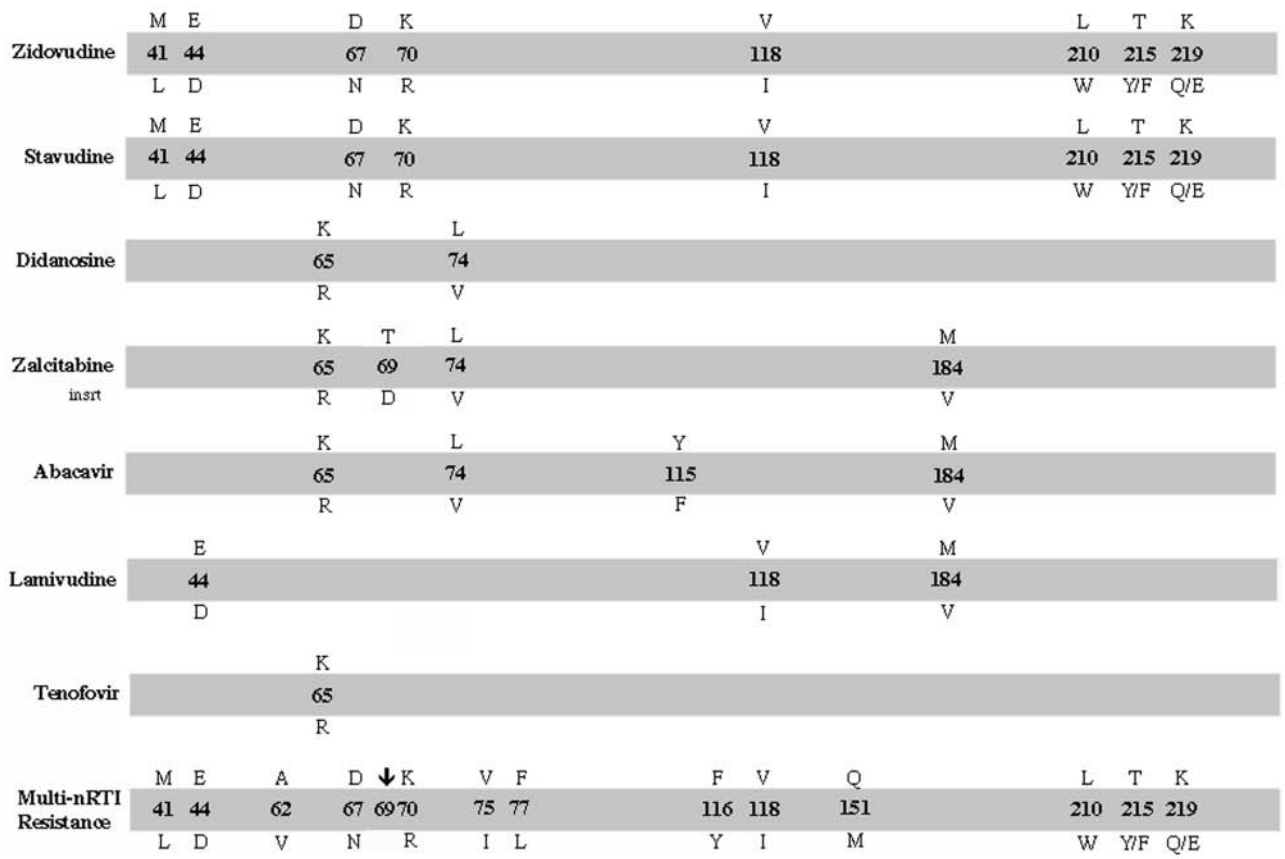

FIG. 34.4a.

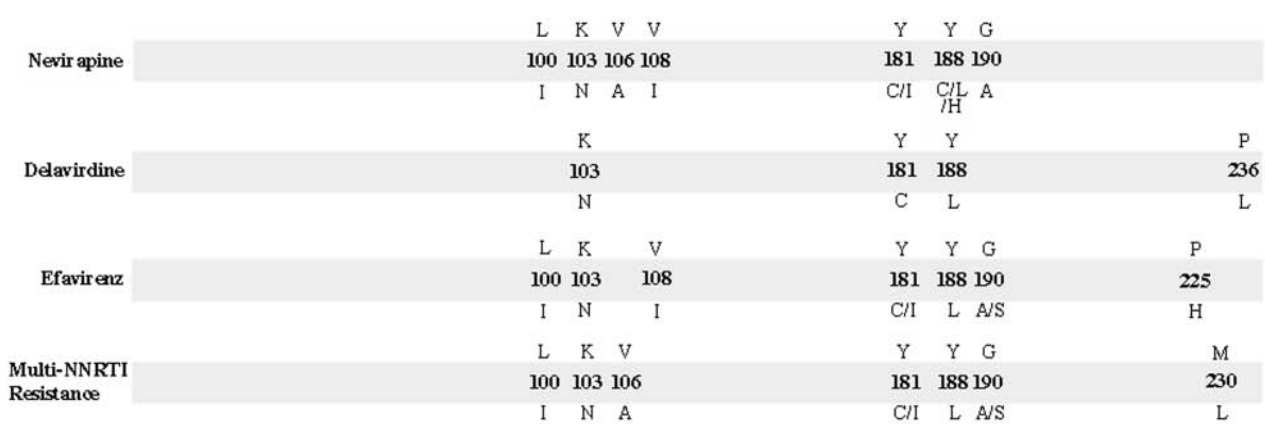

FIG. 34.4b.

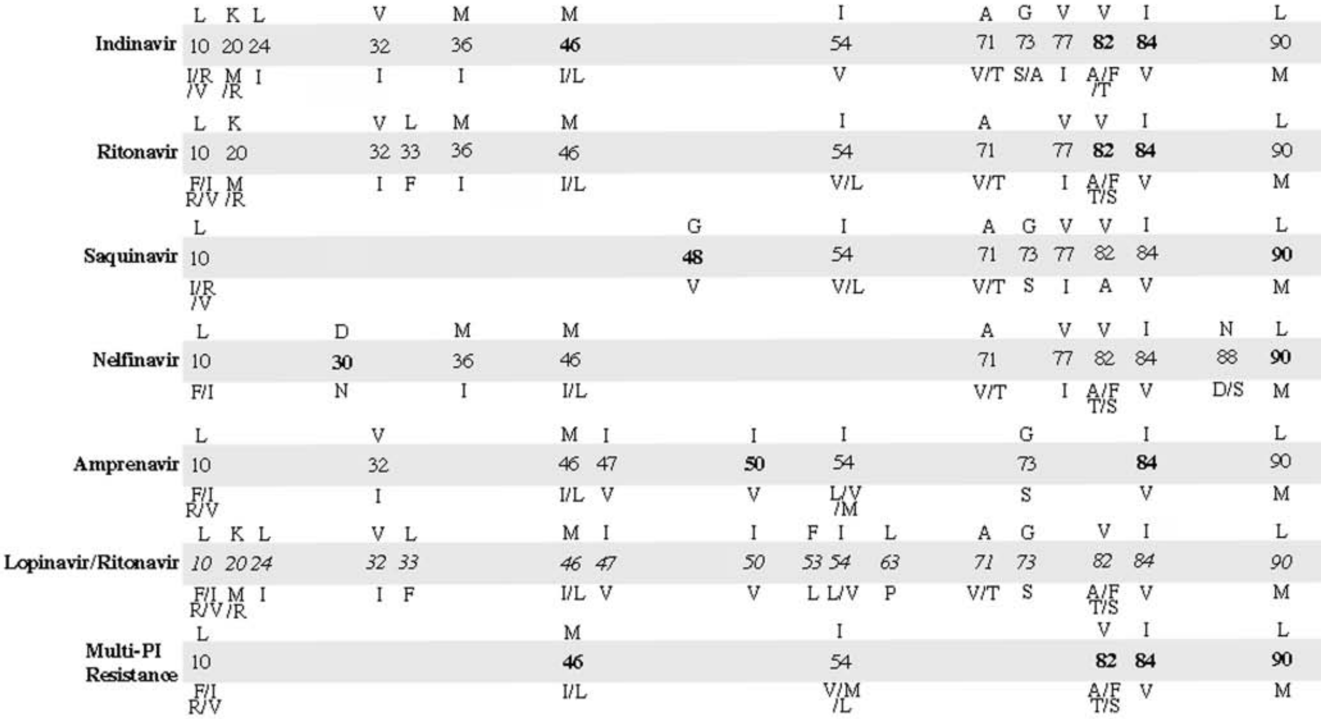

FIG. 34.4c.

therefore not reßect the contemporaneous replicating viral population in plasma (25,61). However, some data suggest that this may not be major problem in practice (53).

A related assay utilizes HeLa cells, which have been engineered to express CD4, to test drug susceptibility (1,62). It scores the amount of HIV infection of HeLa cells by counting syncitial plaques formed by infected cells. Like the PBMC assay, a titered viral isolate must be prepared. However, the readout for infection is relatively simple and less expensive than quantifying p24 production. Because HeLa cells are more homogeneous than donor PBMC, the reproducibility of the assay tends to be better. However, this assay can only be performed with syncitia inducing virus (i.e. those that use the CXCR4 co-receptor) and hence has limited utility for primary virus isolates, which more often do not induce syncitia.

Recombinant Virus Assays

The large time requirement of conventional phenotypic drug susceptibility testing has led to the development of Òrecombinant-virusÓ assays that measure *in vitro* phenotypic susceptibility but can be performed rapidly enough to have clinical utility (63Ð67). These assays utilize virus chimeras that are created from the target gene(s) of interest from clinical specimens using molecular techniques. These assays have advantages over conventional phenotypic assays since they do not require time consuming viral isolation and they minimize the potential for alteration of viral characteristics during *in vitro* cell culturing that can occur during virus isolation (25,61). Several commercial assays have been developed using this strategy.

In one recombinant virus assay, the Phenosense[a] (Virologic, South San Francisco) the HIV gene(s) of interest (in current application, the HIV protease and Þrst 340 bases of the RT) are cloned into a vector which encodes an indicator gene, the Þreßy enzyme, luciferase, under the transcriptional control of the HIV-1 LTR. This chimeric construct and a separate vector that provides the murine leukemia virus envelope (a-MLV) are co-transfected into a cell line, which permits production of pseudotyped virions (Figs. 34.5aÐc). These virions are

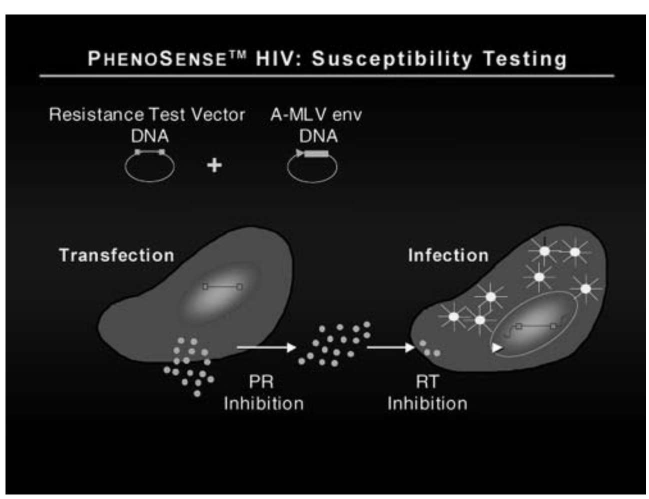

FIG. 34.5a.

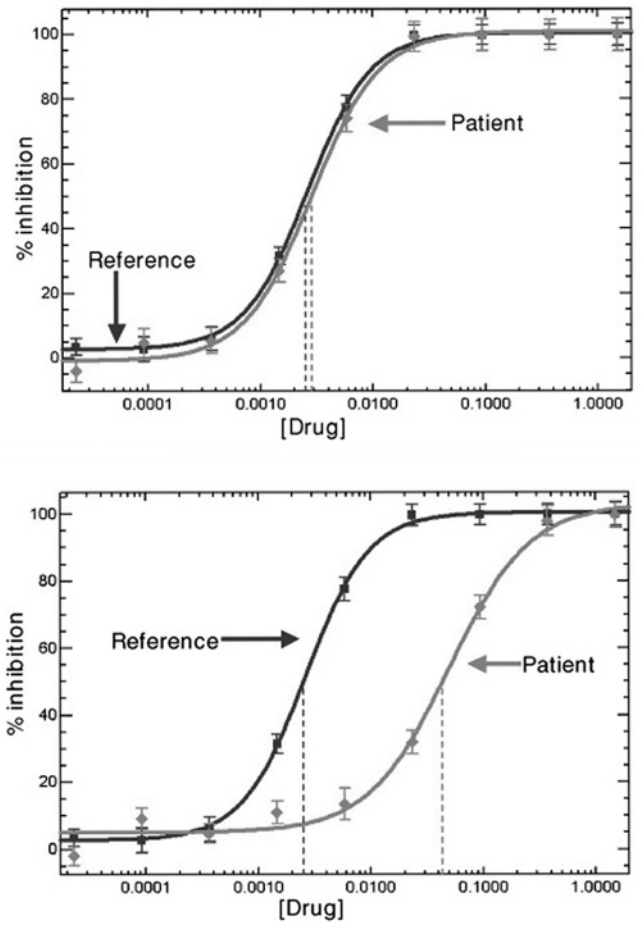

FIG. 34.5b.

either produced in the presence of drugs (protease inhibitors) or are exposed to inhibitors during the infection step (reverse transcriptase inhibitors). Successful infection of a second cell line is quantitatively assayed by detection of luciferase activity in cell lysates. These are compared with no-drug controls and are used to calculate drug concentrations where 50% or 90% of virus replication is inhibited, the inhibitory concentration$_{50}$ (I.C.$_{50}$) or inhibitory concentration$_{90}$ (I.C.$_{95}$). Comparisons with values for wildtype virus are used for calculation of a fold change over wildtype. (Figs. 34.6a and b showing readout from Phenosense assay and inhibition curves) (67). The two other commercial phenotypic assays, the Antivirogram[a] (Tibotec-Virco, Belgium) (63,65,68) and the Phenoscript[a] (Bioalliance, Paris) (68,69) differ from the Phenosense[a] in that they use homologous recombination rather than cloning to construct the recombinant viruses. cDNA corresponding to the viral protease and Þrst 3 to 400 codons of RT derived from the patient sample is co-transfected into a cell line along with a vector containing a laboratory clone of HIV missing the corresponding region in the HIV pol. A complete viral genome is reconstituted by homologous recombination within the target cell by the cellular machinery and results in virus production. The virus is harvested and used in assays of infectivity and virus growth at varying concentrations of drugs. Calculation of I.C.$_{50}$ and I.C.$_{95}$ and fold-change in drug susceptibility are similarly calculated (Fig. 34.6).

Interpretation of these phenotypic assays depends on three assay characteristics: assay reliability, biologic measures and clinical measures. Cutoffs for reliability are determined by observations on assay to assay variability (i.e. consistency of replicate assays on the same sample). Biologic cutoffs are based on the range of results on samples from patients not receiving therapy. Clinical cutoffs are based on relationship between change in I.C.$_{50}$ for a drug and the likelihood of a clinical response. This last and arguably most important cutoff has been determined for some but not all drugs.

The automation of these assays now permits their rapid and reproducible performance and simultaneous testing against all 16 of the licensed antiretroviral agents available in the U.S. Such studies using conventional approaches for phenotypic testing would be hard to perform in a timely manner. However, commercial phenotypic assays are expensive compared to genotypic assays with costs ranging from three to four times more. It remains to be determined when phenotypic testing carries sufficient advantages over genotyping to be indicated. Scenarios where phenotypic testing may provide potentially useful information for clinical management include cases of highly experienced patients whose viral genotypes indicate no available therapeutic alternatives. In such cases, phenotypic testing with precise endpoints may be able to distinguish drugs with little or no effect from drugs that retain some activity. Furthermore, subtle increases in I.C.$_{50}$ for some drugs such as didanosine, stavudine and possibly tenofovir appear to correlate with loss of *in vivo* inhibitory activity even when genotypic resistance is not apparent (70,71). In other cases, mutations to one drug may enhance susceptibility to another rendering genotypically resistant virus phenotypically susceptible. Finally, phenotypic assays can indicate loss of susceptibility to drugs due to novel mutations or combinations of resistance mutations not yet characterized.

Biochemical Assays

Other phenotypic assays, which do not depend on measures of viral infection, assess the biochemical activity of RT in the presence of varying concentrations of inhibitor (72–74). In the system described by Heinene and colleagues, HIV RT present in virions from patient plasma are used to reverse transcribe a heterologous RNA template belonging to the encephalomyocarditis virus (ECMV) in the presence of varying concentrations of RT inhibitors (73). Like genotypic assays and recombinant phenotype assays, the biochemical assays have the advantage that virus isolation is unnecessary, saving time. This assay has an additional advantage in that minor populations of resistant virus in frequencies as low as 10% can be

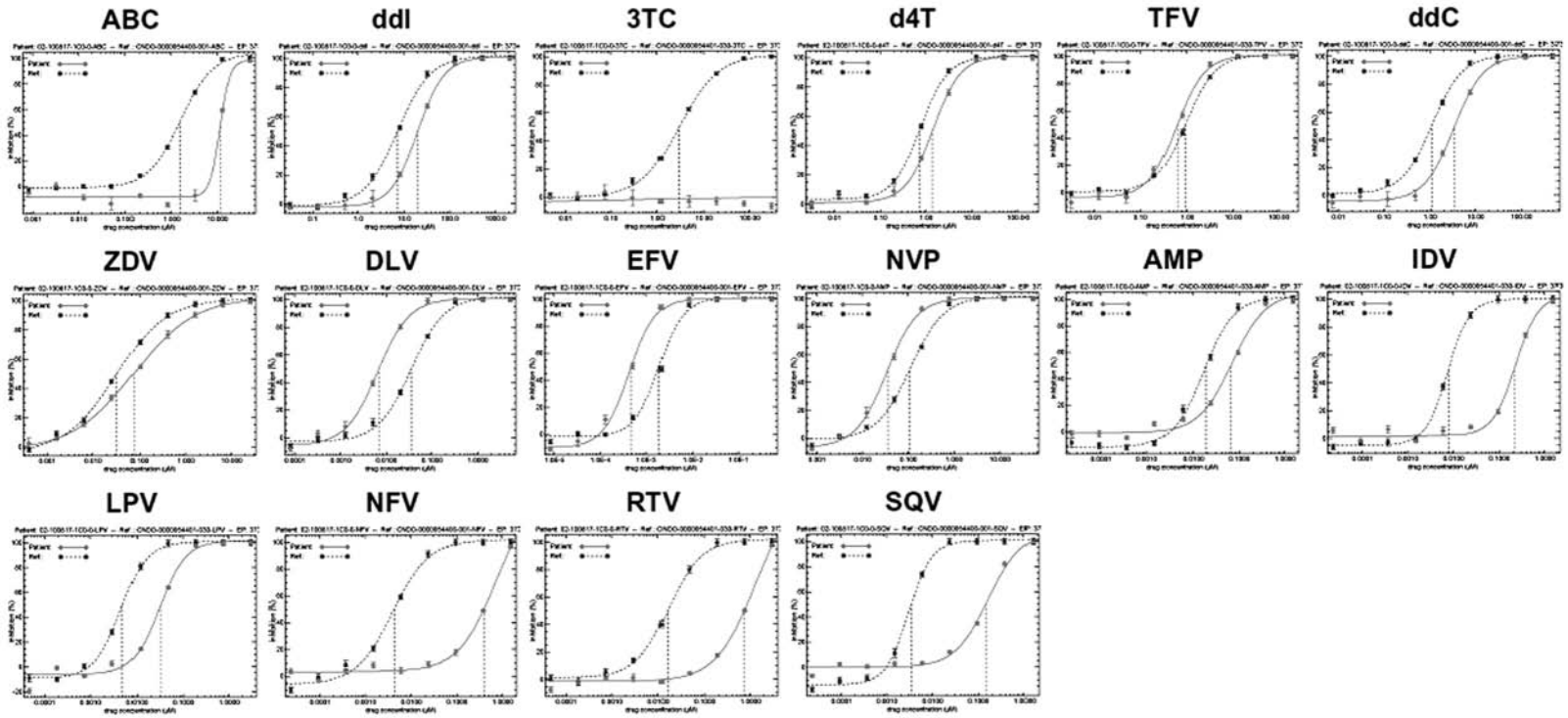

FIG. 34.5c.

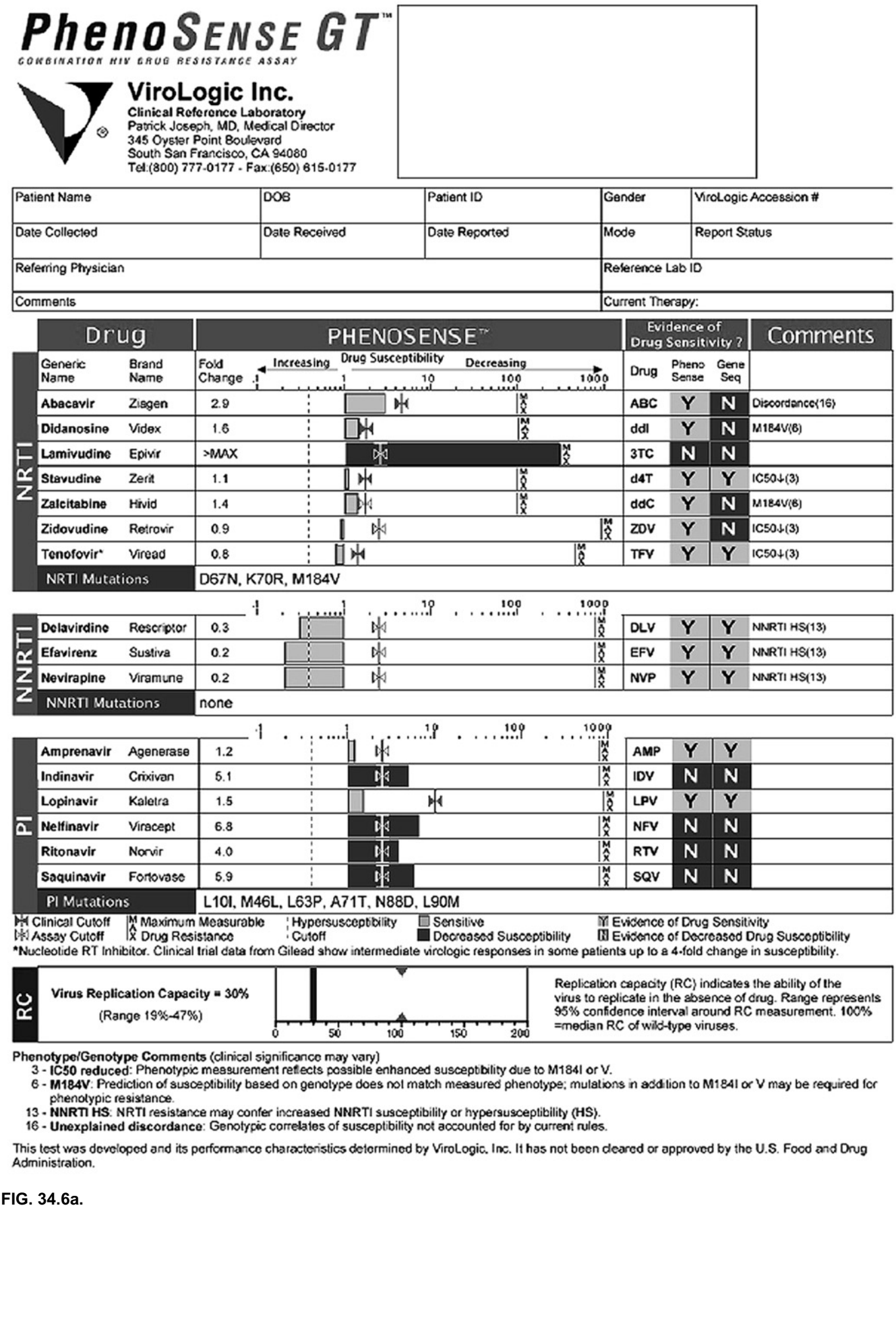
PhenoSense GT™
COMBINATION HIV DRUG RESISTANCE ASSAY
ViroLogic Inc.
Clinical Reference Laboratory
Patrick Joseph, MD, Medical Director
345 Oyster Point Boulevard
South San Francisco, CA 94080
Tel:(800) 777-0177 - Fax:(650) 615-0177
Patient Name
DOB
Patient ID
Gender
ViroLogic Accession #
Date Collected
Date Received
Date Reported
Mode
Report Status
Referring Physician
Reference Lab ID
Comments
Current Therapy:
Drug
PHENOSENSE™
Evidence of Drug Sensitivity ?
Comments
Generic Name
Brand Name
Fold Change
Increasing
Drug Susceptibility
Decreasing
1
10
100
1000
Drug
Pheno Sense
Gene Seq
NRTI
Abacavir Ziagen 2.9 ABC Y N Discordance(16)
Didanosine Videx 1.6 ddI Y N M184V(6)
Lamivudine Epivir >MAX 3TC N N
Stavudine Zerit 1.1 d4T Y Y IC50↓(3)
Zalcitabine Hivid 1.4 ddC Y N M184V(6)
Zidovudine Retrovir 0.9 ZDV Y N IC50↓(3)
Tenofovir* Viread 0.8 TFV Y Y IC50↓(3)
NRTI Mutations
D67N, K70R, M184V
NNRTI
Delavirdine Rescriptor 0.3 DLV Y Y NNRTI HS(13)
Efavirenz Sustiva 0.2 EFV Y Y NNRTI HS(13)
Nevirapine Viramune 0.2 NVP Y Y NNRTI HS(13)
NNRTI Mutations
none
PI
Amprenavir Agenerase 1.2 AMP Y Y
Indinavir Crixivan 5.1 IDV N N
Lopinavir Kaletra 1.5 LPV Y Y
Nelfinavir Viracept 6.8 NFV N N
Ritonavir Norvir 4.0 RTV N N
Saquinavir Fortovase 5.9 SQV N N
PI Mutations
L10I, M46L, L63P, A71T, N88D, L90M
Clinical Cutoff
Assay Cutoff
Maximum Measurable
Drug Resistance
Hypersusceptibility
Cutoff
Sensitive
Decreased Susceptibility
Evidence of Drug Sensitivity
Evidence of Decreased Drug Susceptibility
*Nucleotide RT Inhibitor. Clinical trial data from Gilead show intermediate virologic responses in some patients up to a 4-fold change in susceptibility.
RC
Virus Replication Capacity = 30%
(Range 19%-47%)
0
50
100
150
200
Replication capacity (RC) indicates the ability of the virus to replicate in the absence of drug. Range represents 95% confidence interval around RC measurement. 100% =median RC of wild-type viruses.
Phenotype/Genotype Comments (clinical significance may vary)
3 - IC50 reduced: Phenotypic measurement reflects possible enhanced susceptibility due to M184I or V.
6 - M184V: Prediction of susceptibility based on genotype does not match measured phenotype; mutations in addition to M184I or V may be required for phenotypic resistance.
13 - NNRTI HS: NRTI resistance may confer increased NNRTI susceptibility or hypersusceptibility (HS).
16 - Unexplained discordance: Genotypic correlates of susceptibility not accounted for by current rules.
This test was developed and its performance characteristics determined by ViroLogic, Inc. It has not been cleared or approved by the U.S. Food and Drug Administration.

FIG. 34.6a.

VIRCO

Reported by:
Virco NV
Generaal De Wittelaan L11 B4
B-2800 Mechelen, Belgium

Contact: Paula Mc Kenna
Tel: ++32-15-28.56.05
Fax: ++32-15-28.63.46
E-mail : paula.mckenna@vircolab.com
www.vircolab.com

ELTEST Nr. 114-T

Antivirogram® phenotype

Patient/Sample Details	Test Details	Physician Details
Patient Name	Sample Type Plasma	
Subject ID	Collection Date Sep 20, 2001	
Sample ID	Receipt Date Nov 2, 2001	
Patient ID	Session	
Birth Date	Report Date Nov 23, 2001	
Gender M	Virco ID XYZ00000092	
	Lab ID	

Drug		Susceptibility	Fold change in IC_{50} (Cut-off for normal susceptible range)	Ref.
Trade name	Generic name	Normal susceptible range [1]; Sample within normal susceptible range [1]; Sample above normal susceptible range, but below clinical cut-off [1,2,3]; Sample above normal susceptible range [1]; Fold change in IC_{50} relative to reference virus (log_{10}) 1 10 100		
NRTI				
Retrovir®	Zidovudine		0.8 (4.0)	
Epivir®	Lamivudine		3.6 (4.5)	
Videx®	Didanosine		0.8 (3.5)	
Hivid®	Zalcitabine		1.3 (3.5)	
Zerit®	Stavudine		0.4 (3.0)	
Ziagen®	Abacavir		1.6 (3.0)	
NtRTI				
Viread™	Tenofovir DF		2.5 (3.0)	3
NNRTI				
Viramune®	Nevirapine		> 97.1 (8.0)	
Rescriptor®	Delavirdine		> 340.5 (10.0)	
Sustiva®, Stocrin®	Efavirenz		63.2 (6.0)	
PI				
Crixivan®	Indinavir		0.8 (3.0)	
Norvir®	Ritonavir		0.7 (3.5)	
Viracept®	Nelfinavir		2.0 (4.0)	
Invirase®, Fortovase®	Saquinavir		1.0 (2.5)	
Agenerase®	Amprenavir		0.7 (2.5)	
A component of Kaletra®	Lopinavir		1.6 (2.5)	2

Version 3.5.0b01

FIG. 34.46b.

detected. Furthermore, the assay is independent of sequence type so it is theoretically adaptable to divergent viral forms such as non-B subtype and O group viruses. However, there are a limited number of drugs for which this test has been validated (Nevirapine and 3TC) and some drugs for which this approach does not work (ZDV). Finally, the levels of viremia required are typically higher than for assays that include a genetic amplification step.

Virtual Phenotype

The difficulties with interpretation of genotypic data and the cost and time requirement of many phenotypic assays have led to the examination of large databases of simultaneous genotypes and phenotypes to generate algorithms for predicting phenotype from genetic sequence (Vircogen Virtual Phenotype; Virco) (75,76). In a study of clinical specimens, virtual phenotype and actual phenotype have been found to be in agreement in most but not all cases (77). Prospective comparisons between virtual and actual phenotype are needed to determine clinical utility. Further refinement of the prediction algorithms may be possible by the accumulation of additional "learning" data. Finally, the updating of databases with actual phenotypes for virus with new genotypic resistance patterns developing in response to new and new combinations of antiretroviral drugs will need to be continued.

ROLE OF RESISTANCE TESTING IN CLINICAL PRACTICE

Predictive Value and Clinical Utility

Support for resistance testing in clinical practice is mounting although precise guidelines for which assays to use and how best to use them are not yet available. Abundant retrospective data clearly show the predictive value of the presence of individual and categories of resistance mutations to NRTI (78–85), NNRTI (83,86–88) and PI (76,85,89) on treatment responses and outcomes. These observations suggest the utility of resistance testing in choosing antiretroviral drugs when switching therapy.

Evidence from prospective studies is less clear. Some but not all prospective studies performed to date provide support for the short term benefits of resistance testing. The VIRADAPT study randomly assigned 108 treatment experienced patients either to receive treatment based on genotypic resistance testing or to standard of care (90). By 12 weeks, mean change in plasma RNA was significantly greater in the genotyping arm (–1.04log vs. –0.46log) as was the proportion of patients achieving viral loads <200 copies/ml (29% vs. 14%). Similar results were maintained to the conclusion of the study at six months. In the CPCRA 046 study, genotypic testing along with expert interpretation and recommendations was compared to standard of care in 153 patients failing dual NRTI plus PI therapy (91). Reduction of plasma RNA was greater in the genotype arm than in the standard of care arm (–1.19 log vs. –0.61 log) and the proportion with plasma VL $<$ 500 copies/ml was also greater (55% vs. 25%) (at week 8). However, the difference was no longer statistically significant at week 12 (34% vs. 22%).

Similar results studying phenotypic assays have been reported. In a study of 273 ARV experienced patients randomized to treatment based on a recombinant phenotypic assay (Antivirogram, Virco) or to standard of care, viral load decrease (–1.23 log vs. –0.89 log) and proportion of patients with undetectable viral load (<400 copies/ml) (59% vs. 42%) were significantly better with phenotyping at week 16 (92). However, another study using the same assay reported benefits at week 4 that were lost by week 16 (93). Meynard and colleagues found no overall benefit to either phenotypic or genotypic testing in a study of 541 patients randomized to standard of care, genotyping or phenotyping (in-house assay) at week 12 but in a subset of patients experiencing failure on their first PI regimen, genotyping appeared superior (94). In another study using the Phenosense[a] assay, Haubrich and colleagues noted that baseline phenotype was indeed strongly predictive of outcomes however, at months 6 and 12, viral suppression was similar for those randomized to phenotype and those randomized to standard of care (95). These investigators stressed the need to refine the cutoff values for some drugs such as stavudine and didanosine.

Several important conclusions can be drawn from these prospective studies. First is the need for expert interpretation of resistance data for optimal benefit from resistance testing. In the one study where expert interpretation of resistance data and recommendations were not provided to clinicians, neither genotype nor phenotype provided convincing benefit (94). Computerized algorthims have also been advocated for the interpretation of resistance data, but it remains to be seen how these compare with results based on the recommendations of experienced clinicians and virologists. Another factor that appears to affect the utility of resistance testing is the range of treatment options available to patients failing therapy. Patients with many treatment options based on treatment history and those with no options may receive less benefit from resistance testing than would patients who are in between these extremes (6,94,95). The development of appropriate clinical cutoffs for each drug should also enhance the benefits from resistance testing.

IAS Consensus Recommendations

The International AIDS Society expert panel has recently recommended the use of resistance testing to help guide the selection of new drugs following treatment failure and for the selection of drugs for HIV infected, pregnant women. The panel also recommends consideration of resistance testing of drug naïve chronically infected

patients prior to initiation of therapy in areas where the prevalence of drug resistance is high. Finally, testing is strongly advocated in patients with primary HIV-1 infection who are about to undergo treatment because of the increasing incidence of primary drug resistance (96).

Viral Resistance in Tissues

Drug resistance testing of virus in blood is currently the only commonly employed clinical strategy. Because the vast majority of virus in blood arises from active viral replication and production lymphoid tissue, it is not surprising that drug susceptibility of virus in blood and lymphoid tissues correlate quite well (97,98). However, virus in other anatomic compartments such as the CNS and genital tract has been shown in small studies to comprise distinct genetic sub-populations (99–102). In the absence of treatment, this may reflect the selective effects of differences in target cell availability and host immune response (reviewed in Blankson) (103). With therapy, variation in drug penetration into different anatomical sites may result in suboptimal drug levels, promoting the evolution of resistance (104), or when no significant drug penetration occurs, give rise to sanctuary sites that permit the replication of drug susceptible virus (25,105–110). The discordance of resistance genotypes between virus in plasma and CSF has been noted in two recent studies (106 107). The clinical significance of these observations has not been determined.

EPIDEMIOLOGY OF DRUG RESISTANCE

Through analysis of samples derived from clinical trials and population-based surveys of resistance in treated patients, the risks of emergence of resistance in those receiving specific drug combinations have been well documented. The largest such database to date has been presented by the Virco group, who assessed the prevalence of specific mutations and phenotypic resistance in over 11,000 samples submitted for routine clinical testing in the U.S. (111). These data demonstrate that less than 25% of patients had a wild type phenotype and over 20% had reduced susceptibility to drugs within all three classes of currently available antiretroviral drugs. In nearly 50% of patients, plasma virus carrying M184V in reverse transcriptase could be detected, representing high level resistance to lamivudine, and a large proportion also had the zidovudine resistance-associated mutations at positions 215, 70, 67 and 41 of reverse transcriptase (Fig. 34.7). Multinucleoside resistance mutations (Q151M and 69 insertions) were only rarely detected, although this may rise as patients become more treatment-experienced. Of note, around 30% of patients also had at least one of the nonnucleoside analog and/or one of the key protease inhibitor resistance mutations.

In the U.K., the Public Health Laboratory Service started monitoring the prevalence of HIV drug resistance in 1998, recruiting patients on a random basis, who were receiving antiretroviral drugs with a viral load > 5,000 copies/mL. Results from the first year identified nearly 50% of 100 patients with resistance to drugs within at least one class of drugs, and approximately 20% having demonstrable resistance to drugs within all three classes. The most common mutations were associated with reduced susceptibility to the nucleoside analogs. These results, from a much smaller data set, are remarkably similar to those from the U.S. discussed above, and demonstrate the problems faced in choosing effective antiretroviral therapies for patients failing treatment.

There is also increasing concern about the possible transmission of drug resistant HIV, so called "primary drug resistance" (112–115). In many developed countries, the incidence of HIV/AIDS related mortality fell dramatically since the mid-1990s, coincident with the introduction of HAART while the number of new HIV diagnoses has remained constant. It is therefore unsurprising that some of these individuals have been infected with resistant virus, with prevalences of 5–20% being reported from Europe, Australia, and the U.S. (112–116).

MECHANSIMS OF ANTIRETROVIRAL RESISTANCE

Antiretroviral resistance develops as viral replication is allowed to continue in the presence of drug selective pressure. For some agents (such as lamivudine and the non-nucleoside agents), a single point mutation induces high-grade phenotypic resistance in a predictable manner. For others (such as zidovudine, abacavir and most of the protease inhibitors), high-grade phenotypic resistance requires the serial accumulation of multiple mutations, and is thus much slower to emerge. A final group of drugs (including didanosine and stavudine) is only associated with low-grade phenotypic resistance, despite the presence of one or more key mutations. Although it was originally thought that this could be associated with persistent *in vivo* efficacy of these agents, clinical trial data now show that this is not the case, and the cut-off for phenotypic resistance to didanosine and stavudine has been lowered to reflect this.

Despite the fact that many antiretroviral agents are available for use, the phenomenon of cross-resistance among drugs in a same class limits the options for second and third-line therapy. For protease inhibitors, it appears that a primary resistance mutation develops, which leads to an increase in drug resistance at a cost of decreased viral replicative capacity or "fitness". As an example, isolates carrying the D30N or M46I mutation in the protease gene may be as much as 20% less fit. The majority of isolates resistant to PIs demonstrate alterations in substrate specificity, which in theory, could be exploited to design

novel protease inhibitors (117) that would retain activity against these less Þt isolates. However, the subsequent accumulation of secondary mutations (such as at codons 46 and 63) may restore Þtness and allow the strain to replicate and persist. Recent data show that these strains (with multiple primary and secondary mutations in the protease and reverse transcriptase genes) can be transmitted, and persist for many years in the absence of drug pressure. It is likely that this phenomenon is not fully explained by changes in the target genes themselves, as interesting experiments have shown that if multi-resistant protease genes are cloned into recombinant backgrounds, they appear much less Þt than when wild-type protease genes are cloned into recombinant backgrounds (118). However, the Þtness of resistant viruses may not parallel that of wild-type isolates, as the persistence of this resistance tended to be associated with more favorable virologic and immunologic set points (30).

The sequential use of PIs is based on the fact that a number of agents in this class have unique primary resistance mutations. This is particularly true of nelÞnavir and amprenavir. Indeed, the I50V amprenavir resistance mutation alters the hydrophobic interaction with the substrate without altering the binding of other drugs in this class (119). This advantage may be lost if the isolates are allowed to continue to replicate under drug pressure, and develop secondary mutations. These would, at the very least, hasten the development of the primary mutations to other agents in the class. One agent that seems to retain activity in this setting is lopinavir/ritonavir, due to the high circulating drug concentrations that are achieved. It may thus retain activity in a situation where other protease inhibitors are no longer effective. Finally, protease cleavage site mutations may be more important than previously appreciated. In one study of drug na•ve patients such mutations were present in 77/473 individuals and were associated with a poorer therapeutic response to protease inhibitor-based HAART (120).

Although most of the mutations associated with NRTI resistance are not at the active site of the enzyme, they do lead to conformational changes that propagate to the active site (121). The fact that the mutations occur in two separate domains leads to two speciÞc mechanisms for resistance: decreased substrate binding and increases

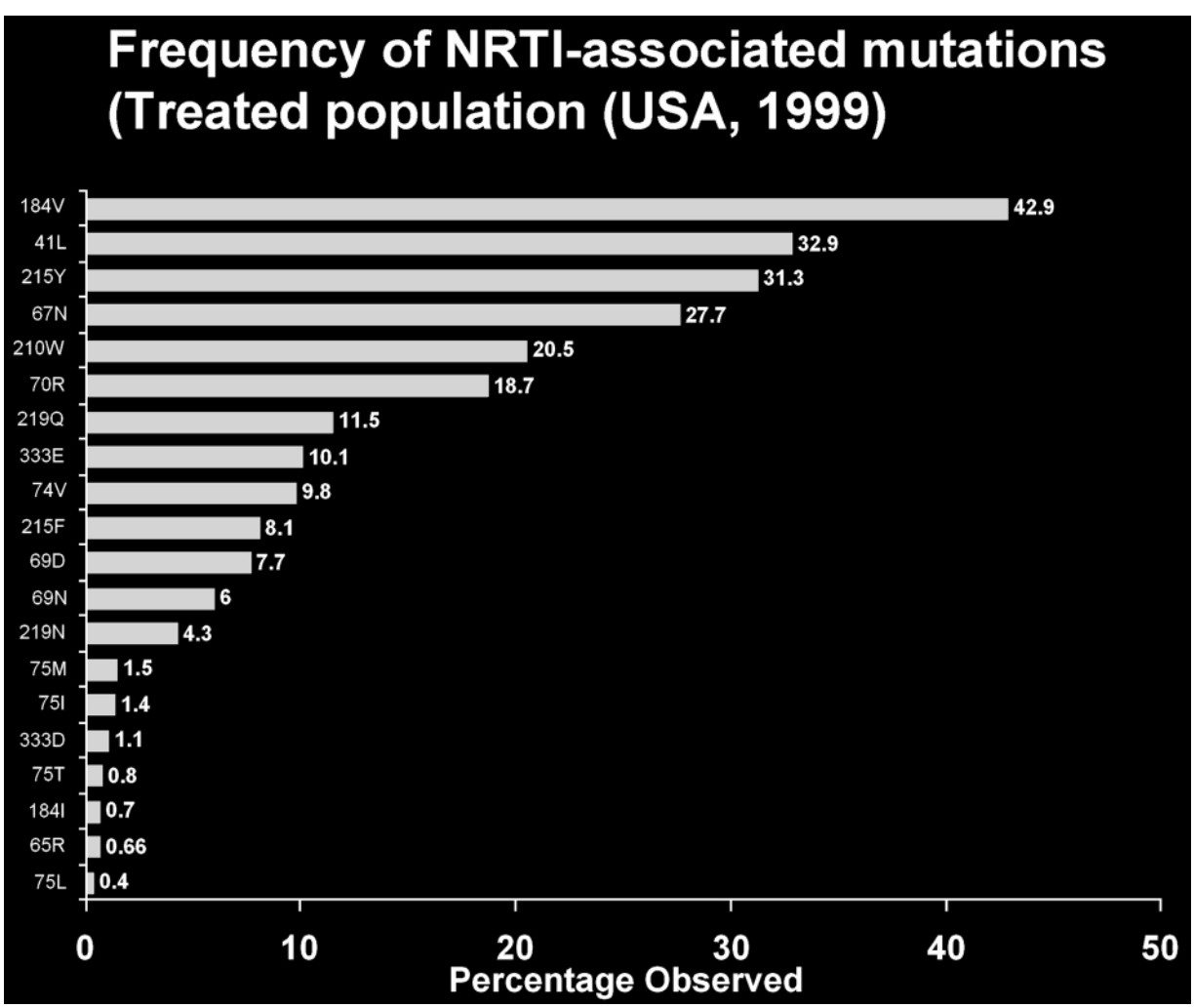

FIG. 34.7.

pyrophosphorolysis, leading to a net decrease in chain termination (122). Resistance to another agent, phospohonoformic acid, reduces pyrophosphorolysis, and partially restores zidovudine susceptibility of resistant isolates, conÞrming the importance of both mechanisms in high-grade zidovudine resistance. For multi-drug resistance three patterns have been identiÞed. Two of these are associated with an inability of the reverse transcriptase enzyme to incorporate ATP and the third (the Q151M complex) leads to substrate-independent inhibition of enzyme function (123). Further, the insertion of a dipeptide between codons 69 and 70 has been shown to enhance the removal of the terminal nucleotide and enhance extension of the unblocked primer (124). These data provide a biochemical explanation as to why the latter pattern is most readily associated with multi-drug resistance within the class. It is worrisome that these isolates may be more Þt than corresponding wild type strains *in vivo*. This is also true of certain strains carrying other multi-drug resistant mutations in the reverse transcriptase genes, suggesting the need to intervene in the course of therapy before such progressive genetic changes occur. A new nucleotide agent, tenofovir, has preserved activity against many isolates carrying multi-nucleoside resistance mutations, but seems less active against those carrying K65R, the T69S insertion or four or more resistance associated mutations (79). The small size of the molecule and its ability to interact with its substrate in multiple conformations likely explain its retained activity against most resistant isolates (125).

The K103N mutation confers resistance to all currently available NNRTIs, presumably by stabilizing the closed pocket form of the enzyme, thus inhibiting the binding of the drug to its substrate (126). The fact that all agents in this class bind to the same area explains the broad pattern of cross-resistance, and should prompt the development of new agents that would interact with this side chain more favorably. Although mutations at codons 181 and 188 confer high-grade resistance to certain drugs in this class such as nevirapine, this is not generalized to newer drugs such as efavirenz, which is a more compact molecule, and these changes do not alter the binding to its substrate (127). Therefore, cross-resistance across this class is not absolute. In practice, as many as 20% or more of patients developing nevirapine resistance will still have isolates that are sensitive to efavirenz (53,128). Although it may be that subsequent exposure to efavirenz will lead to a more rapid development of resistance, than if the baseline isolate were wild-type, which limits the possibility of sequencing of drugs within this class.

Certain mutations may confer resistance to one drug and enhanced susceptibility to another, such as M184V (129,130) and L74V (131), associated with resistance to lamivudine and didanosine respectively, which leads to enhanced sensitivity to zidovudine. M184V may also restore susceptibility to stavudine and adefovir (132,133). Isolates carrying these single changes have been consistently shown to be less Þt. It has been postulated that isolates with M184V mutations have increased Þdelity, thereby decreasing their ability to accumulate new resistance mutations (134). They may also lead to a decrease in pyrophosphorolysis, further enhancing zidovudine susceptibility (135). Indeed in early studies of the combination of zidovudine and lamivudine as double therapy, the development of zidovudine resistance was considerably delayed (129). Based on these data, it has been suggested that the use of lamivudine in salvage therapy settings could help reduce the development of resistance to other agents and enhance the efÞcacy of the overall regimen, a fact that remains to be proven in clinical trials.

Enhanced susceptibility to NNRTIs has been described in association with multiple mutations conferring broad cross-resistance to nucleoside analogues. It now appears that this phenomenon has biological signiÞcance, since its presence enhances the response to efavirenz-based therapy in both uncontrolled (136) and controlled (137) trials. However, the presence of hyper-susceptibility did not appear to delay the emergence of delavirdine resistance in one controlled salvage therapy study (138). This phenomenon may also extend to protease inhibitors, with mutations at codons 30 and 88 possibly conferring hyper susceptibility to amprenavir and ritonavir (139).

Some preliminary data suggest that non-B HIV isolates may have a reduced response to HAART. This may be due to natural polymorphisms in the protease gene, such as M36I, with or without additional changes at codons 71 and 77 (140,141). Secondary mutations that are more frequent in subtype C isolates exposed to protease inhibitors appear to be unique, a Þnding of unclear clinical signiÞcance (142). Additionally, it appears that subtype G isolates develop nelÞnavir resistance through the L90M rather than the D30N pathway, a difference that would confer different patterns of cross-resistance to other agents in this class than we are used to seeing (143). *In vitro* data exist to suggest that subtype C isolates develop resistance to NNRTIs more rapidly (144,145).

FUTURE DIRECTIONS

Clinical Outcomes with Resistance Testing

While available data strongly suggest a role for resistance testing in clinical practice, they also suggest that we currently underestimate the degree of cross-resistance (in particular with NRTIs) and that more accurate clinical cutoffs need to be established for all drugs. Additionally, the most cost effective approach to resistance testing remains to be deÞned and given the rapid pace of new drug development and the ever growing complexity of drug resistance is an issue that will require continued review and revision.

Early Detection of Resistance

In research applications, sequences of individual viral RNA molecules or proviral DNA sequences can be obtained by performing a cloning step (105,146) Such procedures can permit detection of minor populations of drug resistant virus when the bulk sequences only reßect the major variant. How important this might be to clinical practice is not known.

Hypersusceptibility

The precision of some of the automated phenotypic assays have permitted the identiÞcation of genetic polymorphisms of HIV from some patients that exhibit greater susceptibility to drugs than the control susceptible or Òwild-typeÓ strains (136,147Ð150). Some of these polymorphisms represent resistance mutations to other drugs or classes of drugs and are elicited by prior treatment while others appear to be naturally occurring. Whether treatment with drugs for which a patientÕs virus is hypersusceptible translates to a better clinical outcome remains to be determined but these observations suggest an additional potential beneÞt of drug susceptibility assessment to guide drug selection.

Viral Fitness

The recent observation that some drug resistant virus may exhibit attenuated virulence properties (30,151Ð153) adds additional complexity to therapeutic decisions based on drug resistance testing. Continued treatment with drugs against which resistance has developed can still provide some residual viral inhibition and also serves to maintain drug resistant viral populations that may be associated with lower viral replication Þtness and virulence compared to Òwild-typeÓ virus. In net, continued treatment can be associated with prolonged immunologic beneÞt. Under circumstances where therapeutic options are limited, the potential beneÞts of continued treatment need to be weighed against the possibility that continued treatment will result in selection of viral variants with greater resistance and replication Þtness (154,155).

Resistance to New Drug Classes

Inhibitors of the HIV-1 integrase are now in clinical trials and resistance to these agents will doubtless be recognized in the near future. The investigational fusion inhibitor enfuviritide (156,157) is already under review by the FDA and resistance to this drug appears to develop in the gp41 coding region (20, 158). This and future progress in drug development against new viral targets will necessitate the development of new tests for resistance and the adaptation of existing resistance testing methods.

Non-Clade B Resistance

While the Þeld of HIV drug resistance testing has largely been restricted to the study of subtype-B virus, three issues make increased attention to drug resistance in non-B viruses important. First, drug therapy is being introduced into areas of the world where the predominant subtypes are non-B (159,160). While there remain serious Þnancial and other logistical barriers to antiretroviral therapy in the developing world (160,161), the need to begin to build the infrastructure to support treatment in these areas of the world is acknowledged to be great and is a major goal of many governmental and non-governmental agencies. Second, some non-B viruses exhibit natural polymorphisms that appear to confer intrinsic resistance to certain drugs. The need for pre-treatment screening may therefore be greater under these circumstances. Third, the genetic background of these viruses may theoretically result in mutational patterns not previously encountered with clade B virus (162). Because many of the existing assays were developed and reÞned speciÞcally for type B virus, their performance for the testing of non-B virus is only recently being explored (163,164).

CONCLUSION

HIV drug resistance testing is a rapidly evolving Þeld. Technical advances and reÞnement of testing modalities are matched by the growing complexity of therapeutic options and the complex resistance patterns that result. A further challenge will be the application of resistance testing to non-subtype B virus as therapy is introduced into areas outside North America and Europe. Ultimately, analyses of large data bases relating genetic resistance patterns to phenotypic susceptibility, drug treatment and clinical outcomes will be needed to fully optimize and deÞne the use of resistance testing in clinical care. In the meantime, the standard of care for HIV infected patients includes the judicious use of resistance testing along with expert interpretation. Resistance testing has been advocated when switching patients from a failing drug regimen, for choosing drugs for pregnant patients and should be considered in all patients with primary HIV infection or any patient about to embark on therapy in areas where the prevalence of drug resistance is high.

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Chapter 35

The Analysis of HIV Dynamics Using Mathematical Models

Ruy M. Ribeiro and Alan S. Perelson

The use of mathematical modeling and computer simulation in the study of infectious disease and, in particular HIV infection, has proven quite fruitful. The study of the dynamics of HIV infection represents a paradigm of success in the application of mathematical models to further our knowledge of disease pathogenesis (1,2). With these analyses, it was possible to quantify the rapidity of HIV infection and replication, the rate of virion clearance, the lifespan of productively infected cells (3Ð5), and predict the impact of treatment and the appearance of drug resistant mutations (6). Moreover, models have helped clarify controversial issues relating to the mechanism of T cell depletion in HIV infection and motivated new experimental and clinical studies.

In this chapter we will introduce the basic model for the analysis of HIV viral load decay under treatment, and explain the quantitative results obtained by these models when compared with experimental data. In later sections we will discuss extensions of this basic model to the analysis of viral reservoirs, primary infection and immune responses. Finally, we will consider models of cellular turnover, which are closely related to pathogenesis of HIV infection.

BASIC MODEL OF HIV INFECTION

Throughout most of the asymptomatic phase of HIV infection the plasma viral load remains approximately constant, at the set point reached after primary infection. This observation implies that the total amounts of virus produced and cleared in the body are balanced, i.e. production = clearance. If one of the processes in that equation dominated the other, than we would either have uncontrollable growth of virus (if production > clearance), or the virus would be eradicated (if production < clearance). This is a very powerful observation, because it means that by perturbing one side of the equation we can gain insight into the other. Thus, if we decrease the production rate, for example with antiretroviral therapy, then clearance dominates and the viral load decreases. This is exactly what is observed. Importantly, the magnitude and speed of the decrease allows the calculation of the clearance rate. Moreover, once the clearance rate is estimated, then the baseline production rate before therapy was initiated can also be estimated since at baseline, production = clearance. To obtain these estimates we need appropriate models, because the proÞle of the viral decay curve depends on several factors such as the clearance rate, the efÞcacy of the drug, and the death rate of virus producing cells. These models, based on the lifecycle of the virus, can then be used to Þt quantitative data about viral decay under antiretroviral therapy.

We begin by considering a model of the viral lifecycle. Although HIV infects different types of cells, with variable replication rates, there is a consensus that the bulk of HIV production occurs in activated CD4 + T-cells (7). Thus a basic model of HIV infection only includes activated cells (T), productively infected cells (T^*), and free virus (V). It is assumed that cells become activated at constant rate λ, and die at rate d per cell. In the uninfected individual, for most of the time, there is a steady level of these cells, which is given by λ/d. However, in infected patients these cells are susceptible to infection by HIV at a rate proportional to the available numbers of uninfected cells and free virus, with rate constant k. Thus cells become infected at rate kVT, which corresponds to a mass-action term, common in chemical kinetics. Infected cells are created at this same rate, and die at rate δ per cell,

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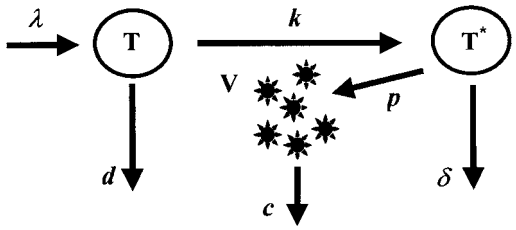

FIG. 35.1. Model used to analyze the dynamics of HIV-1 infection. See text for a full description.

which may be different from the death rate of uninfected cells (d), possibly higher. Finally, the virus is produced from the infected cells at rate p, and it is cleared from the circulation at rate c. Figure 35.1 represents this system in schematic form, and the corresponding equations are:

$$\frac{dT}{dt} = \lambda - dT - kVT \tag{1}$$

$$\frac{dT^*}{dt} = kVT - \delta T^* \tag{2}$$

$$\frac{dV}{dt} = pT^* - cV \tag{3}$$

We note that these terms and rates represent abstractions of possibly many different biological processes. Thus, for example, the clearance rate, c, indicates how fast virus is cleared from the circulation, whatever the mechanismsÑ Þltration by the reticuloendothelial system, opsonization, binding to follicular dendritic cells, phagocytosis, etc.

During the long asymptomatic phase of untreated HIV infection the viral load remains unchanged (8). This suggested initially that HIV replicated slowly. However, there was no quantitative way of measuring the viral turnover. Eqs. (1)Ð(3) show that the viral load can be at a steady state, i.e. unchanged in time ($dV/dt = 0$), as long as the production of virus (pT^*) and the clearance (cV) are balanced ($pT^* = cV$). However, this balance may occur for many different sets of parameters, and thus viral production could be high, if clearance was also large, or those rates could both be small. Knowledge of the rate of production also indicates the burden imposed on the immune system and the opportunity for the virus to mutate.

Treatment of HIV Infection

When treatments with potent antiretroviral drugs, either protease inhibitors or a combination of a protease inhibitor and reverse transcriptase inhibitors, were administered to HIV-1 infected patients, a 1 to 2 log drop in the viral load was observed over the Þrst one to two weeks of therapy. This observation allowed the quantiÞcation of the dynamics of virus production and clearance, using model (1)Ð(3) (3Ð5). Indeed, drug treatment represents a perturbation of the equilibrium by stopping the production of new virus, and the observed changes in viral load with time (dV/dt) are an indirect measurement of the clearance rate of free virions.

To analyze such drug treatment experiments, a slight modiÞcation of model (1)Ð(3) is necessary. The drugs belonging to the class of reverse trancriptase inhibitors (RTI) reduce the ability of the virus to infect new cells, by interfering with the necessary process of reverse transcription of viral RNA into proviral DNA. Thus if the efÞcacy of these drugs is ε_{RT}, then only a proportion $1 - \varepsilon_{RT}$ of new infections will be productive and the infection terms in Eqs. (1)Ð(3), kVT, have to be reduced to $(1 - \varepsilon_{RT})kVT$. On the other hand, drugs belonging to the class of protease inhibitors (PI) interfere with the maturation of new virions, rendering them non-infectious. However, these non-infectious viral particles are still quantiÞed by the commonly used RT-PCR based assays of viral load. Thus, it is important to follow the dynamics of these non-infections particles (V_{NI}). If the protease inhibitor efÞcacy is ε_{PI}, then only a proportion $1 - \varepsilon_{PI}$ of new viruses will be infectious, and a proportion, ε_{PI}, of viral particles will be non-infectious. The equations for the analysis of drug treatment data are then (Fig. 35.2):

$$\frac{dT}{dt} = \lambda - dT - (1 - \varepsilon_{RT})kV_1T \tag{4}$$

$$\frac{dT^*}{dt} = (1 - \varepsilon_{RT})kV_1T - \delta T^* \tag{5}$$

$$\frac{dV_1}{dt} = (1 - \varepsilon_{PI})pT^* - cV_1 \tag{6}$$

$$\frac{dV_{NI}}{dt} = \varepsilon_{PI}pT^* - cV_{NI} \tag{7}$$

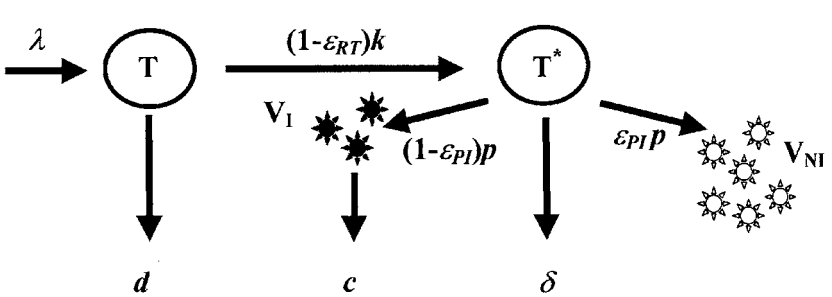

FIG. 35.2. Adaptation of the model in Fig. 1 to the study of antiretroviral drug therapy in HIV-1 infection.

where V_I represents infectious (or more precisely mature protease inhibitor untreated) virus, and V_{NI} represents non-infectious (or immature) virus.

With Eqs. (4)–(7) it is in principle possible to estimate the decay rate of free virus (c) and infected cells (δ), and from these the respective half-lives: $\ln(2)/c$ and $\ln(2)/\delta$. (Here $\ln(2)$ represents the natural logarithm of 2 and it is approximately 0.693.) It may also be possible to estimate the efficacies of the drugs, ε_{RT} and ε_{PI}. However, estimates of all these parameters demand frequent sampling of viral loads, from the start of therapy, which is not always possible due to ethical considerations.

Experimental Results

The model in Eqs. (4)–(7) was used to fit experimental data of viral decay under drug treatment in HIV infection. With a 100% effective PI regimen, and assuming that the uninfected cells (T) remain constant during the short period of analysis, the number of viral particles ($V_I + V_{NI}$) per ml of blood decays as:

$$V(t) = V_0 e^{-ct} + \frac{cV_0}{c-\delta}\left[\frac{cV_0}{c-\delta}(e^{-\delta t} - e^{-ct}) - \delta t e^{-ct}\right] \quad (8)$$

where V_0 is the pre-treatment (baseline) steady state viral load.

Estimates obtained by this method indicated that the half-life of free virus is less than 6 h, and that of infected cells is less than 1.6 days (3). The estimates are upper bounds because they are based on the assumption that drug therapy was 100% effective. In reality therapy is not so effective and residual viral production occurs. Thus, residual virus that is not accounted for in the model must also be cleared, and hence the true rates of virus and infected cell loss must be higher than is estimated by assuming a perfect drug. In Fig. 35.3 we show typical fits of the model solution, given by Eq. (8), to data for three patients. Since the virus is cleared rapidly, frequent sampling is needed at the initiation of treatment. Indeed for this experiment blood samples were initially obtained every 2 h, and still the estimated half-life of 6 h is approximately the limit of detection since it depends on so few initial data points, and is in fact just an upper bound.

The half-life of free virus has also been estimated using a different experimental method: plasma apheresis (9). In this method plasma with suspended viral particles is removed from the patient, and the same volume of fluid is re-infused. This technique again induces a perturbation of the equilibrium, since the clearance of HIV is no longer just cV, as in model (1)–(3), but larger due to the apheresis process. Frequent measurements of the decline in viral load with knowledge of the rate of removal of the virus by apheresis allow the estimate of c, and thus the half-life of free virus. This method yielded half-lives of about 1h, i.e. even shorter than those estimated by application of drug therapy.

A half-life can be biologically interpreted as the time it takes for 50% of the free virus (infected cells) to be removed from circulation. Since before treatment, the viral load is in equilibrium, it follows that the viral (infected cell) production has to equal the removal rate. Thus a half-life of 1 h indicates that a typical individual with a set-point viral load of 10^5 HIV RNA copies/ml (or equivalently 1.5×10^9 HIV RNA in the 15 liters of body water in a 70 kg person) has a total body daily production of between 10^{10} and 10^{11} viruses. The total body load of productively CD4+ T-cells was estimated to be about 4×10^7 (10). If these cells have a half-life of 1.0 day, then 4×10^7 CD4+ T-cells are destroyed and produced each day. (This half-life of one day is a recent estimate obtained with a very potent four-drug protocol (11).)

These large turnovers have implications for the generation of mutations in the viral genome. The estimated mutation rate of HIV is 3.4×10^{-5} mutations per base per replication cycle (12), and the viral genome is about 104 bases. Thus, it can be estimated that, on average, all one-point mutations will be produced many times each day, and that a proportion of all viable two-point mutations will also be produced. Depending on the selective disadvantage of these mutants, many will be present at low levels in the patient's viral population, allowing for further accumulation of mutations (13,14). This explains the quasi-species nature of HIV and its rapid evolution *in vivo*. One consequence of this evolution is the rapid generation of viral variants resistant to all the available drugs when used alone, and this provides a rationale for combination therapy.

Potent combination antiretroviral therapy leads to a sustained viral decay over long periods of time until the viral load becomes undetectable by conventional assays. However, after the first week or two, the decay slows down and it no longer corresponds to the decline of short-lived productively infected cells, which have a half-life of about 1.0 day.

MODELS FOR THE SECOND PHASE OF DECAY

In the basic model (Section 1) HIV is only produced from productively infected CD4+ T-cells. However, to understand the second phase of decline observed in patients undergoing HAART, models with additional sources of HIV had to be developed (15). After productively infected CD4+ T-cells are removed by treatment, these other sources would become dominant and their slower clearance leads to the observed slower decline in the levels of HIV RNA. Different possible putative sources were considered in the context of modeling: long-lived productively infected cells, latently infected cells, and tissue reservoirs, such as follicular dendritic cells (FDC) or drug sanctuaries (i.e. cell

populations or tissues where drug penetration is sub-optimal).

Data Þtting to HIV RNA decline cannot distinguish between those possible sources, since the Þts for different models are equally good. For example, one can not distinguish between long-lived productively infected cells or latently infected cells being the source of second phase of decay. However, Þtting HIV RNA and infected cell decay simultaneously indicates that the second phase of decay is more likely due to long-lived productively infected cells. These cells were estimated to have an average half-life of about 14 days (16), whereas latently infected cells without integrated provirus were estimated to have a half-life of about 8.5 days (16). With these estimates, we have calculated that productively infected cells contribute 93Ð99% of the virions produced at steady state, before therapy, whereas long-lived infected cells contribute only 1Ð7%, and latently infected cells less than 1% (16). HIV infects not only CD4 + T-cells but also other cell types such as macrophages and dendritic cells, which are candidates for the long-lived productively infected cell pool. However, it is difÞcult to eliminate other contributions to the second phase of decline, and instead of a long-lived infected cell pool the second phase of decay

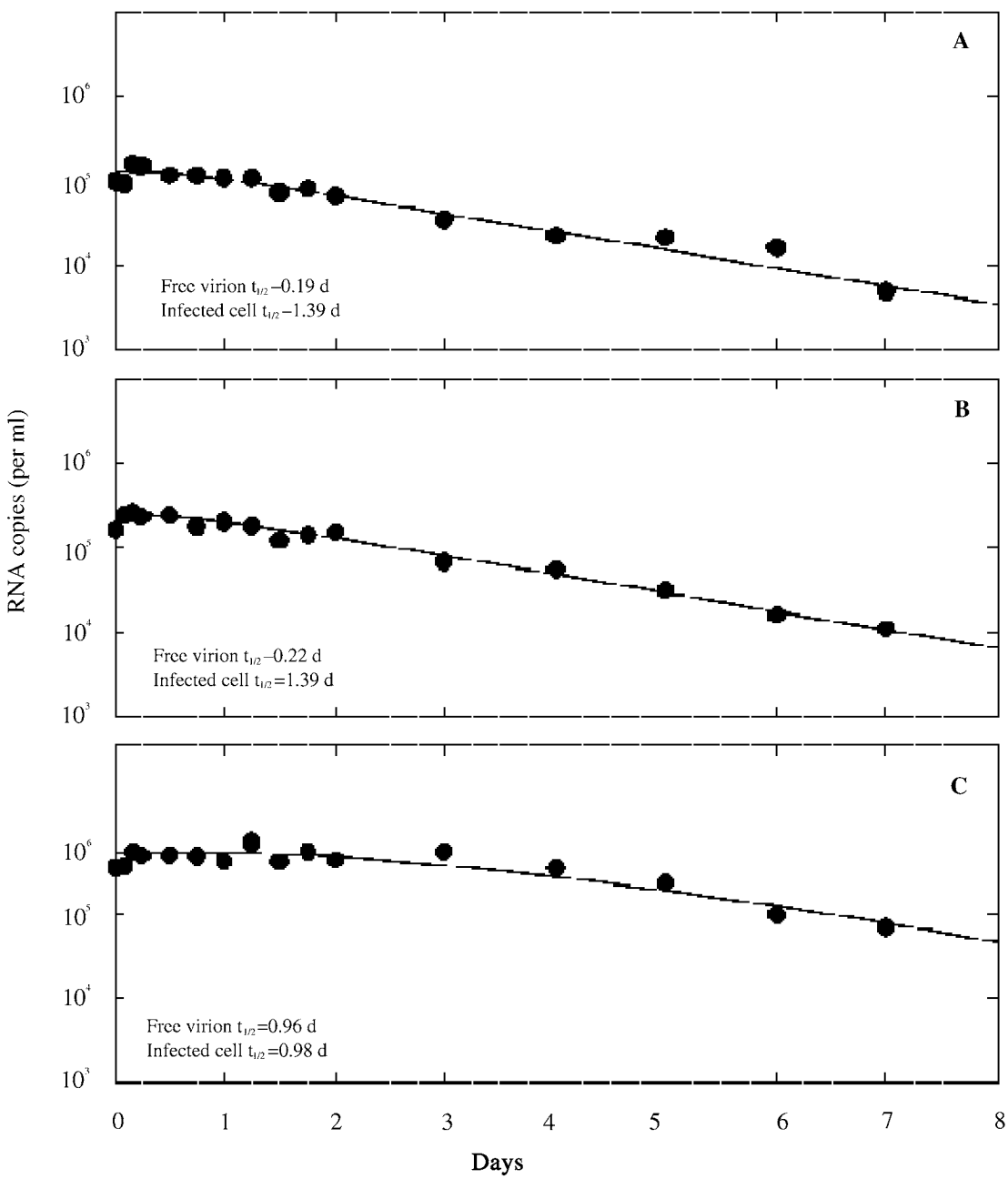

FIG. 35.3. Fit of Eq. (8) to data of HIV-1 treatment, for three individuals (A, B, C), studied in (3). The black dots are the data-points and the solid lines the theoretical ts, from Eq. (8).

could actually correspond to release of virions trapped on follicular dendritic cells (FDC) (17) (Fig. 35.4).

There are large amounts of potentially infectious virions attached to FDC, and these may be released in a biphasic mode, if binding is reversible and multivalent (18). Thus, it is conceivable that these virions are an important source of HIV RNA during drug treatment (19). If this is the case, then the estimates of viral clearance and infected cell death rates would have to be revised upward, because these rates would have to compensate not only for the residual production of virus from infected cells, but also for the release of virus from FDC. Moreover, virus may remain bound to FDC for prolonged periods of time, perhaps decades (18). If they maintain their infectiousness, then they represent a serious obstacle to current therapies, since they could re-seed a productive infection as soon as therapy is stopped.

Finally, detailed measurements of HIV provirus integrated in resting memory CD4+ T-cells indicate that this cell population can constitute an inducible long-lived latent reservoir (20–23). Measurements of the decay of PBMC infectiousness by limiting dilution analysis suggests that this reservoir could lead to a third, even slower, phase of decay of plasma HIV RNA. Analysis of the decay of the latent reservoir in patients treated with potent combination therapy, with models that assume exponential decay of the reservoir, show that the half-life of cells carrying replication competent viral genomes varies between six and 44 months (20–23). Since, it is possible that this latent pool is replenished whenever there is active production of virus, for example due to temporary incomplete suppression of replication by the drug regimen, it is unclear whether the long half-lives in this range represent the true rate of decay of the reservoir or are artificially raised due to reseeding of the reservoir.

EXTENSIONS OF THE BASIC MODEL

Most of the models used to analyze HIV RNA decay do not take into account the immune response mounted against the virus. However, several lines of evidence point to the importance of cytotoxic T lymphocyte (CTL) control of the virus. These include viral evolution and escape under CTL pressure (24), the rise of viremia in CD8+ T-cell depletion studies in macaques (25,26), and the concomitant appearance of a HIV-specific CTL response and the decline of virus in primary infection (27).

It is worth pointing out that models without an immune response can account for the general dynamics of virus during primary infection (28,29). In these models, the viral load increase and subsequent decrease is controlled by the availability of target CD4+ T-cells for the virus to infect and spread (28). However, when data are analyzed over periods longer than 100 days after infection, in some patients the predicted viral set point by the basic model is larger than observed, suggesting an effect of immune control. A way to model immune control by CTL-mediated cytolysis is simply to assume that the death rate of infected cells (δ) in Eq. (2) is not constant (29). For example, Stafford et al. (29) modeled δ as the sum of a constant part, δ_0, and a part, $\delta_1(V)$, that increases with viral (antigen) load, i.e. $\delta = \delta_0 + \delta_1(V)$. The use of models that allowed an immune response provided a better fit to the viral load data obtained from some but not all primary infection patients studied by Stafford et al. (29). Changing the rate of death of productive infected cells is only one of a number of possible implementations of the effect of the immune response. Another possibility is that the antiviral effect of CD8+ T cells is due to secretion of soluble factors that inhibit viral production (30). In this case, it is the production of virus by infected cells, p, in Eq. (3) that

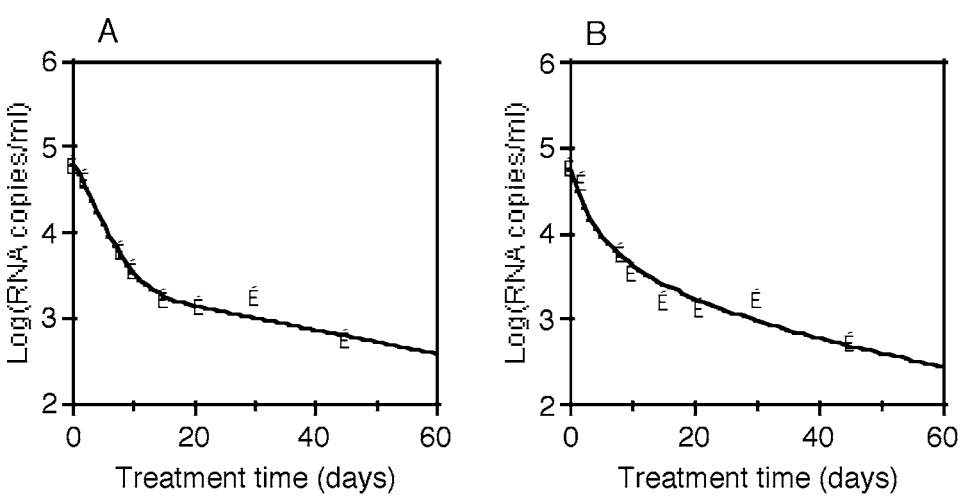

FIG. 35.4. Fit of data from the same patient with two different models. (A) A model assuming a population of long lived infected cells (15), and (B) A model that takes into account the decay of FDC bound virions during treatment (17). The circles are the data-points and the solid lines the theoretical fits. Notice that both models fit the data equally well.

is not constant, but rather decreases as CD8+ T cells increase (1,29).

Models of immune response in HIV infection have also been developed in other contexts. For example, to explain the two phase initial decay of viral load under therapy (31), to explore the cytopathicity of HIV (32), to explain HIV pathology and pathogenesis (33), to study viral evolution and diversification (34), and the role of CD4+ T-cell help in maintaining the immune response (35).

MODELS OF T-LYMPHOCYTE DYNAMICS

An important question in HIV infection has been what is the effect of infection in T-lymphocyte turnover. Several experimental methods have been used to try to look at this question (36–43). For the quantitative interpretation of these experimental results, the use of mathematical models became crucial. Here we will only refer to two methods, which attempt to directly quantify turnover of T-cells. Both involve the incorporation of a label into the DNA of replicating T-cells. In one the label used is bromodeoxyuridine (BrdU), and in the other it is deuterated glucose (D-glucose).

The most informative way to use these labels is in pulse-chase experiments with frequent measurements to assess the kinetics of acquisition and loss of label. For modeling purposes, these methods differ mainly in the way measurements are taken. In the BrdU case, the fraction of labeled cells is measured, whereas in the deuterated glucose case, the fraction of labeled DNA is measured (44). This subtle difference has important implications in the definition of the model. For example, the pool of unlabeled cells is reduced both by proliferation (cell acquires label) and death (cell is removed), when one measures cell labeling with BrdU (45). However, if one uses DNA labeling, then death of a cell will reduce the pool of unlabeled DNA, but proliferation of a cell does not reduce the pool of unlabeled DNA. This is because replication of DNA is accomplished by copying the original unlabeled DNA strand template, which thus is still present in the progeny (46). Thus, when an unlabeled DNA strand, U, is copied, the result is one unlabeled and one labeled, $U+L$ strand (Fig. 35.5). This difference between the two methods is easily seen in the equations used to analyze each of the experiments (see Table 35.1).

In Table 35.1, in the BrdU method, U and L refer to unlabeled and labeled cells; and in the D-glucose method, the same letters refer to unlabeled and labeled DNA, respectively. In these equations, d and p are the death and proliferation rates of lymphocytes, whereas s_U and s_L represent potential sources of unlabeled and labeled material (cells or DNA) into the proliferating pool, where measurements are taken. As can be seen in Table 35.1, the equations to describe D-glucose labeling can be reduced to expressions involving only the death rate d and the sources, whereas in the BrdU expressions both death and proliferation rates are present, in addition to the sources. Thus the D-glucose data can be fitted with a model with fewer parameters. Both models assume that the cell population being labeled is at steady state (T), so that the total number of cells and total amount of DNA is constant. Thus, for each method $U+L=$ constant and the fraction labeled, $f_L(t)$, can be determined from either $U(t)$ or $L(t)$, as shown in Table 35.2. In this table, T_0 represents the total number of cells in the BrdU method, and the total amount of DNA in the case of D-glucose labeling.

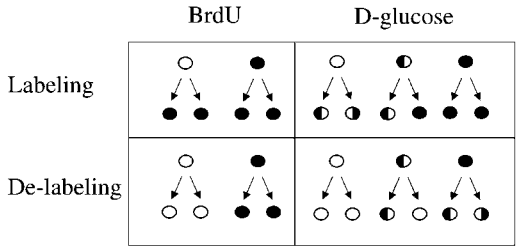

FIG. 35.5. Diagram of the labeling of T-cells under two protocols: BrdU labeling of dividing cells, and D-glucose labeling of the DNA of dividing cells. Labeled cells or both strands of DNA labeled are shown as filled circles, and half-filled circles indicate that only half of the DNA strands are labeled. Notice that in the D-glucose case the amount of unlabeled/labeled DNA remains constant with division during the labeling/de-labeling period, respectively.

The use of these types of models has shown that in untreated HIV infection the average proliferation and death rates are increased threefold or more, and that treatment reduces these rates (40–43). Moreover, this reduction is dependent on the time since the initiation of treatment and after one year of antiretroviral therapy, the values for the proliferation and death rates are nearly equal to those of uninfected subjects (41).

One drawback of these models is that to obtain good agreement with the data one needs to assume a source of unlabeled cells (DNA) into the labeling population. However, the exact nature of this source is not known, and it is indeed controversial (40,41,47). We have built a more realistic model, based on two populations of resting and dividing T-cells, to study this issue (46,47). In this model, resting T-cells can be activated into the dividing pool, and the death and proliferation rates in the activated population are the same. In this way, the model allows for the differentiation between the dynamics of proliferation and

TABLE 35.1. *Equations for the analysis of T-cell labeling experiments*

	BrdU method	D-glucose method
Labeling	$\frac{dU}{dt} = s_U - pU - dU$	$\frac{dU}{dt} = s_U - dU$
De-labeling	$\frac{dL}{dt} = s_L + pL - dL$	$\frac{dL}{dt} = s_L - dL$

TABLE 35.2. *Solution for the equations presented in Table 1*

	BrdU method	D-glucose method
Labeling	$\left(1-\frac{s_U}{(d+p)T_0}\right)(1-e^{-(d+p)t})$	$\left(1-\frac{s_U}{dT_0}\right)(1-e^{-dt})$
De-labeling	$\left(f_L(t_e)-\frac{s_L}{(d-p)T_0}\right)e^{-(d-p)(t-t_e)}+\frac{s_L}{(d-p)T_0}$	$\left(f_L(t_e)-\frac{s_L}{dT_0}\right)e^{-d(t-t_e)}+\frac{s_L}{dT_0}$

death, and the dynamics of activation. Surprisingly, the same model applied to data on CD4+ and CD8+ T-cells, that look similar leads to very different conclusions. The analysis shows that in the CD8+ T-cells the fraction of activated cells is increased in relation to healthy individuals, even though the proliferation and death rates of activated CD8+ T-cells are the same in infected and uninfected people. On the other hand, in the CD4+ T-cell compartment the proliferation and death rates of activated cells are increased in relation to healthy individuals. Even though these short-term experiments that assume equilibrium cannot explain the long-term dynamics of T-cells in HIV infection, this result helps explain why CD4+ and CD8+ T-cells show different behavior during the course of infection.

CONCLUSIONS

Modeling has become an important tool in the analysis and interpretation of experiments in HIV research. The use of these models has allowed the estimation of important kinetic parameters that allow us to understand the rapidity of HIV infection, to test hypotheses, and to make new predictions. Overall, the use of models has resulted in a signiÞcantly better understanding of viral dynamics, the appearance of drug resistant mutations, and the pathogenesis of infection (49). In the future, collaborations between experimentalists and modelers should lead to new ways to plan, conduct, and analyze experiments.

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Chapter 36

Practical Therapeutics

Stephen C. Piscitelli, Scott R. Penzak and Charles Flexner

Treatment regimens for HIV infection have evolved dramatically over the past 15 years. In 1985, treatment was limited to management of opportunistic infections, prophylactic trimethoprim-sulfamethoxazole, and zidovudine monotherapy. Clinicians now have an arsenal of sixteen antiretroviral drugs to choose from along with a variety of adjunctive and supportive medications. However, increases in therapeutic options have come at the price of regimens that are increasingly complex. Food restrictions, drug interactions, scheduling, adherence and overlapping side effects have become important factors to consider when constructing an antiretroviral regimen. Keeping abreast of this information can be overwhelming for the clinician. This chapter will outline a number of practical considerations in the construction of an antiretroviral regimen.

SCHEDULING

Food Restrictions and Spacing of Medications

Scheduling medication intake around meals remains one of the most common complaints of HIV-infected patients. SpeciÞc food restrictions, whether they be with meals or on an empty stomach, have a large impact on quality of life. Food may dramatically affect the systemic availability of several drugs commonly used in HIV-infected patients. Nonadherence with food restrictions or requirements can lead to suboptimal or excessive plasma concentrations; reduced efÞcacy or increased toxicity may result. Most nucleoside reverse transcriptase inhibitors (NRTIs) can be taken without regard to food. However, didanosine tablets and sachets are formulated with a buffer that requires administration on an empty stomach because a low gastric pH leads to degradation of the drug (1). Although not buffered, didanosine enteric coated capsules also must be given on an empty stomach since administration with food decreases the Cmax and AUC of the drug by 46% and 19%, respectively (2). Bioavailability of the other nucleosides is not signiÞcantly affected by food, although peak absorption may be delayed. For example, a high-fat (3) or high-protein (4) meal slows the rate of absorption of zidovudine and may reduce its maximum concentration; however, the extent of zidovudine absorption is unaltered, making this interaction clinically inconsequential.

Several protease inhibitor regimens are particularly problematic in terms of food effects. When used alone, indinavir must be given on an empty stomach or with a light meal containing less than 2 gm of fat (5). When combined with ritonavir, indinavir can be administered without regard to food (6). Conversely, the bioavailability of both formulations of saquinavir (Invirase¨ and F ortovase¨) is impro ved with high-calorie, high-fat foods, and these drugs must be given within two hours of a full meal. Both Lopinavir-ritonavir (Kaletra¨) and nelÞna vir also require administration with food for optimal absorption. While ritonavir capsule absorption is only somewhat increased in the presence of food, administration with a meal is recommended to decrease its gastrointestinal side effects (7).

In general, food does not markedly affect the systemic availability of non-nucleoside reverse transcriptase inhibitors (8). Neither nevirapine nor delavirdine plasma concentrations are appreciably altered by food. Antacids, however, may impair delavirdine absorption and the two medications should be separated by at least one hour. Efavirenz plasma concentrations are increased approximately 50% (11Ð126%) when administered after a high fat meal (1070 Kcal; 82 g of fat) (9). Because enhanced efavirenz exposure may predispose patients to increased central nervous system side effects, efavirenz should not

Stephen C. Piscitelli, Ph.D.: **AfÞliation ???**
Scott R. Penzak, Ph.D.: **AfÞliation ???**
Charles Flexner, MD: **AfÞliation ???**

TABLE 36.1. *Food-drug interactions with HIV-related medications*

Drug	Food Effect	Recommendation
Atovaquone	High-fat meal may increase bioavailability up to 3-fold	Administer with food
Didanosine (tabs)	Administration with food results in 55% decrease in AUC	Administer on empty stomach at least 30 min before a meal
Didanosine (caps)	Administration with food results in 20–25% decrease in AUC	Administer on empty stomach at least 30 min before a meal
Ganciclovir (caps)	High-fat meal results in 22% increase in AUC	Administer with food
Indinavir	High-fat caloric meal results in 77% decrease in AUC	Administer on empty stomach or with low-fat, light meal
Itraconazole (caps)	Signi cant increase in bioavailability when taken with a full meal	Administer with food
Itraconazole (solution)	Maximal absorption when taken under fasting conditions	Recommended without food if possible
Nel navir	AUC 2- to 3-fold higher when given with food	Administer with meal or light snack
Ritonavir	Food results in 15% increase in AUC	Recommended to be taken with meals
Saquinavir	Marked increase in AUC following high-fat meal	Administer within 2 hr after a full meal
Saquinavir	Grapefruit juice increases AUC by 50–200%	Use caution or avoid coadministration
Efavirenz	High-fat meal results in 50% increase in AUC	Avoid taking after high-fat meals
Amprenavir	High-fat meal results in 21% decrease in AUC	Avoid taking after high-fat meals
Lopinavir-r (caps)	Moderate fat meal results in 48% increase in AUC	Administer with food
Lopinavir-r (solution)	Moderate fat meal results in 80% increase in AUC	Administer with food
Tenofovir DF	Food enhances bioavailability from 27% to 40%	Administer with food

AUC, area under the concentration-time curve.

be taken along withÑ or immediately after, a high fat meal. In summary, a variety of HIV-related medications have speciÞc food restrictions stemming from the possibility of increased or decreased absorption from the gastrointestinal tract; these restrictions are summarized in Table 36.1. A review of food restrictions should be an important part of patient counseling for a new or modiÞed drug regimen.

Certain antiretrovirals cannot be taken concomitantly and require separation. For example, the antacids in didanosine tablets and sachets may decrease the absorption of indinavir and delavirdine (10). Thus, a minimum 30-minute separation is required between didanosine tablets or sachets and indinavir; one hour separation is required for delavirdine. In the case of indinavir and didanosine tablets (or sachets), both agents are generally taken on an empty stomach and the patient may have to wait several hours before a full meal can be eaten. Such regimens are particularly difÞcult for patients to take and may result in nonadherence with the regimen. Didanosine is currently FDA-approved for once-daily dosing, and this schedule may alleviate the problem. The new enteric coated formulation of didanosine does not alter indinavirÕs absorption and can be given concomitantly.

Concentrations of some protease inhibitors in dual combination may differ depending on whether they are given concomitantly or in a staggered fashion. A study in healthy volunteers evaluated single doses of dual combination of protease inhibitors (saquinavir, ritonavir, or nelÞnavir) given either simultaneously or when separated by four hours. The AUC of saquinavir was markedly lower when given four hours before ritonavir or nelÞnavir, as compared to simultaneous dosing. NelÞnavir and ritonavir exposure did not appear to be affected by separation (11). As such, the simultaneous administration of protease inhibitor combinations is currently being exploited to enhance the pharmacokinetic proÞle of one of the coadministered agents (usually the non-ritonavir protease inhibitor).

A number of dual protease inhibitor combinations are being evaluated for once daily dosing (12,13) Such regimens may be particularly useful in selected populations such as prison inmates, patients enrolled in methadone maintenance programs, and those requiring directly observed therapy. Although these regimens would be more convenient, they might also require perfect adherence, since protease inhibitor concentrations at the end of the 24-hour dosing interval may be near the IC50 value for the patientÕs virus. Therefore, a missed dose would result in suboptimal plasma concentrations for a prolonged period, leading to increased risk of recurrent viral replication and drug resistance. Furthermore, because most protease inhibitors exhibit large inter-patient pharmacokinetic variability, a small proportion of patients may

present with inadequate plasma concentrations for durable viral suppression despite optimal adherence (5).

DRUG INTERACTIONS

The management of drug interactions in HIV-infected patients has signiÞcantly changed in the past few years. When protease inhibitors and non-nucleoside reverse transcriptase inhibitors became available, interactions were a concern that had to be addressed.

A number of medications used in HIV-infected patients can produce adverse drug interactions (14). A review of product information for the 16 available antiretrovirals reveals over 200 potential drug interactions (2,9,10, 15Ð27). Some common antiretroviral drug interactions are shown in Table 36.2. Although many of these interactions may be minor in nature, some are potentially serious, leading to severe toxicity or treatment failure. Fortunately, most interactions can be easily recognized, prevented, and corrected. In addition, some of the drugs that caused life-threatening interactions have been removed from the U.S. market. Minor alterations in scheduling or selection of an appropriate alternative are usually all that is required to avoid a potential interaction. On the other hand, some interactions increase the systemic exposure of antiretroviral medications and are therapeutically beneÞcial.

Drug interactions can generally be classiÞed as pharmacokinetic, that is, affecting drug concentrations, or pharmacodynamic, that is, affecting drug activity. Pharmacokinetic interactions involve changes in absorption, distribution, metabolism, or excretion, whereas pharmacodynamic interactions may involve additive, synergistic, or antagonistic effects.

TABLE 36.2. *Clinically signiÞcant drug interactions in HIV-infected patients*

Affected Drug	Interacting Drug(s)	Effect	Recommendations
APV IDV RTV NFV SQV	Rifampin	Protease inhibitor AUC decreased by 70–92%	Avoid concomitant use
NFV IDV RTV NFV SQV LPV/RTV	Phenytoin Carbamazepine	Protease inhibitor concentrations potentially decreased; phenytoin and carbamazepine concentrations potentially increased or decreased	Monitor anticonvulsant levels; consider measurement of protease inhibitor levels
APV IDV SQV LPV/RTV	Nevirapine Efavirenz	Protease inhibitor AUC decreased by 28–62%	Use with ritonavir or increase dose
Methadone	Efavirenz Nevirapine	Methadone concentrations decreased	Monitor for symptoms of opiate withdrawal and adjust dose if necessary
	Ritonavir	Methadone concentrations possibly decreased	Monitor for symptoms of opiate withdrawal and adjust dose if necessary
	Nel navir	Methadone concentrations possibly decreased	Monitor for symptoms of opiate withdrawaland adjust dose if necessary
Rifabutin	APV IDV NFV	Rifabutin AUC increased two to three-fold	Decrease dose to 150 mg/day, increase IDV dose to 1000 mg tid
Atorvastatin	RTV/SQV LPV/RTV	Atorvastatin AUC increased four to ve-fold	Use low dose and increase with caution
Simvastatin	RTV/SQV	Simvastatin AUC increased 32-fold	Avoid concomitant use
Pravastatin	RTV/SQV	Minimal change in pravastatin AUC	No dose adjustment necessary
Sildena l	IDV SQV RTV	Sildena l AUC increased 2 to 11-fold	Use 25 mg dose, with ritonavir do not repeat for 48 hours
MDMA (Ecstasy)	RTV	Potential Increase in MDMA concentrations	Avoid concomitant use
GHB (hydroxy butyrate)	RTV RTV/SQV	Potential increase in GHB concentrations	Avoid concomitant use

AUC, area under the concentration-time curve; APV, amprenavir; IDV, indinavir, NFV, nel navir; RTV, ritonavir, SQV, saquinavir; LPV, lopinavir.

Pharmacokinetic Interactions

Altered Drug Absorption

Impairment of drug absorption can lead to a marked reduction in the bioavailability of certain agents. One of the most common interactions affecting drug absorption is chelation, the binding of drugs to substances in the gastrointestinal tract. The concomitant administration of a ßuoroquinolone antibiotic with a di- or trivalent cation such as calcium, magnesium, aluminum, or iron results in a greater than 90% decrease in the area under the concentration-time curve (AUC) of the ßuoroquinolone, possibly leading to therapeutic failure (28Ð30). Didanosine formulations with a calcium and magnesium buffer (tablets) or citrate-phosphate buffer (sachet) can alter ßuoroquinolone disposition. Concomitant administration of didanosine with ciproßoxacin has been shown to decrease the ciproßoxacin AUC from 15.5 to 0.26 μg/hr/ml (31). A similar interaction would be expected with other products such as antacids, sucralfate, or iron preparations. Administration of these agents should be separated from the ßuoroquinolone by at least two hours, and the ßuoroquinolone should be administered Þrst, followed by the cation, to ensure adequate absorption. The newer enteric coated formulation of didanosine does not alter ciproßoxacin pharmacokinetics and can be given concomitantly (2).

A change in gastric pH may affect the absorption of azole antifungals such as ketoconazole and itraconazole. An acidic environment is required for absorption of ketoconazole; thus its administration should be avoided with concomitant histamine-2 antagonists, proton pump inhibitors, and antacids (32,33). Administration of ketoconazole with sodium bicarbonate and cimetidine resulted in therapeutic failure in a patient with cryptococcal infection (34). If clinically appropriate, ßuconazole may be an acceptable alternative in patients requiring agents that raise gastric pH, or in those with achlorhydria; this is because ßuconazole absorption is not dependent on gastric pH (35). For itraconazole capsules, the presence of food is the most important determinant of drug absorption, while low gastric pH is also beneÞcial. If itraconazole capsules cannot be coadministered with food, an acidic beverage such as Coca-Cola¨ may be given to improve absorption (36). Counterintuitively, acidic citrus juices such as orange juice and grapefruit juice do not enhance the absorption of itraconazole capsules (37,38). The oral solution of itraconazole is best absorbed on an empty stomach (39).

Altered Drug Metabolism

Intestinal Metabolism and P-Glycoprotein

The cytochrome P-450 (CYP) enzyme system consists of at least 12 families of enzymes common to all mammals, and represents the major enzyme system involved in drug metabolism (40). In humans, the CYP1, CYP2, and CYP3 families are primarily responsible for drug metabolism, with the CYP3A subfamily involved in the metabolism of the largest number of drugs including most available protease inhibitors. CYP-mediated metabolism takes place primarily in the liver, although CYP enzymes are also present in other sites including enterocytes in the intestinal wall (40). Thus, inhibitors of CYP3A4 may alter drug absorption or hepatic metabolism. The 20-fold increase in plasma concentrations of saquinavir produced by ritonavir is likely caused by inhibition of CYP3A4 at both sites (41). Grapefruit juice contains various substances that inhibit CYP3A4- mediated metabolism in the gut wall, mainly by selective down regulation of the CYP3A4 protein (40). In quantities normally consumed by the public (< 6 glasses daily), CYP3A4 inhibition by grapefruit juice occurs solely at the intestinal level; larger quantities and highly concentrated preparations of grapefruit juice may also inhibit hepatic CYP3A4 (42). The area under the curve for saquinavir is increased by 1.5 to 2.5-fold during concomitant administration of grapefruit juice (19). Conversely, grapefruit juice does not appreciably alter the pharmacokinetics of indinavir (43,44). As such, grapefruit juice cannot be relied upon to increase the plasma concentrations of protease inhibitors because the amounts of P450 inhibitors in the juice vary widely between brands and are affected by factors such as how much and how often the juice is consumed (42).

P-glycoproteins (P-gp) is the product of the *mdr1* gene Þrst described as a mediator of resistance to cancer chemotherapy. Enterocytes in the intestinal mucosa are a major site for expression of P-gp, one of several membrane-bound proteins that increase efßux of drugs from cells (45). P-gp appears to contribute to the low bioavailability of some drugs, including certain PIs. P-gp in the brush border cells of the intestine can pump drug back into the gastrointestinal lumen, decreasing absorption. In the liver, P-gp pumps drug into bile; it is also present in the blood-brain barrier, where it can limit the uptake of drugs into the central nervous system. Lastly, P-gp is also expressed in hematopoietic progenitor cells, lymphocytes, and macrophages, where it may inßuence intracellular drug exposure and/or immunologic and virologic response to antiretroviral treatment (46).

Several protease inhibitors are substrates for and inhibitors of P-gp. In theory, inhibiting P-gp could be used to increase protease inhibitor concentrations in target sites such as the CNS (47). However, P-gp expression has a profound negative impact on HIV replication. In two separate sets of experiments, cells expressing P-gp produced at least 40- to 70-fold less HIV, than control cells. This was thought to be primarily due to inhibition of HIV entry and/or membrane fusion (48,49). Recently, patients with low P-gp expression (TT genotype) were found to have a greater rise in CD4+ cells compared to patients with high P-gp expression (CC genotype) six months after

beginning antiretroviral treatment; this occurred despite lower plasma antiretroviral concentrations among patients with the TT genotype (50). The clinical implications of these Þndings highlight a dilemma regarding how (or whether) P-gp should be altered in HIV-infected patients, particularly since the inßuence of P-gp expression on drug exposure, HIV replication, immune recovery, and the interrelationship between these factors, is not fully understood.

In the intestine and liver, cytochrome P450 enzymes and P-glycoprotein can present a barrier to oral drug absorption and contribute to interactions between medications. The overlap of tissue distribution and substrate speciÞcity of CYP3A4 and P-glycoprotein complicates deÞnition of the speciÞc mechanisms of some drug interactions. Many drugs that are modulators of P-glycoprotein are also inhibitors of CYP3A4 (51). The effect of these two pathways on antiretroviral drug concentrations remains a fertile area for additional research.

Enzyme Induction

CYP enzyme inducers increase the rate of hepatic metabolism, usually through increased transcription of mRNA, and decrease serum concentrations of other drugs metabolized by the same hepatic isoenzyme. Rifampin and rifabutin are classic examples of enzyme inducers that decrease plasma concentrations of coadministered CYP substrates. Both drugs can decrease concentrations of protease inhibitors. The Centers for Disease Control and Prevention have issued guidelines for concomitant use of rifampin or rifabutin with HIV protease inhibitors in patients with tuberculosis (52). Rifampin should be avoided with all single protease inhibitors but may be used with caution in patients receiving saquinavir plus ritonavir (52). Patients receiving indinavir or nelÞnavir should receive a reduced dose of rifabutin and a slightly increased PI dose (52,53). With regard to non-nucleoside reverse transcriptase inhibitors, rifampin should be avoided in patients receiving nevirapine and delavirdine (52). Rifampin may, however, be coadministered with efavirenz although some clinicians advocate giving a higher efavirenz dose in patients receiving this combination (52,54). Rifabutin may be taken along with nevirapine without dosage adjustment of either agent; however, its use with delavirdine is not recommended. Higher rifabutin doses are necessary when the drug is given concurrently with efavirenz, however efavirenz dose-adjustment is unnecessary (9,52,54).

Nevirapine is a mild to moderate hepatic enzyme inducer, and decreases the AUC of saquinavir and indinavir by 27% and 28%, respectively, but has a minimal effect on ritonavir and nelÞnavir (55,56). It is currently recommended that the indinavir dose be increased to 1,000 mg q8h with nevirapine, although clinical studies have not veriÞed the effect of this combination on surrogate markers or clinical endpoints. Efavirenz is a mixed inducer/inhibitor that decreases concentrations of amprenavir, saquinavir, and indinavir, necessitating increased doses of these drugs or the addition of ritonavir (57).

Ritonavir and nelÞnavir are also moderate enzyme inducers, and can increase hepatic glucuronidation as well as CYP activity. The AUC of the oral contraceptive ethinyl estradiol is decreased by approximately 40% with these agents (and also with the lopinavir-ritonavir combination product (Kaletra¨)), necessitating an alternative form of birth control (25,58). Ritonavir is also an inducer of CYP1A2 which is involved in the metabolism of theophylline and the antipsychotic medications clozapine and olanzapine (59,60). Concomitant administration of ritonavir was noted to reduce theophylline and olanzapine AUCs by 43% and 53%, respectively (59,60). Patients receiving these drugs in combination with ritonavir should be warned to watch for symptoms of reduced therapeutic effects with theophylline and olanzapine; theophylline levels should be monitored.

Enzyme Inhibition

There are a number of inhibitors of CYP that decrease the rate of hepatic metabolism and increase plasma concentrations of other drugs metabolized by the same isoenzyme. HIV protease inhibitors can be both CYP inhibitors and substrates, increasing concentrations of some metabolized drugs and having their own concentrations increased by other CYP inhibitors. Most of the currently available protease inhibitors are primarily metabolized by CYP3A4 (5). These agents differ in the number and magnitude of potential drug interactions. Ritonavir is associated with the greatest number of drug interactions, while saquinavir is the weakest enzyme inhibitor and, has less propensity to alter concentrations of other drugs. In general, the combination of lopinavir and ritonavir has a similar drug interaction proÞle to that of ritonavir (25). Amprenavir, nelÞnavir, and indinavir inhibit CYP3A4 metabolism to a lesser extent than ritonavir. For example, all three drugs increase the rifabutin AUC approximately two-fold, necessitating a 50% reduction in the rifabutin dose (14). Ritonavir increases the rifabutin AUC by four-fold, necessitating a marked reduction in the rifabutin dose when these agents are coadministered (61,62). Nonetheless, although dosage adjustments are necessary, rifabutin can usually be safely coadministered with protease inhibitors in patients requiring the drug for anti-tuberculosis therapy.

In patients in whom MAC prophylaxis is required, clarithromycin or azithromycin are commonly prescribed. The clarithromycin AUC is increased 77% with ritonavir and 53% with indinavir, although a dosage adjustment is unnecessary in patients with normal renal function (63). Azithromycin is primarily excreted by the biliary route and does not interact with inhibitors of CYP (64).

Deleterious Drug Interactions

Historically, the most serious potential interactions with CYP inhibitors involved concomitant administration with certain metabolized drugs such as terfenadine and cisapride, antiarrhythmics, and ergot alkaloids. Terfenadine and cisapride have been removed from the U.S. market and a number of non-sedating antihistamines without drug interaction potential are now available. In the case of cisapride and terfenadine, these combinations led to cardiotoxicity, with the potential for life-threatening arrhythmias (65). Administration of protease inhibitors with some benzodiazepine sedative-hypnotics can result in exaggerated side effects such as oversedation, but serious adverse effects such as respiratory depression are unlikely.

BeneÞcial Drug Interactions

Drug interactions were initially viewed as a complication to be avoided in HIV-infected patients. The concept of using two protease inhibitors concomitantly to increase plasma concentrations or improve convenience was Þrst recognized with the combination of saquinavir and ritonavir. Simultaneous administration of two protease inhibitors takes advantage of beneÞcial pharmacokinetic interactions, and may circumvent many of the drugsÕ undesirable pharmacologic properties (6). In addition, dual protease inhibitors decrease inter-patient variability, making drug concentrations more predictable. Dual protease inhibitor regimens have now been widely prescribed and are included in published treatment guidelines despite not being FDA approved. A number of potentially beneÞcial metabolic drug interactions exist for combinations of two HIV protease inhibitors. One drug is used to inhibit the metabolism of the second agent, producing increased bioavailability, decreased clearance, or both. Two-way interactions also exist, in which the pharmacokinetic proÞle of each drug is enhanced.

Ritonavir-Saquinavir

Administered as a single protease inhibitor, saquinavir possesses a number of disadvantages including poor bioavailability, three times daily dosing, and a pill burden of 18 capsules per day. However, when combined with even small doses of ritonavir, there is a marked increase in saquinavir bioavailability and a decrease in clearance, allowing twice daily dosing and a decreased dose from 1800 mg T.I.D. to 400 mg B.I.D. In a single-dose, crossover study in healthy volunteers, ritonavir increased the saquinavir AUC by 50- to 132-fold, and increased the saquinavir Cmax by 23- to 35-fold (66). Since ritonavir is a P450 inducer and undergoes autoinduction during the Þrst 10Ð14 days of therapy (67), steady-state concentrations of saquinavir should be lower when these two drugs are combined. Indeed, multiple-dose pharmacokinetic interaction studies found that the steady-state saquinavir AUC was increased only 20- to 30-fold (68). Nonetheless, this increase in saquinavir exposure remains sufÞcient to be pharmacologically beneÞcial.

The effect of ritonavir dose on saquinavir plasma levels was evaluated in 120 patients receiving various saquinavir/ritonavir combinations (69). Data from two dose-ranging trials of saquinavir/ritonavir given either twice daily or once daily were included in the analysis. A wide range of saquinavir doses (400Ð1,800 mg) and ritonavir doses (100Ð400 mg) were evaluated. The investigators used multivariate linear and nonlinear regression to correlate steady-state saquinavir pharmacokinetic parameters (Cmin, Cmax, and half-life) with saquinavir and ritonavir doses. This model showed a strong effect of ritonavir on saquinavir Cmax and Cmin; however, these parameters were correlated only with saquinavir dosage, and the increase in saquinavir concentrations was similar for ritonavir dosages over the range of 100Ð400 mg twice daily. In this analysis there was not a dose-dependent effect of ritonavir on saquinavir concentrations.

The poor oral bioavailability of saquinavir (1Ð12%, depending on formulation and conditions) likely reßects extensive Þrst-pass metabolism rather than poor absorption. The increase in saquinavir concentrations with ritonavir is the result of improved bioavailability, perhaps to as much as 100%, with little effect on post-absorptive systemic clearance. The large magnitude of ritonavirÕs effect on the oral bioavailability of saquinavir may be accounted for by ritonavirÕs dual inhibitory effects on intestinal CYP3A4 and P-gp, which are involved in the presystemic metabolism and transport of saquinavir, respectively (70).

Although the doses of 400 mg ritonavir/400 mg saquinavir twice daily have been commonly used for a number of years, additional dosing regimens are under evaluation that employ a lower dose of ritonavir to improve tolerability and reduce effects on plasma lipids. One single-dose study in healthy volunteers found that combining 200 mg of ritonavir with 600 mg saquinavir increased the saquinavir AUC by an average of 74-fold, an effect similar to that seen with 400 mg ritonavir plus 400 mg saquinavir (66). Clinically, doses of 1000 mg saquinavir/ 100 mg ritonavir B.I.D. are being evaluated as well as 1600 mg saquinavir/100 mg ritonavir administered once daily (12,13).

RitonavirÑIndinavir

When used as a sole protease inhibitor, indinavir possesses a number of limitations including an every-eight-hour dosing regimen, food restrictions, hydration requirements, and large inter-individual pharmacokinetic variability (5). The combination of indinavir, even with small doses of ritonavir, alleviates many of these disadvantages.

In a steady-state pharmacokinetic interaction study in healthy volunteers involving 14 days of ritonavir treatment, the combination of 200 or 400 mg ritonavir with 400 or 600 mg indinavir increased the indinavir AUC by three- to six-fold compared to indinavir 800 mg alone (71). This mechanism primarily involves inhibition of hepatic CYP 3A4 with reduced Þrst-pass metabolism making a minor contribution. Ritonavir increased the indinavir Cmax up to two-fold, and increased the indinavir concentration eight hours after dosing by 11- to 33-fold (71). Decreased ritonavir dose and increased indinavir dose were examined in a separate study. In healthy volunteers administered ritonavir for 14 days, the 24-hour indinavir AUC with a 100 mg B.I.D. ritonavir/800 mg B.I.D. indinavir regimen was four-fold higher than with 800 mg q8h indinavir alone. In the same study, the 24-hour AUC of indinavir with a 400/400 B.I.D. ritonavir-indinavir regimen was 40% lower than with the 100/800 regimen and 55% lower than with a 200/800 regimen (72). However, the mean 12-hour trough concentrations of the 400/400 regimen and the 100/800 regimen were nearly the same.

In two studies, co-administration of ritonavir and indinavir abolished the effect of food on indinavir bioavailability. A high-fat meal reduced the bioavailability of oral indinavir by up to 85% (72). Doses of 100, 200, or 400 mg of ritonavir B.I.D. reversed the effect of a high- or low-fat meal on indinavir pharmacokinetics, as compared to 800 mg indinavir given in the fasted state (72). In theory, ritonavir could enhance indinavir oral bioavailability in the presence of food through inhibition of intestinal cytochrome P450 or drug transporters such as P-glycoprotein. However, the respective roles of P-gp and intestinal CYP3A4 in limiting the oral availability of indinavir appear to be minimal (73), suggesting that inhibition if these enterocyte proteins by ritonavir does not signiÞcantly alter the Þrst-pass metabolism of indinavir (6).

There is some concern that the high peak concentrations achieved with indinavirÐritonavir combinations may lead to an increased risk of nephrolithiasis. In patients taking indinavir 800 mg q8h, a higher AUC and Cmax were associated with increased risk of IDV nephrotoxicity in one study (74). Increasing indinavir Cmax and Cmin were associated with an increase in nephrolithiasis in patients taking indinavir/ritonavir B.I.D. combinations of 400/100, 400/400, 600/100, or 800/100 mg; these regimens were associated with short-term nephrotoxicity risks of 0, 2, 6, or 10%, respectively (75). In 17 Thai patients receiving indinavir 800 mg plus ritonavir 100 mg every 12 hours, a 12-hour indinavir AUC > 60 mg*hr/L and Cmax > 13 mg/L were associated with nephrotoxicity (76). Indinavir/ritonavir regimens of 400/400 mg B.I.D. were not associated with an increased incidence of nephrolithiasis compared to indinavir 800 mg q8h without ritonavir, although tolerability may be an issue with the higher ritonavir dose (77). Of note, one study found that indinavir Cmax > 7 mg/L was associated with increased CD4+ cell response, perhaps due to inhibition of CD4+ cell apoptosis with higher indinavir Cmax; further study is warranted to conÞrm this theory (78).

The inßuence of efavirenz on indinavir disposition was analyzed under steady-state conditions in 18 healthy male volunteers receiving indinavir 800 mg plus ritonavir 100 mg twice daily (79). Efavirenz co-administration resulted in a 19% reduction in the indinavir AUC and a 48% reduction in Cmin. Based on these results, ritonavir should be administered at doses 200 mg twice daily when used in combination with indinavir and efavirenz; this is particularly true in treatment-experienced patients who no longer harbor wild-type HIV virus.

LopinavirÐRitonavir

Lopinavir-ritonavir (Kaletra¨) represents the Þrst use of ritonavir in a co-formulated product to increase concentrations of a second protease inhibitor. Each dose of Kaletra¨ contains 400 mg of lopinavir along with 100 mg of ritonavir (133/33 mg capsules × 3 caps) which is given twice daily. Lopinavir is a highly active protease inhibitor but its bioavailability is low and its clearance rapid when given alone. However, in combination with low doses of ritonavir, lopinavirÕs AUC is increased by greater than 100-fold (80) due to inhibition of the drugÕs CYP3A4-mediated metabolism in the liver and gastrointestinal tract. RitonavirÕs effect on the lopinavir AUC was several-fold greater than ritonavirÕs effect on the saquinavir AUC (6).

This beneÞcial pharmacokinetic interaction was conÞrmed in human volunteers; when dosed at 12-hour intervals (q12h) with ritonavir, mean trough concentrations of lopinavir were approximately 30-fold higher than the *in vitro* IC50 for HIV (81). In 101 HIV-infected patients taking lopinavir 200 or 400 mg B.I.D. with ritonavir 100 or 200 mg B.I.D. plus two nucleoside analogs for 48 weeks, HIV viral load was suppressed to < 400 copies/mL in 93Ð100% of patients and to < 50 copies/mL in 83Ð86% (82).

The addition of efavirenz to lopinavirÐritonavir results in a decrease of 40% in the lopinavir AUC (83). Although concentrations are still relatively high despite this reduction, the manufacturer suggests the dose be increased to 533/133 (four capsules) mg B.I.D. when combined with efavirenz or nevirapine in order to offset the enzyme induction effects of the NNRTIs, especially if the patient is treatment-experienced and resistance is suspected (25).

AmprenavirÐRitonavir

Amprenavir is a twice daily protease inhibitor but has the disadvantage of an oral formulation requiring 16 capsules per day. The combination of amprenavir with varying ritonavir doses has been evaluated in a number of pharmacokinetic studies in patients and healthy volunteers. Administration of amprenavir with low-dose

ritonavir increases the amprenavir AUC two- to three-fold and increases the trough concentration by approximately 5-fold (84). In one study, patients received amprenavir 1200 mg B.I.D. with either 200 mg or 500 mg B.I.D. of ritonavir. The patients receiving the higher ritonavir dose did not achieve higher amprenavir concentrations (84). Thus increasing the ritonavir above 200 mg likely will only be associated with more adverse effects and will not provide higher plasma concentrations.

A number of amprenavirÐritonavir regimens have been proposed based on pharmacokinetic studies in volunteers and simulation of plasma concentrations (85). Although clinical data are lacking, many clinicians employ a 600/100 mg amprenavir/ritonavir B.I.D. regimen, although the amprenavir dose may need to be increased in treatment-experienced patients with suspected resistance. However, in patients also receiving nevirapine or efavirenz, 100 mg B.I.D. of ritonavir does not appear to prevent a decrease in amprenavir concentrations from the enzyme inducing properties of these NNRTIs. In a small study, patients receiving 450 mg of amprenavir with 200 mg B.I.D. of ritonavir were switched to a 600 /100 mg regimen and amprenavir trough values were decreased by 80% (86). A separate study demonstrated no decrease in amprenavir Cmin when efavirenz was added to an amprenavir/ritonavir regimen containing 200 mg B.I.D. of ritonavir (84). Thus, there appears to be a threshold dose of at least 200 mg of ritonavir that is required to prevent NNRTI-induced drug interactions.

Once daily dosing regimens of 1200 mg amprenavir/ 200 mg ritonavir have been recently evaluated in HIV-infected patients. At the end of a 24-hour dosing interval, amprenavir concentrations were approximately 3-fold higher with this regimen than with amprenavir1200 mg B.I.D. alone (87).

RitonavirÐNelÞnavir

Originally marketed as a T.I.D. regimen, nelÞnavir was studied in combination with ritonavir to reduce its dosing frequency, and the potential for once-a-day dosing. While ritonavir does increase nelÞnavir concentrations modestly, this combination is generally not widely used because of poor tolerability. Dose limiting diarrhea or gastrointestinal adverse effects make this regimen less attractive than other dual protease inhibitor combinations.

A single-dose drug interaction study in healthy volunteers showed that ritonavir increased the nelÞnavir AUC by 152%, while nelÞnavir increased ritonavirÕs AUC by only 9% (22). A steady-state pharmacokinetic interaction study in HIV-infected volunteers evaluated the combination of 400 mg ritonavir B.I.D. with 500 or 750 mg nelÞnavir B.I.D. After Þve weeks of dosing, ritonavir use was associated with a 162% increase in the 500 mg nelÞnavir 24-hour AUC (dose-normalized), and a 62% increase in the 750 mg AUC, as compared to historical controls taking only nelÞnavir 750 mg T.I.D. (88). At the same time, the change in ritonavirÕs dose-normalized 24-hour AUC was +13% with nelÞnavir 500 mg, and Ð13% with the 750 mg regimen.

This pharmacokinetic interaction is more complicated than others, because both drugs are CYP450 inducers as well as inhibitors (6). The fact that nelÞnavirÕs AUC did not increase signiÞcantly when the dose was increased from 500 to 750 mg B.I.D. may reßect increased induction with the higher nelÞnavir dose. In addition, there was a trend for nelÞnavir to reduce ritonavirÕs trough concentrations at the higher 750 mg nelÞnavir dose (88). This may have decreased the magnitude of ritonavirÕs beneÞcial impact on nelÞnavir pharmacokinetics.

NelÞnavir is the only HIV protease inhibitor known to produce an active metabolite, the hydroxy-butylamide M8 (AG1402), which is the major metabolite of nelÞnavir in humans and has equipotent anti-HIV activity *in vitro* (89). Ritonavir had a more signiÞcant beneÞcial impact on the pharmacokinetics of M8 than on nelÞnavir itself. After Þve weeks of dosing, ritonavir use was associated with a 430% increase in the 500 mg M8 24-hour AUC, and a 370% increase in the 750 mg M8 AUC, as compared to historical controls taking nelÞnavir 750 mg T.I.D. alone (6). This is likely due to impaired CYP-mediated metabolism of M8 by ritonavir, as opposed to increased production of the metabolite.

A recent study evaluated the effect of low-dose ritonavir (100 or 200 mg B.I.D.) on nelÞnavir pharmacokinetics in healthy volunteers (90). Overall, the AUC of nelÞnavir was increased by approximately 30% in both ritonavir dosing groups. The morning steady-state pre-dose concentrations of nelÞnavir were increased substantially in a dose-dependent fashion with a 45% and 90% increase in the 100-mg and 200-mg arms, respectively. The half-life of nelÞnavir was not altered by ritonavir, and the active nelÞnavir M8 metabolite was increased by approximately 75% for Cmax and 90% for AUC. The magnitude of the M8 increase was similar for both ritonavir doses (90).

NelÞnavirÐSaquinavir

In single-dose pharmacokinetic interaction studies, nelÞnavir increased the saquinavir AUC by up to Þve-fold, with no effect of saquinavir on nelÞnavir concentrations (5). However, nelÞnavir is an inducer of CYP450 3A, and at steady-state the magnitude of this interaction was substantially reduced. Combining nelÞnavir 750 mg T.I.D. with 800 mg T.I.D. of the soft-gel formulation of saquinavir produced a saquinavir AUC equivalent to 1200 mg T.I.D. at steady-state (91). This combination was well-tolerated and was highly active against HIV in patients who were also taking two nucleoside analogs (92). However, this combination lacks many of the pharmacologic and clinical beneÞts of other dual protease inhibitor combinations. With a trend to convert all protease inhibitor

regimens to B.I.D., the T.I.D. dosing regimens of nelfinavir and saquinavir appear to be less relevant to clinical practice.

Nelfinavir–Indinavir

Combining nelfinavir with indinavir produced a 50% increase in the indinavir AUC and an 80% increase in the nelfinavir AUC in single-dose studies in healthy volunteers (5). However, when these two drugs were administered to patients in a B.I.D. steady-state regimen, there was little pharmacokinetic enhancement and a disappointing anti-HIV effect, with only 10 of 21 patients suppressing their plasma HIV RNA to <400 copies/mL (the lower limit of quantification) after 32 weeks (93). Presumably hepatic enzyme induction by nelfinavir resulted in reduced concentrations of both drugs, and no real pharmacokinetic benefits.

Indinavir–Saquinavir

This combination was reported to be antagonistic when used to inhibit HIV replication *in vitro* (94). While the clinical relevance of this finding is unknown, this combination has not been pursued further *in vivo*, even though indinavir increased saquinavir concentrations by five-fold in single dose studies (5).

Lopinavir/Ritonavir–Other Protease Inhibitors

Drug interaction studies between lopinavir-ritonavir and other protease inhibitors have generally been conducted in healthy volunteers under non-steady-state conditions (25). Because of these limitations in study design, it is not possible to make definitive dosing recommendations for lopinavir–ritonavir in combination with other protease inhibitors. Nonetheless, based on the data that is available, the Kaletra® manufacturer recommends reduced doses of indinavir, saquinavir, and amprenavir when these drugs are coadministered with lopinavir–ritonavir (400 mg/100 mg twice daily) (25). Recent data suggest that plasma concentrations of both amprenavir and lopinavir may be reduced when they are coadministered; however, limitations in study design mandate that these results be confirmed before they are widely accepted (95).

Other Antiretroviral Combinations

Another strategy for increasing concentrations of protease inhibitors through enzyme inhibition includes administration of the CYP3A4 inhibitor delavirdine. Delavirdine significantly increased the AUC of saquinavir by 520%, indinavir by 72%, and nelfinavir by 92% (96,97). Other CYP3A4 inhibitors evaluated for their ability to increase plasma protease inhibitor concentrations include ketoconazole, itraconazole, and erythromycin. Despite encouraging data in healthy subjects, ketoconazole and erythromycin increased saquinavir concentrations only modestly (69% and 99%, respectively) in patients with HIV infection (98). In a separate study in HIV-infected patients, saquinavir concentrations were not appreciably altered by concurrent itraconazole administration (99).

Interactions with Herbal Therapies

Herbal remedies and nutritional supplements are widely used in HIV-infected patients although little attention has been paid to the pharmacokinetic effects of these compounds since they are considered benign. An increasing number of studies have shown that certain alternative therapies may cause drug interactions with agents used in the treatment of HIV infection. In healthy volunteers, St. John's wort decreased the AUC of indinavir by over 50% (100). In a case series of five HIV-infected patients, St. John's wort appeared to increase nevirapine clearance by 35% (101). The mechanism of these interactions is complex and appears to be mediated by both induction of CYP3A4 and P-glycoprotein (102,103). This herb should be avoided in patients taking protease inhibitors and non-nucleoside reverse transcriptase inhibitors, although there currently are no data on whether ritonavir can reverse this interaction.

Garlic supplements are sometimes used by HIV-infected patients because of their touted effects on lowering cholesterol. Raw garlic and garlic supplements inhibit the activity of CYP3A4 in vitro and in animals, and case reports have documented ritonavir-related gastrointestinal toxicity in two people after they ingested uncooked garlic preparations with ritonavir (104). However, a study in healthy volunteers showed that garlic capsules taken B.I.D. for three weeks led to a mean decrease in saquinavir concentrations of approximately 50% probably as a consequence of reduced bioavailibility (105). Even after a 10-day washout period, AUC values returned to only 60–70% of baseline suggesting a prolonged effect. In contrast to the findings with garlic and St. John's wort, silymarin, (milk thistle) did not significantly alter the pharmacokinetics of indinavir in healthy volunteers (106). Other herbs with reported in vitro effects on CYP450-mediated metabolism include ginseng and skullcap although clinical data are lacking (107). Clinicians need to include alternative medicines in their drug histories and consider them when adverse effects or treatment failure appear with no other cause.

Drug-Cytokine Interactions

The effect of cytokines on antiretroviral drug concentrations has received recent attention with the advent of immune-based therapies for HIV infection. Cytokines may

be either synthesized recombinantly and administered as therapy or they may be produced *in vivo*. Pro-inßammatory cytokines including interleukin (IL)Ð6, IL-1, and tumor necrosis factor (TNF)Ðα are released during periods of stress, trauma, or infection. A number of in vitro and clinical studies have shown that IL-6 and TNF-α inhibit cytochrome P450-mediated metabolism. This mechanism is not competitive but is a metabolic interaction at the level of transcription of cytochrome P450 messenger RNA (108).

Several immunodulating agents are being evaluated for the treatment of HIV-infection. One of the best studied is IL-2. Its exogenous administration has been shown to increase CD4 cells, but results in a profound release of pro-inßammatory cytokines that are one likely cause of its problematic side effect proÞle. In HIV-infected patients receiving a Þve-day continuous infusion of IL-2, indinavir clearance signiÞcantly decreased and the area under the curve increased 88% as compared with baseline values before IL-2 adminsitration (109). The short term administration (Þve days) of IL-2 makes this interaction less clinically signiÞcant, although drug-cytokine interactions should be considered as additional investigational agents are used in a more chronic fashion; this rings particularly true since recent data suggest that, in addition to CYP3A4, IL-2 may also down-regulate CYP1A2, CYP2C, and CYP2E1, increasing the likelihood of drug interactions with this cytokine (110).

Altered Excretion

Drug interactions may also be caused by alterations in renal elimination, although in general these interactions are not often clinically signiÞcant for antiretrovirals. This can be a consequence of either inhibition of tubular secretion or impairment of renal function. Probenecid and trimethoprim are inhibitors of renal tubular secretion, which may increase concentrations of some renally cleared drugs. The lamivudine AUC is increased by 44% with concomitant trimethoprim-sulfamethoxazole (111). The acyclovir AUC is increased 40% with concomitant probenecid (112). Recently, the AUC of didanosine, administered in tablet form, was found to be increased by 44% with concurrent administration of the nucleotide reverse transcriptase inhibitor, tenofovir (113). Although the mechanism of this interaction is unknown, it is hypothesized to result from impaired renal excretion of didanosine by tenofovir. Inhibition of renal secretion may be a useful strategy to increase plasma concentrations of antimicrobials such as acyclovir, to increase the likelihood of a successful response or to offset poor oral absorption.

Intracellular Interactions

The nucleoside reverse transcriptase inhibitors are prodrugs that must undergo phosphorylation intracellularly to their active forms. While these drugs are not generally affected by CYP450 interactions, there may be competition for intracellular activation pathways that result in clinically relevant drug interactions. Ribavirin decreases the phosphorylation of zidovudine and stavudine in vitro, resulting in decreased concentrations of the active compound (114,115). AIDS patients with hepatitis C may be treated with Rebetron¨ , a ribavirin-interferon combination formulation that may result in decreased efÞcacy of zidovudine. Similarly, zidovudine may impair the intracellular phosphorylation of stavudine (115), and this combination is associated with unfavorable clinical outcomes as compared with other regimens containing two nucleoside reverse transcriptase inhibitors (116). Lamivudine inhibits zalcitabine phosphorylation and this combination should be avoided (117).

Penetration of HIV protease inhibitors into CD4+ cells and macrophages may be regulated in-part by P-gp. As such, compounds that inhibit P-gp may modulate intracellular protease inhibitor concentrations and enhance their antiretroviral activity. Ritonavir, a potent P-gp inhibitor, was recently shown to signiÞcantly raise intracellular amprenavir concentrations above those achieved when amprenavir was administered by itself (118). Whether higher intracellular protease inhibitor concentrations are associated with improved virologic and/or immunologic response is unknown and requires further study.

Recognizing and Circumventing Drug Interactions

Strategies for recognizing and avoiding drug interactions are shown in Table 36.3. A careful review of the patientÕs medication proÞle is essential to monitor for drug interactions. Patients should be asked to disclose all their medications, because they may often seek treatment from more than one health care provider. In addition, a medication history should include both prescription and nonprescription drugs, as well as any herbal, investigational, or alternative therapies. Clinicians should be

TABLE 36.3. *Strategies for recognition and avoidance of drug interactions*

Thorough review of patient medication pro les
Include over-the-counter, herbal therapies, investigational drugs, alternative medications
Counsel patients to discuss with clinician before starting new prescriptions
Recognition of “red ag” drugs with high interaction potential
Protease inhibitors, azole antifungals, NNRTIs, rifampin, etc.
Recognition of drugs with overlapping toxicities
Selection of alternative regimen if possible
Use of supportive medications for treatment of toxicity
Proper staggering and scheduling of medications
Counseling of dietary restrictions with certain medications
Selection of agents with fewer drug interactions if clinically appropriate

familiar with those agents most commonly associated with drug interactions. Any patient receiving Ò red ßagÓ drugs such as rifampin, ritonavir, or ketoconazole requires extra attention, and patients should be given a list of drugs that should not be administered concomitantly.

Finally, drug interactions can be avoided by simplifying drug regimens whenever possible. Selection of a therapeutically equivalent agent with fewer drug interactions may be wise. Azithromycin could be substituted for erythromycin or clarithromycin if appropriate for the clinical situation. Similarly, it may be advantageous to replace ritonavir with another protease inhibitor for patients requiring medications with the potential for serious interactions. Care provider and patient need to work together to develop a tolerable regimen that meets therapeutic goals.

OVERLAPPING SIDE EFFECTS

Many of the drugs used in the HIV-infected patient share similar toxicity proÞles. Some common examples are shown in Table 36.4. Although it would be ideal to prescribe drugs with non-overlapping adverse effects, the limited choices for antiretroviral therapy and opportunistic infection treatment often preclude this. In the nucleoside analog class, stavudine, didanosine, and zalcitabine are all associated with the development of peripheral neuropathy. However, a clinical study examining the combination of stavudine and didanosine showed that only two patients developed peripheral neuropathy after one year of therapy (119). Regardless, patients receiving these drugs should be counseled on the signs and symptoms of peripheral neuropathy. Other examples include a ßu-like syndrome with some recombinant cytokines (interferon, interleukin-2) or pancreatitis with didanosine and pentamidine. When the use of these combinations is unavoidable, reduced dosages or supportive medications (i.e. antidiarrheals, antiemetics) should be considered to lessen certain adverse effects.

A common dilemma is trying to identify which drug is actually causing an adverse effect. For example, ritonavir, zidovudine, and didanosine are all associated with nausea and other gastrointestinal side effects. One strategy would involve stopping all medications until the side effects resolve and adding drugs back sequentially as tolerated. Note that this strategy may be unwise for patients taking combination antiretroviral therapy. Alternatively, single drugs could be removed and/or substituted in the regimen to see if the gastrointestinal symptoms resolve. This situation may be more difÞcult in the case of neutropenia, in which the adverse effect could be caused either by medications (Table 36.4) or by disease processes such as *M. avium* infection. For particularly toxic combinations such as zidovudine and ganciclovir, supportive care with granulocyte colony-stimulating factor may be a useful adjunctive therapy to allow continuation of the regimen. Another common adverse effect that crosses multiple drugs and classes is rash. The clinician again must consider treating through the rash or guessing which agent is causing the problem and discontinuing the proposed offending agent. As some rashes can be serious in nature, this involves close monitoring of the patient.

TABLE 36.4. *Common examples of overlapping toxicities of HIV-related medications*

Rash
Abacavir
Amprenavir
Delavirdine
Efavirenz
Nevirapine
Bone Marrow Suppression
Cytotoxic chemotherapy (i.e. doxorubicin, cyclophosphamide, vinblastine, etc.)
Dapsone
Flucytosine
Ganciclovir
Interferon-□
Pentamidine
Sulfadiazine/pyrimethamine
Trimetrexate
Trimethoprim sulfamethoxazole
Peripheral Neuropathy
Didanosine
Isoniazid
Stavudine
Zalcitabine

MEDICATION ADHERENCE

Health care providers generally assume that patients take most or all of their prescribed medication. Numerous studies indicate that adherence to prescribed medication regimens varies greatly, and that few patients take all of their prescribed doses of drug. Imperfect adherence has probably been a medical fact of life since the dawn of civilization.

Using the most accurate means available to assess adherence, studies with single drugs suggest that fewer than 10% of patients take all prescribed doses of medication. Most patients are moderately compliant, taking between 70% and 90% of prescribed doses. About one third of patients take less than 60% of prescribed doses (120,121). These adherence estimates hold true in HIV-infected patients taking combination antiretroviral therapy (122). or treatment for opportunistic infections (123). Adherence assessment of combination regimens is far more complicated because adherence behavior may be different for different agents prescribed at the same time (124).

Even intelligent and highly motivated individuals with medical backgrounds have difÞculty with adherence. A

study in 36 medical students randomized to receive a B.I.D. or T.I.D. placebo for 14 days found that the mean number of doses taken was only 71% of those prescribed (125), a result similar to that seen in AIDS or epilepsy patients. Furthermore, less than 30% of dosing intervals were correct, regardless of assigned regimen. The two most common reasons cited for improper compliance in this study were "hectic schedule" and "irregular routine."

Health care providers cannot with great precision predict which patients will be adherent and which will not. Patient self-report and pill count also provide unreliable and inaccurate information (121,126,127). Socioeconomic factors, racial and ethnic background, and disease state, which seem as if they ought to be related to degree of adherence, generally are not (128). Depression and active substance abuse, however, are frequently recognized as barriers to adherence (129,130). Therefore, care providers need to anticipate noncompliance and whenever possible avert any problems it may cause.

Recent studies suggest that poor adherence may be a major factor in the development of resistance to HIV protease inhibitors (131). Overall adherence rates of less than 80% were associated with a significant increase in the risk of treatment failure (132,133). The risk of treatment failure increased with decreasing adherence rate in patients taking protease inhibitors plus two nucleoside analogs (133). In one study, 78% of patients taking 95% or more of prescribed protease inhibitor doses had undetectable viral loads, while only 20% of those taking less than 80% of prescribed doses had undetectable viral loads (133). Viral suppression dropped off substantially with each decile fall in adherence rate (133). It should be noted that most subjects evaluated in these studies were taking only a single protease inhibitor without ritonavir enhancement, and therefore most were using a T.I.D. regimen. Whether similar stringent adherence requirements would occur with simplified dual protease inhibitor regimens remains to be demonstrated.

A number of interventions can promote better adherence (122). Regimens involving fewer daily doses and fewer agents are easier to take. Once-a-day regimens promote adherence best; B.I.D. regimens are only marginally better than T.I.D. regimens in recently published studies (121,124,125), although these studies were conducted in patients taking only a single medication. An additional consideration is that the consequences of noncompliance are more severe for agents dosed infrequently. For example, if a drug is dosed T.I.D., then the daily therapeutic coverage is reduced by 33% if a single dose is missed; however, if a drug is dosed qd, then daily therapeutic coverage is reduced 100% if a single dose is missed.

Effective pharmacotherapy for AIDS in the future may benefit from incorporation into medical practice of means of improving adherence. Previous studies in patients with epilepsy and hypertension show that adherence can often be improved by modifying the drug regimen, physician practice, and patient behavior. Steps include increasing recognition of the problem, counseling and educating the patient about the importance of good adherence and the dangers of poor adherence, providing environmental cues to habituate the taking of medicines, incorporating medication taking into established routines, monitoring outcomes and providing feedback to reinforce success, minimizing polypharmacy, and using B.I.D. or qd regimens whenever possible.

THERAPEUTIC DRUG MONITORING

Therapeutic Drug Monitoring (TDM) refers to the adjustment of drug doses based on measured plasma concentrations to attain values within a "therapeutic window." While TDM has been used for years for drugs such as cyclosporin and digoxin, the concept has generally not been used for chronic infectious diseases. TDM may be useful in HIV infection since HIV is lifelong and treatment options can rapidly decrease with the development of resistance. A monitoring tool that can assure optimal plasma concentrations would help clinicians in constructing an effective regimen. Some antiretrovirals share many of the characteristics of drugs that require monitoring of plasma levels, including variable intersubject pharmacokinetics, serious consequences if there is a lack of effect or drug toxicity, and documented relationships between concentration and effect or toxicity. Assays for antiretrovirals are available but need to be sent to a reference laboratory for analysis. A number of other inherent problems must be resolved before TDM becomes a standard practice.

It is clear that there are relationships between antiretroviral drug concentrations and antiviral effect (134–140). What is less certain is whether adjustment of patient doses can lead to improved outcomes. Results from two prospective, randomized trials of TDM in HIV infected patients have been reported. ATHENA is an ongoing study of 600 patients randomized to TDM or no TDM. In treatment-naïve patients, indinavir and nelfinavir doses were adjusted based on the "concentration ratio," a measure of the patient's drug level compared to the mean expected drug level in the population at any time during the dosage interval. The TDM group had a significantly improved clinical outcome at one year as defined by a viral load < 500 copies; those who discontinued the drug were also considered treatment failures (141,142). Improved outcome with TDM for indinavir was primarily driven by reduced toxicity leading to fewer discontinuations, while improved outcome with TDM of nelfinavir was primarily due to improved efficacy. Results in treatment-experienced patients are not yet available. Conversely, PHARMADAPT, which used trough plasma PI concentrations to modify salvage therapy, did not show a significant improvement in virologic outcomes at 12 weeks (143). Modification of PI therapy occurred for only 22% of

patients receiving PI TDM and dosage modiÞcations did not occur until eight weeks into therapy. In addition, wild type IC_{50}s were used as target concentrations in PHARMADAPT, and this target may have been too low in this antiretroviral-experienced population.

Recent studies have evaluated the phenotype or Òvirtual Óphenotype used along with the plasma concentration as a potential tool to optimize drug therapy. The ratio of the Cmin (trough level) to the protein-adjusted IC_{50} is often called the Inhibitory Quotient or IQ. Preliminary trials demonstrate a correlation between the IQ and virologic outcome as measured by VL after 24 to 48 weeks, but larger, longer term studies are needed to validate this approach (144Ð146). These studies suggest that integration of the drug level and virus susceptibility to the drug may provide more complete information than either measurement used alone. Ongoing trials will evaluate if the IQ ratio will be a useful monitoring tool in HIV-infected patients.

A number of practical and logistical challenges may limit the widespread use of TDM for antiretroviral therapy. A primary limitation of TDM is that it does not provide information on long term adherence. A patient could not have taken their drugs correctly for weeks but may do so for the two to three days immediately before their clinic appointment if they know a TDM sample will be taken. The drug concentration in such a patient would appear to be adequate although the patient may be failing therapy due to nonadherence. It is important that any TDM program be combined with adherence counseling and monitoring so that results can be easily interpretable. Intrapatient variability also appears to be large for some antiretrovirals, suggesting that clinical decisions should be made only after two or more trough levels are collected and not after a single determination. The deÞnition of a true Òtherapeutic rangeÓ will also need to be determined for each drug, knowing that the target value may be very different for na•ve and treatment-experienced patients. Finally, accurate sample collection, timely processing, storage and shipping of plasma, and rapid turnaround times for assay results must be assured.

FORMULATION AND STORAGE ISSUES

Despite improved formulations of several antiretrovirals, many of the current drugs remain less than optimal in terms of patient convenience. A major problem is the actual number of capsules or tablets that must be swallowed through the course of the day. Approved protease inhibitor regimens vary from 6 capsules per day for indinavir or (Kaletra¨) to 18 capsules per day with saquinavir (Fortovase¨ formulation). Table 36.5 lists the number of pills for some common anti-HIV regimens. In patients taking two or more protease inhibitors, the number of pills per day can be quite large. Some recent improvements in formulation include the combination of zidovudine (300 mg) with lamivudine (150 mg; Combivir¨), and the combination of zidovudine, lamivudine and abacavir (Trizivir¨). This product represents the Þrst triple combination HAART regimen that requires only two tablets per day. This dramatically decreases the number of pills compared to taking these drugs separately.

TABLE 36.5. *Number of pills in combination regimens using standard doses*

Regimen per day	No. of Pills
Trizivir	2
COMB, NVP	4
COMB, EFV	5
COMB, IDV 800/RTV100	8
COMB, LPV/RTV	8
ZDV, d4T, LPV/RTV	10
COMB, NFV (bid)	12
ZDV, d4T, IDV 800/RTV 100, EFV	13
ZDV, d4T, APV 600, RTV 100	14
COMB, SQV 400, RTV 400, EFV	17

COMB, Combivir; d4T, stavudine; IDV, indinavir; LPV/RTV, lopinavir + ritonavir; NFV, nel navir; RTV, ritonavir; SQV, saquinavir (Fortovase); ZDV, zidovudine, EFV, efavirenz, NVP, nevirapine.

Didanosine is now available in a 200 mg tablet, and two of these tablets can be taken once daily. Didanosine can also be administered as an enteric coated capsule given once daily. A signiÞcant problem with the previous formulation of nelÞnavir was that it was difÞcult to swallow; the new Þlm coated tablet has generally alleviated this problem (22). Of note, ritonavir formulations contain ethanol and should be used with caution in patients receiving disulÞram, or drugs that can precipitate disulÞram-like reactions (e.g. metronidazole and certain cephalosporins) (20). A formulation consideration with amprenavir is that the capsules each contain 109 IU of vitamin E. As such, patients should be instructed to avoid vitamin E supplements and to exercise caution when taking the amprenavir along with anticoagulants. Amprenavir also contains a sulfonamide moiety and should be used with caution in patients with a history of sulfonamide allergy (24). Finally, amprenavir capsules and solution are not interchangeable on a milligram per milligram basis due to differences in formulation between the two products (24).

Two different combination products exist for the treatment of tuberculosis. Rifamate¨ is a product containing 300 mg of rifampin and 150 mg of isoniazid, while Rifater¨ contains 120 mg of rifampin, 50 mg of isoniazid, and 300 mg of pyrazinamide. The use of these combination products provides a simple and convenient alternative if clinically warranted.

Many products are available in liquid formulations for use in pediatric patients or in adults with dysphagia. Antiretrovirals with liquid or reconstitutable formulations include didanosine, stavudine, zidovudine, lamivudine, ritonavir, amprenavir and nelÞnavir. The taste of the

ritonavir and amprenavir solutions are often described as unpleasant, and may cause administration problems. Ritonavir can be mixed with chocolate milk, Advera¨ , or Ensure¨ to mask the taste. In addition, many of the medications for opportunistic infections, such as azithromycin, clarithromycin, and trimethoprim-sulfamethoxazole, are available as liquids.

Table 36.6 lists the storage requirements and stability of the Food and Drug Administration-approved antiretrovirals. Clinicians should remind patients to follow general precautions regarding storage of all medications (147). Drugs should be kept out of the reach of children and outdated medications should be discarded. In the absence of speciÞc storage recommendations, medications should

TABLE 36.6. *Storage and stability of antiretrovirals*

Drug	Trade Name	Dosage Form	Storage and Stability
Abacavir	Ziagen	Tablets	Store at room temperature
		Solution	Store at room temperature. May refrigerate, do not freeze.
Amprenavir	Agenerase	Capsules	Store at room temperature
		Solution	Store at room temperature
Delavirdine	Rescriptor	Tablets	Store in tightly closed containers at room temperature. Protect from high humidity. If dispersion in 3 oz water is prepared, consume within 1/2 hr.
Didanosine	Videx	Tablets	Store at room temperature in tightly closed bottles. If dispersed in water or apple juice, stable for 1 hr at room temperature.
		EC caps	Store at room temperature
		Sachet	Store packets at room temperature. If dissolved in water, stable for 4 hr at room temperature.
		Suspension	Store bottles at room temperature. After mixing with antacid, store in refrigerator in tightly closed container for up to 30 d.
Efavirenz	Sustiva	Capsules	Store at room temperature
Indinavir	Crixivan	Capsules	Sensitive to moisture; must be stored and dispensed in original container with desiccant.
Lamivudine	Epivir	Tablets	Store at room temperature in tightly closed bottles.
		Solution	Store at room temperature in tightly closed bottles.
Lopinavir/ritonavir Kaletra		Capsules	If refrigerated, stable until expiration date. At room temperature, use within two months
		Solution	If refrigerated, stable until expiration date. At room temperature, use within two months
Nel navir	Viracept	Tablets	Store at room temperature.
		Oral powder	Store at room temperature. Once mixed with liquid, use within 6 hr.
Nevirapine	Viramune	Tablets	Store at room temperature in tightly closed bottles.
Ritonavir	Norvir	Capsules	Store in refrigerator, protect from light. Discard if left at room temperature for > 12 hr.
		Solution	Storage in refrigerator recommended but not required if used within 30 d and stored at room temperature. Keep in tightly closed, original container away from excessive heat.
Saquinavir	Fortovase	Capsules	Stored in refrigerator in pharmacy until dispensed. For patients, refrigerated capsules stable until expiration date on label. If stored at room temperature, stable for up to 3 mo.
Saquinavir	Invirase	Capsules	Store at room temperature in tightly closed bottles.
Stavudine	Zerit	Capsules	Store at room temperature in tightly closed bottles.
		Suspension	After reconstitution with water, store in refrigerator in tightly closed, original container for up to 30 d.
Tenofovir DF	Viread	Tablets	Store at room temperature.
Zalcitabine	Hivid	Tablets	Store at room temperature in tightly closed bottles.
Zidovudine	Retrovir	Capsules	Store at room temperature and protect from moisture.
		Tablets	Store at room temperature.
		Solution	Store at room temperature.
Zidovudine/ lamivudine	Combivir	Tablets	Store at room temperature.

be kept in a cool, dry area, away from excessive heat, light, and moisture. They should not be stored in places that may be damp, such as the bathroom or near the kitchen sink.

SUMMARY

Antiretroviral regimens must be individualized to the patient. An understanding of the patientÕs lifestyle, work day, eating habits, and level of adherence is important when constructing a treatment plan. Counseling of the patient is critical to treatment success and even the simplest of regimens require a detailed explanation of food effects, potential drug interactions, adherence and scheduling.

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Chapter 37

Immune-based Therapies for HIV Infection

Maria C. Allende and H. Clifford Lane

Due to the introduction of highly active antiretroviral therapy (HAART), HIV infection is no longer the often fatal disease that it was a decade earlier. Cessation of virus-induced immune system dysfunction and clinically relevant immune system improvement are responsible for HAARTÕs success.

Despite these successes there are still many challenges. One cannot achieve complete eradication of HIV nor total restoration of immune system function. Virological rebound invariably occurs following discontinuation of antiretrovirals, even after prolonged viral suppression with the most potent drug regimens (1Ð3).

SigniÞcant progress has been made in understanding the mechanisms of immune dysfunction in HIV infection over the last decade. HIV infection leads to a state of immune deÞciency caused by progressive loss of CD4+ T cells (a main target of the virus) coupled with a generalized immune activation that results in immune suppression (4). A number of qualitative and quantitative immunologic abnormalities occur in the setting of HIV infection that are potentially amenable to direct therapeutic intervention. These include strategies aimed at expanding or restoring the CD4+ T cell pool; strategies designed to counteract immune system activation/immunosuppression and strategies to enhance HIV-speciÞc immunity (Table 37.1). The current article will review the progress made in these areas over the last year.

IMMUNOLOGIC ABNORMALITIES IN PATIENTS WITH HIV INFECTION POTENTIALLY AMENABLE TO IMMUNE BASED THERAPIES

The immunodeÞciency state in HIV infection is characterized by a decline in the CD4+ T-lymphocyte count. The correlation between the CD4+ T cell count and the level of immunodeÞciency is illustrated by the association of lower CD4+ T cell counts with an increased frequency of opportunistic infections. While the level of HIV RNA in plasma is the strongest predictor of long-term clinical outcomes in HIV infection, the CD4+ T cell count is the best marker of immunodeÞciency and the best tool to use as a guide in the initiation or discontinuation of prophylaxis against opportunistic infections. Treatment with highly active antiretroviral therapy (HAART) leads to increases in CD4+ T cell counts due to a combination of redistribution and expansion of T cells. Genetic analyses of T cell receptor repertoire and studies of immune responsiveness to neoantigens, however, have revealed evidence of incomplete recovery of the immune system in HIV-infected patients treated with HAART. In one study some HIV-infected patients treated with HAART demonstrated abnormal *in vivo* CD4+ T cell responses after immunization with the primary antigen Bacteriophage Phix 174, suggesting the persistence of some degree of immunodeÞciency (5). The therapeutic strategies that

TABLE 37.1. *Immunologic approaches to the treatment of patients with HIV-1 infection*

I. Strategies to increase the CD4+ T cell pool
A. Cytokines: IL-2; IL-7, IL-15, CSFs
B. Adoptive transfer of CD4+ T cells
II. Strategies to reverse immune system activation
A. Corticosteroids
B. Cyclosporin A
C. Mycophenolate mofetil
D. Hydroxyurea
E. Thalidomide
F. IL-10
III. Strategies to enhance HIV-speci c immune responses
A. Immunization with HIV vaccine candidates
B. Adoptive transfer of HIV speci c T cells or antibodies
C. Structured treatment interruptions

Note: CSF – colony stimulating factor.
IL – interleukin.

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target the CD4+ lymphopenia component of HIV infection include cytokines such as interleukin-2 and T-cell transfers.

The immune systems of patients with HIV infection are also characterized by a profound polyclonal activation of T cells and B cells. This immune activation in turn enhances HIV replication and leads to increased destruction of CD4+ T cells. Rates of CD4(+) and CD8(+) T cell turnover are directly correlated with changes in viral load. In a recent study, rates of T cell turnover increased four-fold Þve weeks after termination of HAART and declined to pre-HAART-termination levels eight weeks after reinitiation of therapy (3,6). These data are consistent with the hypothesis that HIV-1 infection induces a viral burden-related, global activation of the immune system. Lack of proliferation of antigen-speciÞc CD4+ T cells in response to antigen is also seen as a consequence of high viral load and immune system activation. This rapidly reverses when levels of virus are suppressed (7). The rapid improvement in pre-existing opportunistic infections as well as the immune reactivation syndromes seen following the institution of HAART also suggest that HIV-associated immune system activation is associated with immunosuppression. Among the interventions that have been studied clinically to reduce immune activation are steroids, cyclosporin A, thalidomide, hydroxyurea, mycophenolate mofetil, and interleukin-10.

HIV-speciÞc CD4+ and CD8+ T cell responses are elicited in response to HIV infection. Their precise role in containment of HIV infection remains unclear. While it may seem logical to assume the higher the number of circulating HIV-speciÞc cells the better the control of virus, this logical assumption may not be correct. Conßicting results appear in the literature. Most recently, a study of 21 long-term non-progressors, determined that the frequency of CD8+ T cell HIV-speciÞc responses did not correlate with plasma viral load (8). There is strong evidence from other systems that virus-speciÞc CD8+ cytotoxic lymphocytes are crucial in controlling virus replication and that the optimal function of these cells requires a robust virus-speciÞc CD4+ T cell pool. Strategies explored in order to increase HIV-speciÞc immune responses include structured treatment interruptions, vaccines, and adoptive transfer of HIV-speciÞc T cells or antibodies.

Cytokines

Interleukin-2

IL-2 is a glycoprotein produced mainly by human CD4+T-lymphocytes and also by some CD8+T cells and natural killer (NK) cells following mitogen or antigen activation (9). IL-2 is known to play a central role in the generation of immune responses. It induces proliferation and differentiation of T-lymphocytes, natural killer cells and B-cells. IL-2 induces the production of other cytokines including γ-interferon (γIF), IL-1β, IL-5, IL-6, IL-10, TNF α, GM-CSF and M-CSF(10) and acts synergistically with TNF α, IFN γ, IL-1 and IL-6 to activate cytotoxic lymphocytes. IL-2 is licensed for treatment of metastatic renal cell carcinoma and melanoma in 45 countries. The doses approved for these indications are very different from the doses used in the setting of HIV infection.

The initial rationale for investigating the use of IL-2 in HIV infection came from the *in vitro* observation that IL-2 could augment natural killer cell cytotoxicity and cytotoxicity towards CMV infected cells by PBMC from patients with AIDS (11). In addition, IL-2 seemed a logical choice as a therapeutic candidate in patients with CD4+ T cell depletion due to its potential for *in vivo* T cell expansion. Despite the fact that the CD4+ T cell pool of patients with HIV infection shows approximately a three-fold increase in the rate of T cell turnover when compared to cells from healthy controls, this increased rate is not enough to compensate for the increased CD4+ T cell loss induced by HIV infection.

Phase I studies of IL-2 in patients with HIV-1 infection were initiated in 1983. Treatment modalities explored since then include daily SQ IL-2 in doses <3.0 MIU/d, intermittent IV or SQ IL-2 in doses ranging 6Ð18 MIU/d in cycles of three to Þve days every four to eight weeks, and PEG IL-2 in single or multiple infusions cycles, IV or SQ. A summary of recent studies is provided in Table 37.2.

A single randomized, controlled trial in 115 HIV infected patients examined the effects of daily SQ IL-2 at doses of 1.2 MIU/m2/d (12). Fifty-six asymptomatic HIV infected patients with <300 CD4+T cell counts and stable viral load received IL-2, while Þfty-nine patients received HAART alone. There was a statistically signiÞcant increase at week 26 in the mean percentage of CD4+ T cells (3.52% increase) when compared to control patients (1.33% increase) (p=0.001). This included a preferential expansion of the naïve phenotype (CD45RA+) component of the CD4+ T cell pool. The differences in total CD4+ T cell count between treatment groups did not reach statistical signiÞcance. The most common side effects of IL-2 were injection site reactions (73% of patients), asthenia (53%), ßu syndrome (46%), nausea (36%) and diarrhea (27%). Less than 13% of patients in the control group experienced these adverse events.

Fifteen randomized, controlled phase II studies of intermittent IL-2 therapy for HIV infection have been conducted over the last Þve years. These studies have documented a series of critical Þndings. The conclusions from these studies can be summarized as follows:

- A dose of 7.5 MIU BID is more efÞcacious than 1.5MIU BID(3) in increasing the CD4+ T cell counts.
- Patients with higher baseline counts appear to have a greater CD4 cell response to sc IL-2 therapy (13).

TABLE 37.2. *Randomized, controlled trials of Interleukin-2 plus antiretroviral agents in HIV infected patients performed over the past six years*

Stage of HIV infection	Study Authors, Year	Patient characteristics at study entry: CD4 count	Patient characteristics at study entry: Viral load	N-Number of patients: Treatment arms with IL-2 starting dose and schedule*	Change in CD4 + cell count Mean or **Median**: 6 months	Change in CD4 + cell count Mean or **Median**: 1 year	Change in viral load	Notes: P value for CD4 counts (IL-2 vs. control)
Advanced (CD4 T cell counts < 300)	Saravolatz et al. 1996	100–300	Any	• 19-ART + CIV IL-2, 12MIU qd × 5 days q8wk • 20-ART + CIV IL-2, 12MIU qd × 4 days q8wk • 21-ART + CIV IL-2, 12MIU qd × 3 days q8wk • 21-ART		+ 80 + 14 − 16 − 45	No difference	Dual nucleosides P0.02
	Arno et al. 1999	< 250	< 500	• 13-ART + SQ IL-2, 3MIU qd × 5 days q4wk × 6 cycles • 12-ART		+ 105 + 30	No difference	PI treated > 6 mos. P < 0.05
	Mitsuyasu, 2000	50–350	< 5,000	• 48-ART + CIV IL-2, 9.0MIU qd × 5 days q8wk • 49-ART + SQ IL-2, 7.5MIU bid × 5 days q8wk • 45-ART		**+ 309** **+ 240** **+ 97**		Median change in CD4 count at week 60 P < 0.001
	Lalezari, 2000*	< 300	Stable	• 56-ART + SQ IL-2, 1.2MIU/m2 daily for 6 months • 59-ART	+ 66 + 33		No difference	Mean change from baseline P = NS
	Katlama, 2000	< 200	< 1,000	• 34-ART + SQ IL-2, 4.5MIU bid × 5 days q6wk • 36-ART	**+ 65** **+ 18**		No difference	Median increase P < 0.01
Early (CD4 T cell counts > 300)	Kovacs et al. 1996	> 200	Any	• 30-ART + CIVQD IL-2,18MIU qd × 5 days q8wk • 29-ART		+ 412 –48	No difference	Dual nucleosides P < 0.001
	Carr et al. 1998	200–500	Any	• 27-ART + CIV IL-2, 12MIU qd × 5 days q8wk • 58-ART + SQPEG IL-2, □1MIU two doses q8wk • 30-ART		**+ 359** **+ 44** − 46	No difference	Dual nucleosides. P < 0.0001
	Hengge et al. 1998	200–500	Any	• 22-ART + SQ IL-2, 9MIU qd × 5 days q6wk • 20-ART + SQ IL-2, 9MIU qd × 5 days if CD4 < 1.25 baseline • 19-ART		+ 122 + 104 + 24	No difference	ART therapy with PI P < 0.01
	Levy et al. 1999	250–550	Any	• 22-ART + CIV IL-2, mean 8.8, 12MIU qd × 5 days q8wk • 24-ART + SQ IL-2, 3MIU/m2 bid × 5 days q8wk • 22-ART + IVPEG IL-2, 2MIU/m2 IV bolus • 26-ART		+ 676 + 564 + 105 + 55	No difference	Dual nucleosides (naïve patients) P < 0.0001
	Davey et al. 1999	> 500	Any	• 24-ART + SQ IL-2, 7.5 MIU bid × 5days q8wk or q4wk • 25-ART + SQ IL-2, 1.5MIU bid × 5 days q4wk or q8wk	+ 696 + 156		No difference	1.5 vs. 7.5 MIU bid P < 0.001
	Tambussi et al. 1999	200–500	Any	• 15-ART + CIV IL-2, 12MIU qd × 5 days q8wk x2 cycles, then • 15-ART + SQ IL-2, 7.5MIU bid × 5 days q8wk x6 cycles • 16-ART + SQ IL-2, 3MIU bid × 5 days q4wk x 12 cycles • 15-ART		+ 698 + 625 + 726 + 103	No difference	Above baseline P < 0.001

TABLE 37.2. *Continued*

Stage of HIV infection	Study Authors, Year	Patient characteristics at study entry: CD4 count	Patient characteristics at study entry: Viral load	N-Number of patients Treatment arms with IL-2 starting dose and schedule*	Change in CD4 + cell count Mean or **Median**: 6 months	Change in CD4 + cell count Mean or **Median**: 1 year	Change in viral load	Notes P value for CD4 counts (IL-2 vs. control)
	Losso et al. 1999	> 350	Any	• 12-ART + SQ IL-2, 7.5MIU bid × 5 days q8wk • 12 ART + SQ IL-2, 4.5MIU bid × 5 days q8wk • 12-ART + SQ IL-2, 1.5MIU bid × 5 days q8wk • 37-ART	+ 520 + 354 + 81 + 29		No difference	Time-weighted mean change from baseline above control group (IL-2 vs. control) P < 0.0001
	Ruxrungtham, 2000	> 350	Any	• 12-ART + SQ IL-2, 7.5MIU bid × 5 days q8wk • 12-ART + SQ IL-2, 4.5MIU bid × 5 days q8wk • 12-ART + SQ IL-2, 1.5MIU bid × 5 days q8wk • 36-ART	+ 462 + 205 + 88 + 36		No difference	Time-weighted mean change from baseline above control group (IL-2 vs. control) P < 0.0004
	Abrams, 2000	> 300	Any	• 126-ART + SQ IL-2, 7.5MIU bid × 5 days q8wk • 130-ART + SQ IL-2, 4.5MIU bid × 5 days q8wk • 255-ART		+ 289 + 264 + 83	No difference	Mean cell difference from baseline in IL-2 group vs control P < 0.0001
	Davey, 2000	200–500	< 10,000	• 39-ART + SQ IL-2, 7.5MIU bid × 5 days q8wk • 43-ART		+ 384 + 64	–0.28log –0.09log	Mean viral load change: P = 0.03 CD4 changes: P < 0.001

* All studies included intermittent IL-2 with the exception of the Lalezari study, which used once daily infusions.
Key:
ART = antiretroviral therapy
CIV = continuous intravenous
MIU = million international units
SQ = subcutaneous
PI = protease inhibitor

- Five-day treatment cycles are more effective than three-day cycles (14) in raising CD4+ T cell counts.
- Intervals of eight weeks seem preferable to four weeks since they have similar efficacy and less toxicity (3).
- Cycles of more than five days are not better than five days due to the tachyphylaxis observed beyond this duration (15).
- PEG IL-2 has not shown similar activity (16,17)
- There are no clinically significant changes in viral load.
- Administration of IL-2 is associated with a series of predictable, often dose-related side effects.

Most of these trials have compared IL-2 with antiretrovirals with a control arm treated with antiretrovirals alone. The follow up of 157 patients from the first three randomized controlled trials of this approach revealed a statistically significant long term reduction of HIV viral load (mean change from baseline HIV RNA −0.98 vs. −0.63 log/copies/mL, $p = 0.004$) in patients randomized to the IL-2 arm. The median followup was 31 months for the IL-2 group (range 4–44) and 28 months for the control group (range 9–42) (18). The results of seven recent randomized controlled studies, four of which were done in patients with <350 CD4+ T cell counts, show no statistically significant differences in viral load measurements between study groups at one year follow-up. An eighth study (19) revealed a slightly lower viral load in the IL-2 treated group (Table 37.2).

A study of 36 HIV-infected patients that were randomized to receive IL-2 without antiretrovirals at doses of 4.5 MIU or 7.5 MIU BID ($n = 24$) for five days every eight weeks or no treatment ($n = 12$) was presented at an international conference (20). Patients were treatment naïve and had CD4+ counts >350 cells/mm^3. At six months, a CD4+ count increase of 108 cells/mm^3 was observed in the IL-2 arm. Control patients received neither antiretroviral therapy nor IL-2 and exhibited an increase in CD4+ count of 11 cells/mm^3 ($P = 0.002$). IL-2 did not cause a sustained or significant increase in HIV replication in this study. Further studies of this type will be useful in evaluating IL-2 therapy alone or with pulse antiretrovirals as an alternative to chronic antiretroviral treatment.

In the pre-HAART era, the use of intermittent IL-2 in patients with lower baseline CD4 counts produced smaller CD4+ T cell count increases and greater toxicity (21). Three randomized, controlled studies of intermittent IL-2 done during the HAART era in HIV-infected patients with CD4+ T cell counts of <350 (22–24) have revealed that IL-2 is safely tolerated by patients with lower CD4+ T cell counts and stable, controlled viral loads and can lead to CD4+ T cell expansions (Table 37.2).

In vivo, IL-2 produces an immediate polyclonal T-cell proliferation with expansion of both naïve and memory cells. It also leads to an immediate mean increased cell death as reflected in rates of apoptosis of CD4 and CD8 T lymphocytes (25). In a study of apoptosis in HIV-infected patients treated with antiretrovirals and IL-2, cytofluorographic determination of DNA breaks ("TUNEL" assay) was used to examine the impact of IL-2 on cell death. In contrast to what is seen during the administration of IL-2, at the end of the treatment period (24 weeks) the percentage of PBMCs undergoing apoptosis was significantly decreased in the IL-2 treated group (26). These observations suggest that IL-2 induces a proliferation of the CD4+ T cell pool that is accompanied early by increased apoptosis but later results in decreased (normalized) immune activation.

The ability of IL-2 to activate the T cell pool led some investigators to postulate that IL-2 could be used to "flush" the reservoirs of latently infected T cells by promoting their activation, proliferation and death. A study of 18 HIV-infected patients with CD4(+) T cell counts >350 cells/microliter and viral load below the limits of detection for >1 year while on HAART revealed that all 18 patients developed viral loads >50 copies following discontinuation of therapy. Twelve of these 18 patients had previously received IL-2 and viral load rebound occurred despite prior IL-2 treatment (2,3). This approach was also tested in at least two other studies. In one study, 18 months of five antiretroviral agents, three cycles of IL-2 and two courses of γIFN, failed to prevent an increase in plasma HIV RNA levels after therapy interruption (27). In another study, treatment with IL-2 plus OKT3, combined with a five-drug HAART regimen (28) led to induction of viral replication and depletion of the CD4 pool. Thus, a consistent result from several studies is that high-level T cell stimulation in the setting of HAART has failed to reduce viral reservoirs.

To better understand the nature of the transient "viral burst" associated with IL-2 therapy, a detailed virological evaluation was performed in 11 patients undergoing intermittent IL-2 treatments for a year in the pre-HAART era (29). Six patients showed a >0.5 log increase in plasma HIV during at least one cycle. These increases were transient with levels returning to baseline within 7–10 days. Quasi species analysis from plasma and tissue in a separate cohort demonstrated that the virus induced by IL-2 most commonly resembled pre-IL-2 plasma quasi species. Thus, intermittent IL-2 therapy induced viremia does not result in sustained increases in plasma or tissue levels of HIV and does not produce a sustained expression of a previously silent quasispecies.

As noted above, five-day cycles of IL-2, spaced by at least four weeks, lead to prominent and sustained polyclonal increases in CD4+ T cell counts. This selective expansion of the CD4 pool appears to be associated with a selective induction of the alpha chain of the IL-2 receptor (CD25) on CD4+ T lymphocytes and is associated with immediate increases in rates of cell growth and cell death. Increased delayed-type hypersensitivity responses to tetanus and tuberculosis antigens were seen in patients that had weaker but present responses before IL-2 therapy (16). No new antigen-specific responses were generated with

IL-2 treatment, suggesting that deleted clones were not recovered. In a randomized, controlled trial, proliferative responses to tuberculin and CMV improved to a greater degree in the IL-2 treated patients than in the control group (24). Clinical endpoint studies with longer followup will determine if these observations will translate into clinical beneÞt.

In 1999, two large phase III clinical endpoint studies of IL-2 in HIV-infected patients were open to enrollment. ESPRIT (Evaluation of Subcutaneous Proleukins¨ in a Randomized International Trial) is a six-year study that will enroll a total of 4,000 patients with CD4 counts >300. SILCAAT (A Study of IL-2 with Low CD4 counts on Active Anti-HIV Therapy will enroll and follow for four years 1,400 patients with CD4 counts between 50 and 200. It is hoped that these studies will answer the question of the therapeutic role, if any, of IL-2 in patients with HIV infection. The websites for these studies are: www.e-spritstudy.org and www.silcaat.com.

Other Cytokines

G-CSF is capable of increasing neutrophil number in patients with HIV-infection and can allow treatment with higher doses of myelosuppressive agents in HIV-infected patients by minimizing the resulting neutropenia (30). A randomized, multicenter controlled trial (31) of 258 patients with HIV infection and ANC between 750–1000 showed that G-CSF treated patients had 31% fewer bacterial infections and 54% fewer severe bacterial infections than the control group. Another randomized, placebo-controlled trial examined the long term effects of 300mcg G-CSF three times a week on 30 HIV-infected patients treated for 12 weeks. SigniÞcant increases in absolute numbers of CD34+ cells and total white count were detected in the treated group. There was a small but statistically signiÞcant increase in CD4+ T cell count in the G-CSF treated group at week 12 (+58 vs +3, $p=0.03$). No correlation was seen between the numbers of myeloid progenitors and the CD34 counts. Thus, G-CSF appeared to be acting by increasing the number and differentiation of myeloid progenitors (32) ; rather than by expanding the pool of stem cells. No increases in HIV replication were seen with G-CSF administration. In contrast, a randomized, double blind, controlled study of G-CSF plus HAART in treatment na•ve HIV infected patients with <300 CD4+ counts was terminated prematurely after a case of severe encephalopathy occurred in the G-CSF group. At the time the study was closed, the G-CSF plus HAART treated group showed a less pronounced decrease in plasma HIV RNA of about 1 log ($p=0.02$) compared to the placebo plus HAART group. All patients reported bone pain. Thus, the use of G-CSF as a treatment for HIV infection remains controversial (33).

Compared to placebo, GM-CSF reduced the incidence of overall infections (78% vs. 67%, $p=0.03$) and the time to Þrst infection (56 days vs. 97 days, $p=0.04$) in a phase III randomized, double-blind, placebo-controlled trial of 309 subjects with no prior AIDS-deÞning illness and CD4 cell counts ≤ 50, or a prior AIDS-deÞning illness and CD4 cell counts ≤ 100. All patients were on stable antiretroviral therapy (34). No statistical difference in the cumulative number of opportunistic infections was observed between groups. In this study, GM-CSF signiÞcantly increased CD4 T cell counts with a mean change from baseline of approximately 40 vs. 20 cells ($p=0.004$). Neutrophil counts increased (median increase of 800×10^6 cells/l at two weeks vs. no increase in the control group) and there was a decrease in virological breakthroughs and overall infection rate in subjects with advanced HIV disease during the followup period of 24 weeks. Changes in viral load were not signiÞcantly different between the groups. Another use of GM-CSF in HIV infection was explored by Herranz and colleagues (35). Three patients with recurrent aphthous ulcerations who had failed to respond to other treatments (two of whom had contraindications for thalidomide use) underwent therapy with an oral solution containing recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF, 400 microg in 5% glucose 200 mL). All three patients experienced marked improvement and showed no relapses on a prolonged followup. GM-CSF appears capable of increasing neutrophil counts without adversely affecting HIV viral load in patients on HAART.

IL-10 use has been explored in HIV infection because of its inhibitory effects on proinßammatory cytokines (IL-1 α, IL-1 β, TNF α and IL-6) that increase HIV replication *in vitro* and are overexpressed in uncontrolled HIV infection. A prospective, randomized, double-blind and placebo-controlled trial of 39 HIV infected subjects was done with four different doses of IL-10 subcutaneously three times a week for four weeks (36). The follow-up was done up to four weeks after completion of therapy. Compared to baseline, no changes in CD4+ or viral load were seen in any of the groups. These results do not encourage the development of IL-10 as an immune therapy for HIV infection.

IL-12 is essential in the generation of CTL responses and induces IFN γ production by macrophages. IL-12 production by macrophages in response to antigen stimulation has been shown to be reduced in HIV infection (37). A phase I study of a single dose of recombinant human IL-12 (rhIL-12) in 47 HIV-infected patients with CD4+ counts between 100–500 (38) revealed no effects of rhIL-12 on plasma HIV RNA or absolute CD4(+) T cell counts. Dose-related increases in absolute CD8(+) T and NK cells were observed in subjects assigned to rhIL-12 doses of 30–300 ng/kg. Single rhIL-12 doses of 30–300 ng/kg were well tolerated. In an *in vitro* study, CD40 ligand trimer (CD40LT) and IL-12 were shown to enhance CD4+ T cell proliferation and production of IFN γ in response to p24 by PBMCs of HIV infected individuals

(39). CD40LT exerted its effect through B7-CD28 dependent and IL-12 and IL-15 independent mechanisms. These findings indicate the potential for CD40LT and IL-12 as immune-based therapies for HIV infection. Clinical studies need to be developed to followup on these findings.

Interferon-α is approved in some countries for treatment of hepatitis C and for therapy of Kaposi's sarcoma. Its use in HIV infection has been explored in followup of *in vitro* evidence of its ability to block HIV replication. In the treatment of Kaposi's sarcoma, the correlation between higher CD4+ counts and successful clinical outcome suggests that the effects of IFN α treatment are due to immunomodulation rather than direct anti tumor effects. In a seeming paradox, high levels of IFN α have been found in patients with advanced disease and some authors have suggested a pathogenic role for IFN α in HIV infection. In one study, HAART led to marked decreases in levels of IFN α and IFN-inducible protein 10 (40).

A randomized trial of IFN α was performed in 256 HIV infected subjects with CD4+ counts between 300–500/ml and not more than 14 weeks of prior antiretroviral therapy. All subjects received AZT and ddC and half of the subjects also received IFN α (3MIU SQ/day) (41). Evaluation at 48 weeks demonstrated that IFN-α inhibited HIV-1 replication but attenuated the CD4+ cell response to dual therapy with AZT and ddC. The mean HIV RNA changes from baseline at week 48 were −0.65 and −1.12 log 10 copies/ml for the control and IFN group respectively ($p = 0.01$). The use of IFN α with ribavirin for hepatitis C led to no significant changes in mean HIV plasma RNA levels irrespective of the hepatitis C response to treatment (42).

PEG*ylated* IFN α is a polyethylene glycol derivative of recombinant IFN α. It was studied in a multiple-dose clinical trial in HIV-infected patients with suboptimal viral suppression during antiretroviral therapy. Thirty subjects with CD4+ counts $>200/mm^3$ were enrolled to receive SQ PEG-IFN (1, 1.5, 2.25 or 3.5 mcg/kg) weekly for eight weeks (43). HIV viremia decreased by a median of 0.92 log^{10} at week 8, from a baseline median viral load of 4.47log^{10}. The responses were poorer in HIV-infected individuals with higher baseline activation markers (CD38+CD8 cells >40%). Future trials will be needed to assess the role of IFN α in HIV infection in combination with other agents.

IFN γ during the mid 1980s did not show clinical benefit in AIDS patients. Its potential use in adults has not been pursued. It has been the focus of one recent report in pediatric patients (44). Nineteen HIV+children receiving AZT or ddI were treated with $50mcg/m^2$ of recombinant IFN-γ subcutaneously three times a week for 12 weeks, and with twice that dose in same schedule during weeks 13 to 24 in a phase I/II trial. In this study, IFN-γ was well tolerated, however, it produced no changes in viral load or CD4 counts. No changes were noted *in vivo* in neutrophil bactericidal activity. There is little continued enthusiasm for the study of this cytokine in HIV-1 infection at this time.

Interleukin-7 may play an important role in T-cell immune reconstitution. A study evaluated plasma IL-7 levels and noted a correlation among IL-7 levels, thymic mass on computed tomography scan, HIV viral load and subpopulations of T cells in 175 HIV-infected individuals (45). Higher levels of IL-7 were seen with higher viral loads, lower thymic mass and lower CD4 counts, suggesting a positive feedback on thymopoiesis in patients with lower CD4 counts due to HIV infection. An *in vitro* study showed that IL-7 can increase bcl-2 expression and inhibit apoptosis in peripheral blood mononuclear cells (46). Based on these data, IL-7 would seem a promising agent for future clinical trials.

Interleukin-15 has similar effects to IL-2, using a different alpha chain in its high-affinity receptor while sharing the beta and gamma chains. *In vitro*, it has been shown to enhance neutrophil chemotaxis and fungicidal ability (47) and to enhance survival and expansion of NK cells from HIV-infected patients (48). IL-15 priming of pathogen stimulated PBMCs did not lead to increased IL-12 and IFN γ in HIV positive patients as it did in HIV negative ones (49). This suggests that IL-15 alone may be insufficient to correct the cellular defects seen in HIV infection. IL-15 has also been shown to reduce apoptosis of lymphocytes of HIV-infected patients by suppressing the down-modulation of Bcl-2 (50). IL-15, as IL-2, has failed to inhibit CD95 induced lymphocyte apoptosis *in vitro* (51). When compared with IL-2 effects *in vitro*, increases in lymphocyte survival were greater with equivalent doses of IL-15 (52). These data provide a preclinical basis for IL-15 immunotherapy in HIV infection.

Interleukin-16 is a proinßammatory cytokine synthesized mainly by CD8+ cells. IL-16 binds to the CD4 receptor; however, CD4 expression is not required for IL-16 to exert its actions (53). IL-16 leads to enhanced expression of the alpha and beta chains of the IL-2 receptor and enhances T cell proliferation in response to IL-2 or IL-15. *In vitro*, IL-16 was found to have an inhibitory effect on HIV replication at the level of viral transmission (54). T cells responding to IL-16 become unresponsive to antigen and less susceptible to activation-induced cell death. Lower *in vivo* levels of IL-16 correlated with advanced HIV disease (55). The enhancing effects on IL-2 and IL-15-induced proliferation, as well as its antiviral effects and ability to decrease activation-induced cell death make IL-16 an attractive molecule to be studied further in HIV infection.

Cell Transfers

Autologous CD4+ and/or CD8+ T cells expanded *in vitro* have been given to HIV+ patients in a variety of different clinical trials. Ex-vivo expansion of CD4+ T cells from infected patients using anti-CD3/CD28 antibodies has been more effective than using anti-CD3 alone.

This technique increases T-cell numbers in a polyclonal fashion. The resulting cell preparations are relatively resistant to HIV infection due to chemokine receptor downregulation. In a recent study by Bernstein et al. (56), eight patients received a mean of Þve infusions of autologous ex-vivo expanded anti-CD3CD28 co-stimulated CD4+ T cells. Doses started at 3×10^9 cells with a six week interval between infusions. The patients had a median baseline CD4 count of 442 cells/mm^3. The median viral load in the four of the eight patients with detectable levels was 24,000 copies/ml. At the end of the study, there were no changes in viral load. A median CD4+ T cell increase of 38% were seen in seven of the eight patients. All patients were taking antiretroviral agents.

Transfers of HIV-speciÞc CTLs between HIV-discordant syngeneic twins and autologous, expanded CD8+ T-cell transfers have also been attempted. One study assessed the survival of HIV-speciÞc CD8+ T cells and their *in vivo* migration using both a quantitative ßuorescent probe technique and PCR in situ hybridization (57). The cells were gene-modiÞed to contain a neomycin phosphotransferase gene. The number of circulating CD8+ T cells ranged from 1.6Ð3.5% shortly following infusion to $< 0.5\%$ (< 1 per 200) two weeks later. Concurrent analysis of blood and lymph node by these two techniques showed that modiÞed CTL were present in the lymph node at a 4.5- to 11-fold higher levels in lymph node than peripheral blood. The gene-marked CTL localized within parafollicular regions of the lymph node adjacent to cells expressing tat-env fusion transcripts, a correlate of virus replication. Thus, it appears that HIV-speciÞc CD8+ T cells expanded ex-vivo are capable of homing to sites of viral replication *in vivo*.

In an attempt to create CD4+ T cells resistant to HIV infection, studies have been carried out using genes that can disrupt the HIV life cycle by interfering with regulatory or structural genes of HIV. Inhibitors of HIV genes can be RNA-based or protein-based. RNA-based strategies include anti-sense trans-activation response (TAR) element inhibitors and ribozymes engineered to cleave speciÞc sites in the HIV genome (58). Ribozymes targeted to the HIV-1 tat gene have been shown to inhibit HIV-1 replication in tissue cultures. A phase I study(59) using an anti-tat ribozyme has been carried out. In this study cells from four healthy twins were transduced with an anti-tat ribozyme and infused into the corresponding HIV negative twin. Results at 10 months showed persistence of the transduced cells (with two different vectors) in all four twin pairs. There were no signiÞcant changes in CD4+ counts or viral load and no serious toxicities. Protein-based inhibitors include a dominant-negative mutant Rev protein (RevM10), engineered intracellular antibodies, and chemokines that bind and sequester speciÞc cellular coreceptors (60). A report from an ongoing study utilizing an antisense TAR (trans-activation response element) along with a transdominant rev noted that transduced cells have remained present in the circulation three years after infusion. These cells have shown a survival advantage compared to cells infused at the same time and marked with a control gene (61).

In an attempt to increase the number of CD4+ and CD8+ T cells capable of binding to HIV-infected cells, studies have been carried out in which a second T cell receptor consisting of the CD3 zeta chain of the T cell receptor fused to the sp120-binding portion of CD4 has been inserted to confer binding to HIV gp120. Genetically engineered CD4+ and CD8+ autologous T cells, modiÞed with the insertion of this fusion protein (CD4-zeta) were infused into 24 subjects (62), with or without IL-2 at a dose of 6MIU/day for Þve days. All patients were on stable antiretroviral therapy for more than eight weeks, had CD4+ counts □50 and HIV viral loads of □1,000 copies/mL. The T cells were autologous and had been stimulated ex vivo with anti-CD3/CD28 and expanded for approximately two weeks. CD4zeta was detected in 1Ð3% of blood mononuclear cells at eight weeks and 0.1% at one year. There were no signiÞcant changes in plasma HIV RNA or blood proviral DNA in either treatment arm. An increase of CD4+ counts of 73 cells/microL was noticed at eight weeks in the group receiving IL-2.

In a similar study CD4+ and CD8+ lymphocytes were obtained from healthy HIV negative syngeneic twins and transduced with the chimeric T cell receptor gene (CD4/CD3-zeta) mentioned above. These cells were then infused to the HIV+ twin (63). Transduced cells peaked in the circulation by 24Ð48 hs (10^4 to $10^5/10^6$ cells) and decreased by 2Ð3 logs in eight weeks. Sustained fractions of approximately 10^3 to 10^4 modiÞed cells/10^6 total CD4(+) or CD8(+) cells persisted for at least one year. Lymphoid tissue biopsies revealed the presence of modiÞed cells in proportions equivalent to or below those in the circulation. There were no signiÞcant changes in CD4 count or viral load in these patients. Transduction techniques used in these cell transfers have an efÞciency of 15Ð30%, which limits the efÞcacy of this kind of treatment and creates the need for a higher number of transfusions. The number of cells infused and the intervals used has varied among these studies, making comparisons difÞcult. Thus far, none of these approaches has achieved signiÞcant increases in CD4 or decreases in viral load. Higher numbers of cells, more frequent infusions and/or different targets are needed. One of the limitations of this area of research has been the development of immune responses directed towards the new genes.

Several groups have studied the potential role of genetic modiÞcation of stem cells as a way of creating T cells resistant to HIV infection. This area faces several hurdles, including poor transduction efÞciencies and limited differentiation via T cell pathways. The *in vivo* survival of transduced stem cells of HIV-infected subjects was evaluated in one study (64). Approximately 10^8 PBSC (from mobilized HIV-1 infected patients after leukopheresis and enrichment of the CD34+ cell population) were transduced with HGTV-43, a retrovirus-based vector,

followed by autologous infusion without host conditioning. The transduced cells were expressing an HIV-1 antisense RNA and were resistant to HIV infection *in vitro*. Measurements of the antisense RNA was performed using real-time RT-PCR of PBMC RNA. Antisense RNA was observed in CD4+ cells at six months. This observation is an example of the survival of transduced hematopoietic cells in nonablated human subjects.

Immunosuppressants

Corticosteroids

Corticosteroids have been evaluated in HIV infection as a temporary treatment directed to reduce the state of immune activation and inßammation that accompanies HIV disease. Their use is recommended as an adjunct to speciÞc antimicrobial therapy in patients with severe *Pneumocystis carinii* pneumonia (PCP). Among the actions of corticosteroids of possible beneÞt in HIV infection are a decrease in the levels of proinßammatory cytokines leading to a decrease in HIV replication and protection of CD4+ T cells from activation-induced cell death (65). In an early study, oral prednisolone at 0.5mg/kg for six months given to patients with CD4+ counts between 200Ð799 cells/ml produced increases in CD4+ T cells (median increase at one year, 119 cells/microL) with no changes in viral load after one year (66). Another study, done on four patients with HIV wasting syndrome, demonstrated a mean weight increase of 3.5 kg along with a transient decrease in viral load (67). A controlled study, designed to evaluate the effects of corticosteroids on IL-2 induced toxicities, found that although side effects were signiÞcantly decreased, the rise in CD4+ T cells was diminished (61). Recent studies describing an increased incidence of avascular hip necrosis in HIV-infected patients and identifying corticosteroids as a signiÞcant risk factor (68), as well as the growing evidence of insulin resistance and lipid metabolism abnormalities found with HAART, have created additional doubt as to the role of corticosteroids as a maintenance therapy in patients with HIV infection (69).

Hydroxyurea

Hydroxyurea (HU) inhibits ribonucleotide reductase, the rate-limiting enzyme for dNTP synthesis. HU decreases intracellular dNTPs, which are essential for cell division as well as HIV replication. This action creates a competitive advantage for the incorporation of nucleoside reverse transcriptase inhibitors in the HIV replication cycle. Thus, HU has both antiviral and cytostatic properties. It has been postulated that the cytostatic effects might control the dissemination of HIV early in primary infection. HUÕs predominant combination with didanosine rather than other nucleoside analogues in clinical trials is based on HUÕs ability to deplete deoxyadenosine triphosphates to a greater extent than deoxythymidine triphosphates. This likely increases intracellular concentrations of ddI metabolites relative to concentrations of dATP. The use of hydroxyurea at 1,000 mg/day in patients with CD4 counts between 100Ð350 that were on didanosine monotherapy resulted in a viral load reduction of 0.6 log10 after four weeks of treatment. No increases in CD4 counts were seen (70). A randomized study of 144 patients compared ddI plus d4T plus hydroxyurea to ddI plus d4T plus placebo. After 12 weeks of therapy, patients in the HU group had greater decreases in viral load (2.3 log10 vs 1.7 log10) with an increase in the CD4 count of 28cells/ml compared to 38cells/ml in the placebo group. A variety of side effects (neutropenia, thrombocytopenia, diarrhea and neuropathy) were increased in the HU group (71).

Interest in the potential role of HU in patients with HIV infection increased with the report of the ÒBerlin patientÓ (72). This patient was started on therapy with HU, ddI and indinavir two months after acquiring HIV infection. Treatment was then discontinued on two occasions. The plasma viral load was < 50 copies/ml 17 months after the last discontinuation of therapy. In follow-up of these Þndings, a 19 patient study was carried out in patients with primary HIV infection comparing four-drug HAART not containing HU with four-drug HAART that contained HU (73). At 36 weeks of therapy, the antiretroviral potency as well as the changes in CD4 were similar in both regimens.

More recently, a randomized trial of more than 200 patients (ACTG5025) compared ddI-d4T-indinavir with Hu to ddI-d4T-indinavir without HU. After 24 weeks of follow-up, patients in the HU arm had signiÞcantly higher treatment-limiting toxicities, particularly neuropathy, with two fatalities due to pancreatitis. It is unclear if these side effects were due to interactions with ddI and/or d4T exclusively or if the addition of indinavir might have produced additional interactions that resulted in increased toxicity in the HU group. In view of these toxic interactions and the fact that HU combination regimens do not seem to be superior to other HAART combinations in efÞcacy for primary infection treatment, the role of HU in HIV infection remains unclear.

Mycophenolate mofetil

Mycophenolate mofetil is the prodrug of mycophenolic acid (MA). This drug limits the *de novo* synthesis of guanosine nucleotides by reversibly inhibiting inosine monophosphate dehydrogenase. Thus, proliferation of monocytes and lymphocytes, which are dependent on de novo synthesis of purines, is inhibited by mycophenolic acid. MA also induces apoptosis of activated T cells (74). MA can directly inhibit HIV-1 replication *in vitro* at concentrations that are lower than those used for organ transplantation (75). This antiviral effect is obtained by

depleting one of the substrates (deoxy guanosine triphosphate) required for reverse transcription. By increasing the ratio of drug : native substrate, MA potentiates the antiviral activity of abacavir, a guanosine analogue. In contrast, the combination of MA with the thymidine analogues zidovudine or stavudine was antagonistic *in vitro*. A non-randomized controlled clinical study assessed the effects of mycophenolate mofetil (MM) in eight HIV-infected patients with viral loads <5 copies/ml treated with abacavir and amprenavir. No liver or renal toxicities were noted. MM induced a rapid decrease of both CD4 and CD8 T cell production. Daily CD4+ T cell production was suppressed by approximately 60% compared to that of HIV negative subjects. Therefore, MM may substantially reduce the number of dividing CD4+ T cells *in vivo* (74). Randomized clinical trials with long-term followup are needed to delineate the clinical effects of mycophenolate mofetil and its interactions with reverse transcriptase inhibitors. In transplant patients this agent has been associated with the development of Kaposiõ sarcoma, cytomegalovirus and fungal infections.

Cyclosporin-A

Cyclosporin-A (CyA) reduces T-cell activation by inhibiting IL-2 release. In this way, HIV replication is reduced at the expense of a reduction in T cell number. Initial studies before HAART and using higher doses (7.5mg/kg/day) had disappointing results in HIV-infected patients. In one early study no signiÞcant changes in CD4 count (440 vs. 404, $p=0.1$) and a signiÞcant increase in HIV p24 antigen levels (62 vs. 275, $p=0.001$) were found after one year of CyA treatment (76). While the CD4 decline was more rapid following the discontinuation of cyclosporin A it is difÞcult to assess the importance of this in the absence of a control group. More recently, two studies were presented at international meetings. Rizzardi et al. (77) treated nine patients with primary HIV infection with CyA at the lower dose of 0.6Ð1.2 mg/kg/day for eight weeks in combination with HAART and compared virologic and immunologic responses with 29 patients treated with the same HAART regimen without CyA. At week 24, the viral load reduction was similar in both groups, although the proportion of patients with viral loads <50 was higher in the CyA group (88% vs. 55%, $p=0.05$). A striking Þnding in this study was a sustained and marked increase in CD4 counts in the CyA treated group at week 64 (1,443 vs. 712 cells/mm^3). A randomized and placebo controlled study, ACTG 334, evaluated the use of 4mg/kg/day of CyA for 12 weeks in 28 patients treated with no antiretrovirals ($n=15$) or stable dual nucleoside therapy ($n=13$). Overall, therapy was safely tolerated. Total CD4+ and CD8+ lymphocytes numbers did not differ between the groups. No major differences were noted in viral load in preliminary analysis. A formal analysis is still pending (78). These studies support the further evaluation of CyA at low doses along with HAART in patients with HIV infection. HIV infected patients with autoimmune diseases or undergoing organ transplantation may be good candidates for future studies.

Thalidomide

Thalidomide was initially evaluated in HIV infection due to its ability to interfere with the production of TNF-α, a proinßammatory cytokine that can enhance HIV replication *in vitro*. The only current FDA-approved indication for thalidomide is for the treatment of erythema nodosum leprosum. A previous randomized, placebo-controlled trial (79) supported the use of thalidomide in the treatment of HIV-associated oral ulcers at a dose of 200mg/day. Sixteen of 29 patients in the thalidomide group (55%) had complete healing of their aphthous ulcers after four weeks, as compared with only two of 28 patients in the placebo group (7%; odds ratio, 15; 95% conÞdence interval after adjustment for group sequential testing, 1.8 to 499; unadjusted $P<0.001$).Unexpectedly in this study, this clinical beneÞt on oral ulcers was accompanied by increases in viral load that were statistically signiÞcant in comparison with the control group (0.42log10 vs. 0.05log10, $p=0.04$) and increases in serum TNF levels. More recently, a multicenter, double-blind, randomized, placebo-controlled study was conducted to determine the safety and efÞcacy of reduced, intermittent doses of thalidomide for preventing recurrences of oral and esophageal aphthous ulcers in HIV-infected patients (80). Forty-nine HIV-infected patients whose ulcers previously had healed as a result of thalidomide therapy were randomly assigned to receive either 100 mg of oral thalidomide or placebo three times per week for six months. Ulcers recurred in 61% of thalidomide-treated patients compared to 42% of placebo treated patients. The time to recurrence was not signiÞcantly different between groups. A randomized, placebo-controlled trial of thalidomide in 103 patients with HIV-associated cachexia (81) at doses of 100mg/day or 200mg/day for eight weeks demonstrated a signiÞcant increase in weight (2.2kg, $p=0.008$ and 1.5kg, $p=0.019$, respectively), in each thalidomide group compared to controls. Approximately half of this weight gain was fat-free body mass (bioimpedance analysis). There was an increase in viral load in the treatment group compared to controls, with no signiÞcant changes in CD4+ or CD8+ cell counts. More recently, other uses of thalidomide have been explored. Thalidomide was not able to decrease the toxicities or transient viral load increases seen with IL-2 (82). In another study, twenty patients aged 29 to 49 years with a median CD4 count of 246 cells/mm^3 (range, 14 to 646 cells/mm^3) and taking antiretroviral therapy were enrolled in a phase II dose-escalation study of oral thalidomide in Kaposiõ sarcoma (KS) (83). The median thalidomide dose at the time of response was 500 mg/d (range, 400 to 1,000 mg/d). Oral thalidomide was tolerated in this population at doses up to 1,000 mg/d for as long as 12

months. The median duration of drug treatment was 6.3 months, and the median time to progression was 7.3 months. Partial anti-KS responses were noted in eight of 17 evaluable patients (47%).

Overall, thalidomide appears to have a role in the treatment of HIV-associated oral ulcers. Additional work is needed to determine if there is beneÞt in other settings in HIV-infected patients. ThalidomideÕs serious teratogenic effects and its effect on the viral load require a careful selection of patients as well as frequent follow-up.

Therapeutic Vaccines

Among the candidate vaccines that have been evaluated in HIV-infected individuals are a series of recombinant envelope and gag proteins; killed virus; recombinant viruses and DNA vaccines. Vaccine research for HIV has two potential areas of application: prevention and therapy of HIV infection. Therapeutic vaccines for HIV infection are intended to promote HIV-speciÞc responses that would theoretically lead to better immunologic control of the disease and hopefully reduce the need for antiretrovirals in individuals already infected with HIV.

Recombinant Envelope Proteins

Several candidate envelope vaccines have been evaluated in HIV infected patients. In most cases, HIV-speciÞc proliferative T cell responses were induced. However, clinical beneÞt measured by disease progression, or signiÞcant drop in viral loadÑ the only surrogate marker of antiviral activity validated so farÑ has not been observed.

A large phase II efÞcacy trial with HIV-1 recombinant envelope glycoprotein gp160 (rgp 160) in 608 HIV-infected, asymptomatic individuals with CD4+ T cell counts >400 cells/mm^3 was reported this past year. This trial was multicenter, randomized, placebo-controlled and double blind, with a Þve year follow-up. It was designed to evaluate the time to AIDS deÞning events and/or mortality. The vaccine was given intramuscularly at days 0, 7 and then monthly at months 1, 2, 4 and 6 initially and every four months thereafter (84). Previous use of AZT for >1 year or within three months of study entry were exclusion criteria. After day 240, all approved antiretroviral drugs could be used. There were no signiÞcant differences in time to antiretroviral use between groups. Even though the study had a 17% loss of follow-up, there was sufÞcient power (90%) to detect 50% reduction in the primary clinical outcomes at the conclusion of the study. There was no statistically signiÞcant difference in time to primary endpoint between vaccine and placebo groups ($p=0.94$). Time to secondary endpoints (30% decline in mean on-study CD4+ T cell count from baseline) was not signiÞcantly different between arms ($p=0.23$). While increases in median viral load were noted for the whole cohort (3.91 to 4.22 logs over the observation period, $p=0.0001$ compared to baseline), there were no differences between study arms at any time point. Cellular immune responses, measured by lymphoproliferative assays, increased in the vaccine group from 31% at baseline to 88% at the end of year 1. Cellular reactivity was unchanged in the placebo arm. Overall, the vaccine was well tolerated without signiÞcant serious adverse events. Two other large phase II placebo controlled randomized studies of rpg160 have also been recently reported. One included 278 subjects with CD4 counts >500. The majority of them were on no antiretroviral therapy. Approximately 20% of subjects in each group were receiving AZT monotherapy (85). The other study involved 835 HIV-1-infected patients with CD4 counts >200. In this study, approximately 50% of subjects in each group were receiving antiretroviral therapy (86). The Þrst study showed no differences in the rate of CD4 decline, plasma RNA viral load, incidence of opportunistic infections or death at two year follow-up. The second study showed a higher CD4 count among vaccine recipients at six months than at baseline compared to those in the placebo group and there was a reduction in mortality at the two year follow-up in the vaccinees. However, results at the three-year follow-up failed to reveal this advantage. There were no differences in the rate of CD4 cell count decline, progression to AIDS or HIV viral load at that point.

In a recent review, the results from two studies, ACTG 209 and ACTG 214 (87), were reported. These studies were designed to compare the safety and immunogenicity of several candidate recombinant HIV-1 envelope vaccines and adjuvants. They were conducted between 1993 and 1995. Overall, immunogenicity was detected in $<30\%$ of subjects and there were no signiÞcant differences with placebo arms regarding disease progression. In most patients there was evidence of ongoing viral replication. A correlation between the baseline plasma HIV-1 RNA and the ability to elicit new cellular immune responses was demonstrated in these studies. In view of the partial immune restoration and improved T cell homeostasis achieved with HAART there is renewed interest in these approaches in patients with sustained viral load suppression.

A new approach to develop more potent and broadly directed antibodies is the generation of ÒfusionÓ-competentÓ immunogens (88). These have been generated by Þxing cells in the transient stage of fusion, involving the interaction of envelope, CD4 and coreceptor. In murine studies, high levels of cross-reactive neutralizing antibodies have been elicited. No clinical studies in humans have been developed yet.

Remune

One of the most extensively studied therapeutic vaccine candidates is the HIV-1 Immunogen, *Remune*, a gp120

depleted whole virus vaccine. Initial studies demonstrated enhanced HIV-p24-speciÞc lymphocyte blast transformation responses in HIV-infected patients immunized with Remune. Unfortunately, a phase III clinical endpoint trial of this candidate vaccine failed to prove differences in clinical progression or death between the treatment arm and placebo controls (89). This study was designed to assess the immune effects of the addition of HIV-1 Immunogen to patients on antiretroviral therapy by comparison with a antiretroviral-only treated control group receiving placebo injections. A total of 2,527 patients were randomized (1,262 received Immunogen and 1,263, IFA placebo). Entry CD4 T cell counts ranged between 300Ð549 x 10^6/L. There were no viral load entry criteria. The primary endpoint was AIDS-free survival (time to development of AIDS deÞning illnesses or death). At the time of randomization, 27% of the subjects were not receiving antiretrovirals, 5% were on monotherapy, 37% on dual therapy and 31% were on a protease inhibitor. During the course of the study the use of PIs increased from 31% to 59%. Changes in antiretroviral therapy were similar between treatment groups. Up to 13 vaccinations were administered intramuscularly at 12 weeks intervals. The mean followup was 119 weeks in the treatment group and 121 in the placebo group. The loss to follow-up was similar for both groups (approximately 18%). Even though the HIV-1 Immunogen elicited at least a Þve-fold increase in the lymphocyte proliferation assay to HIV-1 p24 antigen at week 24 and week 48 (45% and 34% of patients randomized to HIV-1 Immunogen, respectively, compared with 1% and 1% in the control group, ($p = < 0.001$ for both comparisons), there were no signiÞcance differences between the HIV-1 Immunogen and control group with respect to changes in viral load ($p = 0.59$) or clinical progression (53 events in each group, $p = 0.89$). There was a statistically signiÞcant increase of 10 CD4 T cells at 48 weeks in the treatment group compared to the control group ($p = 0.02$). A total of 42 subjects died, 23 in the HIV-1 Immunogen group and 19 in the control group ($p = 0.49$). While it failed to demonstrate a beneÞt of therapeutic immunization, this study provided important new information on the rate of disease progression after the introduction of protease inhibitors. The rate of 1.8 per 100 person-years was one-third of what had been previously reported in the literature and has important implications for ongoing studies with clinical endpoints in patients with early disease. Several additional trials of HIV-1 Immunogen with a modiÞed design are either in progress or planned to reassess the efÞcacy of this approach.

Poxvirus Vaccines

Recombinant Canarypox vectors, which are similar to vaccinia virus but have a limited ability to replicate in human cells, are being evaluated in human trials. The vectors may express sequences from env, gag and pol and CTL epitopes from nef and pol. Data on immunogenicity or viral load are yet available. Studies in HIV negative persons have shown that these vectors are able to induce CD4 and CTL responses as well as antibodies and that these responses are more robust in regimens that include a boost with recombinant protein vaccines (90).

Polypeptide Vaccines

This vaccine design strategy involves the use of polypeptide sequences from HIV-1 selected for their capacity to induce CTL responses and/or neutralizing antibodies. At least two vaccine candidates in this category, PCLUS and C4-V3 PV, are being studied in phase I trials for safety and immunogenicity. PCLUS are synthetic envelope cluster peptides. In a study of eight HIV-infected patients who received six subcutaneous injections, HIV-speciÞc T helper responses increased greater than four fold in four of eight patients, with smaller increases in three other patients at 36 weeks (adjusted $p < 0.05$) (91). Serum HIV-1 MN-neutralizing antibody titers increased in each of the three patients who had low titers prior to immunization. Plasma HIV RNA levels and CD4 cell counts did not change signiÞcantly during the study period.

C4-V3 PV is an immunogen that consists of four peptides containing T-helper epitopes from the fourth constant region (C4) of gp120 of the HIV-1 MN virus and B-cell neutralizing epitopes from the gp120 third variable region (V3) of four clade B viruses. In one study of 10 HIV-1-infected patients, (92) follow-up at 52 weeks showed a four-fold rise in serum neutralizing antibody titers in four out of eight subjects that received the vaccine. Five out of eight had a Þve-fold rise in peripheral blood mononuclear cells lymphocyte blast transformation assays. No signiÞcant changes in CD4 T cell counts or viral load were observed during this follow-up.

p24 VLP

The immunogen p24 VLP, contains codons 100Ð308 of the gag component of HIV-1. This agent has been studied in a phase II randomized, double-blind placebo controlled phase II trial (93). Sixty-one patients with CD4 > 400 received vaccinations with or without AZT monotherapy. At both 24 and 52 weeks there were no signiÞcant differences between the treatment groups in terms of antibody responses to p24, CD4 T cell counts or viral load, despite induction of antibody and proliferative responses to the carrier protein of the vaccine.

DNA Vaccines

DNA vaccines are currently being studied in phase I trials. The potential advantage of this strategy over others is that it allows expression of whole proteins from HIV

(gag, pol, tat, nef and rev, for example) by the body's own cells and thus facilitate processing via MHC I pathways. A large number of epitopes (small peptides) can be encoded in these plasmids and thus be available to produce a stronger immune response with a prolonged persistence of the antigens. Human trials of a DNA-based vaccine have been conducted in small groups of patients. In one study, (94) nine HIV-1-infected asymptomatic patients were immunized with DNA constructs containing the nef, rev or tat genes of HIV-1. The same vaccine was used in another study of nine patients, four of whom received concomitant HAART (95). Both studies have shown enhanced specific lymphocyte proliferative and cytotoxicity functions in the vaccinees, without significant changes in viral load or CD4 counts. Larger studies are needed to assess these responses and the impact of the use of HAART. DNA vectors that encode regulating cytokines as well as immunogenes are also being studied as a strategy to enhance immune responses.

Recombinant live attenuated bacterial or viral vectors, such as BCG, salmonella and rabies, can be engineered to contain fragments of HIV that are recognized by T cells during vector replication *in vivo*. This approach would potentially increase the number of epitopes available and the time of antigen exposure. These vaccines are currently in preclinical phases of development. Whether or not the exogenous administration of antigens will lead to a better immune resposne than that elicited during the course of natural infection remains to be determined.

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Chapter 38

Immunizations, Vaccine-Preventable Diseases, and HIV Infection

Allyn K. Nakashima and Ida M. Onorato

Advances in antiretroviral therapy for human immunodeÞciency virus (HIV) infection have delayed progression to the acquired immunodeÞciency syndrome (AIDS) and reduced mortality (1). There is evidence of partial immune restoration after therapy, and this may have impact on response to immunizations (2Ð4).As HIV infection takes on the characteristics of a chronic disease and the incidence of AIDS and opportunistic infections declines, other causes of morbidity and mortality, including vaccine-preventable diseases, may become more important.

Immunization is generally thought to be safe and beneÞcial for persons infected with HIV. The Þrst recommendations for immunization for HIV-infected children and young adults were published in 1986 (5) and revised in 1993 (6). Current recommendations for routine immunization of children including those infected with HIV have been recently updated and are shown in Table 38.1 (7,8). This chapter focuses on recommendations for adults. The American College of Physicians and the Advisory Committee on Immunization Practices (ACIP) have published recommendations for immunization of adults, including those who are HIV-infected (6,9,10). In addition, the U.S. Public Health Service and Infectious Diseases Society of America Prevention of Opportunistic Infections Working Group has updated recommendations for prevention of opportunistic infections, including those that are vaccine preventable (11). Generally recommended vaccines at present for HIV-infected adults and adolescents include 23-valent polysaccharide pneumococcal vaccine, inactivated trivalent inßuenza virus vaccine, hepatitis A vaccine, and hepatitis B vaccine.

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TABLE 38.1. *Recommendations for routine immunization of children with HIV infection, United States**

Vaccine	Recommended
DTaP	Yes
OPV	No
IPV	Yes
MMR	Yes[a]
Hib	Yes
Pneumococcal	Yes
In uenza	Yes
Hepatitis B	Yes
Hepatitis A	Yes[b]
Varicella	Possibly[c]
BCG	No

* See References 7 and 8. DTaP, diphtheria and tetanus toxoids and acellular pertussis vaccine; OPV, poliovirus live oral, trivalent: contains poliovirus types 1, 2, and 3 (OPV is no longer recommended for routine immunization of any children in the United States.); IPV, inactivated poliovirus vaccine: contains poliovirus types 1, 2, 3; MMR, measles, mumps, and rubella virus vaccine; Hib, *Haemophilus inßuenzae*, type b; BCG, bacille Calmette-Guérin vaccine.

[a] The Advisory Committee on Immunization Practices (ACIP) currently recommends withholding measles vaccine from HIV-infected persons with severe immunosuppression, de ned as 1) CD4 T-lymphocyte counts < 750 for children aged < 12 months, < 500 for children aged 1–5 years, or < 200 for persons aged □6 years; or 2) CD4 T-lymphocytes constituting < 15% of total lymphocytes for children aged < 13 years or < 14% for □13 years. MMR is recommended for HIV-infected persons without evidence of measles immunity who are not severely immunocompromised (25).

[b] ACIP recommends hepatitis A vaccine for routine use in some states and regions and for persons in certain high risk groups (e.g. travelers to endemic areas) (7).

[c] ACIP recommends that varicella vaccine should not be administered to persons who have cellular immunode ciencies, including HIV. However, after weighing potential risks and bene ts, varicella vaccine should be considered for asymptomatic or mildly symptomatic HIV-infected children in CDC class N1 or A1 with age-speci c CD4 + T-lymphocyte percentages > 25% (252).

In spite of the substantial morbidity and mortality of hepatitis B, inßuenza, and pneumococcal disease in older age groups, immunization levels of recommended vaccines for adults in general have been low (12). Immunization coverage among HIV-infected persons and those at risk for HIV is also low. In a study conducted during 1994Ð1998, only 9% of 3,432 MSM aged 15Ð22 years, a group for whom hepatitis B vaccine has been recommended since 1982, had been immunized (13). An ongoing medical record review study of HIV-infected adults and adolescents in care in over 100 health care settings found that only 14% of 13,012 persons had received >1 dose of hepatitis B vaccine (14). In other analyses from the same surveillance cohort, 40% of 19,081 persons had received inßuenza vaccination during follow-up (15) and 38% of 30,086 persons had documented receipt of pneumococcal vaccine (16); there was no temporal trend in vaccination rates. The increasing numbers of persons living with HIV infection in the United States (17) and their prolonged life expectancy necessitate an understanding of the epidemiology of vaccine-preventable diseases in this group and a renewed priority on immunization.

Several concerns about immunizing HIV-infected persons have been raised: the safety of the vaccines themselves; potential adverse effects on the progression of HIV infection; the indications for vaccines; and the immunogenicity and efÞcacy of vaccines in this population. These issues are reviewed in this chapter.

SAFETY OF IMMUNIZATION OF HIV-INFECTED PERSONS

Historically, live virus (e.g. oral poliovaccine (OPV); measles, mumps, and rubella (MMR) vaccine; smallpox vaccine) and live bacterial (i.e. Bacille Calmette-GuŽrin (BCG)) vaccines have not been recommended for immunocompromised persons because replication of live, attenuated agents may be enhanced, while inactivated bacterial and viral vaccines (e.g. diphtheria and tetanus toxoids and acellular pertussis vaccine (DTaP), inßuenza vaccine, pneumococcal vaccine, *Haemophilus inßuenzae* type b vaccine (Hib), inactivated poliovaccine (IPV), hepatitis A vaccine (HAV), and hepatitis B vaccine (HBV)) are recommended. In the 1960s, for example, a child with leukemia immunized with a less attenuated strain of measles vaccine than is used in the United States today developed measles pneumonia (18).

Vaccine-associated poliomyelitis, like infection with wild poliovirus, is more frequent and severe in persons with immunologic abnormalities (e.g. severe combined immunodeÞciency syndrome, agammaglobulinemia, or hypogammaglobulinemia) (19,20). However, only two cases of vaccine-associated paralytic poliomyelitis in HIV-infected children have been documented in the literature (21,22), and a case-control study in Zaire suggested that HIV infection may not be a risk factor for paralytic poliomyelitis (23). Current guidelines recommend that OPV not be administered to persons with immune systems compromised by HIV, or given to their healthy household contacts (24). In addition, as poliomyelitis control has been achieved in the United States, the ACIP has recommended exclusive use of the inactivated vaccine (IPV) essentially eliminating the problem of vaccine-associated paralytic poliomyelitis in this country (24). (OPV is recommended only under certain circumstances, e.g. outbreak control.) In contrast, extensive experience has shown that the attenuated viruses in MMR are not transmissible to close contacts. Therefore, all family and other close contacts of HIV-infected persons should be vaccinated with MMR vaccine, unless they have been previously immunized (25).

A corollary concern is the risk of vaccine-associated poliomyelitis in HIV-infected household contacts of recently immunized children. Because most HIV-infected children acquire HIV prenatally, their mothers are infected as well. Vaccine poliovirus strains are excreted by healthy vaccinees for up to eight weeks, but viral shedding may persist for years in some immunodeÞcient persons (26). Liste et al., in a study of HIV-infected children in Venezuela, although they concluded that there was Òno evidence of prolonged excretion of poliovirusÓ, two of 11 HIV-infected children who had received OPV were excreting poliovirus Þve to nine months after their last dose of OPV (27). Studies are being conducted to further assess the likelihood of prolonged poliovirus excretion in HIV-infected children and adults in countries where OPV continues to be used (28).

The safety of immunization in HIV infection has been studied mostly in children. The New York City Department of Health conducted a retrospective survey of 221 HIV-infected children with valid immunization records to evaluate the frequency of vaccine-associated adverse events (29). No adverse reactions were noted after receipt of a total of 468 doses of OPV and 70 doses of MMR or measles vaccine, some of which were given after the onset of AIDS. Prospective studies of cohorts of children born to HIV-infected mothers in Africa, the United States, and other countries provide further evidence of the safety of vaccines in HIV-infected children. For example, in the best studied cohorts in Zaire and Rwanda, 656 children with HIV infection and 1,088 non-infected children were vaccinated with BCG, DTP, OPV, and/or measles vaccine and observed for adverse events (30,31). Rates of common adverse reactions, speciÞcally fever and rash, were similar in both groups of children and no serious reactions were noted. National surveillance in the United States for adverse reactions to immunizations noted no increase in serious adverse events due to measles vaccine and no cases of vaccine-associated poliomyelitis in HIV-infected persons in the United States (CDC, unpublished data). An investigation has documented progressive vaccine-associated measles pneumonitis in a 20 year old AIDS patient

who received a second dose of measles vaccine to fulfill college requirements (32,33). The patient was severely immunosuppressed (CD4 T-lymphocytes "too few to enumerate"), although he was asymptomatic at the time of his vaccination one year earlier. The measles virus isolate recovered from the patient's lung tissue was genetically identical to the Moraten vaccine strain that he had received. Because of this case, evidence of diminished antibody response to measles vaccine among severely immunocompromised HIV-infected persons, and morbidity related to measles vaccination of severely immunosuppressed persons without HIV, the ACIP does not recommend measles vaccine for severely immunosuppressed HIV-infected persons (25). Severely immunocompromised patients and other symptomatic HIV-infected patients who are exposed to measles should received immune globulin prophylaxis regardless of vaccination status because they may not be protected by the vaccine (25). Serious illness associated with administration of rubella or mumps vaccines to HIV-infected persons has not been reported (25).

Adverse effects from vaccination have occurred in HIV-infected adults receiving vaccines not currently recommended for general use in the United States. An asymptomatic HIV-infected military recruit who received smallpox vaccine during basic training, later developed disseminated vaccinia and AIDS (34). Disseminated *Mycobacterium bovis*-BCG disease and regional lymphadenitis have occurred in persons with HIV infection who received BCG (35–38).

HIV-infected adults have been immunized with influenza and pneumococcal vaccines, as well as multiple other simultaneous vaccines, in a number of prospective studies. No unusual or more frequent adverse reactions have been observed in the immediate postvaccination period among HIV-infected persons (39–41). However, a recent large randomized, double-blind, placebo-controlled study of 23-valent pneumococcal vaccine in 1,392 HIV-infected adults in Uganda showed excess pneumonias in the vaccine recipients, including pneumonias with vaccine serotypes, and concluded that the vaccine should not be recommended in sub-Saharan Africa (42). The authors suggested a direct harmful effect of pneumococcal polysaccharides on polysaccharide-responsive B-cell clones as a possible cause. The HIV-infected population in sub-Saharan Africa where antiretroviral therapy is generally unavailable may be substantially different from the HIV-infected population in the United States. In addition, observational or case-control studies in the United States have not shown increased risk of pneumonias associated with pneumococcal vaccination and have shown benefit in HIV-infected populations with variable access to antiretroviral therapy (16,43,44). Therefore, most experts in this country believe the potential benefit of pneumococcal vaccination outweighs the risk and continue to recommend its use (7,11).

Data suggest that chronic hepatitis B surface antigen (HBsAg) carriage may be an unexpected consequence of hepatitis B vaccine in HIV-infected adults. In one study, HIV-infected men who received vaccine at the time they developed new hepatitis B infection were at increased risk of developing chronic HBsAg carriage compared to exposed unvaccinated HIV-infected men (45). Eighty percent of those who received their first dose and 56% of those who received a second dose at the time of hepatitis B infection became carriers compared with 21% of exposed unvaccinated men. This suggests that hepatitis B vaccine may temporarily impair the immune response to hepatitis B virus infection in HIV-infected men.

INFLUENCE OF VACCINATION ON HIV REPLICATION AND DISEASE PROGRESSION

Concern has been expressed that simultaneous administration of multiple antigens (even inactivated vaccines) may accelerate the progression of HIV disease by increasing HIV replication and activating CD4 cells (46,47). Retrospective review of clinical disease in a group of HIV-infected children who had received routine immunizations did not reveal any correlation between the number of vaccines received and subsequent progression to AIDS (29). Historically, prospective follow-up of HIV-infected men immunized with influenza and pneumococcal vaccines who received multiple simultaneous vaccines showed no clinical deterioration, although the observation periods were short (one to six months) for the majority of vaccinees (39,41,48,49). A cohort of 489 homosexual men enrolled in hepatitis B vaccine trials in 1978–1980 were retrospectively studied to determine progression to AIDS following hepatitis B infection or vaccination after seroconversion to HIV. When controlling for duration of HIV infection, there were no significant differences between 30 HBsAg carriers and non-carriers, 22 hepatitis B-infected and non-infected men or 56 hepatitis B vaccine recipients versus unvaccinated men in rates of progression to AIDS (50).

Laboratory indicators of increased HIV replication or adverse effect on immune function following vaccination have been studied by many investigators. Vaccination of 13 HIV-infected adults with a booster dose of tetanus toxoid resulted in activation of the immune system and transient increase in the expression of the HIV (47). Increases in HIV viral load following hepatitis B vaccination were not seen in ten patients who had moderate-to-severe immunosuppression (51). However, in another study, 20 patients with CD4 cell counts $>200/\mu l$ and receiving antiretroviral therapy were given standard hepatitis B vaccination (three doses), which was repeated (additional three doses) if they did not respond to the initial vaccination; transient viral load increase was seen in three patients, and a sustained increase in two patients (52). Increases in HIV viral load have been noted after

inßuenza or pneumococcal vaccination by some investigators (53Ð58). A double-blind trial of 47 HIV-infected patients randomized to receive inßuenza vaccine or saline placebo showed that Þve of 23 vaccine recipients had clinically signiÞcant increases in viral load compared with only one of 24 placebo recipients in at least one follow-up measurement up to one month after vaccination (58). However, a later trial by the same group that included 102 HIV-infected patients found no effect of inßuenza vaccination on plasma HIV-1 RNA levels or CD4+ cell counts (59). Other investigators have also found no increases in viral load following pneumococcal or inßuenza immunization (60Ð66). Viral load increases, when observed, appeared to be transient in most patients and did not appear to affect disease progression (55,64,65). More recent studies of HIV-infected patients undergoing highly active antiretroviral therapy (HAART) suggest that inßuenza (67Ð70) and pneumococcal (71) vaccination is safe and does not appear to induce any clinical deterioration. For example, Kroon et al. showed that viral load measured before, and nine and 30 days after, inßuenza vaccination was not increased in 13 of 17 patients on HAART with baseline viral loads of <400 copies/ml; of the remaining four, none had signiÞcant clinical change after administration of inßuenza vaccine (67). However, one study of 34 patients with undetectable viral loads at the time of inßuenza vaccination showed that seven had increased viral load after vaccination. Two of these patients had evidence of mutational changes in the HIV viral genome that might have contributed to resistance to antiretroviral therapy, and one patient failed therapy after vaccination (72). Although the effect on viral load of one immunization may be minimal, another concern has been the safety of reimmunization. A study of reimmunization with pneumococcal vaccine did not show changes in CD4+ cell counts or increases in viral load (73). A study of immune response to varicella vaccine in HIV-infected persons (who never had CD4+ cell counts <400) previously infected with varicella, found live attenuated varicella vaccine to be safe (74).

Similar concerns have been raised about reduction in CD4+ cell counts or increase in frequency of HIV-infected CD4+ cells following vaccination. Nine of 10 HIV-infected persons had a transient increase in frequency of infected CD4+ cells two weeks after inßuenza vaccination, which was greater in persons with high viral loads (75). A one-year prospective study of 90 HIV-infected men who received hepatitis A vaccine compared with 90 HIV-infected men (matched for CD4+ lymphocyte percentage at baseline) who were not vaccinated showed no differences in development of AIDS, deaths, or mean decline in CD4+ cell counts (76). Some studies have shown declines in CD4+ counts in HIV-infected adults vaccinated with pneumococcal or inßuenza vaccine (54,58), while others have not (57,59,61Ð66). A large observational cohort study of more than 36,000 HIV-infected persons showed no negative long-term effect of inßuenza vaccine on CD4 counts, HIV RNA levels, or progression to AIDS or death (15). They concluded that physicians should not withhold inßuenza vaccine because of concerns about long-term detrimental effects on disease progression.

In summary, transient viral load increases and CD4 declines shortly after immunization with some vaccines might be expected as a part of the normal immunologic response to antigenic stimuli and may be variably detected depending on the timing of follow-up examinations. At present, the effect on disease progression appears minimal. Nevertheless, vaccines may cause harm to HIV-infected persons. Therefore, the risks and beneÞts must be carefully weighed; in doing so, most experts and the ACIP continue to recommend inßuenza, pneumococcal, hepatitis A, hepatitis B, diphtheria, and tetanus as safe for most HIV-infected adults (11,77Ð81).

VACCINE-PREVENTABLE DISEASES IN HIV-INFECTED ADULTS

HIV infection is associated with profound defects in cell-mediated and humoral immunity, resulting in increased susceptibility to a variety of infectious agents.

Pneumococcus and *Haemophilus inßuenzae*

Polyclonal activation of B cells caused by HIV and the resulting decreases in speciÞc antibody responses and opsonization render HIV-infected persons susceptible to infection with the encapsulated bacteria, *Streptococcus pneumoniae* and *Haemophilus inßuenzae*. The increased incidence and severity of these infections in HIV-infected adults have been documented by examining mortality reports and pneumonia and inßuenza surveillance data. U.S. death rates for pneumonia and inßuenza (excluding *Pneumocystis carinii* pneumonia) in men 25Ð44 years had been declining for two decades until 1981. After 1981, rates increased each year with a 176% increase in deaths attributable to pneumonia or inßuenza in cities with the highest AIDS incidence (82). Pneumococcal and *H. inßuenzae* pneumonias were reported in homosexual men and intravenous drug users with AIDS early in the epidemic (83Ð85). The Þrst case reports of invasive pneumococcal disease in persons with AIDS, in 1984, also raised a question about the efÞcacy of pneumococcal vaccine in AIDS patients since one of the Þve cases had recently been immunized (83). Estimates of the annual incidence of community-acquired pneumococcal pneumonia in patients with symptomatic HIV infection were 17.9/1,000, 45.5/1,000, and 95/1,000 in three hospital-based clinical case series (83,86,87). These rates were several-fold higher than those in the general population or in non-AIDS patients admitted to the same hospitals (75,78,82). AIDS patients in San Francisco also had a

higher rate of pneumococcal bacteremia (9.4/1,000 person-years) than splenectomized patients (0.92–2.1/1,000 person-years), adults with sickle-cell disease (0.86/1,000 person-years), or community-based rates reported before the AIDS epidemic (7.5–16.4/100,000) (88). In New Jersey, the annual rate of invasive pneumococcal infections among persons 25–44 years was 0.0033% for the general population, 0.53% for persons developing AIDS within three years and 1.07% among AIDS patients (89). It is estimated that the rate of pneumococcal bacteremia among hospitalized patients with AIDS may be more than 100 times higher than that found in age-matched populations (90). Among a cohort of methadone-maintenance clients followed prospectively for one year, asymptomatic HIV-infected clients were five times as likely to be hospitalized with pneumococcal or *H. inßuenzae* pneumonia than HIV-seronegative drug users (91).

Since the advent of HAART, the incidence of invasive pneumococcal disease may have declined. A study of AIDS patients in San Francisco showed a decline in incidence from 10.6 to 4.2 cases of invasive pneumococcal disease per 1,000 person-years between October 1994 and June 1997; the incidence among persons without known HIV infection remained relatively stable during this time period at about 0.2 cases per 1,000 person-years (92). In this study, however, persons with HIV infection accounted for 54% of patients aged 18 to 64 years who had pneumococcal disease, and the risk for invasive pneumococcal disease for persons with AIDS was 46 times higher than for persons without known HIV infection. A study in Baltimore, Maryland, of 1,898 HIV-infected persons with CD4+ cell counts $<200/\mu L$ showed a decreased incidence of bacterial pneumonia from 22.7 episodes/100 person-years in 1993 to 9.1 episodes/100 person-years in 1997; use of protease-inhibitor containing antiretroviral regimens was associated with a decreased risk of bacterial pneumonia (93). In New York City, a study of hospitalization rates of HIV-infected persons showed that the incidence of hospitalizations with bacterial pneumonia decreased steadily between 1995–2001 from nearly 14 to three diagnoses per 100 patient-years (94). A study conducted in a hospital in Australia between 1996 and 2000 among HIV-infected patients, most of whom were on antiretroviral therapy, showed an incidence of invasive pneumococcal disease of only 1.9 per 1,000 person years. Because of low incidence and low vaccine efficacy (16 of 34 patients with invasive, presumptive or possible pneumococcal disease had been vaccinated), the authors suggested that the recommendation for routine pneumococcal vaccination of HIV-infected persons in Australia should be re-examined (95). In contrast, a large study of HIV-infected persons in care in the United States, showed an incidence of invasive pneumococcal disease of 8.2/1,000 person-years during 1990–1998, a period that spans the introduction of HAART; no significant decline in incidence was seen between 1994 and 1998 (16,96). Factors associated with an increased risk for invasive pneumococcal disease in that study included injection drug use, blood transfusion, black race, AIDS, and alcoholism; factors associated with a decreased risk included antiretroviral therapy and pneumococcal vaccination. A retrospective review of charts of HIV-infected patients seen in a pulmonary and critical care medical service showed that overall visits declined and *Pneumocystis carinii* pneumonia was less commonly diagnosed in persons taking HAART compared to the pre-HAART era (18% vs. 36%), while bacterial pneumonia was more common (47% vs. 29%) (97). In an Italian study comparing community-acquired bacterial pneumonia pathogens from 149 HIV-infected patients in the pre-HAART era (1995–1996) to 130 HIV-infected patients in the post-HAART era (1997–1998), both *H. inßuenzae* (4.7% vs. 6.1%) and *S. pneumoniae* (3.4% vs. 6.9%) were more commonly isolated post-HAART, although these differences were not statistically significant (98). In a county-level analysis of data from the Centers for Disease Control and Prevention's Active Bacterial Core Surveillance of Emerging Infections Program Network, the number of cases of invasive pneumococcal disease among adults 18–64 years of age, was directly associated with higher number of AIDS patients and larger total black population (99).

Two population-based studies have reported annual cumulative incidence rates of *H. inßuenzae* disease in HIV-infected persons. The study conducted in San Francisco reported rates of 79.2/100,000 in men with AIDS and 14.6/100,000 in those with HIV infection that had not progressed to AIDS (100). A study conducted in Atlanta reported an annual incidence of 41/100,000 in HIV-infected men regardless of AIDS status (the corresponding rate from the San Francisco study was 22.7/100,000) (101). The observed rate of 79.2/100,000 in men with AIDS in San Francisco is approximately 100 times the rate of invasive *H. inßuenzae* disease in the general adult population (102,103). Serotyping of isolates from the San Francisco study revealed that 33% were type b, and thus potentially preventable by current vaccines. In a study of 26 HIV-infected patients with *H. inßuenzae* pneumonia, 73.1% had a CD4+ cell count $<100/\mu L$ and mortality was 11.5% (104).

Clinical complications and recurrent infections are also more frequent in HIV-infected persons. In one study, AIDS patients with pneumococcal pneumonia had a survival rate of less that 50% compared to 75% for patients without HIV infection (105). Pneumonia due to penicillin-resistant pneumococci has also been reported (106, 107). In a study of colonization by *S. pneumoniae,* 25 of 34 isolates from 102 HIV-infected persons were penicillin-resistant compared with one of eight isolates from 39 non-HIV-infected controls (108). Mortality following invasive pneumococcal disease may be increased in HIV-infected persons depending on degree of immunosuppression. In a study of patients with pneumococcal bacteremia, 44 of 111 men and three of 41 women seen between 1989 and 1994 were

HIV-infected; 8.5% of HIV-infected patients and 3.8% of other patients died (109). A study of mortality from invasive pneumococcal pneumonia during 1995–1997 showed that AIDS (but not HIV infection without AIDS) was among the underlying diseases associated with elevated mortality; mortality was not elevated in most infections with β-lactam-resistant pneumococci (110). In contrast, a review of 153 episodes of invasive pneumococcal disease showed that HIV-infected persons had an increased rate of recurrence (15% vs. <1%); overall mortality was 12%, but this did not vary by HIV serostatus (111). An examination of U.S. death certificates showed dramatic overall declines in deaths with HIV or AIDS diagnoses after 1995 but found that the proportion of these deaths with unspecified pneumonia (non-*Pneumocystis carinii*) remained stable at about 14% (1% was specified as bacterial pneumonia) (112).

Thus, although the incidence of invasive disease due to *S. pneumoniae* and *H. influenzae* may be declining in the era of HAART, these infections remain an important cause of morbidity and mortality for HIV-infected persons. Effective prevention (if possible) of pneumococcal and *H. influenzae* infections would substantially decrease morbidity and mortality (83–86,90,103–115).

Pertussis, Influenza

Although *Bordetella pertussis* is increasingly recognized as a cause of respiratory illness in adults, and immunocompromised persons are known to be at risk of complications of influenza, these pathogens have rarely been reported in HIV-infected patients (116,117). Several case reports document the isolation of *B. pertussis* from HIV-infected persons with persistent cough which initially was incorrectly attributed to other respiratory pathogens (118–120). However, despite the increasing recognition of *B. pertussis* as a cause of respiratory disease in adults, pertussis appears to be rarely diagnosed in HIV-infected adults. In addition, HIV-infected persons do not appear to be a persistent reservoir for *B. pertussis* in the community. Among 60 HIV-infected adults, of whom 20 had a cough illness lasting >14 days, none had a positive isolation of *B. pertussis* from nasopharyngeal swabs (121).

Influenza viruses have also been isolated from HIV-infected patients initially suspected of having *Pneumocystis carinii* pneumonia (116,122,123). The increases in mortality in pneumonia and influenza surveillance data noted above occurred in the winter, concurrent with influenza activity, but the relative contributions of influenza and bacterial pneumonia are not known. Case series of HIV-infected patients diagnosed with influenza have been described (124,125). A series of 12 HIV-infected patients followed for five months after influenza was diagnosed showed no negative impact on CD4+ cell count, viral load, or clinical progression (125). Recent studies of HIV-infected persons with influenza showed no unusual clinical manifestations, although the hospitalization rate was higher than for HIV-negative persons (126,127). In a study of women aged 15–64 years, Neuzil et al. found influenza incidence rates and excess acute cardiopulmonary events requiring hospitalization higher among those who were HIV-infected (128). Excess mortality due to pneumonia or influenza has been found during influenza seasons among persons with AIDS (129).

Measles

Measles is known to be severe in immunocompromised persons with defects in cell-mediated immunity, including HIV-infected children both in the United States and in developing countries (130–132). Severe complications of measles include pneumonitis, measles-associated neurologic disorders (acute and subacute encephalitis), and myelopathy (133–137). Measles and measles-associated deaths in HIV-infected adults have been reported (133–138). Seven of 19 persons who died from measles complications in New York City during 1990–1991 were HIV-infected (138). A review of measles in HIV-infected persons reported the absence of rash in 27% of cases, and documented a case-fatality rate of 33% (136).

Because of the current epidemiology of measles in the United States, characterized by recurrent outbreaks of measles in inner city areas, HIV-infected persons may be at increased risk of exposure to measles. In 2000, 86 measles cases were reported in the United States; 57% were reported from three states—New York, California, and Nevada (139). Most cases were epidemiologically linked to international sources and the majority were in persons ≥5 years of age. In a review of seroprevalence studies of measles in HIV-infected adults, detectable measles antibody prevalence ranged from 79% to 99%, indicating that in those with pre-existing measles immunity, antibody is not lost despite progressive HIV-induced immunosuppression (140). Globally, the HIV epidemic has important implications for the control and eradication of measles because of primary and secondary failure of measles vaccine in HIV-infected children and adults, and issues of safety of vaccine for HIV-infected persons, including the theoretical risk that HIV-infected persons could become chronic carriers of measles (140,141).

An interesting phenomenon was recently reported by Moss et al. in a prospective study of 33 HIV-infected children hospitalized with acute measles in Zambia; they found that the median HIV RNA level was 5,339 copies/mL at entry, 60,121 copies/mL at discharge, and 387,148 copies/mL at one-month follow-up, and concluded that HIV replication was transiently suppressed in these children during acute measles (142).

Poliomyelitis

The Western hemisphere has been certified to be free of indigenous poliovirus since 1994, and substantial progress towards eradication has been reported from all regions (28). In the United States, except for religious communities opposed to vaccination, immunity to poliomyelitis is high among adults due to either vaccinations or natural infection, and no cases of poliomyelitis have been reported among HIV-infected adults. However, exposure to poliovirus could occur through reintroduction by visitors to the United States or travel to the developing world. In an outbreak of wild type 1 poliovirus among predominantly unvaccinated gypsy children in Romania in 1991, four (31%) of 13 paralytic cases occurred among HIV-infected children (143). In addition, the first published report of vaccine-associated paralytic poliomyelitis in an HIV-infected child was from Romania in 1993 (21); only one other subsequent case has been reported from Zimbabwe (22). It is uncertain whether HIV infection played any role in the etiology of paralytic poliomyelitis in these six children. One possible explanation in the Romanian outbreak was that they were exposed to a risk factor (e.g. non-sterile intramuscular injections) which placed them at increased risk of both HIV infection and provocation paralytic poliomyelitis (143,144). Recent outbreaks of poliomyelitis (including one in Hispanola) due to vaccine-derived poliovirus have important implications for current and future vaccination strategies for eradication of polio worldwide and stress the importance of maintaining vaccination coverage, especially among travelers to endemic areas (145).

Hepatitis A

Many persons at risk for HIV infection (i.e. men who have sex with men, injecting and non-injecting substance users, and high risk heterosexuals) are frequently also at risk of acquiring hepatitis A (146–153). For example, seropositivity for hepatitis A virus antibody in Baltimore was found in 32% of 300 homosexual men, 66% of injecting drug users and 14% of 300 blood donors (152). A study of 185 homosexual and 70 heterosexual men seen in an outpatient genitoruinary medicine clinic in the United Kingdom showed that 32.4% and 30.0%, respectively, were seropositive for hepatitis A; 48.1% of the homosexual men were HIV-positive compared with 4.3% of the heterosexual men (150).

A description of nine HIV-infected persons with relatively well-preserved immune systems (CD4 count range of 239–774 cells/μL) who acquired hepatitis A all had spontaneous recovery without progression of their HIV disease (154). Antiretroviral therapy was suspended for the seven patients who were receiving it, however, the utility of stopping antiretroviral therapy is not known (155). Increases in HIV viral load and declines in CD4 counts during acute hepatitis A have been observed in HIV-infected patients who were previously well-controlled but stopped their antiretroviral therapy during hepatitis A illness (156,157). Ida et al. reported an outbreak of hepatitis A in homosexual men in Japan; 51 (38%) of 137 homosexual men were positive for anti-HAV IgG antibodies. Twenty-three patients had typical symptoms of acute hepatitis A and 28 were asymptomatic; of the asymptomatic patients, 11 had evidence of recent seroconversion (158). The difficulties of vaccinating adults in high risk groups was illustrated by a survey of 210 men who have have sex with men (MSM) in Georgia following an outbreak of hepatitis A and a vaccination campaign in this community; only 66% were aware of the recent outbreak and of the 178 MSM with no previous vaccination or history of hepatitis A, only 19% received the vaccine during the campaign (159).

Fulminant hepatitis associated with hepatitis A superinfection in patients with chronic hepatitis C has been reported (160). The increased risk for both infections in many HIV-infected persons, this observation further supports hepatitis A vaccination for those with hepatitis C co-infection.

Hepatitis B

As for hepatitis A, behaviors such as illicit substance use and high risk sexual practices that place individuals at risk for HIV infection also increase their risk for hepatitis B, so co-infection is common (161–166). In addition, HIV-infected persons are at increased risk of becoming chronic HBsAg carriers once exposed. Hadler et al. (45) reported that among exposed HIV-uninfected men, 7% became carriers compared with 21% of 14 exposed HIV-infected men. Bodsworth et al. (167) found a similar increased risk of HBsAg carriage in HIV-infected men. Similar findings were reported by Taylor et al. (168); 20% of HIV-infected men became carriers compared with 6% of those without HIV infection. HIV-infected persons also have higher levels of viremia (169–171). These data indicate that the current epidemiology of HIV will have a major impact on the risk of hepatitis B carriage. In contrast to the early years of the AIDS epidemic, many high-risk persons are exposed to hepatitis B after they are infected with HIV, resulting in higher carriage rates and facilitating transmission of hepatitis B. Among HIV-infected patients, co-infection with hepatitis B or C correlates with reduced survival (172). A recent study of the incidence of acute hepatitis B infection in a large observational cohort of HIV-infected persons in care showed that hepatitis B vaccination and prolonged time on antiretroviral therapy were associated with a decreased incidence of hepatitis B (14).

Varicella Zoster

Since over 90% of adults in the United States have serologic evidence of previous varicella zoster virus exposure, reactivation (herpes zoster) is a frequent manifestation of immune compromise in HIV-infected adults, sometimes with a severe clinical course. A serosurvey of HIV-infected adults found that 95% had antibody to varicella, including patients with < 200 CD4 T-lymphocytes (173). Another serosurvey of 281 HIV-infected pregnant women with no history of chickenpox found that only three women had no varicella antibody. One later developed clinical varicella (174). However, primary varicella infection, documented by seroconversion, was reported in nine HIV-infected adults with no history of varicella (175). One fatality occurred in spite of acyclovir therapy. Although four of the nine patients reported household contact with a child with chickenpox and two were infected by other hospitalized patients, postexposure prophylaxis with varicella immune globulin was not given in any of these cases. HIV-infected persons may be at higher risk for development of recurrent zoster (176,177).

Other Vaccine-Preventable Diseases

There have been no reports of mumps, rubella, respiratory diphtheria, or tetanus in HIV-infected persons. Although often considered childhood illnesses, all these diseases have been reported increasingly in adults in recent years and the more serious complications occur in older persons or their fetuses. During 1985–1992, the age distribution of mumps and rubella cases shifted from the under-10 year old age group in the prevaccination era to adolescents and young adults, a group that is relatively underimmunized (178,179). Outbreaks of mumps and rubella on college campuses and in the workplace have led to recommendations for vaccination of susceptible adolescents on college entry and child-bearing women postpartum (178,179). Recently, an increasing proportion of reported rubella cases have occurred in persons of Hispanic ethnicity; therefore, persons from countries without rubella vaccination programs should be evaluated for susceptibility (180). In 1985–1989, 92% of tetanus and 64% of diphtheria patients were adults who were inadequately immunized (181).

IMMUNOGENICITY OF RECOMMENDED VACCINES IN HIV-INFECTED PERSONS

A number of studies have compared immunogenicity (i.e. the serologic response postvaccination) in HIV-infected persons with that in healthy HIV-seronegative controls (Table 38.2). Difficulties comparing studies include different classifications of HIV disease, differences in antiretroviral treatment and response to treatment, different vaccines used, and different serologic measures of immunity reported. In general, these studies have shown that responses are impaired as HIV-induced immunosuppression progresses and that even in responders, antibody levels achieved may be lower than in healthy persons. Responses to immunization attempted after the onset of HIV infection appear to be lower than responses to antigens encountered prior to HIV infection. Vaccine-induced antibody may also decline over time to a nonprotective level as other immunologic functions deteriorate. Pirofski and Casadevall provide an excellent perspective on immunogenicity and use of licensed vaccines in the immunocompromised host (182). With the advent of HAART, the resulting partial restoration of immune function, and the prolonged lifespans of HIV-infected persons, vaccine immunogenicity must be re-evaluated.

Polysaccharide Vaccines

One of the earliest indications of the B-cell dysfunction characteristic of AIDS was the poor response of AIDS patients to immunization with T-cell-independent antigens, such as pneumococcal polysaccharides. Immunogenicity studies in different groups of HIV-infected people showed that fewer HIV-infected persons respond to vaccinations than seronegative controls (Table 38.2). Pneumococcal vaccine failures in HIV-infected patients with or without AIDS have been reported (83,84,183,184). Response rates are higher in asymptomatic HIV-infected persons and those with higher levels of CD4 counts compared to AIDS patients and those with □500 CD4 cells/μL (39,185), however, immunogenicity may not depend on CD4 cell count (186–188). Kroon et al. observed normal antibody levels post-vaccination in HIV-infected individuals, including those with □100 CD4 lymphocytes (186). Recent HIV seroconverters and HIV-infected persons with CD4 count > 500 cells/μL also appear to have normal responses (64,189). There are differences in immunoglobulin responses by class; HIV-infected patients had nearly normal IgG responses, while IgM and IgA responses were depressed (190,191).

In spite of the poorer response of some HIV-infected persons, there are indications that vaccination may be useful. Klein et al. noted that 87% of all HIV-infected intravenous drug users and persons infected via heterosexual transmission had a rise in antibody to greater than 400 ngAbN/ml to one or more vaccine types (40). Overall, protective antibody levels to 35% of capsular types were achieved in HIV-infected intravenous drug users and to 54% of capsular types in their infected sex partners. At one year follow-up, in another study, there were no significant differences in the proportions of asymptomatic HIV-infected homosexual men and HIV-negative homosexual men who retained antibody after vaccination, although the level of antibody was lower in the HIV-infected men (49).

TABLE 38.2. *Immunogenicity of recommended vaccines in HIV-infected patients compared to seronegative adults*

		HIV-infected patients			Controls	
Vaccine	Ref	Stage of HIV-infection	Number tested	Response[a]	Number tested	Response[a]
Diphtheria	41	Asymptomatic	21	70%	21	90%
	270	Asymptomatic	8	3[d]	6	8
	223	100 CD4 cells	23	0.19[c]	16	3.2[c]
		101–300 CD4 cells	10	0.28[c]		
		> 300 CD4 cells	15	0.51[c]		
	189	recent HIV seroconverters	20	1.56[c]	15	2.02[c]
	64	recent HIV seroconverters	14	10.5[d]	9	12.1[d]
Hib	209	Asymptomatic	79	45.1%[e]	67	40%
		ARC	47	30.2%[e]		
		AIDS	55	32.0%		
	208	Asymptomatic	7	3.1 ± 1[c]	14	42 ± 19[c]
	189	Recent HIV seroconverters	20	24,896[c]	15	21,620[c]
	64	Recent HIV seroconverters	14	4.0[d]	9	2.7[d]
Hepatitis A	243	Hemophiliacs, asymptomatic/AIDS	17	76%, delayed response	6 [g]	100%
					23	100%
	244	NA	14	77% after 3 doses	20	100%
	245	Asymptomatic/AIDS	83	88% after 2 doses	39	100%
Hepatitis B plasma-derived	249	Asymptomatic/PGL	17	53%	18	94%
	237	NA	16	56%	68	91%
	250	Asymptomatic	4	50%	69	93%
	251	NA/hemophiliacs	12	50%	29	93%
Hepatitis B recombinant	252	NA	10	40%	19	91%
	253	Asymptomatic	14	43%	37	86%
	250	Asymptomatic	9	22%	72	81%
	259	CD4 500 cells on HAART	6	83%	28	95%
		200–499 CD4 cells on HAART	25	57%		
		< 200 CD4 cells on HAART	7	29%		
In uenza	213	AIDS/ARC	13	1.5[d]	23	2.8
		Asymptomatic	11	2.1		
	39	PGL	25	72–96%[f]	19	58–95%
		Asymptomatic	10	60–100%		
	48	AIDS	15	13–40%[f]	38	71–100%
		AIDS, on AZT	10	17–50%		
		ARC	14	15–54%		
		Asymptomatic/PGL	27	32–89%[f]		
	212	AIDS	7	13–37%	31	25–100%
		AIDS, on AZT	30	0–43%		
		ARC	9	11–78%		
		Asymptomatic/PGL	32	22–84%		
	186	< 100 CD4 cells	21	10–24%[f]	10	30–90%
		100–300 CD4 cells	14	21–71%		
		> 300 CD4 cells	16	31–94%		
	276	AIDS/ARC/ Asymptomatic	19	42–89%[f]	30	64–90%

TABLE 38.2. *Continued*

		HIV-infected patients			Controls	
Vaccine	Ref	Stage of HIV-infection	Number tested	Response[a]	Number tested	Response[a]
	215	< 200 CD4 cells	19	21–42%[f]	76	70–75%
		200–500 CD4 cells	21	24–52%		
		> 500 CD4 cells	10	50–70%		
	63	< 300 CD4 cells, most on non-HAART antiretroviral therapy	22	27–59%[f]	9	44–100%
		> 300 CD4 cells, most on non-HAART antiretroviral therapy	10	70%		
	68	100–800 CD4 cells, viral load < 50, on HAART	11	9–47%[f]	5	0%
		100–800 CD4 cells, viral load > 50, on HAART	10	12–30%		
	69	All stages, mean CD4 count of 237 cells on HAART; majority had detectable viral load	13	Mean IgG: In uenza A 7.88 U/ml. In uenza B 18.00 U/ml	12	Mean IgG: In uenza A 34.77 U/ml. In uenza B 32.72 U/ml
	216	< 200 CD4 cells, most on HAART	10	50–70%[f]	337	81–83%
		200–500 CD4 cells, most on HAART	38	50–68%		
		> 500 CD4 cells, most on HAART	24	46–63%		
IPV	222	Asymptomatic	5	9–10 ± 3[c]	8	11–12 ± 3[c]
	271	AIDS/ARC/PGL	5	63–102[c]	3	5,955–9,410[c]
		Asymptomatic	12	5,250–6,277[c]		
	223	□100 CD4 cells	23	37–196[c]	16	511–1486[c]
		101–300 CD4 cells	10	84–223[c]		
IPV		> 300 CD4 cells	15	287–1,299		
	260	Asymptomatic hemophiliacs	34	100%	43	100%
Pneumococcal polysaccharide	272	AIDS	18	51%	20	99%
	273	PGL	25	36%	10	100%
	213	AIDS/ARC	13	15%	23	100%
		Asymptomatic	11	18%		
	39	PGL	25	10/12 types[b]	39	12/12 types[b]
		Asymptomatic	15	11/12 types[b]	53	
	49	AIDS	21	61%	53	88%
		Asymptomatic	27	63%		
	40	Asymptomatic	21	7/12 types[b]	23	11/12 types[b]
	274	AIDS/ARC	10	0.78–1.41[d]	112	1.5–2.6[d]
	185	□500 CD4 cells	39	24%	25	74%
		> 500 CD4 cells	12	75%		
	275	AIDS/PGL/ Asymptomatic	23	50%	11	100%
	186	< 100 CD4 cells	21	5.0[d]	10	5.8[d]
		100–300 CD4 cells	14	6.2[d]		
		> 300 CD4 cells	15	5.8[d]		
	189	recent HIV seroconverters	20	4/5[b]	15	5/5[b]
	64	recent HIV seroconverters	14	10.1[d]	9	5.3[d]
Pneumococcal polysacchride	193	< 300 CD4 cells	40	68%	25	92%
		> 300 CD4 cells	20	94%		
	198	< 200 CD4 cells	42	24–38%[a]		
		□200 CD4 cells	38	26–71%[a]	48	63–79%

TABLE 38.2. *Continued*

		HIV-infected patients			Controls	
Vaccine	Ref	Stage of HIV-infection	Number tested	Response[a]	Number tested	Response[a]
Pneumococcal conjugate	198	< 200 CD4 cells	43	5–44%[a]	48	50–96%[a]
		□200 CD4 cells	44	11–66%[a]		
Tetanus	186	< 100 CD4 cells	20	90%	10	100%
		100–300 CD4 cells	12	84%		
		> 300 CD4 cells	15	93%		
	41	Asymptomatic	21	86%	21	86%
	273	PGL	25	5.25%[d]	12	5.85
	222	Asymptomatic	5	1.7[d]	9	8.6
	270	Asymptomatic	8	6.5[d]	6	10
	223	□100 CD4 cells	23	1.5[c]	16	35.7[c]
		101–300 CD4 cells	10	4.1[c]		
		> 300 CD4 cells	15	13.8[c]		

HIV, human immunode ciency virus; AIDS, acquired immunode ciency syndrome; ARC, AIDS-related complex; PGL, persistent generalized lymphadenopathy; Hib, *Haemophilus inßuenzae* type B polysaccharide (PRP) and conjugate vaccines; IPV, inactivated poliovirus vaccine; HBV-plasma, plasma derived hepatitis B vaccine; HBV-recomb, hepatitis B recombinant vaccine; NA, not available; AZT, zidovudine; HAART, highly active antiretroviral therapy.

[a] De nition of response varied by study and type of serologic test performed. Percent indicates proportion attaining speci ed antibody level or signi cant titer rise. When a range is given, the percentage varied by serotype.

[b] Geometric mean titer (GMT) in HIV-infected persons equal to controls for speci ed number of capsular types.

[c] Mean titer of antibody postvaccination in μg/ml or reciprocal log dilutions. When a range is given, titers varied by serotype.

[d] Ratio of post vaccination to prevaccination titers.

[e] From < 1.0 ug/ml to > 1.0 ug/ml of PRP determined by ELISA.

[f] Percentage of patients who had □4-fold increase in hemagglutination antibody titers over baseline. When range is given, the percentage varied by antigen.

[g] HIV-negative controls with hemophilia.

Other data indicate that AIDS patients who were treated with zidovudine for a mean of 17.7 weeks prior to vaccination had a signiÞcantly increased response compared to untreated AIDS patients (aggregate GMT 770 vs. 369)(192). However, Loeliger et al. did not Þnd any correlation between antiretroviral therapy and response to vaccination (193). Immunologic response to pneumococcal vaccine in the context of HAART-associated immune reconstitution needs further assessment (194).

HIV-infected persons who do not initially respond to pneumococcal vaccine do not appear to beneÞt from re-vaccination (73,195). Among those who do respond, speciÞc anti-pneumococcal antibodies also rapidly wane after vaccination (188). Talesnik et al. found that about half of initial responders maintained an immune response after one year (196). In another study, follow-up of HIV-infected responders at one and two years after immunization showed similar IgG levels compared with healthy controls (195). Kroon et al. found that immune response is impaired in HIV-infected persons with CD4 counts < 200/μL and this results in a low concentration of antibodies, which declines below the level considered to be adequate for protection within three years (197). They suggested that re-vaccination every three years should be considered for HIV-infected persons. This recommendation is more frequent than current guidelines and needs further evaluation (11).

Unlike the experience with conjugated *H. inßuenzae* vaccines, pneumococcal glycoprotein conjugate vaccine did not elicit higher antibody levels than the polysaccharide vaccine (198). However, two recent studies have been conducted to examine the possibility of enhancing and prolonging the immune response to pneumococcal vaccine in HIV-infected individuals by using a conjugate vaccine followed by a polysaccharide vaccine (199,200). Both studies showed higher antibody concentrations after sequential vaccination with conjugate vaccine and polysaccharide vaccine compared with polysaccharide vaccine alone. Feikin et al. concluded that although the beneÞts of adding conjugate vaccine were not dramatic, a dose of conjugate vaccine and a dose of 23-valent polysaccharide vaccine may be optimal (200).

Over 85% of pneumococcal serotypes causing invasive disease in HIV-infected persons are contained in the currently licensed 23-valent vaccine (90,109), however, because of the relatively poor immunogenicity reported in many studies and waning immunity, the efÞcacy of pneumococcal vaccine in preventing invasive pneumococcal in HIV-infected persons remains controversial (80,95,98,114,201). A large prospective clinical efÞcacy trial in HIV-infected persons was recently conducted in Uganda and showed no protective effect; on the contrary, all-cause pneumonia was signiÞcantly more frequent in the vaccine arm (42). A meta-analysis of randomized controlled trials in adult populations (HIV-infected

patients were included in some of these trials, but were not the primary focus) showed pneumococcal vaccinaton to be efÞcacious in low-risk adults but not in those at high-risk (202). A small study of 48 HIV-infected drug users, some of whom were treated with HAART, also showed no protective effect of pneumococcal vaccine against all-cause pneumonia (203). A recent study in Australia questioned the cost-effectiveness of pneumococcal vaccination because of the low incidence of pneumoccocal disease in patients on antiretroviral therapy and the lack of evidence for efÞcacy; of 34 patients with invasive, presumptive, or possible pneumococcal disease, 16 (47%) had received pneumococcal vaccine (95). Chang et al. have suggested that a possible mechanism for pneumococcal vaccine failure in HIV-infected persons is an aberrant response to pneumococcal vaccine by peripheral B cells (204). Support for pneumococcal vaccine efÞcacy in the United States comes from retrospective case-control studies and longitudinal cohort studies. A case-control study of 85 HIV-infected cases of pneumococcal infection in which the comparison group consisted of HIV-infected patients without pneumococcal disease, showed signiÞcant protection for the pneumococcal vaccine in persons with CD4 > 200 cells/μL (43). Guerrero et al. conducted a retrospective case-control study of more than 2,000 HIV-seropositive patients followed in a large outpatient clinic from 1990 though 1998 (205). Cases of pneumonia (other than *Pneumocystis carinii* pneumonia) were matched by CD4 cell counts to controls without pneumonia. Pneumococcal immunization was associated with 70% reduction in risk of pneumonia. Breiman et al. conducted a case-control study of 176 HIV-infected patients with documented invasive pneumococcal disease compared with age- and CD4 count-matched controls and found overall that pneumococcal vaccine effectiveness was 49% (44); effectiveness among whites was signiÞcant at 76%, but not among blacks at 24%. In a large observational cohort study of nearly 40,000 HIV-infected persons with more than 70,000 person-years of observation, Dworkin et al. found vaccine to be protective in persons with CD4 cell count □500 cells/μL at the time of vaccination, but not in those with lower CD4 counts (16). Pneumococcal vaccination has been found to be cost-effective for HIV-infected persons in two analyses (206,207).

The rationale for immunizing HIV-seropositive adults against *Haemophilus inßuenzae* type b (Hib) has been debated due to the lower incidence of Hib infections in adults compared with children (113). Although asymptomatic HIV-infected men immunized with Hib polysaccharide vaccine had lower levels of antibody post-vaccination than HIV-negative controls, levels in both groups were protective (□1.0 ug/ml) (208). A trial of Hib polysaccharide vaccine (PRP) and ribosylribitol phosphate conjugated with diphtheria toxoid (PRP-CRM) Hib vaccine reported that these vaccines were most immunogenic in descending order among persons who were: (1) HIV-negative; (2) HIV-positive but asymptomatic; (3) diagnosed with AIDS-related complex; and (4) AIDS (209). One month after vaccination, 100% of HIV-negative persons had antibody levels of > 1.0 ug/ml, compared with 97.5% of HIV-positive but asymptomatic persons, 95% of patients with AIDS-related complex, and 80% of patients with AIDS. The conjugated Hib vaccine (PRP-CRM) was more immunogenic (e.g. mean titers, percent > 1.0 ug/ml) than the unconjugated Hib vaccine (PRP) among all groups, except in patients with AIDS who responded better to the PRP vaccine (207). Kroon et al. also found impaired response to conjugated Hib in HIV-infected persons with CD4 counts < 100/μL (210). A comparison of three licensed Hib vaccines in HIV seropositive adults most of whom were on triple-drug antiretroviral therapy showed that all three vaccines were immunogenic in these patients; response was poorer with lower CD4 cell counts and lower IgG2 levels at baseline (211). A study of recent HIV seroconverters showed that response to Hib conjugate vaccine was similar to that of HIV-negative controls (189).

Inßuenza Vaccine

The inßuence of stage of HIV disease on the ability to respond to immunization is clearly demonstrated in prospective studies of inßuenza vaccination of HIV-infected patients with a spectrum of clinical manifestations (Table 38.2). Interestingly, at all stages of HIV disease, responses to inßuenza A hemagglutinins H1 and H3 were higher than responses to inßuenza B antigen (48,212). The proportion of symptomatic HIV-infected patients who respond to monovalent inßuenza A and trivalent inßuenza A and B vaccines was much lower than for seronegative controls (48,186,212,213). The number of CD4 cells present at vaccination was related to antibody titers achieved postvaccination (48,65,186,214). Higher viral load has also been found to correlate with poorer response to vaccination, but on multivariate analysis only CD4 count remained predictive (65). AIDS patients receiving zidovudine had similar CD4 counts as AIDS-related complex (ARC) patients and their responses to immunization were intermediate between those of untreated AIDS patients and ARC patients. The proportions of asymptomatic patients and those with persistent generalized lymphadenopathy who acquired protective levels of antibody were equal to controls for all three vaccine antigens (inßuenza H1 and H3 and inßuenza B) in two studies (39,212) and for one or two antigens in the other two reported studies (48,213). A study of HIV-seropositive drug users showed that those with CD4 counts > 500 cells/μL responded to inßuenza vaccine as well as HIV-seronegative drug users; however, those with CD4 counts < 500 cells/μL showed impaired reactivity (215). GŸnthard et al. found that HIV-infected patients on HAART therapy with relatively high CD4 cells counts responded as well as or better than healthy controls to

inßuenza vaccine (68). However, other investigators have found that HIV-infected patients on HAART, especially those with with higher viral loads and lower CD4 counts, responded less well than healthy controls (69,216,217). Thus inßuenza vaccination, if given early in the course of HIV infection, should provide a measure of protection.

In an attempt to improve vaccine responses, investigators (186,212) studied the effect of a booster dose of vaccine given one month after the Þrst dose. In general, the booster dose did not improve responses in HIV-infected persons. The booster dose increased antibody to protective levels ($\geq$ 1 : 64) in only one asymptomatic HIV-infected person and had no effect on levels in ARC patients. Of 30 AIDS patients on zidovudine therapy, the proportion who reached a protective level of antibody increased from 30% to 33% for H1 antibody, from 37% to 47% for H3 antibody, and from 13% to 17% for antibody to inßuenza B after the booster dose. In a more recent study with three-year consecutive follow-up for annual re-vaccination, signiÞcantly higher prevaccination antibody was seen in the second and third year of vaccination in healthy controls, but not in HIV-infected individuals (218). The authors concluded that annual vaccination of HIV-infected persons with a CD4 cell count $>100/\mu L$ seemed worthwhile.

Tasker et al. evaluated the clinical efÞcacy of inßuenza vaccine in a randomized, double-blind, placebo-controlled trial of 102 HIV-infected persons (the majority had CD4 counts >200 cells/μL and were on antiretroviral therapy) during the fall of 1995Ð1996 inßuenza season (59). Ten placebo recipients and no vaccine recipients had laboratory-conÞrmed symptomatic inßuenza; thus, protective efÞcacy was 100% although response to the vaccine was only 12% for A/Texas (H1N1), 29% for A/Johannesburg (H3N2), and 36% for B/Harbin components.

Diphtheria and Tetanus Toxoids

After primary immunization with tetanus toxoid in childhood, almost all HIV-infected adults maintain protective levels of antibody ($\geq$ 0.01 ug/ml) after the onset of HIV infection (219). Similar experiences have been reported from Africa and Haiti, where HIV-infected women were found to have the same levels of tetanus antibody after two doses of vaccine given during pregnancy as HIV-negative women (220,221). All of 78 Danish HIV-infected men, who probably received primary vaccination with diphtheria and tetanus toxoids before they contracted HIV infection, had tetanus antibodies above the protective level (219). However, 24 (31%) of these same men were unprotected (<0.01 ug/mL) against diphtheria compared to 10% of men of similar age in the general population. Thus, despite adequate vaccinations during childhood, HIV-infected adults appear more likely to be susceptible to diphtheria than non-HIV infected adults. Increases in antibody levels after booster doses of diphtheria and/or tetanus toxoid vaccine were lower in asymptomatic HIV-infected persons than in controls, however, almost all HIV-infected persons maintain protective levels of anti-tetanus toxin antibody (41,186,222). Decreased response in HIV-infected men correlated with the lack of proliferative responses of peripheral blood mononuclear cells postvaccination when exposed to tetanus toxoid *in vitro* (222). However, when tetanus toxoid was coupled to agarose beads creating a T-cell-independent antigen, *in vitro* responses did occur. HIV-infected adults with CD4 lymphocyte counts <300 appear to have impaired secondary (booster) antibody responses to diphtheria and tetanus toxoids (186,223). Talesnik et al. found that only 23% of HIV-infected patients had antibody response to tetanus toxoid at two months after a booster dose, which waned to below minimum protective levels by 12 months (196). Valdez et al. evaluated the recall response to tetanus toxoid in 31 HIV-infected patients receiving HAART for at least 48 weeks; 48% had a four-fold increase in antibody concentration and all patients developed protective levels (3). They recommended that clinicians consider re-immunization of HIV-infected patients after initiation of HAART to restore lost responses to recall antigens. Takano et al. found that fully immunized HIV-infected children without protective antibody levels at baseline had good response to tetanus and diphtheria booster after receiving HAART (224). In another study, children receiving HAART responded well to tetanus toxoid but not to diphtheria immunization; response correlated with immune status (225). The effect of intermittent cycles of interleukin-2 (IL-2) on response to immunization was evaluated in HIV-infected patients on HAART (226). In this study, while IL-2 increased the number of circulating CD4 cells, response to tetanus toxoid was not improved.

Measles, Mumps, and Rubella Vaccine

Most data on the response to measles vaccine are from children in developing countries (132,227,228). There are few data for rubella vaccine and none for mumps. In one study in Zaire, response rates after measles vaccination at nine months of age were related to severity of HIV infection. Among HIV-uninfected, asymptomatic HIV-infected, and symptomatic HIV-infected children, 89%, 77%, and 36% responded to vaccine, respectively (132). In a study in the United States, only three (12.5%) of 24 vaccinated HIV-infected children studied retrospectively had antibody to measles virus; however, a larger number of children had antibody when tested by a more sensitive assay (229). In the same study, among children studied prospectively, only two (25%) of eight responded to measles vaccination. In a larger study, among 127 HIV-infected children, only 35% had documented vaccination

with measles-mumps-rubella (MMR) (230). Of the 80 children whose pre-vaccination serology results were available, 56% were measles antibody-negative and 40% were antibody-positive; after re-vaccination 36% remained antibody negative. Arpadi et al. studied 81 perinatally HIV-infected children and found that 83% who were tested within one year of measles vaccination had detectable antibody compared with 52% who were evaluated more than a year after vaccination (231). The proportion of children with detectable measles antibody was also higher among those with no or moderate immunosuppression. Brena et al. found that at two months post-MMR vaccination, 11 of 20 HIV-infected children had adequate antibody to measles while 12 of 13 HIV-seroreverters responded (232).

Most HIV-infected adults will either have received MMR vaccine during childhood or have had natural disease. Several studies have found that most HIV-infected adults have protective levels of measles antibody (233–237). However, lower levels of measles antibody were reported from two studies of prison inmates (238,239), among women (240), and in persons born in the United States in 1957 or later (237). Data suggest that after primary immunization or disease in childhood, HIV-infected adults retain protective measles antibody regardless of progression of HIV disease (237,241). Only one study has evaluated the response of HIV-infected adults to measles and rubella vaccination. In this study, only three of 39 HIV-infected and two of 17 healthy vaccinees were measles seronegative at the time of vaccination. Of those who were measles seronegative, none of the HIV-infected adults and one of the healthy adults seroconverted (238). Four HIV-infected and three healthy vaccinees were rubella seronegative at the time of vaccination: three and two, respectively, responded to vaccination.

Antiretroviral therapy may improve serologic response to measles vaccine. A recent study of 28 HIV-infected children who lacked measles antibody after initial measles vaccination showed that nine (64.3%) of 14 children undergoing HAART responded to an additional MMR vaccination compared with only three (21.4%) of 14 children undergoing non-HAART regimens. The HAART group had lower mean viral load (27,700 copies/mL) compared with the non-HAART group (86,000 copies/mL); there was no difference in CD4 counts between the groups (242).

Hepatitis A Vaccine

The immunogenicity of hepatitis A vaccine in HIV-infected adults has been evaluated in several studies (243–246). In a study of 25 HIV-infected persons with hemophilia, eight HIV-negative hemophilic controls, and 25 HIV-negative healthy adults, all persons in both control groups responded to one dose of vaccine; however, among HIV-infected persons response was delayed (243). Of the 17 HIV-infected persons who completed all three doses of vaccine and follow-up serologies, four (24%) failed to respond. In a hepatitis A vaccine study in 14 HIV-positive homosexual men compared with 20 HIV-negative men, 77% and 100%, respectively, responded after seven months; mean antibody titres were higher in the HIV-negative men (244). In another similar study, 88% of 90 HIV-infected compared to 100% of 44 HIV-negative homosexual men responded to two doses of hepatitis A vaccine; HIV-infected men who responded had significantly higher CD4 counts at baseline than those who did not (245). Wallace et al. conducted a randomized trial of hepatitis A vaccine compared with placebo in 90 hepatitis A IgG seronegative HIV-infected persons (equal numbers having CD4 count >300 cells/μL and <300 cells/μL). In the vaccinated group, 64% had antibody to hepatitis A by week 4 and 96% by week 28 (246). Changes in antiretroviral therapy were discouraged during the trial; there were no differences noted between vaccine and placebo groups in viral loads or CD4 counts. However, in another study, Kemper et al. found that 68% of HIV-infected persons with CD4 count >200 cells/μL responded to hepatitis A vaccine compared to only 9% of those with CD4 count <200 cells/μL (247).

Hepatitis B

Several studies have demonstrated an impaired response to hepatitis B vaccine in HIV-infected adults (Table 38.2). Three studies in HIV-infected homosexual men, using plasma-derived vaccine, found that 50–56% of HIV-infected adults seroconverted compared with over 80% of HIV-uninfected controls (248–250). Similar results have been reported in studies of HIV-infected hemophiliacs (251). Studies of recombinant vaccine have found similarly low immunogenicity in HIV-infected persons (248,252–255). Re-vaccination of HIV-infected persons who do not respond to the three-dose primary series produces poor results. In a small study, one of six HIV-infected persons, who did not respond to the initial vaccine series, seroconverted after a second series compared with 50% of uninfected adults (256). Among those who responded to vaccine, no difference in geometric mean titers between HIV-infected and uninfected vaccinees was reported by Carne et al. (249); however, lower levels were found in other studies (248,252). In addition to low seroconversion rates in HIV-infected persons, accelerated loss of hepatitis B surface antibody in responders has been reported (257). Thirty-six percent of 75 men without HIV infection lost protective levels of antibody within five years of vaccination compared with 63–86% of 21 men who acquired HIV infection before or during the hepatitis B vaccination series. However, good persistence was reported in another study (258). Among HIV-infected persons who responded to vaccine, vaccine may not

protect against infection but may protect against serious illness: among 17 HIV-infected responders who were subsequently infected with hepatitis B virus, none had severe illness or became chronic carriers (45). Rey et al. enhanced overall response rate to hepatitis B vaccine in HIV-infected patients from 55% with the standard three doses, to 90% by giving initial nonresponders three additional doses (52). Veiga et al. showed that humoral response to hepatitis B vaccination diminishes with decreasing CD4 cell count in HIV-infected persons on HAART and suggested that individuals with CD4 count >200 cells/μL would beneÞt most from vaccination (Table 38.2) (259).

Poliovirus Vaccines

In two small studies, all but one of 22 HIV-infected patients had antibody to all three poliovirus types prior to vaccination with inactivated poliovaccine (IPV) (Table 38.2). The patients had all been vaccinated with poliovirus vaccine in childhood or were exposed to wild polioviruses. Following vaccination, asymptomatic HIV-infected men all responded similarly as uninfected men with substantial increases in antibody. However, none of the symptomatic HIV-infected men had a booster response to IPV. In a study comparing the antibody response to a booster dose of IPV among HIV-infected vs. uninfected hemophiliac patients, both groups responded with increased antibody titers to the three poliovirus types; however; HIV-negative patients exhibited higher titers than the HIV-positive group (260).

RECOMMENDATIONS FOR IMMUNIZATION OF HIV-INFECTED ADULTS

Recommendations for immunization of HIV-infected persons have been made and regularly updated by the Advisory Committee on Immunization Practices (ACIP) (6,10) and the U.S. Public Health Service/Infectious Diseases Society of America (USPHS/IDSA) (11). These are summarized in Table 38.3. Recommendations from disease-speciÞc ACIP guidelines are also included.

Pneumococcal and *H. inßuenzae* type B Vaccines

The use of inactivated vaccines poses no risk of serious adverse events and may be beneÞcial for HIV-infected persons. Although the response to immunization may be less than optimal in HIV-infected persons, the USPHS/IDSA recommends that all HIV-infected persons with CD4+ T-lymphocyte count of >200 cells/μL be immunized with a single dose of 23-valent polysaccharide pneumococcal vaccine if they have not received this vaccine during the previous Þve years (11). Immunization should be considered for persons with CD4 count of <200 cells/μL although clinical efÞcacy has not been conÞrmed for this group. Re-vaccination can also be considered for persons with CD4 counts <200 cells μ/L at initial immunization but whose CD4 count has increased to □200 cells/μL on HAART. The duration of the protective effect of pneumococcal vaccine is unknown. Because rapid declines in antibody levels may occur in persons with HIV infection, a single re-vaccination can be considered after Þve years. However, clinical beneÞts of re-vaccination have not been proven (11). Studies to date have shown no beneÞcial effects of re-vaccination in initial non-responders (195). Sequential immunization of HIV-infected adults with pneumococcal polysaccharide vaccine after prior immunization with conjugate pneumococcal vaccine resulted in enhanced antibody response in one study which may have implications for future vaccination policies (190).

Since the incidence of *H. inßuenzae* type B disease is low, *H. inßuenzae* type B vaccine is not generally recommended for adult use in this country (11). If given, the greatest beneÞt from immunization will occur when HIV-infected patients are immunized before the onset of immunocompromise and possibly after immune restoration with HAART. In studies of recent HIV seroconverters (duration of HIV infection <18 months and mean CD4 cells >500), responses to pneumococcal and *H. inßuenzae* type B conjugate vaccine were equal to those of uninfected controls (189). Others have demonstrated that immune response to *H. inßuenzae* type B vaccine is related to CD4 count with a deÞnite impairment in response at CD4 <100 cells/μL. Although the USPHS/IDSA guidelines do not recommend the vaccine, because incidence of *H. inßuenzae* type B disease may be higher among some HIV-infected adults (100,101), the ACIP recommends the vaccine be considered while taking into account the risk of disease (6).

Inßuenza Vaccine

Inßuenza vaccine should be given annually, because the prevalent virus strain and the vaccine composition changes each year (78). In addition, medical personnel and household members who may expose HIV-infected persons should be vaccinated to prevent transmission of inßuenza virus. Because vaccine efÞcacy cannot be assured, if exposure to inßuenza has occurred or is suspected, chemoprophylaxis with one of the following antiviral agents may be considered: amantidine (inßuenza A), rimantidine (inßuenza A), oseltamivir (inßuenza A or B), or zanamivir (inßuenza A or B). Chemoprophylaxis should be considered for HIV-infected patients (especially those with advanced disease) who may be expected to have a poor response to immunization, although the efÞcacy of these agents in preventing inßuenza among severely immunocompromised persons is unknown (78).

TABLE 38.3. *Recommendations for immunization and prophylaxis of HIV-infected adults, United States*

Disease	Vaccine	Type	Prophylaxis	Recommendation for vaccination
Pneumococcal	Pneumococcal	23-valent polysaccharide		Recommended for patients with CD4 □200 cells; offer to patients CD4 with < 200 cells. Vaccinate as close to diagnosis as possible when CD4 counts are highest. Consider re-vaccinating once if > 5 years have elapsed since initial vaccination.
H. inßuenzae type B	*H. inßuenzae* type B (Hib)	Polysaccharide conjugate		Not recommended by USPHS/IDSA; ACIP recommends considering vaccine for patients at high risk of *H inßuenzae* type B disease.
In uenza	Trivalent in uenza	Inactivated viral	Amantadine or rimantadine (for in uenza A only); oseltamavir or zanamivir (for in uenza A or B)	Recommended for all patients each year.
Tetanus	Tetanus-diphtheria (Td)	Toxoid	Tetanus immune globulin	Primary series (usually in childhood) followed by booster every 10 years.
Diphtheria	Td	Toxoid		Primary series (usually in childhood) followed by booster every 10 years.
Poliomyelitis	Inactivated poliovirus vaccine (IPV)	Inactivated viral		Routine vaccination of adults residing in the United States is not necessary. Consider for persons at risk of exposure to polioviruses, e.g. travel to an endemic area.
	Oral poliovirus vaccine (IPV)	Live viral		Contraindicated.
Measles	Measles-mumps-rubella (MMR)	Live viral	Immune globulin (IG)	Consider for adults without severe immunosuppression and born after 1957 if they have no documentation of previous vaccination or are seronegative for measles antibody; contraindicated for those who are severely immunosuppressed.[a]
Mumps	MMR	Live viral		Consider for adults without evidence of immunity, without documentation of vaccination, or born after 1957. Same contraindication as for measles.
Rubella	MMR	Live viral		Offer to women of childbearing age without documented evidence of rubella immunity. Same contraindication as for measles.
Hepatitis A	Hepatitis A	Inactivated viral	IG	Offer to susceptible persons at high risk of expsoure (see Text). Two doses given 6–12 months apart.
Hepatitis B	Hepatitis B	Recombinant, inactivated viral	Hepatitis B immune globulin	Offer to all susceptible persons. Three doses: one month between 1st and 2nd dose; six months between the 2nd and 3rd dose.
Varicella	Varicella	Live viral	Varicella zoster immune globulin (VZIG)	Vaccine is not recommended for adults at this time.

ACIP, Advisory Committee on Immunization Practices; USPHS/IDSA, U.S. Public Health Service and Infectious Diseases Society of America.

[a] CD4 + T-lymphocyte count < 200 cells/μL or CD4 + T-lymphocytes < 14% of total lymphocytes.

During nosocomial outbreaks, chemoprophylaxis should be given to all institutionalized patients regardless of previous inßuenza vaccination. Chemoprophylaxis may also be given during the two-week period after inßuenza vaccination and before the development of antibodies if an inßuenza outbreak has begun in the community. Finally, during periods of inßuenza A activity, chemoprophylaxis may be given to unvaccinated or recently vaccinated institutionalized HIV-infected persons and to those who will be exposed in the community. There are almost no data on the safety of these agents in HIV-infected persons (116,78). No data are available on possible interactions with other drugs used in the management of patients with HIV infection. Patients should be carefully observed for adverse drug reactions, especially when neurologic conditions or renal insufÞciency is present. Dosages should be adjusted in patients with renal insufÞciency or severe hepatic dysfunction (78). During inßuenza outbreaks, unvaccinated hospital and clinic personnel and household care-givers should also receive prophylaxis to prevent transmission of virus to HIV-infected persons.

Diphtheria and Tetanus Toxoids

Diphtheria and tetanus toxoids and inactivated poliovirus vaccines may be used for immunization of unvaccinated or inadequately vaccinated HIV-infected adults or for boosters as recommended for immunocompetent adults (6). A history of immunization with tetanus and diphithera toxoids (which is often not known for adults) may not indicate present immunity and regular booster doses of combined tetanus and diphtheria toxoid should be considered for adolescents and adults, regardless of HIV infection status, particularly in countries experiencing a resurgence in diphtheria. Prophylaxis with tetanus immune globulin (TIG) and a tetanus booster are recommended for HIV-infected adults who sustain a signiÞcant wound exposure (6).

Polio Vaccine

In the United States, only inactivated poliovirus vaccine (IPV) is recommended for routine vaccination of adults and children in order to eliminate the risk of vaccine-associated paralytic poliomyelitis (VAPP) (24). Oral poliovirus vaccine (OPV) may be used for mass vaccination to control polio outbreaks, however, OPV should never be used to immunize immunocompromised persons because they are at substantial risk for VAPP. Children residing in households or having close contact with known HIV-infected adults should receive inactivated polio vaccine (IPV) for primary immunization (6,9,24). If OPV is inadvertently administered to a household contact of an immunodeÞcient person, the OPV recipient should avoid close contact with the immunodeÞcient person for about four to six weeks after vaccination (224). If this is not possible, rigorous handwashing and avoidance of contact with feces and saliva should be instituted.

Routine polio vaccination of adults residing in the United States is not necessary. Most adults have a minimal risk for exposure to polioviruses in this country, and most are immune as a result of vaccination during childhood. Some unvaccinated adults (including those who are HIV-infected) at greater risk of exposure to polioviruses than the general population (e.g. those who travel to polio-endemic areas; members of groups with disease caused by wild polioviruses; laboratory workers; health care workers in close contact with patients who might be excreting wild polioviruses; and unvaccinated adults with children receiving OPV) should be vaccinated with IPV. If they have no documentation of vaccination, they should receive a primary series consisting of two doses of IPV at intervals of four to eight weeks; a third dose should be administered six to 12 months after the second. Adults who have had a primary series of OPV or IPV and who are at increased risk of exposure can receive a single booster dose of IPV (24).

Measles Vaccine, MMR

All adults born in or after 1957 should have evidence of immunity to measles vaccine by documentation of receipt of measles vaccine after the Þrst birthday, laboratory evidence of immunity, or history of physician documented measles disease. Adults born before 1957 can generally be considered to be immune from natural disease (25). The ACIP does not recommend measles vaccination of HIV-infected persons with evidence of severe immunosuppression, deÞned as CD4+ T-lymphocyte <200 cells/μL or $<14\%$ of total lymphocytes for persons ≥ 13 years for the following reasons: a case of progressive measles pneumonitits occurred in a person with AIDS after receipt of MMR; evidence of diminished antibody response among severely immunocompromised HIV-infected persons; morbidty related to measles vaccination in non-HIV-infected persons with severe immunosuppression; and the low incidence of measles in the United States (25). The ACIP continues to recommend measles vaccine for HIV-infected persons without evidence of measles immunity who are not severely immunocompromised. Exposure to measles is not a contraindication to vaccination; MMR administered within 72 hours of exposure may provide some protection and should be to administered in HIV-infected patients who are not severely immunocompromised (25). Because of the reduced immunogenicity of measles vaccine in HIV-infected persons, previously vaccinated persons, as well as those with no history of vaccination or disease, should receive prophylaxis with immune globulin within six days of measles exposure (25). Persons receiving regular (e.g. monthly) intravenous immune globulin for HIV infection or other indications

may not respond to MMR due to presence of high levels of passive antibody (25).

Hepatitis A Vaccine

Hepatitis A vaccination is recommended for susceptible HIV-infected persons at increased risk for hepatitis A including men who have sex with men, injecting and non-injecting drug users, hemophiliacs, travelers to developing countries, travelers to endemic areas, those with chronic liver disease (e.g. due to hepatitis B or C), and others (11,261). Persons recently exposed to hepatitis A virus who have not been previously vaccinated, should be given a single dose of immune globulin intramuscularly as soon as possible, but not more than two weeks after the last exposure (261). If indicated, hepatitis A vaccine may be administered simultaneously with immune globulin at a separate anatomic site (261).

Hepatitis B Vaccine

Indications for the use of hepatitis B vaccine in HIV-infected persons are the same as for uninfected persons (6,11). A routine three-dose schedule (with an interval of one month between the Þrst and second doses and six months between the second and third doses) is recommended for HIV-infected persons who lack evidence of prior immunity (antibody to hepatitis B surface or core antigens (anti-Hbs or anti-Hbc)). A larger than normal vaccine dose, 40 μg which is recommended for some immuno-compromised persons, may be considered but has not been studied in HIV-infected adults. Post vaccination serologic testing is recommended for all HIV-infected persons between one and six months after completion of the vaccination series. Re-vaccination with one or more doses should be considered if the anti-Hbs is below 10 MIU/ml. Postexposure recommendations for HIV-infected persons are the same as those for uninfected persons (6). Hepatitis B immune globulin and initiation of the hepatitis B vaccination series is recommended for all nonimmune HIV-infected persons who have accidental percutaneous or mucous membrane exposure to blood or body ßuids containing hepatitis B surface antigen or who have sexual exposure.

Varicella Zoster Postexposure Prophylaxis

Susceptible HIV-infected patients who have a signiÞcant exposure to varicella should be given prophylaxis with varicella immune globulin (VZIG) within 96 hr of exposure, as recommended for other immunocompromised persons (6,11). HIV-infected adults who are believed to have had varicella on the basis of a carefully obtained history by an experienced interviewer can be considered immune. However, a past history of varicella, a previous positive serologic test for varicella antibody, or recent administration of VZIG does not guarantee immunity (9). Recently, varicella vaccine has been licensed in the United States (262). Varicella vaccine is not currently recommended for HIV-infected adults at this time (262). However, the ACIP does recommend that varicella vaccine should be considered for asymptomatic or mildly symptomatic HIV-infected children (263). Susceptible medical personnel and household members of HIV-infected persons should be immunized to decrease the risk of transmission to HIV-infected contacts.

HIV-Infected Travelers

HIV-infected persons who wish to travel to developing countries pose a special problem. They should be made aware of the risk of travel to areas in which many diseases are endemic and that lack supportive medical care. A recent survey of 89 HIV-infected adults found that, in a period of two years, 45% had traveled to a median of three U.S. destinations for at least a week and 20% traveled to at least one international destination (mean duration 20 days) (264). There are no data concerning adverse reactions, immunogenicity, or efÞcacy of vaccines usually recommended only for travel outside the United States. The live attenuated virus vaccine for prevention of typhoid is contraindicated for HIV-infected persons (6). Live attenuated vaccines for measles and varicella may be administered to some HIV-infected persons as discussed previously. For necessary travel to countries requiring proof of vaccination, a waiver letter should be provided by the travelerÕs physician. For persons who cannot avoid possible exposure to yellow fever, the physician should inform the patient of the risks and beneÞts of immunization and offer the choice of vaccination (265). Vaccinees should be monitored for possible adverse effects. Inactivated typhoid vaccines are available and theoretically may be safe alternatives for symptomatic and asymptomatic HIV-infected persons (266). Persons at risk for exposure to typhoid should be administered an inactivated parenteral typhoid vaccine instead of the live-attenuated oral vaccine (9). Prior to travel, determination of an adequate antibody response to vaccination is desirable. All HIV-infected persons should be advised to avoid exposure to mosquitoes by using barrier methods of protection. Other vaccines containing killed antigens (including diphtheria-tetanus, hepatitis A, hepatitis B, Japanese encephalitis, rabies, plague, and anthrax) do not pose a risk to HIV-infected persons and should be used for the same indications as for uninfected persons (6,265). Because immunity to diphtheria wanes with time since the last dose of diphtheria vaccine, a booster dose of combined tetanus and diphtheria toxoids should be administered to adults wishing to travel to countries currently experiencing a resurgence of diphtheria. This recommendation is particularly important for HIV-infected adults who may have lower antibody

levels against diphtheria than non-HIV infected adults. The available cholera vaccine is not recommended for persons with a routine tourist itinerary, even if travel includes countries reporting cases of cholera (11).

Other vaccine-preventable diseases, including rabies, Japanese encephalitis, meningococcal infection, and plague, are rare in American travelers. Recent reviews of these vaccines and their use in immunocompromised persons have been published (81,182). These vaccines are inactivated and could be considered in the unusual circumstance when exposure cannot be avoided.

Bioterrorism-Related Vaccines

Following terrorist incidents in New York City and Washington, D.C. on September 11, 2001, and intentional release of anthrax in October 2001, the Centers for Disease Control and Prevention has recommended heightened surveillance for illnesses that may be indicative of bioterrorism (267). Although the safety and efficacy of anthrax vaccine in HIV-infected persons has not been evaluated, because it is an inactivated bacterial vaccine, the risks to this population may be expected to be minimal should vaccination be recommended. In contrast, severe complications after vaccinia (smallpox) vaccination in HIV-infected persons have been reported (34,268). Although smallpox vaccination has been discontinued for many years, recommendations for use of smallpox vaccine in the situation of intentional release are being revised (269). Careful consideration must given on how to identify HIV-infected persons and others who are risk for severe complications to this vaccine (269).

CONCLUSION

As the number of HIV-infected persons continues to increase, as more patients are identified earlier in their disease, and as survival is increased, there will be more opportunity for acquisition of vaccine-preventable diseases. Current vaccines are most likely to be effective when HIV-infected persons are immunized before the onset of immune compromise. Past experiences with adult immunizations indicate that provision of recommended vaccines and immuno- and chemoprophylaxis to HIV-infected persons needs to be much more aggressive. Additional techniques to improve responses to immunization are necessary. Research should include modifications of vaccination schedules including higher vaccine doses, booster or multiple dose schedules, vaccination concurrent with anti-HIV therapy, and development of more immunogenic vaccines.

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Chapter 39

Progress in the Development and Testing of HIV Vaccines

Marta-Louise Ackers, Bradford N. Bartholow and Timothy D. Mastro

Since the start of the human immunodeÞciency virus (HIV) epidemic in the late 1970s, more than 60 million HIV-1 infections are estimated to have occurred worldwide.(1) In general, behavioral interventions on a national level have had limited success; however, these efforts in Thailand, Uganda, and Senegal have slowed the epidemic substantially (2Ð5). Antiretroviral medications, although capable of controlling HIV viremia and slowing the rate of disease progression, do not eradicate HIV, have substantial adverse effects, are expensive, and are not widely available globally. The development and subsequent widespread administration of a safe, effective, and affordable HIV preventive vaccine is essential to halt continuing spread of the epidemic.

The Þrst U.S. HIV-1 vaccine human clinical trial started in 1988 (6). However, 14 years later, despite more than 80 phase I/II clinical trials of various vaccine constructs conducted worldwide and the involvement of numerous U.S. and international government agencies, nongovernmental organizations, universities, and industry, we still do not have an efÞcacious vaccine. In comparison, two other viral diseases, hepatitis B and hepatitis A, had licensed vaccines available 16 and 22 years respectively, after pathogen identiÞcation (7Ð8).

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Since the correlates of human protection are still unknown, current scientiÞc opinion advocates development of a preventive HIV-1 vaccine capable of inducing both humoral and cellular immunity. A vaccine should induce broad cross-neutralizing antibodies against primary isolates from differing HIV-1 genetic subtypes and against viruses of both major phenotypes: lymphotropic, syncytium-inducing viruses that use the CXCR4 coreceptor and macrophage-tropic non-syncytium inducing viruses that use the chemokine CCR5 coreceptor. Also, the vaccine should elicit cross-subtype reactive cytotoxic T lymphocyte (CTL) responses, helper CD4 responses, mucosal immunity, and long-term protection. Although development of such a vaccine appears to be a daunting task, this does not imply that these expectations are unrealistic. This chapter describes obstacles hindering the development of a preventive HIV vaccine, progress toward developing and evaluating vaccine candidates, clinical trials, the social and ethical issues involved in conducting HIV vaccine trials, and plans for future efÞcacy trials.

OBSTACLES TO THE DEVELOPMENT OF AN HIV VACCINE

Three obstacles are widely considered to be responsible for hindering the development of an HIV-1 vaccine. These obstacles are: (1) the extensive antigenic variability of HIV; (2) uncertainty regarding which immune responses are correlated with protection; and (3) the lack of an ideal animal model in which to test vaccine constructs.

Antigenic Variability

AIDS is caused by two different lentiviruses, HIV-1 and HIV-2; HIV-1 accounts for most AIDS cases. Phylogenetic analyses of the nucleotide sequences of the envelope (*env*)

and core (*gag*) genes of a large number of HIV-1 isolates have identified at least 11 different genetic clades or subtypes (A, A2, B, C, D, F1, F2, G, H, J and K) (9). These subtypes form the major group of HIV-1, group M, and are of approximately equal genetic distance from one another. In addition, two other groups distantly related to group M have been identified in patients from Cameroon (subtypes O and N), Gabon (O), and Guinea (O) (10,11).

The majority of this genetic variability is due to the high error rates of the HIV-1 reverse transcriptase. Most genetic variation appears to occur in the envelope (*env*) gene. The extent of genetic variability increases over time as HIV-1 spreads within populations. Furthermore, genomic recombination between one or more different subtypes is common, giving rise to various circulating recombinant forms (CRF): e.g. CRF01_AE, CRF02_AG, CRF03_AB, CRF04_cpx (12,13). Currently, the importance of genetic HIV-1 subtypes for vaccine-induced protection from infection or disease is not known. Could a vaccine designed for HIV-1 subtype B protect against a subtype C infection or will subtype specific vaccine formulations be required? Furthermore, if immune protection is dependent on a specific, variable HIV sequence, then HIV vaccine development may require continuous modification as new genetic strains evolve.

Correlates of Protection

In the past, scientists studied persons who became infected with a pathogen and survived in hope of identifying immunologic features among these survivors that protected them from re-occurrence of disease. Vaccines were designed and developed to elicit the immune responses of these survivors. However, for HIV there appear to be no known survivors who clear the virus. Clinical research has focused on natural history studies of HIV-infected persons, including HIV-infected persons in whom HIV-related disease does not develop (long-term non-progressors), persons whom have had multiple exposures to HIV yet remain uninfected, and discordant and concordant mother-infant pairs (14), examining both viral and genetic host factors. However, to date, no single immune correlate has been identified. Rather, the data suggest that immunity from HIV will require activity from multiple immune components. Consequently, vaccine immunogenicity studies strive to elicit a wide range of humoral and cellular immune responses, including both neutralizing antibodies and CTLs, in an attempt to include multiple modes of protection.

Humoral antibody responses have previously been shown to be important in preventing disease. Other viral diseases (e.g. polio, inßuenza, rabies, and hepatitis B) have been prevented through the administration of immunoglobulin containing protective antibodies or vaccines designed to induce a humoral antibody response. Therefore, early HIV-1 vaccine constructs focused on subunit proteins designed to induce neutralizing antibodies and thereby prevent HIV infection. However, other epidemiologic and immunologic studies among long-term non-progressors (15), highly exposed persistently seronegative African and Asian sex workers (16,17), and acutely exposed infants (18) led to the belief that CTL responses are important in controlling virus replication during acute infection. Subsequent vaccine constructs were designed to induce these cell-mediated responses. However, these latter constructs did not induce a humoral response, leading to the development of a prime-boost concept whereby a CTL-inducing construct is administered first, followed by an antibody-inducing subunit protein boost (19).

Animal Models

Pharmaceutical products are commonly tested using animal models before advancing to human clinical trials. Suitable animal models are those that develop human signs of disease after infection with a pathogen known to cause human illness. HIV has posed an additional challenge to investigators in that no non-primate animal model has thus far been identified in which an AIDS-like illness develops from HIV infection. Chimpanzees are the only animal species to have demonstrated susceptibility to HIV-1 infection; however, they are not susceptible to infection with all strains of HIV-1 and rarely exhibit an AIDS-like illness once infected (20–22). Severe restrictions on the supply and research use of chimpanzees further limits the use of this model.

Consequently, most HIV vaccine research among animals has been performed using a common primate species, the Asian rhesus macaque. Macaques can be infected with an HIV-related lentivirus, simian immunodeficiency virus (SIV), and demonstrate signs of an AIDS-like illness leading to death within a few weeks or months (23,24). Consequently, SIV-based vaccine constructs have been developed and administered to macaques, and subsequent challenges are performed with SIV strains. Many of these SIV vaccine/macaque protection experiments have demonstrated impressive results (25–27). However, it is unknown how closely these macaque experiments may approximate HIV vaccine clinical trials in humans.

In the early 1990s, SIV/HIV hybrids (SHIV) that carry and express the HIV-1 *env*, *tat*, and *rev* genes within SIV were developed (28,29). These early SHIV strains replicated well within primates but did not induce disease. However, further studies demonstrated that serially passaged SHIV produced an AIDS-like illness in macaques leading to death within weeks (30–33). Recent SHIV vaccine constructs and protection experiments in macaques have demonstrated the ability to contain pathogenic SHIV infection (34,35). However, as with the SIV-based model, it is unknown how well the SHIV-macaque model predicts the HIV-human situation.

CANDIDATE HIV-1 VACCINE CONSTRUCTS

To date, over 100 different HIV-1 vaccine constructs have been developed and tested in animal models. These constructs have been developed using various approaches or strategies (Table 39.1). The approaches that appeared the most promising (recombinant subunit, live vector, DNA, and live-attenuated) have progressed the furthest. However, only the Þrst three have progressed to human clinical trials.

Recombinant Subunit Vaccines

Early HIV-1 vaccine development focused on the production of recombinant protein subunit vaccines capable of eliciting neutralizing antibodies. These HIV protein subunit vaccines generally were from envelope proteins (e.g. gp120 and gp160) and were expressed or produced through mammalian cells, baculovirus, or insect cells. These vaccine constructs were developed from laboratory T-cell line adapted (TCLA) strains and showed good neutralizing antibody response (against TCLA strains) and protection of chimpanzees against infection with homologous HIV-1 strains (36Ð38). Results from numerous human clinical trials indicated subunit vaccines to be both safe and capable of eliciting a neutralizing antibody response in humans (39,40).

However, advancement to large-scale efÞcacy trials was controversial due to subsequent study results and new scientiÞc discoveries that found the recombinant subunit approach lacking. Later studies indicated that neutralizing antibody activity generated by subunit vaccines was limited to only laboratory-adapted viruses, and that primary or wild-type HIV isolates were not neutralized with sera from subjects with neutralizing antibodies (41). Other studies indicated the importance of the cell-mediated immune response and CD8+ CTL activity (42,43). Although the subunit vaccine constructs stimulated lymphoproliferative responses to HIV-1 antigens and antibody-dependent cytotoxicity, they did not induce CTL responses (39,44). Both cell-mediated immune responses and the neutralization of wild-type isolates were widely considered to be important for vaccine efÞcacy; the inability of subunit vaccines to induce these responses was perceived as a major drawback. In June 1994, a U.S. government panel (the AIDS Research Advisory Committee) decided not to fund a large-scale efÞcacy trial of recombinant (r)gp120 protein subunits that had advanced through early human clinical trials (45), and directed subsequent research towards vaccine constructs that would elicit both neutralizing antibody activity against primary isolates and cell-mediated responses. However, one biotechnology company, VaxGen, Inc., (Brisbane, Ca) decided to proceed and privately sponsored large-scale efÞcacy trials of a rgp120 subunit construct (AIDSVAX) in North America, the Netherlands, and Thailand (46).

Live Vector Vaccines

The perceived importance of cellular immunity and the CTL response encouraged the development of live vector vaccines. In these vaccines, various HIV-1 genes (e.g. *env, gag, pol*) were incorporated into a replicating system through live vector constructs such as adenovirus, *Salmonella*, alphaviruses, canarypox, vaccinia, or attenuated vaccinia vectors (i.e. modiÞed vaccinia Ankara or MVA), resulting in antigen expression. Preliminary studies using canarypox or vaccinia-based constructs appeared promising in that they induced HIV-1-speciÞc CTL responses and indicated that cross-subtype killing did occur (47). However, when administered alone, these constructs elicited poor neutralizing antibody responses. Since 1994, efforts

TABLE 39.1. *Approaches to HIV-1 vaccine development*

Recombinant subunit vaccine: a vaccine produced by genetic engineering, mostly generated from laboratory-adapted HIV-1 strains, simulating a part of the outer surface envelope or other part of HIV
Peptide vaccine: HIV-1 internal proteins or synthetic peptides that contain relevant epitopes of HIV-1 envelope
Live vector vaccine: a live bacteria or virus that is harmless or can be modi ed to be harmless to humans that contains various combinations of HIV-1 genes (*env*, *gag*, *pol*, and *nef*). Current vectors include vaccinia virus, canarypox, adenovirus, *Salmonella*, bacille Calmette-Guerin, poliovirus, and Venezuelan equine encephalitis virus
DNA immunization (naked DNA or nucleic acid vaccine): segments of genome or plasmid that undergo transcription to express proteins
Whole-killed or inactivated vaccine: live HIV that has been inactivated by chemicals, irradiation, temperature, or other means to render it noninfectious but that retains its antigenicity and the ability to stimulate the immune system. Such formulations would theoretically require additional doses of vaccine or “boosters” to provide long-lasting immunity
Live-attenuated vaccine: live HIV that has had its virulence or pathogenicity reduced or removed through the deletion of genes responsible for viral replication or disease, usually accomplished by repeated passage of the wild-type or disease-causing strain through different hosts or cell lines. Live-attenuated vaccines produced for other diseases (e.g. polio) have demonstrated long-lasting immunity. Generally considered to be too great a risk because of the potential to revert to the disease-causing form.

have focused on a combination (prime-boost) regime consisting of the administration of a live vector construct "prime" followed by a subunit protein "boost" to enhance the humoral immune response.

Subsequently, numerous small and medium-sized human clinical trials conducted in the United States and abroad that employed canarypox or vaccinia vector construct primes plus subunit boosts demonstrated these constructs to be safe and immunogenic. However, consistent and durable CTL responses among all vaccinated individuals have been difficult to achieve; only 15–30% of vaccine recipients have had detectable CTL activity at any given time point (19,48–50). In addition, the evaluation of CTL responses has been complicated by the lack of a simple, reproducible assay, although results from studies using a new enzyme-linked immunospot (ELISPOT) assay look promising (51). Despite these difficulties, two large-scale efficacy trials using canarypox constructs with and without subunit protein boosts were originally proposed for initiation in 2002 and 2003 in Thailand and North/Latin America (52,53). The North/Latin American trial, designed to have statistical power to evaluate correlates of protection, was subsequently canceled after data from two phase II trials indicated that this outcome would not be possible; however, the Thai trial, powered to show efficacy, will proceed as scheduled (54).

DNA Vaccines

Advances in technology have promoted the exploration of DNA-based constructs as potential preventive vaccines for malaria and HIV-1. HIV DNA vaccine constructs encode for virus proteins that are expressed by cells of the vaccine recipient and stimulate neutralizing antibodies to circulating free virus, as well as prime cytotoxic T-cells, to recognize HIV and destroy infected cells. DNA vaccines are an attractive option because they are potentially highly immunogenic, relatively inexpensive, and easy to manufacture and administer (55).

Initial HIV DNA vaccine studies demonstrated that sole administration of DNA constructs, referred to as "naked DNA," elicited antigen specific lymphocyte proliferative responses and antigen specific production of interferon gamma and beta-chemokines. However, these responses were not persistent or consistent among individuals (56), and CTL and humoral immune responses were relatively weak. Subsequent animal studies demonstrated that both cell-mediated and humoral immune responses improved with the development of more complex and immunogenic DNA vaccine constructs, including naked DNA with cytokine enhancement (34), combination prime-boost regimens (i.e. naked DNA prime plus recombinant protein boost (57–59) naked DNA prime plus vaccinia boost (58,60) and DNA prime plus MVA boost (35,61,62)) and replicon-based vaccines using various alphaviruses: e.g. Sindbis virus, Semliki Forest virus, and Venezuelan equine encephalitis virus (VEE) (63,64).

Several SHIV DNA animal vaccine/challenge studies in macaques failed to demonstrate sterilizing immunity but did show the ability of these constructs to control infection by maintaining a low viral load (34,35,65). Several small human clinical trials of combination DNA prime-boost construct regimens have recently been initiated or are in the early planning stages to evaluate safety and immunogenicity in humans (65,66).

Live Attenuated Vaccines

Live attenuated vaccines developed against polio, measles, and mumps are examples of efficacious vaccines capable of providing long-lasting immunity. Attempts to develop a live attenuated HIV-1 vaccine have been made despite the inherent risks of such a vaccine being pathogenic or spontaneously reverting to a highly pathogenic form. The most impressive protection from a SIV challenge to date has been established by a live attenuated SIV vaccine administered to macaques (67,68). However, subsequent animal studies indicating that a SIV strain constructed with triple deletions (*nef*, *vpr*, and a negative regulatory element) was pathogenic in neonate and adult macaques (69) has halted further progression of a live attenuated HIV-1 construct to human clinical trials.

CLINICAL TRIALS

After animal studies showed HIV-1 vaccine constructs to be safe and immunogenic, investigators initiated human clinical trials to evaluate the most promising candidates. Human HIV-1 vaccine clinical trials are generally performed in three steps or phases.

Categories of Clinical Trials

In phase I trials, vaccine constructs are administered to approximately 10–40 persons at low risk for HIV infection to assess safety and immunogenicity. These trials may involve the use of placebo, randomization, and blinding of participants. Phase II trials are generally conducted among 50–400 persons at low risk (although can include some at higher risk), and additional safety and immunogenicity data are collected. Placebo, randomization, and blinding are generally used in these trials. Phase I and II trials may sometimes be combined if certain constructs have been satisfactorily tested in prior phase I trials. Phase III (large-scale efficacy) trials are performed after successful phase I and II results. They are conducted among thousands of persons at high risk to assess safety, immunogenicity, and efficacy. These trials involve control groups (possibly a placebo), randomization, and blinding of both investigators and participants (double-blind). Phase III trial results provide data leading to vaccine licensure and the

recommendations for its use among the public. After phase III trials, vaccine bridging studies and/or phase IV trials may be performed to examine different immunization schedules and assess the vaccineÕs protective effects against various HIV strains, within different population groups, and when coadministered with other vaccines or drugs.

Major Organizations Involved in Clinical Trials

From 1986 through 2001, there have been more than 80 HIV-1 phase I and phase II trials conducted worldwide evaluating more than 30 vaccine constructs from a wide variety of vaccine developers and only one phase III trial. The vast majority of HIV-1 vaccine trials have been conducted in the United States and France (Table 38.2) and have consisted largely of subtype B vaccine constructs. Only 15 trials have been conducted in developing countries; African countries have participated in three trials. Numerous governmental agencies, non-governmental organizations, universities, clinical sites, and industry representatives have been involved in the support, coordination, and evaluation of these vaccines. A few of these organizations are described below.

U.S. Federal Agencies

In the United States, HIV vaccine clinical trials are predominantly sponsored either through the National Institutes of Health (NIH) or privately through individual pharmaceutical or biotechnology companies (e.g. Aventis Pasteur, VaxGen, Inc., Merck Research Labs). From 1988 to 2000, NIH sponsored over 50 phase I and II clinical trials through the AIDS Vaccines Evaluation Group (AVEG) or the HIV Network for Prevention Trials (HIVNET). AVEG consisted of a group of six U.S. university-based clinical testing sites formed in order to evaluate HIV-1 vaccine candidates in phase I and II clinical trials. HIVNET, composed of both international and domestic sites, was created to assess the feasibility and design of future efficacy trials in high-risk uninfected individuals, to develop clinical trial-related infrastructure, and to eventually conduct clinical trials to evaluate the safety and efficacy of interventions to prevent sexual, perinatal, and parenteral HIV transmission. HIVNET research areas included: HIV preventive vaccines, topical microbicides, sexually transmitted disease (STD) treatment, prophylaxis to prevent mother-to-infant transmission, hormonal contraception, and behavioral risk-reduction strategies.

In May 2000, AVEG and HIVNET were merged to form the international HIV Vaccine Trials Network (HVTN). HVTN is a comprehensive network of clinical sites located in the United States, sub-Saharan Africa, Asia, Latin America, and the Caribbean that will develop and test preventive HIV vaccines (70). Within this framework, HIVNET protocol 026, a phase II trial of a subtype B-based canarypox construct (ALVAC vCP1452) with a gp120 subunit protein boost (AIDSVAX B), was initiated in Haiti, Trinidad and Tobago, and Brazil (71). This study was the first HIV vaccine clinical trial for both Haiti and Trinidad.

Two other federal agencies, the Department of Defense through the Walter Reed Army Institute of Research (WRAIR) and the Centers for Disease Control and Prevention (CDC) are also active in vaccine development and clinical trials. WRAIR supports basic science research, nonhuman primate studies, and human clinical trials, with a particular focus on non-subtype B candidate vaccines. It has been active in Thailand and East Africa and worked with Thai officials on plans for a possible phase III trial of a subtype B/E-based canarypox construct (ALVAC vCP1521) plus a bivalent rgp120 (AIDSVAX B/E) for late 2002 (72). Planning and implementation of the trial and other WRAIR vaccine activities will be funded through the NIH at the end of 2002. CDC is collaborating with VaxGen, Inc. on the two ongoing AIDSVAX phase III efficacy trials in the United States and in Thailand. In addition, CDC has been collaborating with Emory University scientists and public health officials from the CÔte dÕIvoire, West Africa, to develop and prepare to evaluate a DNA vaccine based on HIV-1 A/G viruses (CRF02_AG) from CÔte dÕIvoire.

France and Thailand

Although several foreign countries have been involved in vaccine clinical trials, of special note are France and Thailand. In France, lÕAgence Nationale de Recherches sur le SIDA (ANRS) was established in 1989 to promote, fund, and coordinate HIV research. The agency contains three research sections: basic science, clinical studies and epidemiology (includes vaccine and other clinical trials), and social and behavioral science. ANRS-sponsored phase I and II vaccine clinical trials have been performed only in France, but ANRS plans to conduct future international vaccine trials through its collaborative clinical research program with the French Secretary of State for Overseas Cooperation in Africa and southeast Asia (73).

Thailand has staged eight preventive HIV-1 vaccine clinical trials since 1994 (Table 39.3) including one of the first two phase III efficacy trials (74). These trials have been coordinated by Thai officials and have been conducted by a variety of Thai institutions in collaboration with international partners and industry. In the face of a rapidly expanding HIV-1 epidemic in the early 1990s, Thailand took several steps to prepare for HIV vaccine trials. With the assistance of the World Health Organization (WHO), Thailand developed a national plan for HIV vaccine development and evaluation. This plan was very helpful in setting the course for subsequent trials. To complement this plan, efforts were made to strengthen

TABLE 39.2. *Candidate HIV vaccines evaluated in human clinical trials in HIV uninfected volunteers*

Vaccine Construct	Developers[a]	Trial Sites	Trial Phase	Date of Initial Trial
Recombinant subunit				
rgp160 (6,39,218,219)	MicroGeneSyn, Immuno-AG, Aventis Pasteur, U Libre de Bruxelles	Zaire, U.S., France	I	1987
rgp120 (env 2–3) (220,221)	Biocine	Switzerland, U.S.	I	1988
rgp120 (29,121,122,142,222–226)	Biocine, Smith Kline Beecham, Chiron Vaccines, Genentech	U.S., Thailand, U.K.	I, II, III	1991
Tat, chemically inactivated toxoid (227,228)	University Pierre et Marie Curie in Paris/ Institute of Human Virology[b]	Italy, U.S.	I, II	1997
Peptide/Particles				
HGP-30/HGP-30W (229–233)	Viral Technologies, Inc.	U.K., U.S.	I, II	1989
Mixed env (219,234,235)	U Libre de Bruxelles	France	I	1989
V3 peptide, MAPS (multiple antigen presenting system) (236–240)	United Biomedical Inc.	France, U.S., Thailand, China, Brazil, Australia	I, II	1992
V3 peptide-PPD conjugate (241)	Swiss Serum and Vaccine Institute	Switzerland, Israel, U.S.	I	1992
Mixed env on keyhole limpet hemocyanin carrier (242)	Yokohama City University	Japan	I	1992
Ty.p24.VLP (243,244)	British Biotechnology Ltd	U.S., U.K.	I	1992
MN-V3, V3-p24 (CLTB-36) (245,246)	Pasteur-Merieux Connaught	France	I	1994
Gag-lipopeptide (P3C541b) (244,247)	United Biomedical, Inc.	U.S.	I	1995
Gp120 C4-V3 peptides (244,247)	Wyeth-Lederle Vaccines and Pediatrics	U.S.	I	1997
Lipopeptide mixture (nef-LAI, gag-LAI, peptide V3)(245,248)	ANRS	France	I	1996
V3 multi-epitope polypeptide (249,250)	Centro de Ingenieria Genetica y Biotecnologia	Cuba	I	1996
p24 (247)	Chiron	U.S.	I	1997
Live Vector				
Vaccinia-rgp160 (219,251–256)	Bristol Myers Squibb, Institute Jacques Monod, University of Jussieu	Zaire, U.S., France	I	1986
Canarypox gp160 (vCP125) (257–259)	Aventis Pasteur	U.S., France	I	1992
Vaccinia-env/gag/pol TBC-3B (244)	Therion Biologics	U.S.	I	1994
Canarpox-env/gag/prot (vCP 205) (48,246,260,261)	Aventis Pasteur	U.S., France, Uganda	I, II	1994
Canarypox env/gag/ protease + pol/nef epitopes (vCP300) (244,262)	Aventis Pasteur	U.S., France	I	1995
S. Typhi, CVD 908 (244,247)	University of Maryland Center for Vaccine Development, Chiron Corporation	U.S.	I	1997
Canarypox eng/gag/prot + pol/ nef epitopes (vCP1433) (244)	Aventis Pasteur	U.S.	I, II	1998
Canarypox env/gag/prot + pol/ nef epitopes + 2 vaccinia coding sequences (vCP1452) (71,244)	Aventis Pasteur	U.S., Brazil, Haiti, Trinidad & Tobago	I, II	1998**
Canarypox vCP1452 + LIPO-5 (5 lipoproteins of gag/nef/ pol) + LIPO-6T (LIPO-5 and a lipopeptide sequence expressed in tetanus toxoid) (262)	Aventis Pasteur	France	I	1999
Canarypox gp120 subtype E TM, gag/pro + rgp160 THO23/ LAI-DID (vCP1521) (262)	Aventis Pasteur	Thailand	I	1999
Adenovirus (gag) (65)	Merck Research Labs	U.S.	I	2001

TABLE 39.2. *Continued*

Vaccine Construct	Developers[a]	Trial Sites	Trial Phase	Date of Initial Trial
DNA				
APL 400–003 env/rev (263)	Wyeth Lederle	U.S.	I	1996
APL 400–047 gag/pol structural proteins (244,264)	Wyeth Lederle	U.S.	I	1997
Naked DNA (65,265)	Merck Research Labs/Vical	U.S.	I	1999
DNA + MVA boost Gag gene (gag, pol, nef, env epitopes) (66)	Cobra Pharmaceuticals Impfstoffwerke Dessau-Tornau, GmbH (IDT)	U.K., Kenya	I	2000
Modi ed gag-pol protein (pGag(del fs)Pol delta PR delta RT delta IN/h) VRC4302 (247)	National Institutes of Health	U.S.	I	2001
DNA + adenovirus (Ad5) boost (266)	Merck Research Labs	U.S.	I	2002

[a] Developers' current name and previous name(s)

Current Name	*Previous Names*
Aventis Pasteur	Pasteur-Merieux/Connaught, Connaught Laboratories, Pasteur Merieux Connaught
Bristol-Myers Squibb	Bristol-Myers Squibb/Oncogen
Chiron Corporation	Biocine, Chiron/Biocine, Chiron, Chiron Vaccines
IMMUNO AG	IMMUNO-AG, IMMUNO Clinical Research Corporation, IMMUNO-US
Protein Sciences Corporation	MicroGeneSys
VaxGen, Inc.	Genentech, Inc.
Wyeth Lederle Vaccines	Apollon, Wyeth-Lederle Vaccines and Pediatrics

[b] Soon to be manufactured by Aventis Pasteur.

TABLE 39.3. *HIV-1 preventive vaccine constructs evaluated in Thailand*

Vaccine Construct	Collaborating Organizations	Trial Phase	Trial Enrollment	Year Trial Began
Synthetic peptide MN-V3 (236)	Chulalongkorn University, United Biomedical, Inc.	I	30	1994
Gp120 (142)	Mahidol University, Genentech, Inc., BMA[a], WHO[b]	I/II	33	1995
Gp120 (223)	TAVEG,[c] Chiron	I	52	1995
Gp120 B/E (267)	TAVEG, Chiron	II	368	1997
Gp120 B/E (268)	BMA, Mahidol University, VaxGen, Inc., WHO	I/II	92	1998
Gp120 B/E (122)	BMA, Mahidol University, VaxGen, Inc., CDC[d]	III	2,500	1999
ALVAC vCP1521 + gp120 B/E or gp160 (269)	TAVEG, Chiron, Aventis Pasteur	I	130	1999
ALVAC vCP1521 + gp120 B/E (270,271)	TAVEG, Aventis Pasteur, VaxGen, Inc.	I/II	125	2000

[a] Bangkok Metropolitan Administration.
[b] World Health Organization.
[c] The Thai AIDS Vaccine Evaluation Group consists of the Armed Forces Research Institute of Medical Sciences (AFRIMS, a long-standing collaboration between the U.S. and Royal Thai Armies) Chiang Mai University, and Mahidol University).
[d] Centers for Disease Control and Prevention.

Thailandõ scientiÞc and ethical review process, conduct epidemiologic and behavioral studies of at-risk populations, characterize the molecular epidemiology of HIV-1, and build laboratory and data management capacity. In response to these efforts, three companies (Chiron Corp., VaxGen Inc., and Aventis Pasteur) made candidate vaccines based on the predominant HIV-1 subtype circulating in Thailand, HIV-1 subtype E (CRF01_AE).

International Organizations

WHO, the Joint United Nations Programme on HIV/AIDS (UNAIDS), and the International AIDS Vaccine Initiative (IAVI) support the international research and development of vaccine constructs. In 1991 WHO announced that four countries (Brazil, Rwanda, Thailand, and Uganda) would be developed and supported as HIV-1 vaccine testing sites (75). Subsequently, WHO assisted Brazil, Thailand, and Uganda with the development of National AIDS Vaccine Plans. In addition, WHO/UNAIDS has supported the development of an African AIDS Vaccine Program, sponsored workshops and conferences for developing country researchers, and developed ethics guidelines on the conduct of HIV vaccine trials in developing countries (76). All activities are conducted in collaboration with the private and public sectors of both developed and developing countries. In January 2000, the joint WHO/UNAIDS HIV Vaccine Initiative was established to expand the activities initiated and implemented by WHO/UNAIDS in the preceding years and to increase efforts to promote and facilitate vaccine development and evaluation for developing countries (77).

IAVI was formed in 1996 to promote the development of safe, effective, accessible, preventive HIV vaccines for global use. Its work focuses on four areas: accelerating scientiÞc progress, mobilizing public support through advocacy and education, encouraging industrial involvement in vaccine development, and working to assure global access to a vaccine (78). To accomplish these objectives, IAVI assisted in forming partnerships between the private research sector (vaccine manufacturers) and developing country researchers to speed the progress of promising vaccine candidates into clinical trials. As of 2001, Þve main vaccine development partnerships were being supported by IAVI: the Oxford/Kenya (DNA vaccine with MVA boost) (79, 80), AlphaVax/South Africa (VEE replicon particle vaccine) (79,80), Targeted Genetics/ChildrenÕs Research Institute/South Africa (AAV vector), the Institute for Human Virology/Uganda (Salmonella vector), and Therion Biologics/India (MVA vector) (81,82). The Oxford/Kenya partnership was the Þrst group to begin phase I clinical trials; these trials began in the United Kingdom in August 2000 and in Kenya in March 2001.

Phase III (EfÞcacy) Trials

Phase III trials evaluate the efÞcacy of a pharmaceutical product in humans. These trials require a large number of volunteers to provide sufÞcient statistical power to demonstrate efÞcacy and are conducted as placebo-controlled, double-blinded, randomized studies. Trial size is determined by the incidence of the disease of interest and the trial endpoints. For example, phase III vaccine efÞcacy trials for other infectious diseases such as hepatitis B, Japanese encephalitis, and Haemophilus inßuenzae required 1,000 to 60,000 participants and lasted approximately two years (83Ð85).

Endpoints and Effects of HIV Vaccine EfÞcacy Trials

Preventive HIV vaccine efÞcacy trials are designed to determine if a candidate vaccine is able to achieve speciÞc, desirable, measurable parameters or Òendpoints.Ó HIV vaccines, like most vaccines for infectious agents are designed to prevent infection, disease, or both. Consequently, endpoints in efÞcacy trials would be measures of HIV infection, measures of HIV-related disease, or both. EfÞcacy trials can also measure various serologic and immunologic parameters to identify laboratory correlates of protection from infection or disease, adverse clinical effects to evaluate the safety of a candidate vaccine, and effects on HIV transmission.

Endpoints and Correlates of Protection

When phase III HIV vaccine efÞcacy trials were initially considered in the early 1990s, the main efÞcacy endpoint considered was HIV infection, as deÞned by serologic assays and HIV-speciÞc nucleic acid tests (86). This endpoint or the complete prevention of HIV infection, termed Òsterilizing immunity,Ó is a type of protection thought to be conferred by neutralizing antibodies.

Recent data from phase I and II HIV vaccine trials in humans (87,88) and from trials in macaques (34,35) suggest that the goal of providing sterilizing immunity in humans may be difÞcult to attain. Consequently, there has been increasing interest in assessing the ability of HIV vaccines to induce cellular immune responses that control HIV replication, resulting in a mitigation of HIV-related disease in those vaccine recipients who do become HIV infected (89). HIV viral load as determined by plasma HIV RNA levels, is the most readily measurable indicator of the degree of HIV infection. The plasma viral load Òset point,Ó established by six months after infection, is the best single predictor of progression to AIDS and death (90). Plasma viral load is also closely associated with the rate of CD4 T-lymphocyte decline. Consequently, it is hypothesized that a lowered HIV viral load (compared with the viral load of placebo recipients) resulting from HIV vaccination will be associated with less severe HIV-related disease and delayed progression to AIDS and death. HIV vaccine efÞcacy trials are now being designed to assess HIV RNA levels as an important endpoint and a surrogate marker for severity of HIV infection. However, it remains to be established whether a vaccine-induced reduction in viral load has the same beneÞcial effects as a natural reduction in viral load.

The parameter most commonly used to assess HIV disease progression is the CD4 T-lymphocyte count.

Decline in CD4 cell count is closely associated with the onset of HIV-related opportunistic infections and AIDS and is the main laboratory indicator for the initiation of antiretroviral therapy (90). Therefore, like viral load, the CD4 cell count in vaccine recipients who become infected may be a useful endpoint in efficacy trials. An HIV vaccine that slows the rate of decline in CD4 cells would be hypothesized to slow disease progression and delay AIDS and death. An efficacy trial would compare CD4 counts of placebo recipients and vaccine recipients who became HIV infected during the trial. The endpoint could be the rate of decline of CD4 cell count or the time from infection to a CD4 count of a given level; e.g. 500, 350, or 200 cells/mm^3.

Plans for future HIV vaccine efficacy trials will include evaluations of the HIV RNA level and CD4 counts in infected participants. However, each of these endpoints is dramatically altered by antiretroviral therapy. The design of trials will need to account for the prevailing treatment practices, especially if these practices include treating recently identified HIV seroconverters. The early initiation of antiretroviral therapy would make it impossible to interpret the vaccine's effect on subsequent viral load or CD4 cell count determinations.

Determination of readily measurable correlates of immune protection is an important goal of HIV vaccine efficacy trials. Consequently, selected blood specimens are collected to determine serologic and cellular immune responses, and semen specimens and genital secretions may be collected to assess immunologic and virologic factors associated with HIV transmission. In addition to HIV-specific antibodies, current trials are being designed to measure HIV-specific CTLs by traditional assays and by newer ELISPOT assays.

Trial-related Effects

Scientific models have been developed to examine HIV vaccines, their trial-related effects, and potential transmission effects or outcomes. The most traditional type of HIV vaccine efficacy is related to susceptibility to HIV infection (VEs) and is measured by the endpoint of HIV infection status in the vaccine and placebo recipients. The VEs is equal to 1 minus the attack rate in the vaccine recipients divided by the attack (HIV incidence) rate in the placebo recipients; the equation is: $VEs = 1 - (ARv/ARp)$ (91). The endpoint of HIV infection is relatively easy to determine. It is also possible to measure the vaccine efficacy for disease progression (VEp). The endpoints related to progression could be HIV RNA levels, decline in CD4 cell counts, or time to HIV-related disease or AIDS. However, as discussed above, these endpoints would be modified by antiretroviral therapy and may require a longer period of observation to clearly establish a meaningful endpoint.

Another important effect of an HIV vaccine would be the reduction in the infectiousness of vaccine recipients (91,92). Such an effect could profoundly affect the course of the epidemic in a community (93). The vaccine efficacy for infectiousness (VEi) can be determined by novel partner augmentation trial designs (91,92). In such a trial, the sex partners of the vaccine trial participants are assessed to determine if there is differential transmission from vaccine recipients and placebo recipients to the respective partners. Plasma and genital fluid HIV RNA levels are also assessed as they are associated with sexual transmission to partners (94).

The assessment of vaccine efficacy for susceptibility (VEs), disease progression (VEp), and infectiousness (VEi) would provide a comprehensive profile of the beneficial effects of a preventive HIV vaccine.

Current Phase III Trials

Despite the large number of constructs developed and tested in phase I and II trials, only one product (two formulations), a recombinant subunit protein product, rgp120, (AIDSVAX (VaxGen, Inc., Brisbane, Ca.)) has advanced to phase III trials.

Genentech, Inc., a biotechnology firm located in South San Francisco, California, began research to develop a preventive HIV-1 vaccine in 1984 and subsequently developed a recombinant gp120 protein subunit vaccine shown to protect chimpanzees against HIV infection. In 1995, after NIH decided not to proceed with the funding of gp120 vaccine efficacy trials, a new company named VaxGen, Inc. was formed and maintained a relationship with Genentech, Inc. By 1997, VaxGen, Inc. had produced two bivalent rgp120 vaccines (AIDSVAX B/B and AIDSVAX B/E). Both formulations contained the gp120 sequence of the subtype B, T-cell lymphotropic strain "MNÓ in addition to the gp120 sequences from a macrophage-tropic strain (GNE8 (subtype B) or A244 (subtype E)). Both vaccines were demonstrated to be safe and immunogenic in human volunteers in phase I and II studies (46,95).

In June 1998, a phase III trial evaluating AIDSVAX B/B was initiated in North America and the Netherlands, and in March 1999 a second trial evaluating AIDSVAX B/E was started in Thailand (96,97). The AIDSVAX B/B phase III trial is a randomized double-blinded, placebo controlled (2 : 1 vaccine : placebo ratio) trial conducted in 5,109 HIV-1 uninfected men who have sex with men (MSM) and 309 uninfected women at high risk recruited from 61 clinical sites in the United States, Canada, and the Netherlands. Study participants attend 16 study visits, receive HIV counseling and testing at six-month intervals, and are administered seven intramuscular AIDSVAX B/B immunizations over three years (at months 0, 1, 6, 12, 18, 24, and 30). The AIDSVAX B/E trial is being conducted among 2,545 HIV-1 uninfected injection drug users (IDUs) attending seventeen drug treatment clinics in Bangkok, Thailand and, with the exception of a 1:1

vaccine : placebo ratio, has a study protocol similar to that of the AIDSVAX B/B trial.

An independent data safety and monitoring board (DSMB) composed of American and Thai members oversees both trials and reviews study data every six months to assess safety and trial conduct. Community advisory boards (CABs) are used for the AIDSVAX B/B trial; a Community Relations Club was established in Bangkok. Composed of a variety of community members, a CAB serves as an advocate for the study volunteers and represents the perspectives of the affected communities and people at risk for HIV infection. For the AIDSVAX B/B trial, there is one national VaxGen Study CAB and approximately 25 local CABs. Participants who become infected during the trial are referred for medical care by the trial sites in the AIDSVAX B/B trial or followed up by the Bangkok Metropolitan Administration (BMA) in Thailand. The AIDSVAX B/B trial will be completed in late 2002, while the AIDSVAX B/E trial will be completed in late 2003.

Criteria for Phase III Trials

Identifying or developing the appropriate trial conditions and infrastructure within a country for an HIV-1 vaccine phase III efficacy trial requires substantial time and effort (Table 39.4). Initial steps include developing a national plan for the conduct of HIV-1 vaccine research development and evaluation within a specific country and gaining national political and institutional support for vaccine trials. Once support is established, various locations should be evaluated for their potential to serve as vaccine trial field sites.

Site Identification

An ideal field site has access to a population that meets well-defined epidemiologic and social-behavioral criteria and a research and clinical infrastructure that provides appropriate laboratory and data management facilities and trained personnel. In addition, this site would have the involvement of the community, and a trial staff that is aware and recognizes the social and ethical issues involved so as to ensure the protection of human rights for trial participants. It is rare to identify a field site that meets all these criteria. Most often areas that have a high HIV-1 prevalence and/or seroincidence are identified, and further assessment is required to determine their feasibility as vaccine trial field sites.

Site Preparation

If field assessments are favorable, additional study is essential to characterize the populations and to prepare both participants and communities for a potential vaccine trial. It is not unusual for field site preparation to require two to five years of preliminary epidemiologic study and infrastructure development before a vaccine trial can be initiated. Epidemiologic characterization involves identifying high-risk persons and risk behaviors within that population, determining HIV seroincidence with prevention efforts in place, genetic sequencing of prevalent and incident HIV strains, and assessing the ability to enroll and follow up persons at risk. Infrastructure development includes identifying or developing several review boards to oversee the ethics, science, and participant needs throughout the trial; training clinical research personnel; and developing or improving trial facilities and procedures (e.g. data management, laboratory, medical referrals).

TABLE 39.4. *Preparing for a HIV-1 vaccine phase III trial*

National items
- Plan for HIV-1 vaccine research, development, and evaluation
- Political will and institutional support for HIV-1 vaccine trials

Epidemiologic and social-behavioral features
- Well-characterized population with high HIV-1 incidence rates despite ongoing prevention efforts
- Molecular characterization of the circulating HIV strains in the community
- Recognition of factors in uencing the recruitment, enrollment, and retention of potential participants

Infrastructure requirements
- Scienti c review process
- Ethical review process
- Community advisory board to address participant and community concerns and needs
- Clinical research facility with adequate laboratory and data management support
- Trained clinical research personnel (doctors, behavioral scientists, nurses, lab technicians, etc.)

Social issues
- Protection of human rights through discussion, identi cation and awareness of ethical issues pertaining to trial participation (e.g. con dentiality, stigma, etc.)
- Procedures to assure comprehension of informed consent
- Procedures in place to refer or offer medical care to persons who become HIV infected within the trial

Community Preparation

Both communities and potential vaccine trial participants should be provided with information on the conduct of HIV vaccine trials and participantsÕ rights. Discussions should occur to identify ethical issues and to determine how to address issues related to conÞdentiality and stigma that may arise during the trial. Studies performed in communities and among potential participants to determine trial acceptability and willingness to participate in vaccine trials are commonly referred to as vaccine willingness-to-participate studies, and the epidemiologic and behavioral studies are termed vaccine feasibility studies or vaccine preparedness studies. Willingness-to-participate and vaccine feasibility studies are not mutually exclusive and have been performed simultaneously or concurrently among a variety of communities, researchers, and potential vaccine trial participants (98Ð118).

Participant Selection

Identifying a study population or potential cohort can be challenging. Participants must be from a population that has sufÞcient HIV seroincidence, be interested in participating in an HIV vaccine efÞcacy trial, and be available for enrollment and follow-up. These cohorts may be difÞcult to locate, enroll, and follow up (110,119). Female commercial sex workers and young MSM, selected because of their populationsÕ high HIV seroincidence, proved to be difÞcult populations to enroll or retain in several vaccine feasibility studies (114,120,121). Conversely other studies that successfully enrolled and retained cohorts of high risk persons experienced decreases in HIV seroincidence over time because of early seroconversions in persons at higher risk, selective loss to follow-up, and decreases in risk activity due to changes in risk behavior over time or HIV risk-reduction counseling and prevention measures (99,115,122).

Paths Leading to the Current Phase III Trials

Advancement towards a phase III HIV-1 vaccine efÞcacy trial can be envisioned as two stone columns of an arch (Fig. 39.1). Numerous steps or stones must be placed in order to develop a suitable cohort and to evaluate and prepare a vaccine construct for efÞcacy trials. Once both cohort and vaccine construct are ready, they can be linked together through a phase III efÞcacy trial. A phase III efÞcacy trial cannot take place without completion of both columns.

In the AIDSVAX phase III efÞcacy trials, VaxGen, Inc. constructed the right column by scientiÞcally developing gp120 constructs, successfully conducting animal studies and human phase I and II clinical trials, and satisfactorily meeting the U.S. Food and Drug Administration and Thai approval processes to advance to phase III trials in the United States and Thailand. However, successful initiation and implementation of the trials required the simultaneous construction of the left column of the arch: the collaboration of many people and institutions to address the epidemiologic and behavioral factors, infrastructure requirements, and social issues.

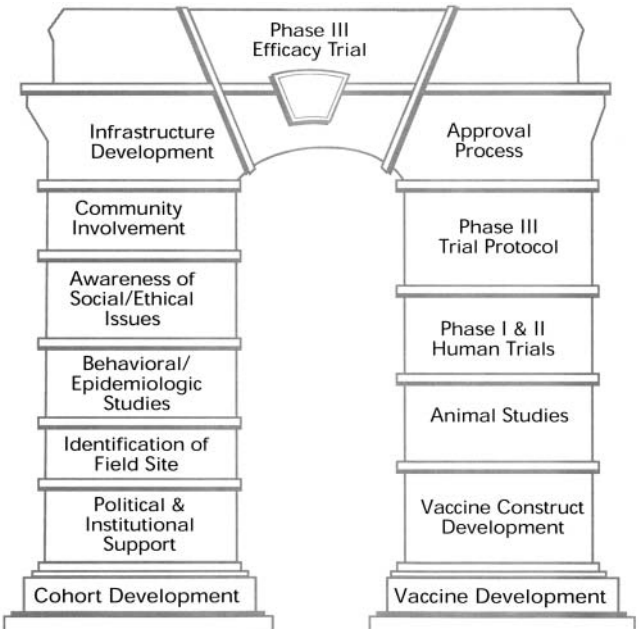

FIG. 39.1. Steps toward a phase III HIV vaccine ef cacy trial.

United States

The United States began AIDS surveillance in 1981, identifying high-risk populations and risk factors for infection. Molecular epidemiologic studies identiÞed the predominant HIV-1 subtypes and strains (123,124); seroincidence and vaccine preparedness studies among cohorts of MSM, IDUs, and other high-risk groups identiÞed factors related to recruitment, enrollment, and retention of potential vaccine participants (99,108,125Ð129). One of the largest studies, the HIVNET Vaccine Preparedness Study (VPS), successfully enrolled and retained 4,892 HIV-negative persons at high risk for HIV in nine U.S. cities between 1995Ð1997 (115).

Participation in AVEG and other industry-sponsored phase I and II clinical trials provided trained clinical research staff at community and academic sites. Procedures to address social and ethical issues, (e.g. conÞdentiality, stigma, and access to HIV-related medical care during HIV vaccine trial participation) had evolved through vaccine preparedness studies and clinical trials (130Ð132). Many AVEG and HIVNET clinical sites had formed local CABs to ensure that the investigators attended to the communityÕs needs and concerns.

Throughout the 1990s, social support and political will for vaccine trials had slowly been gaining momentum. Organizations, such as the American Foundation for AIDS Research (amFAR), the Treatment Action Group (TAG), and the AIDS Vaccine Advocacy Coalition (AVAC) encouraged research and evaluation of HIV vaccine candidates. In May 1997, President Clinton called for the development of a successful vaccine within 10 years, by 2007 (133).

Thailand

Thailand has had well-established national HIV surveillance since 1989. Multiple epidemiologic studies performed early in the epidemic identiÞed and described various at-risk populations; e.g. female commercial sex workers, male army conscripts, STD clinic patients, IDUs, and pregnant women (134Ð137). In 1991, WHO selected Thailand as a potential developing country site for preventive HIV vaccine efÞcacy trials and later supported investigation of Bangkok IDUs as a potential cohort. Subsequent epidemiologic and molecular studies documenting risk factors for infection, a high HIV-1 seroincidence rate, prevalent and incident HIV-1 strains, and the ability to enroll and retain IDUs (109,138Ð141), as well as the presence of the BMA, a public agency that oversees the administration of 17 drug treatment centers in Bangkok, made this location and population an attractive site for efÞcacy trials.

Complementary to the development of this cohort was the presence of a strong medical and public health research infrastructure. There had been high-level national support for HIV vaccine research and evaluation since the early 1990s. The National AIDS Committee created a subcommittee on HIV vaccines and, with WHO and UNAIDS support, a national plan was established for HIV vaccine development and evaluation. In addition, from 1994 to 1998, Þve HIV vaccine phase I and II clinical trials had been conducted in Thailand, far outnumbering that of any other developing country. When a 1995Ð1998 preparatory cohort study of Bangkok IDUs in treatment programs indicated that HIV-1 subtype E accounted for 80% of incident infections (138,140), VaxGen, Inc. formulated AIDSVAX B/E for use in this population. The AIDSVAX B/E phase I/II trial, started in 1998, was a collaborative effort involving the BMA, Mahidol University (Bangkok) and VaxGen, Inc. (142). Data from this successful trial led to a proposal for a phase III efÞcacy trial. The Bangkok AIDS Vaccine Evaluation Group (BVEG) was formed by the BMA, Mahidol University, the HIV/AIDS Collaboration (a joint activity of the Thai Ministry of Public Health and the U.S. CDC), and VaxGen, Inc. to conduct this trial. The trial, initiated in 1999, used existing facilities in Bangkok, and a new data management center was developed at Mahidol University.

ETHICAL, SOCIAL, AND BEHAVIORAL ISSUES ASSOCIATED WITH HIV VACCINE TRIALS

The ethical, social, and behavioral issues associated with the conduct of HIV vaccine trials have received substantial attention given the unique nature of HIV infection and the potential for stigma, discrimination, coercion, or other harms related to trial participation. Scrutiny of a 1987 phase I trial of vaccinia-gp160/LAI in Zaire resulted in allegations of human subjectsÕ regulatory violations, causing the NIH to terminate their collaboration in this trial (143). Since then, continued attention has been given to the ethical, behavioral, and social aspects associated with the conduct of HIV vaccine trials (131,144Ð149). A brief review of the bioethics related to clinical trials provides a conceptual framework for considering informed consent, the potential of experiencing social harms as a result of trial participation, possible vaccine-induced antibody responses, HIV risk behavior change, and access to medical care for trial participants who become infected while participating in HIV vaccine trials.

Bioethics and Research Ethics Involving Human Subjects

ScientiÞcally rigorous research involving human subjects did not become common in the United States and Europe until shortly before World War II; thus, interest in human subjects research emerged relatively late in the twentieth century (150). The Nuremberg trials of the Nazi doctors accused of conducting murderous human experiments in concentration camps was a salient catalyst stimulating interest, concern, and action regarding human subjects research (150,151). As a result of these trials, directives for human experimentation were published as the Nuremberg Code in August of 1947. The Nuremberg Code has subsequently been referred to as the most important document in the ethics of medical research (151).

The General Assembly of the World Medical Association (WMA) perceived the disclosures at Nuremberg to be such a threat to the integrity of biomedical research that they drafted an oath for physicians to not use medical knowledge in a way that is contrary to the laws of humanity, published as the Declaration of Geneva in 1948 (151). In 1964, the WMA distinguished between ethical and unethical clinical research in the Declaration of Helsinki (151). It has been said that Nuremberg was the Þrst code prescribed for medicine externally by a court system and the Declaration of Helsinki, the Þrst code prescribed internally by a professional body in medicine (151).

In the 1970s, the revelation that Tuskegee Syphilis Study researchers withheld effective therapy from infected African-American men in order to study the natural

history of syphilis resulted in a national commission to develop principles and guidelines for the protection of research subjects (152,153). These guidelines were published as the Belmont report in 1978 (154). Although compatible with the WMAÕs Declaration of Helsinki, the Belmont report articulated three principles: *respect for persons* (the recognition of the right of persons to exercise autonomy), *beneficence* (the minimization of risk incurred by research subjects and the maximization of benefits to them and to others), and *justice* (the principle that therapeutic investigations should not unduly involve persons from groups unlikely to benefit from subsequent applications of the research) (154).

Another important ethical construct regarding the conduct of clinical trials is that of *equipoise*, defined as a state of genuine uncertainty regarding the comparative therapeutic merits of each arm in a trial (155). Equipoise is considered to be an essential condition for conducting randomized clinical trials and avows that only when there is no known effective treatment is it ethical to compare a potential new treatment with a placebo (156). Furthermore, should it be discovered that participation in one trial arm confers superior therapeutic merit than another, an ethical obligation to offer that treatment is assumed (155). If it becomes apparent during the course of a trial that one treatment is superior, the trial may need to be terminated and all participants and possibly other persons within the same population may have to be offered the superior treatment (155). This occasionally happens when interim analyses demonstrate efficacy, resulting in trial termination, distribution of product to placebo recipients, and commencement with product licensing.

Informed Consent

The Nuremberg code established the foundation for informed consent, stating the voluntary consent of human subjects in research trials is absolutely essential:

> ÒThis means that the person involved should have legal capacity to give consent; should be so situated as to be able to exercise free power of choice, without the intervention of any element of force, fraud, deceit, duress, overreaching, or other ulterior form of constraint or coercion and should have sufficient knowledge and comprehension of the elements of the subject matter involved as to enable him to make an understanding and enlightened decision. This latter element requires that before the acceptance of an affirmative decision by the experimental subject there should be known to him the nature, duration, and purpose of the experiment; the method and means by which it is to be conducted; all inconveniences and hazards reasonably to be expected and the effects upon his health or person which may possibly come from his participation in the experimentÓ(157).

Federal policy stresses two key elements regarding voluntary consent; the decision to participate in research should be made without undue pressure and should be based on sufficient information to confer informed consent (158). Regardless of whether an HIV vaccine is tested in developing or developed nations, informing potential participants about the risks, benefits, and requirements of trial participation may be extremely challenging. Participants attending a 1997 UNAIDS meeting to address the ethical issues of HIV vaccine trials recognized the challenge of explaining to individuals of radically different cultural backgrounds and vastly different levels of education the meaning of randomization, placebo, partial efficacy, adhering to safer sex practices, potential exclusion of early trial participants from future trials, and the potential of trial-related discrimination (147). In Uganda, for example, researchers preparing for HIV vaccine trials found that there were no linguistic equivalents for terms such as, Òbeing HIV infected,Ó Òhaving AIDS,Ó or ÒplaceboÓ (159). A Brazilian pilot study to identify problematic aspects of informed consent found that understanding of ÒrandomizationÓ and the potential of being excluded from future trials because of participation in current trials were lacking (160).

Problems with obtaining truly informed consent have been widely discussed in the literature of various disciplines including epidemiology (161), psychology (162, 163), clinical and international research (156), and HIV vaccine research (158). Expanded guidelines for the protection of human subjects in biomedical research from the U.S. Department of Health, Education, and Welfare have resulted in consent forms that are longer and more difficult to read (164). In one early HIV vaccine trial, 22 pages of HIV vaccine-related educational materials, judged to be at a reading level exceeding that of a significant percentage of the U.S. population, were used to inform participants of trial-related benefits and risks (158). These issues raise concerns about the ability of potential trial participants to adequately comprehend trial-related information presented to them during the informed consent process.

Comprehension of information presented during the informed consent process has been assessed in some HIV vaccine trials; however, little data have been published. The phase III trial of AIDSVAX B/E in Bangkok, Thailand includes a comprehension assessment and excludes the participation of individuals who score below a minimal threshold. Comprehension of trial-related information at enrollment has been found to be high among IDUs participating in a phase II trial (165). Simplified, restructured, and visual materials have been shown to facilitate recall of key trial-related risks and benefits among low-income women at risk for HIV infection (158).

Because limited data about comprehension are available, it is not known how comprehension might change over time or whether diminishing comprehension might pose a risk of harm to participants or threaten trial integrity. Participants in the two AIDSVAX HIV vaccine efficacy trials receive HIV risk-reduction and trial-related counseling at six-month intervals as reinforcement. Including standardized comprehension monitoring and associated education in future trials may reinforce the understanding

of trial-related concepts, mitigate potential risks among participants, and improve trial compliance.

Coercion

The Nuremberg Code asserts that participants should be free to choose without coercion to participate in research (151). Clinical trials, including HIV vaccine trials, that seek to enroll disenfranchised persons at risk for HIV infection (e.g. IDUs or sex workers of low socioeconomic status) or persons from developing countries with few economic or social support resources may inadvertently coerce individuals to participate by offering incentives that would be difficult to refuse given the personsÕ economic circumstances. Similarly, individuals choosing not to participate in trials must not be denied care that would have otherwise been provided if they did participate in a clinical trial. Potential trial participants could also experience social pressure to join a trial from peers, family, or community members who perceive that participation might provide benefit beyond the individual level (145). Sponsors of trials need to be vigilant about the potential for social coercion and should seek to identify and minimize this potential threat using appropriate formative and action research methods.

Social Harms Related to HIV Vaccine Trial Participation

There have been concerns that vaccine trial participation may result in stigmatization of trial participants by identifying them as high-risk individuals or by subjecting them to discrimination related to vaccine-induced HIV antibody (166–168). In a survey of 247 AVEG trial participants enrolled from 1994 to 1995, 18% reported at least one adverse social incident they perceived to be related to their trial participation; the most common (74%) was that disclosure of trial participation sometimes resulted in a mistaken presumption that participants were infected and possible subsequent avoidance or fear of the participant (131). Few problems related to jobs (1.2%), health or life insurance (1.6%), and immigration or travel (0.8%) were perceived by participants (131). Similarly, a subsequent report from 1,516 AVEG participants enrolled from 1995 to 1998 found that 5% reported social harms related to trial participation, primarily related to negative reactions of friends, family or co-workers; less than 10% of reported harms were reported to be associated with HIV testing (132).

Early data from the AIDSVAX B/B phase III trial indicate that between 4% and 8% of trial participants reported Òdisturbances in personal relationshipsÓ at their six- and 12-month visits. Disturbance typically involved negative comments about trial participation from family and friends or misperceptions that a participant was infected (169). Furthermore, trial-related employment or insurance problems accounted for less than 0.4% of reports (169). Similarly, preliminary data on 1300 IDUs participating in the Thai trial of AIDSVAX B/E, only 0.4% reported problems with personal relationships, and no social harms were associated with vaccine-induced antibody test results (170).

Vaccine-Induced Antibody Responses

The antibodies induced by some HIV vaccines can react with standard serologic assays (e.g. EIA and Western blot), resulting in HIV vaccine-induced seropositivity and may be misinterpreted as true HIV infection (168). These false-positive results could lead to social harms and discrimination regarding employment, insurance, travel, blood donation, and joining groups such as the military or the Peace Corps.

If a recipient of a complex HIV vaccine has positive serologic screening test results, it is necessary to perform confirmatory HIV testing with a Western blot and may also require assays to detect proviral DNA from peripheral blood mononuclear cells or HIV RNA from serum or plasma to determine whether or not there is true HIV infection. However, performing DNA or RNA assays, which are relatively complex and expensive, on a routine basis in large-scale HIV vaccine efficacy trials will add greatly to the cost and complexity of a trial. In addition, these assays are not routinely used for HIV diagnostic testing and are not widely available in resource-poor settings. However, given the potential social harms associated with vaccine-induced antibody responses being interpreted as natural infection, the development of new serologic tests or testing algorithms to distinguish between these two situations will be critical.

HIV-Related Risk Behavior

Concerns about the potential risks of trial participation have included speculation that HIV risk behavior might increase (171). Hypothetically, participation in HIV vaccine trials might bestow optimism regarding protection and a lowered perception of HIV risk among participants. If optimism and decreased perception of risk are prevalent among trial participants, HIV risk behavior could increase and result in subsequent infections.

Despite several reports in the literature that higher risk individuals are more willing than lower risk individuals to participate in HIV efficacy trials and that some individuals may participate in trials seeking protection from HIV infection (96,105,112,126,129,172,173), it is not known how participation in a trial actually influences HIV risk behavior. There has been one report of increased risk behavior among phase I/II HIV vaccine trial participants (171), however, methodologic constraints preclude casual

attribution to vaccine trial participation. For example, comparison groups not receiving vaccine injections were not enrolled, reported HIV risk behavior at baseline was signiÞcantly lower than what would be expected of high-risk participants, and factors other than trial participation could account for the slight increase in HIV risk behavior reported in this study (e.g. increased perception of HIV as a chronic manageable disease).

Traditionally, efÞcacy trials are designed to evaluate endpoints for those who receive an experimental product compared with those who receive a placebo or an existing standard of care, not to evaluate the effect of trial participation on risk behavior. If risk behavior increases over time among efÞcacy trial participants, causal attributions to trial participation are not possible unless a contemporary contrast arm that does not receive an injection of vaccine or placebo is built into the trial design. Without such a contrast group, potential confounders such as biased selection, history, maturation, statistical regression, experimental mortality, etc. (174), rather than vaccine trial participation, could account for observed risk behavior change within the context of a trial. For example, increased risk behavior might be due to the availability of and optimism about emerging treatments. Similarly, decreased risk behavior could be due to the fact that the highest risk individuals are enrolled into efÞcacy trials and a decline in risk behavior over time would be expected due to regression toward the mean (174).

Including perception measures within trials may help identify any association between trial participation, related perceptions, and HIV risk behavior. For example, assessing participant perceptions of randomization or of vaccine efÞcacy provides a conceptual basis for determining whether such perceptions are related to risk behavior change within a trial. In fact, these perceptions have been shown to be related to HIV risk behavior within the context of a trial (175); however, the trial participants at highest risk appear more likely to perceive randomization to vaccine arm and the vaccine itself to be of greater efÞcacy from baseline throughout the trial, so the association of risk behavior and perceptions may say more about these participants at highest risk than about the effect of trial participation on risk behavior. Unless control arms that receive no vaccine injections are included in future trial designs, nonequivalent group comparisons may be the only way to gain an understanding as to the potential for trial participation to increase risk behavior. Despite the potential confounders associated with nonequivalent group comparisons, comparing vaccine cohorts with other cohorts enrolled using similar eligibility criteria could provide convergent validity for this methodologic approach.

From a methodologic perspective, differential HIV risk behavior change between vaccinated and unvaccinated participants could confound the determination of vaccine efÞcacy (176). Behavior change could occur if participants discover through HIV antibody testing outside of the trial the presence or absence of vaccine-induced antibody. For example, if participants with vaccine-induced antibody assume protection and increase their risk behavior relative to those without such antibody, the calculation of efÞcacy will be biased. Furthermore, an increase in risk behavior among those who receive vaccine would expose these participants to an increased risk of infection, especially with a low-efÞcacy vaccine. Currently, participants are counseled to avoid testing outside the context of trials and are provided nonscheduled, on-demand antibody testing to minimize the risk of unblinding from outside testing. Through trial-provided testing and counseling, participants are informed about their antibody status related to actual infection but are not told if they have vaccine-induced antibody.

Beyond efÞcacy trials, there is concern that mass HIV vaccination campaigns might lead to increased risk behavior and HIV incidence, increasing the severity of the epidemic (177). Modeling studies suggest that even with extremely effective HIV vaccines, failure to simultaneously implement efÞcacious HIV risk behavior interventions will make prevention of HIV extremely difÞcult (177). Because of these ethical, empirical, and public health concerns, it is important to evaluate HIV risk behavior in the context of actual HIV vaccine efÞcacy trials so that adverse behavioral manifestations may be identiÞed, understood, and prevented in current and future trials and eventual HIV vaccination programs.

Access to Care

Related to the ethical construct of beneÞcence is whether vaccine trial participants are entitled to medical care as a result of participating in trials; and if so, what level of care should be provided? These questions are complex; the answers, controversial. Many trials have been and will continue to be conducted in resource-poor countries with limited health care infrastructures. In the developing country environment, should care be provided commensurate with the local standards or that provided by the sponsoring country? Thus, if the local standard of care does not include antiretroviral therapy, should these drugs be provided to individuals with positive screening test results or to those who seroconvert while participating in the trial? What if the laboratory, monitoring, and clinical capacity are unavailable for long-term follow-up of infected patients?

These issues have been fervently debated in the literature (145,147,152,178,179). The International Ethical Guidelines for Biomedical Research Involving Human Subjects, published by the Council for International Organizations of Medical Sciences (CIOMS), quotes the Declaration of Helsinki statement: ÒIn any medical study, every patientÑ including the control group, if anyÑ should be assured of the *best proven diagnostic and therapeutic method*Ó(180). Because of the complexity and potential

implications of providing the best proven therapeutic methods in the context of the developing world, an alternative proposal is that the *highest attainable standard* of care be provided in the context of HIV vaccine efficacy trials conducted in the developing world (144).

To address these issues, UNAIDS in September 1997 convened a meeting to identify and review the ethical considerations involved with international HIV vaccine trials (181). A plan was developed to conduct regional workshops in countries previously involved in HIV vaccine research, including Brazil, Thailand, and Uganda. In April and May of 1998, regional workshops were conducted that included biomedical and social scientists, community members, members of nongovernmental organizations, activists, persons living with AIDS, ethicists, lawyers, and government representatives (181). Consensus was not reached with regard to level-of-care issues. Some meeting participants felt that care should be provided commensurate with that offered in sponsoring countries for at least the duration of the trial; whereas, others favored providing treatment for those infected during trial participation, but not as a result of the trial, at a level consistent with that available in the host country (147). Concerns were voiced that promising care might constitute unethical and undue inducements to participate; however, one participant declared that providing the best available care would be tantamount to, ÒleaveÓ a Cadillac or Rolls in our country when no one can afford to drive or even repair themÓ (147,181). A final concern was that if the ethical bar is set below the maximum, it becomes easier to lower ethical standards in the future.

Access to care for trial participants will continue to be debated by ethicists, activists, and scientists. In the future, antiretrovirals may become more available as policy efforts establish global funds to support the purchase and distribution of these medications in the developing world. However, it is unlikely in the near future that such efforts will ameliorate the access-to-care debate regarding HIV efficacy trial participants.

The ethical, social, and behavioral questions associated with HIV vaccine trials will continue to be debated, and definitive answers will be elusive. The world of clinical trials is dynamic with regard to experimental context, product development, and the ethical, social, and behavioral implications of both research design and experimental products. Given the dynamic nature of the clinical trial context and the associated ethical, social, and behavioral questions, it will be important to continue to evaluate their effect on individual trial participants, targeted communities, and the conduct of trials.

FUTURE TRIALS

The AIDSVAX efficacy trials were successfully implemented due to the availability of a safe and immunogenic vaccine construct approved for phase III trials, access to suitable cohorts, and an infrastructure created by previous vaccine feasibility studies and clinical trials. However, the perceived importance of cellular immunity and the new discoveries related to HIV viral structure and pathogenesis have promoted further research, new vaccine construct development, and preparations for additional vaccine efficacy trials.

The next proposed phase III efficacy trial may begin in late 2002 in Thailand (52). This trial will evaluate a live canarypox virus vector construct in combination with AIDSVAX, a vaccine regimen that is anticipated to elicit both cellular and humoral immune responses. It is likely that additional trials will be needed to identify a broadly protective HIV vaccine. Other promising vaccine constructs (*Salmonella* vectors, VEE constructs, DNA constructs, and other envelope and regulatory protein strategies) are still in the very early stages of human clinical trials, implying that the next efficacy trials will be at least three to five years away.

The design of future efficacy trials may differ considerably from the AIDSVAX trials. These trials may require substantially more participants if vaccine constructs evaluated in earlier phase III trials demonstrate partial efficacy and additional study arms are required to compare products. In addition, the study endpoints may include reductions in plasma viral levels and rate of disease progression, necessitating longer trials. Importantly, future trials will evaluate non-subtype B vaccine constructs and will subsequently require new field sites, countries, and populations. Even though many new constructs have not yet advanced through phase I and II trials, trial sites and cohorts are being identified. It is estimated that future HIV vaccine efficacy trials could require 5,000 to 60,000 participants, several countries and trial sites, and participant follow-up for three to 10 years, at a cost of millions of dollars. To prepare for these efficacy trials, current efforts are focused on expanding existing and developing new field sites worldwide. Current stages of cohort preparation in various geographic regions follow.

North America

U.S. and Canadian clinical sites successfully recruited high-risk persons into vaccine feasibility studies and the AIDSVAX trial and could probably recruit additional high-risk persons into future efficacy trials (115,182). However, participation in future trials would require expansion of existing sites and populations. Many of the early vaccine preparatory cohorts established in 1994–1995 consisted primarily of white MSM and IDU; few included women and minorities. A substantial proportion of these ÒolderÓ cohorts may have since seroconverted, reduced their risk behaviors, or volunteered for the AIDSVAX trial, and may now be ineligible for future efficacy trials. Meanwhile, the HIV epidemic has

expanded in North America, and U.S. surveillance and study data indicate high HIV seroprevalence rates among minority MSM and heterosexual women (183–185) in addition to white MSM and IDU populations. Future efficacy trials will need to expand existing field sites and add new sites to include all populations at high risk.

Asia

Thailand, India, China, and Russia (usually considered part of Europe) are all potential participants in future HIV vaccine trials. Russia, India, and China have conducted HIV epidemiologic studies in high-risk populations and are at the preliminary stages of vaccine cohort preparation (186–188). In comparison, Thailand, a current phase III trial site, has performed vaccine feasibility and willingness-to-participate studies among numerous groups.

Latin America

Although four Latin American countries (Cuba, Brazil, Haiti, and Trinidad/Tobago) have conducted or are conducting phase I/II HIV vaccine trials, few have identified potential phase III trial cohorts through vaccine feasibility or willingness-to-participate studies. Three noteworthy exceptions are Brazil, Haiti, and Trinidad/Tobago, participating sites in the 2001 HVTN phase II canarypox/gp120 trial. These sites have been working on vaccine cohort development since the early 1990s. Brazil has concentrated its efforts on three MSM/bisexual male cohorts in Rio de Janeiro, Belo Horizonte, and São Paulo and has produced a wealth of HIV epidemiologic and vaccine feasibility data on those cohorts as well as recent data on heterosexual populations at high risk (100,113,189–193). Similarly, in Haiti, researchers have explored STD clinic patients, individuals referred for HIV testing, discordant couples, and commercial sex workers as potential phase III cohorts (102,194). And in Trinidad/Tobago epidemiologic and willingness-to-participate studies have been performed among STD clinic attendees (195–198). It is anticipated that cohorts from these three countries, as well as new cohorts being developed in Argentina (199) and Peru,(200) will participate in future trials.

Africa

Multiple countries (Kenya, Uganda, Tanzania, Malawi, Rwanda, and South Africa) have explored potential cohorts for phase III trials. Numerous HIV seroincidence, vaccine feasibility and willingness-to-participate studies have been conducted among diverse populations, such as commercial sex workers (105,201,202), truck drivers (105,201,203), rural community residents (204–207), police officers (208), factory workers (209,210), military personnel (211,212), family-planning clinic attendees (213), and postpartum women (214–216). Not unexpectedly, some cohorts had lower than anticipated seroincidence rates (122,217) and retention problems. Other cohorts were disrupted due to political instability (Rwanda, Democratic Republic of Congo). In some settings, the stigma associated with HIV and the limited care available to persons identified as HIV infected were associated with reluctance to be HIV tested and to receive HIV test results (208). This latter set of problems is complex and not easily resolved. It will require the concentrated efforts of both the developing and developed countries to address these concerns and to determine strategies to support existing vaccine preparatory cohorts and to encourage additional countries to pursue cohort preparations.

CONCLUSION

Despite the difficulties involved in developing an HIV vaccine, more than 80 vaccine constructs have proceeded to human clinical trials and two large-scale efficacy trials are almost complete. In addition, other new vaccine candidates appear promising and will be entering clinical trials within the next year. Experienced HIV vaccine trial sites have been developed in several countries and contain necessary epidemiologic, data management, laboratory, and ethical infrastructures. There is an increasing awareness among trial investigators regarding the ethical and social obligations to protect trial participants' rights and provide important human rights protections. Future efforts will be necessary to identify financial mechanisms to ensure global access to an effective HIV vaccine and to determine optimal vaccination strategies to bring the HIV/AIDS pandemic under control.

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Chapter 40

The Public Health Response to the HIV Epidemic in the United States

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During the past 20 years, remarkable progress has been made in the treatment and prevention of HIV infection and AIDS. Highly active antiretroviral therapy (HAART), coupled with other advances in HIV treatment, has led to signiÞcant and widespread decreases in AIDS-related morbidity and mortality (1). Substantial gains also have been made in public health efforts to stem the spread of HIV. The licensing of HIV-antibody tests in 1985 made it possible to detect asymptomatic HIV infection, ensuring the safety of the blood supply and giving HIV-seropositive persons the opportunity to seek early medical care, access social services, and prevent transmission to others (2). The use of antiretroviral drugs to reduce perinatal transmission is another major success (3,4). From 1992 to 2000, mother-to-child transmission declined 75% from 697 to 162 reported cases (5,6). Community-based organizations and public health agencies have successfully provided essential information about HIV to the American public, implemented effective behavioral interventions, and motivated widespread behavior change among men who have sex with men (MSM), injecting drug users (IDUs), and other populations at increased risk (7Ð9). Considered as a whole, these efforts have reduced HIV incidence from more than 150,000 cases per year in the mid-1980s to approximately 40,000 cases per year in the late 1990s (10).

Despite these successes, the HIV epidemic continues to pose unprecedented challenges to the individuals, organizations, and agencies responsible for monitoring and protecting the health of the American people. Many persons living with HIV do not know that they are infected, and a substantial number of who learn that they are seropositive delay seeking medical care (11,12). Among those who attempt to seek care, the considerable expense of HIV treatments and racial/ethnic disparities in health care have made it particularly difÞcult for some people to access HAART (13,14). Of those who are able to obtain HAART, many have difÞculty adhering to their prescribed regimen and develop drug-resistant virus that can limit treatment effectiveness (15Ð17).Transmission of drug-resistant HIV has increased and can severely limit the treatment options of newly infected persons. Primary infection with drug-resistant HIV increased in San Francisco from 17% in 1996 to 28% in 2001 (18). Changes in the proÞle of the epidemic continue to present new challenges for HIV prevention. African-Americans, Latinos, and women account for an increasing proportion of HIV and AIDS cases in the United States, which signals a pressing need to re-examine prevention efforts for these populations. As people with HIV infection live longer and healthier lives, the prevalence of HIV infection has increased to an estimated 850,000Ð950,000 people in 2000 (19). The greater prevalence of HIV infection increases the probability that uninfected people will come in contact with HIV-seropositive sex partners and has drawn attention to the relative lack of prevention programs for this population (11). Furthermore, there are ominous signs that previous prevention gains among MSM have started to erode. Rates of unprotected sexual intercourse and sexually transmitted diseases (STDs) have risen among MSM,

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indicating the potential for a resurgence of HIV infections (20Ð22).

This chapter provides an overview of the public health response to meeting the challenges of HIV and AIDS prevention in the United States (23). The six major elements of this response are shown in Fig. 40.1. Surveillance provides comprehensive monitoring of the epidemic, which guides planning activities and can be used to gauge the combined effects of prevention efforts on HIV/AIDS morbidity and mortality. Research provides in-depth information that is necessary for the development and testing of efÞcacious biomedical and behavioral interventions. Local health departments and community-based organizations are primarily responsible for implementing and delivering prevention services. The evaluation of these services provides feedback about the delivery and real-world effectiveness of HIV prevention programs and facilitates the continued improvement of these efforts. Policy guides and shapes the public health response to HIV at the local, state, and national level by setting standards, encouraging adoption of some prevention strategies, and discouraging the use of others. All of these components are supported by capacity building and technical assistance activities that seek to improve the ability of public health ofÞcials, researchers, prevention programs, evaluators, and policy makers to make informed decisions and do their jobs effectively. The model represents a dynamic process in which each element inßuences, and is inßuenced by, all of the other elements. This representation of the public health response to HIV and AIDS does not adequately recognize the extensive collaboration and involvement of a myriad of prevention partners, advocates, and community members. These partners have provided leadership and expertise that kindled and shaped the public health response to HIV (24). Their inßuence has been profound and has signiÞcantly affected all of the components of the model, which are described in detail in the following sections.

HIV AND AIDS SURVEILLANCE

Surveillance activities are a cornerstone of the public health efforts to stop the spread of HIV. Epidemiological surveillance is deÞned as the Òongoing systematic collection, analysis, and interpretation of health data that are essential to the planning, implementation, and evaluation of public health practiceÓ(25). HIV and AIDS surveillance guides public health efforts by providing essential information about trends in HIV-related disease and deaths and by identifying groups that are at increased risk based on demographic, behavioral, and geographic characteristics. This information is used to guide the development and implementation of prevention programs, suggest future directions for epidemiological and behavioral research, evaluate the combined impact of prevention efforts and clinical care, and to formulate public policy (25,26). Surveillance data also play an important role in the equitable allocation of treatment, social service, and prevention funds, including Þnancial resources provided to states and large metropolitan areas under the Ryan White Comprehensive AIDS Resources Emergency Act and federal prevention funding administered by state and local health departments (26).

A comprehensive system of AIDS surveillance was initiated in the United States shortly after the Þrst cases were identiÞed in 1981. Health departments in all 50 states and United States territories report AIDS cases to the Centers for Disease Control and Prevention (CDC) following a uniform case deÞnition (6). AIDS surveillance data are highly complete (27). and have been the primary basis for assessing changes in AIDS incidence and prevalence and identifying unmet public health needs. For example, surveillance data showed that the availability of HAART led to a dramatic decline in AIDS-related morbidity and mortality in 1996 and 1997 that later stabilized in 1998 and 1999 (1). These declines were not equal across all groups, however, and demonstrated a critical need to improve the timely diagnosis of HIV infection and expand access to life-extending treatments among African-American women, women living in the southern United States, and people infected through heterosexual contact.

The surveillance deÞnition of AIDS has been modiÞed several times in response to advances in etiology, diagnosis, and treatment of advanced HIV disease (6,26). These revisions have improved the sensitivity and speciÞcity of the surveillance deÞnition by increasing the number of AIDS-indicator diseases and incorporating laboratory evidence of severe immunosuppression and HIV infection (6). Without these revisions, early advances in the treatment of AIDS (such as effective prophylaxis for *Pneumocystis carinii pneumonia*) would have diminished the ability of surveillance efforts to monitor accurately advanced HIV-related disease. In recent years, advances in antiretroviral treatment have reduced the ability of AIDS surveillance to characterize the course of the HIV epidemic in the United States. These advances have dramatically increased the time from infection to development of AIDS-deÞning conditions, making it difÞcult to estimate reliably trends in HIV incidence and prevalence from AIDS surveillance data.

The decreased utility of AIDS case reports for estimating trends in HIV infections has led to greater recognition of the need for national HIV surveillance. Prior to June 1997, HIV infection was a reportable disease in only 25 states (28). In 1999, the CDC issued guidelines for a national HIV surveillance system that encouraged all states and territories to conduct case surveillance for HIV (29). These guidelines provided standards for the collection, reporting, and protection of patient information from public and private sources of HIV testing and clinical care. The guidelines encouraged the use of name-based data collection systems at the local level but allowed states to

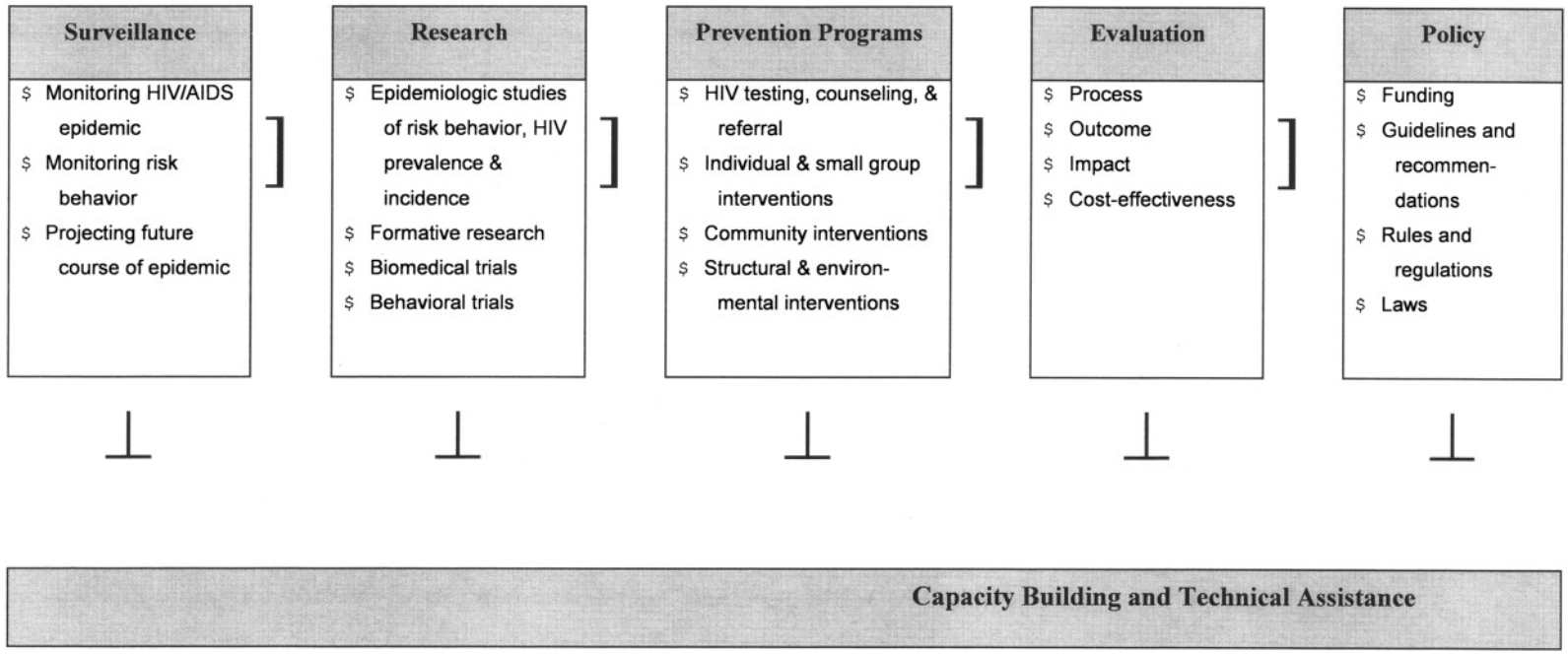

FIG. 40.1. Key elements of the public health response to HIV prevention*
* Although a linear process is ampli ed by the gure, this is not the actual, or the ideal case. All elements are *interrelated* and have the potential to affect all of the other elements of the model. In addition, all elements are in uenced by political, social, and organizational processes that are not re ected in the model.

adopt the use of coded identiÞers to provide additional protection against breeches of patient conÞdentiality or misuse of the data. In addition, the guidelines call for the reporting of rare or previously unrecognized modes of HIV transmission, unusual clinical or virologic manifestations, and other atypical cases of public health importance in order to assess potential changes in the transmission or clinical impact of HIV infection.

As of February 2002, all but three states were conducting HIV case surveillance. Although the reporting of HIV cases provides a better picture of recent trends in the epidemic, they do not provide a complete description of the HIV epidemic in the United States. HIV case surveillance represents a minimum estimate of people living with HIV that is highly dependent upon the availability and utilization of conÞdential HIV testing and treatment. In fact, there has been concern that implementation of HIV surveillance would cause some at-risk persons to avoid HIV testing and further erode the utility of these data (30). Research suggests that name-based reporting has little overall effect on HIV testing rates, but it may deter some persons at elevated risk for HIV infection from being tested (26,29). In order to reduce the impact of reporting on HIV testing rates, CDC has recommended that states provide anonymous testing so that at-risk people have the option of being tested in a setting where they do not have to provide their name or other identifying information (31). In addition, CDC and others have supported the development and adoption of a model law that protects public health information against unauthorized acquisition, use, and disclosure in order to protect the conÞdentiality of people living with HIV infection (32).

The implementation of HIV case surveillance has substantially improved the ability of public health agencies to monitor the epidemic, identify unmet needs, and evaluate the effects of prevention and treatment services. New challenges continue to arise, however, as the epidemic and efforts to limit its impact evolve. These challenges include the need to document the effects of HAART on HIV transmission rates, assess the spread of drug-resistant virus, evaluate the positive and negative effects of HAART therapy, and monitor trends in risk behavior among infected and uninfected persons (33). Addressing these challenges may require additional adaptations to HIV and AIDS case surveillance systems and the integration of other data sources such as sexually transmitted disease (STD) surveillance, HIV seroincidence and behavioral surveillance, and epidemiologic research. STD surveillance provides national information about the incidence and prevalence of STDs that are surrogate markers for unprotected sexual activity and important cofactors for HIV transmission (34). Using the serologic testing algorithm for recent HIV seroconversion (STAHRS), public health agencies can determine whether HIV-seropositive persons have recently acquired HIV and use this information to monitor seroincidence in sentinel sites (e.g. STD clinics, anonymous testing sites) (35Ð37). In addition to data on STD and HIV trends, there is also a need for behavioral surveillance to monitor trends in risk behavior among persons living with HIV infection and those who are at increased risk for HIV infection. To date, however, national behavioral surveillance efforts have been hampered by the lack of population-based studies of persons at increased risk for HIV infection and an over reliance on surveys of the general population that include few at-risk individuals and a limited number of HIV-related questions (38). The broader implementation of these strategies is paramount for establishing a comprehensive system of HIV surveillance that is responsive to changes in the epidemic and better meets the needs of those responsible for planning HIV prevention efforts and reach those at greatest risk for acquiring or transmitting HIV.

EPIDEMIOLOGIC, BEHAVIORAL, AND BIOMEDICAL RESEARCH

Research remains an important element in the public health response to HIV. The continued spread of HIV in the United States indicates a need for additional research on biomedical and behavioral interventions that are more effective and provide at-risk persons with additional prevention options. Given the changing face of the epidemic, there is also much to be learned about behavioral, psychosocial, and environmental factors that facilitate HIV transmission and progression to AIDS in understudied populations. In this section we consider the contributions of four types of research to HIV prevention: (1) epidemiologic studies; (2) formative research; (3) biomedical intervention trials; and (4) behavioral intervention trials.

Epidemiologic studies. Epidemiologic research has provided important information about HIV prevalence and incidence, risk factors for HIV transmission, and barriers to accessing and adhering to care. These studies have documented that rates of HIV infection remain elevated in vulnerable populations and are especially high among those who are African-American or Latino (39Ð42). For example, the Young MenÕs Survey reported that 7% of young MSM (aged 15 to 22 years) were infected with HIV and that prevalence differed signiÞcantly between racial/ethnic groups despite the fact that self-reported risk behaviors were similar across the groups (43). HIV prevalence was 3.3% for white MSM, compared with 6.9% for Latino MSM, and 14.1% for African-American MSM. Epidemiologic studies have also provided important information about HIV risk factors and the ability of condoms and access to sterile needles to reduce HIV transmission. Studies examining the protective effects of condoms have found that consistent condom use is associated with an 80% to 95% reduction in risk in HIV transmission and provide compelling support for condom-based strategies to HIV prevention (44Ð46).

The advent of HAART has directed increased attention to the need to improve access to HIV testing, utilization of medical care, and adherence to care. Despite the availability of free and low-cost anonymous and conÞdential HIV testing, one-quarter of people living with HIV do not know that they are infected (19). Troubling disparities in access and utilization of health care have been reportedÑ a national probability sample of adults receiving HIV care found that some groups, including African-Americans, IDUs, women, and those without insurance, were less likely to have received HAART (13). Descriptive research has also made important contributions to our understanding of adherence to HAART. These studies show that many patients, even those who experience difÞcult life circumstances, can achieve satisfactory levels of adherence and have identiÞed factors associated with poor adherence that are amenable to intervention (15Ð17).

Formative research. The success of prevention programs and clinical trials is often dependent on a thorough understanding of the needs, perspectives, and experiences of community members. Formative research, needs assessments, and other data-based strategies are increasingly being used to guide the development of prevention programs, behavioral intervention trials, and clinical trials. These methods can be used for many purposes including: (1) deÞning and describing target populations; (2) developing sampling and recruitment plans; (3) deÞning program/research objectives; (4) documenting facilitators and barriers to behavior change; (5) assessing the feasibility and acceptability of potential intervention and research strategies; and (6) evaluating potential wording of questions, biologic sample collection techniques, and other aspects of data collection (47,48). These activities draw on research techniques that are commonly used in the social and behavioral sciences such as Þeld observations, case studies, focus groups, qualitative interviews, and quantitative surveys. One approach, the community identiÞcation process, provides a qualitative methodology for rapidly assessing at-risk populations from the perspective of professionals who work with the population, those who interact informally with population members, and members of the target population (49). Formative research has been useful in the planning and implementation of biomedical interventions, including preparation for large-scale HIV vaccine trials. This research has provided valuable information about the concerns of potential participants, their willingness to participate and HIV risk, and the acceptability and feasibility of various data collection methods that will pave the way for Phase III vaccine trials (50Ð52).

Biomedical intervention trials. Randomized clinical trials have provided important information about the efÞcacy of medical and pharmacological strategies to reduce HIV transmission, AIDS incidence, and mortality. These trials, which have been extensively reviewed in this volume and elsewhere, have resulted in medical treatments that reduce viral load, improve immune function, delay progression to AIDS, and increase survival (53). Clinical trials of biomedical interventions have also made signiÞcant contributions to the prevention of HIV transmission. ACTG 076 showed that perinatal transmission could be substantially reduced by providing AZT to HIV-seropositive women during pregnancy and delivery and to their newborns following delivery (3). Subsequent studies have demonstrated that other antiretroviral regimens are also effective (54Ð56).

Research on biomedical interventions to prevent sexual transmission of HIV has been less successful. Efforts to create an effective preventive vaccine have been hampered by an inadequate understanding of the factors that protect against HIV infection, a lack of good animal models, differences between HIV subtypes, and other factors (57Ð59). Although many researchers remain optimistic about their ability to develop an effective preventive vaccine in the next seven to ten years, others believe that it will be much longer before an effective vaccine is available (57,59,60). There have also been considerable setbacks in the development of vaginal and rectal microbicides that can protect against HIV infection. Although a variety of agents that inactivate HIV, inhibit viral entry, or inhibit viral replication have been identiÞed, none has been shown to signiÞcantly reduce rates of HIV infection in a Phase III clinical trial (61,62). Nonoxynol-9, the only product to be tested in large-scale trials, was found to increase vaginal lesions and to increase womenÕs risk of HIV infection or provide no protection compared to a placebo (62,63). Other potential microbicides have been identiÞed and are being tested. As of 2000, 22 products were being evaluated in early trials to evaluate safety and ability to prevent HIV infection *in vivo* (61).

Behavioral intervention trials. Prevention of HIV transmission among at-risk adults and adolescents continues to depend primarily on motivating individuals to change their personal drug-use and sexual practices. Researchers have drawn on epidemiological data, behavioral theory, and formative research to develop interventions for a variety of at-risk populations. Most of these interventions attempt to change the behavior of at-risk individuals by inßuencing self-efÞcacy, perceived norms, attitudes regarding condom or sterile needle use, motivation/intention to adopt risk reduction strategies, or other psychosocial factors articulated in models and theories of human behavior. Typically, intervention activities focus on bringing about changes at the individual or small group level (see Table 40.1). A limited number of interventions have sought to change entire communities (or subgroups within a community) by mobilizing community members, changing social norms, or removing barriers to safer sex or injection practices.

A large number of studies evaluating the effectiveness of behavioral interventions have been published. Although not every intervention has been successful, literature reviews have consistently found evidence indicating that behavioral interventions can motivate reductions in HIV

risk in a wide range of populations including adolescents, MSM, IDUs, and women (64–69). An objective scientific panel convened by the National Institutes of Health in 1997 reached the same conclusion, stating that "behavioral interventions to reduce risk for HIV/AIDS are effective and should be disseminated widely" (70). A comprehensive effort to evaluate the effectiveness of behavioral interventions, the HIV Prevention Research Synthesis

TABLE 40.1. *Typology of HIV prevention interventions*

Level of Intervention	Characteristics	Examples
Individual	• Directly influences knowledge, attitudes, and behavior of people participating in intervention activities • Information delivered in one-on-one setting by professionals, peers, and/or media (e.g. brochures, radio, internet) • Limited number of people reached • Often provides the most flexibility to meet client needs	• HIV counseling and testing • Risk reduction messages given by primary care providers • Prevention case management
Small Group	• Directly influences knowledge, attitudes, and behavior of people participating in intervention activities of newly formed or existing groups of individuals • Activities conducted with couples, small groups, or family members that use professionals, peers, and/or media • Moderate numbers of people reached • Some flexibility to meet needs of individuals	• Interventions for HIV-serodiscordant couples • Single-session and multi-session workshops • Programs that train parents to talk with their children about HIV
Community	• Directly and indirectly influences knowledge, attitudes, and behavior of entire community • Often focus on changing social norms • May have multiple intervention components that use peers or professionals and/or media • Large numbers of people reached • Little flexibility to meet needs of individuals	• Mass media and social marketing campaigns • Peer outreach • Community mobilization
Structural	• Indirectly affect risk behavior by changing structures, laws, or policies that may influence transmission risk or the availability of prevention information or tools • Changing policy/law may be require few resources but implementing structural changes may be expensive • Affects large numbers of people at the city, state, or national level • Not tailored to individual needs	• Needle exchange programs • Reducing cost/increasing availability of condoms • Laws permitting sale of sterile syringes without a prescription • Policies supporting provision of safer sex education and condom availability in schools
Superstructural	• Indirectly affect risk behavior by changing social and economic structures or pervasive societal customs and attitudes that result in unequal advantages to groups, communities, or countries • Can result from governmental effort, social movements, or revolution • Affects large numbers of people at the city, state, or national level • Not tailored to individual needs	• Antipoverty programs • Antidiscrimination programs • Provision of housing to people living with HIV • Licensing of generic versions of antiretroviral drugs
Medical/Technological	• Directly and indirectly affect risk through scientific advances in medical care and other fields that reduce infectivity or provide new/improved prevention technologies • Can affect HIV transmission but rely on other intervention strategies to motivate dissemination and adoption • Can affect large numbers of people but cost and other factors may limit access	• Female condom • Microbicides • Use of antiretrovirals to prevent perinatal transmission • Post-exposure prophylaxis • Preventive vaccine

Adapted from Sweat & Denison (128) and Waldo & Coates (2000) (175).

Project, has reviewed more than 5,000 scientiÞc reports and identiÞed 303 studies that evaluated the effects of behavioral interventions (71,72). Of these, 122 (40%) were conducted in the United States and met standardized criteria for relevance and methodological rigor. Meta-analyses of 61 studies that met these criteria showed signiÞcant reductions in sexual risk among young adults (OR = 0.65, 95% CI = 0.56Ð0.85), MSM (OR = 0.69, 95% CI = 0.56Ð0.86), heterosexual men and women (OR = 0.81, 95% CI = 0.69Ð0.95), and drug users (OR = 0.86, 95% CI = 0.0.76Ð0.98) (66Ð68,71,73). Of the studies reviewed to date, 36 have met additional criteria for effectiveness and have been included in the Compendium of HIV Prevention Interventions with Evidence of Effectiveness (74). The compendium has been widely disseminated to community-based providers and is used as a resource for identifying effective interventions that address local needs.

HIV PREVENTION PROGRAMS IN THE UNITED STATES

The rapid emergence of AIDS and the magnitude of the epidemic led to an unprecedented mobilization of community and public health resources. Responding to a new public health emergency of this size has required overcoming substantial logistical, scientiÞc, social, and political challenges. Addressing these challenges necessitated the development of innovative partnerships that include community members, community-based and national organizations, and local, state, and federal public health ofÞcials. The combined efforts of these groups have resulted in a multitude of HIV prevention programs that seek to increase public awareness, encourage at-risk persons to be tested for HIV infection, and motivate those at increased risk for acquiring or transmitting HIV to reduce their risk. As shown in Table 40.1, these programs can be categorized by whom (or what) intervention efforts are directed toward. Most interventions have focused on individuals or groups of individuals. These interventions include information hotlines, risk reduction counseling, prevention case management, and other strategies that attempt to bring about changes in knowledge, beliefs, motivation, and behavior among people who participate in the intervention. Institutional or community-level interventions focus on larger groups and seek to change risk behavior not only among intervention participants but also among members of an institution or a community who do not participate in the intervention. These programs include peer outreach, social marketing campaigns, and syringe exchange programs that work to change community-wide norms regarding HIV risk reduction or improve access to condoms, sterile syringes, and other risk reduction tools. Changes in public policy and laws act as structural interventions that can make it easier for individuals to adopt reduced risk practices or make it more difÞcult to engage in risky practices. Fundamental changes in the social structure of a community, state, or nation also have the potential to affect risk behavior and HIV transmission. Such programs include those that address economic or social disparities (such as poverty, racism, and homophobia) that are associated with life circumstances that can increase persons risk of being exposed to HIV. Finally, scientiÞc advances in HIV prevention and treatment also act as interventions by reducing infectivity or providing new prevention options that are more effective or easier to use.

Early in the epidemic, public health agencies devoted a great deal of attention to providing basic information about HIV and AIDS to the general public and those at increased risk. Mass mailings, public service announcements, and other large-scale efforts disseminated basic information about HIV, risk behaviors, and risk reduction strategies (75,76). Other programs worked to integrate HIV education into a wide range of community settings including schools, workplaces, churches, beauty parlors, and other locations (75Ð79). These efforts have increased basic knowledge about HIV transmission and prevention (although some myths about casual contagion persist) and helped reduce negative attitudes about people living with AIDS (80Ð82).

Important as these changes are, it was recognized early on that increasing HIV knowledge and awareness would not be sufÞcient to motivate widespread changes in risk behavior. This recognition and the differential risk of HIV infection in well-deÞned subpopulations led to the development of more intensive programs that were designed to meet the needs of those who are at greatest risk. Many different strategies have been developed to encourage people to protect themselves from HIV (see Table 40.1 for examples). Many of these programs are based on interventions that have been shown to be efÞcacious in rigorous research trials. Behavioral interventions do not always originate with researchers. Other HIV interventions have been developed by prevention providers working in health departments and community-based organizations. These interventions have the advantage of being responsive to local needs and the realities of the agencies that implement these programs. Many of these interventions have not been subjected to outcome and impact evaluations due to limited evaluation budgets and methodological challenges, making it difÞcult to identify successful programs and compare their effectiveness. One effort to identify successful programs used an extensive nomination and review process that resulted in the selection of 18 interventions that were considered by prevention providers to be model programs (83). Site visits to these programs revealed important characteristics of the interventions, agencies, and staff that appeared to contribute to their success. The characteristics of effective interventions are summarized in Table 40.2.

For more than 15 years, HIV counseling and testing has been the single most widely available prevention program

TABLE 40.2. *Characteristics of effective HIV prevention programs*

Intervention Characteristics	Staff Characteristics	Agency Characteristics
• Clearly de ned goals, measurable objectives, audience, and intervention activities • Based on behavioral theory, scienti c literature, and needs of community • Culturally competent and client centered • Ongoing monitoring of implementation and client needs • Use evaluation ndings to improve program	• Adequate training and prior experience • High level of commitment • Understand needs of local community • Flexible and able to make changes in response to client needs • Respect clients • Able to listen to, and advocate for, client needs • Do not adopt "top-down" approach • Able to deal with stress	• Support from administrators and managers of agency • Suf cient staff, equipment, space, and other resources • Integration/consistency with agency's mission • Partnership and collaboration with other agencies • Able to retain staff • Credible and respected in community

Sources: Eke (83), Mezoff et al. (176), and Holtgrave et al. (177).

in the United States. The primary goals of HIV counseling, testing, and referral programs are: (1) to promote knowledge of HIV status and early diagnosis of HIV infection; (2) to provide high-quality prevention counseling; and (3) to provide referrals to medical, preventive, and psychosocial support services (31). The CDC has recommended that people being tested for HIV receive pretest and posttest counseling since testing became available in 1985 (84). Initially, this counseling provided information about the test itself and the meaning of positive and negative results, but by 1987, greater emphasis was placed on encouraging risk behavior change (85–87). Early studies found that counseling and testing led to signiþcant reductions in risk among people who learned that they were HIV-seropositive but motivated little change among those who were HIV-seronegative (88–90). Based on these þndings and expert consultation, the CDC recommended a shift to client-centered counseling that emphasizes increasing clientÕs perception of risk and developing a personalized risk-reduction plan (2,91). This approach has been shown to increase condom use and decrease new STDs among STD clinic patients who received client-centered counseling compared to those who received standard risk reduction messages (92). In 2001, the CDC issued new guidelines in an effort to make HIV testing more accessible, address technological advances in HIV testing, recognize resource and provider constraints, and accommodate the diverse needs and preferences of people seeking testing (31).

Federally funded HIV prevention programs are guided by a comprehensive HIV prevention plan that is developed locally through a community planning process. Community planning involves members of affected communities, prevention providers, and others in the on-going development of prevention plans that reßect local needs and are based on sound science and public health practice (93). Members of community planning groups are responsible for monitoring and anticipating the course of the HIV epidemic in their community, assessing and prioritizing prevention needs, and identifying science-based interventions to meet these needs. This value- and science-based decision making process has led to important shifts in the allocation of prevention funds that more adequately reßect the racial/ethnic characteristics of people living with HIV/AIDS, increased funding for health education and risk reduction programs, and provided more resources to community-based organizations (94,95). Other positive outcomes of community planning include greater recognition of the needs of different geographic, cultural, and risk groups, improved coordination of HIV prevention activities, and an increased attention to evaluation (96).

EVALUATION OF HIV PREVENTION EFFORTS

Limited prevention resources and the painful realization that HIV has become endemic in the United States have placed greater emphasis on accountability and the continued need to improve the effectiveness of prevention efforts. Ongoing evaluation of HIV prevention programs is an essential component of the public health response to HIV. It provides useful information regarding the implementation of intervention activities, the ability of interventions to achieve program objectives, the cumulative impact of prevention efforts, and the economic beneþts and relative costs associated with various intervention strategies. In this section, we review four major types of program evaluation: (1) process monitoring and evaluation; (2) outcome monitoring and evaluation; (3) impact evaluation; and (4) economic evaluation.

Process monitoring and evaluation. Process measures are used to describe and assess the delivery of prevention services. Process monitoring refers to information that is collected in order to document and describe the intervention services that are conducted, the characteristics of persons who receive these services, and the resources that were used (97,98). Process evaluation involves the collection and analysis of information about the implementation of program activities (97,98). Process evaluation provides

feedback on the quality of prevention services that are provided, whether those at greatest risk are being reached, and how these programs are implemented within the realities of community-based providers (99). When combined with outcome data, process measures facilitate the identiÞcation of the program elements that are most strongly associated with behavior change (99). Process data have proven to be extremely useful for monitoring the implementation of HIV prevention efforts at local, state, and national levels. At the national level, for example, process data have indicated that the types of prevention services provided in local communities have changed since community planning was initiated and have identiÞed gaps in prevention services for African Americans, MSM, and IDUs (95).

Outcome monitoring and evaluation. Relatively little is known about the effectiveness of interventions that are developed and/or implemented by community providers (100). A number of these interventions have not been evaluated to determine their effects on risk behavior. Other programs are based on interventions that were rigorously tested in randomized trials. Although it is hoped that these interventions would continue to be effective when conducted by community-based providers, this cannot be assumed with certainty. Reasons to question the real-world effectiveness of these interventions include characteristics of the original intervention research (e.g. training of staff, payment for participation, methods for recruiting participants), characteristics of community-based organizations (e.g. staff turnover, limited resources, Þdelity to intervention protocol), and changes in the needs of at-risk persons that may affect the ability of these interventions to motivate behavior change in community settings. These factors, coupled with substantial diversity across interventions in terms of strategies used, settings, and populations served, necessitate the continued evaluation of intervention effects at the local level.

Outcome monitoring measures the effects of an intervention on those who receive it and allows for an evaluation of the extent to which program participants have experienced measurable changes in knowledge, perceptions, or behavior (97,98). Outcome monitoring does not employ comparison or control groups, and because of this, does not rule out the possibility that factors other than the intervention were responsible for the observed changes. Outcome evaluation is a more rigorous, and employs experimental or quasi-experimental research designs to rule out the inßuence of factors other than the intervention that might be responsible for the observed changes. A small number of outcome evaluations of community-based programs have been reported (100), but these represent only a tiny fraction of active prevention programs. In order to address this gap, the CDC now requires jurisdictions that receive one million dollars or more in federal funding each year to monitor outcomes on at least two programs or conduct outcome evaluation of at least one program (97).

Impact evaluation. Measurement of the long-term and combined effects of multiple HIV prevention efforts is the goal of impact evaluation (97,98). This type of evaluation goes beyond the outcome evaluation of a given intervention among program participants by assessing the broader societal effects of multiple interventions on communities as a whole. The cumulative impact of HIV prevention programs may be affected by a number of factors including the number and type of interventions available in the community, the individual effectiveness of each intervention, the number of people reached, and the duration of intervention activity. The impact of prevention programs may also be affected by external factors that are not associated with these efforts such as changes in social norms, technological advances, and historical events.

The most basic and direct measure of the impact of HIV prevention is HIV incidence in the community or population of interest. Other biological, behavioral, service, and sociopolitical measures also can serve as important indicators. A series of consultations sponsored by the CDC identiÞed 37 core indicators in these categories that have been Þeld tested to assess their utility in Þve jurisdictions (101,102). These pilot projects demonstrated the overall usefulness of the core indicators and documented progress toward reaching prevention goals in some populations (102Ð104). All of the indicators were not available in all sites, however, and there were signiÞcant gaps in the available indicators for MSM, IDUs, high-risk heterosexuals, and childbearing women (102).

Economic evaluation. The goal of economic evaluation is to provide information about the costs of prevention efforts and the resources that are required to achieve a given outcome. Cost analysis involves the assessment of the resources that are needed to conduct a speciÞc program (105). Cost effectiveness analysis links the monetary value of the resources needed to deliver an intervention with outcomes (e.g. the cost per HIV infection averted) (97,105). Cost effectiveness analysis facilitates the comparison of interventions with common outcomes, making it possible for decision makers to choose interventions that are likely to have the greatest impact on risk behavior for a given expenditure of resources. Other types of economic evaluation include cost-beneÞt analysis, in which outcomes are assigned a monetary value (e.g. cost of medical care for each HIV infection averted), and cost utility analysis, which converts intervention outcomes into units that represent changes in the quantity and quality of life (e.g. increases in life expectancy, gains in quality adjusted life years) (105). The advantage of these analyses is that they translate intervention outcomes into standard units (i.e. dollars or quality adjusted life years) that can be readily used to help make decisions about the potential monetary or health beneÞts associated with very different types of programs or services (e.g. interventions to reduce unprotected sex versus programs to improve air quality).

A review of the economic evaluation literature identiÞed 28 studies that examined HIV prevention interventions in

the United States (106). Most of these studies (79%) were cost effectiveness evaluations, and provided evidence that HIV interventions can be cost effective or cost saving. Cost effective interventions have been identified for IDUs, MSM, male African-American adolescents, and at-risk women but some populations and promising intervention strategies have been understudied (106–111). The economic evaluation literature is growing rapidly and many other types of HIV prevention interventions are being subjected to cost effectiveness analyses.

PUBLIC POLICY

The main goals of HIV-related public policy are to reduce HIV/AIDS morbidity and mortality by supporting the adoption and maintenance of practices that reduce the spread of HIV, encouraging early identification of HIV infection, and promoting access and adherence to high-quality medical care. Policy is both a product of the public health response to HIV and an external influence that has shaped this response. Policies issued by public health agencies establish standards and provide recommendations for monitoring and responding to HIV/AIDS, conducting prevention programs, and delivering medical care. Other forms of policy, such as laws, regulations, and funding allocations are often generated outside of public health. They indirectly affect morbidity and mortality by influencing the number and types of prevention programs in communities, the availability of condoms, sterile needles and syringes, and other prevention tools, and the rights of people living with HIV and those who are at risk of infection.

All forms of policy are affected by multiple factors including empirical data, expert and public opinion, and political concerns. Although many have argued that policy should be based solely on published empirical evidence, this not achievable (112,113). The rapid emergence of HIV and AIDS required that policies be developed before sufficient empirical data could be collected. Even when data were available, policy decisions were often influenced by ethical, moral, and political values. These values included beliefs about the appropriate role of government in public health, the importance of HIV compared to that of other health threats, the rights and responsibilities of those who are infected with HIV, and the rights and responsibilities of those who are uninfected. The far reaching effects of public health policy makes it undesirable for decision makers to act unilaterally and necessitates the active participation of diverse stakeholders, consideration of intended and unintended consequences, and trade offs between private rights and public good (112). Unfortunately, however, this process can become polarized. Extreme views on either side of a particular issue can lead to inaction or the formulation of public policy that unnecessarily deprives individuals of basic rights, is inconsistent with empirical data, or fails to achieve the goal of protecting the health of the American public.

Many policies and laws have been developed in an attempt to influence the risk behavior of individuals and direct the public health response to HIV and AIDS. These include laws and policies that protect the confidentiality of people living with HIV, encourage the availability of anonymous HIV testing, mandate HIV testing in some circumstances, restrict the types and content of HIV prevention programs, regulate the purchase, exchange, and possession of needles and syringes, increase access to HIV-related medical care, or govern the sexual practices of people living with HIV (12,114–117). There are many strongly voiced opinions about the positive and negative effects of specific policies, but often empirical data are not available to evaluate the relative merit of divergent perspectives on either side of a given issue.

In some cases, extensive empirical data are available to inform the development of public policy or to evaluate its effects on public health efforts to limit the spread of HIV. For example, a considerable amount of research has demonstrated that limiting the availability of sterile needles and syringes increases HIV risk and that efforts to reduce barriers to obtaining sterile injection equipment decreases the sharing of contaminated needles and syringes among IDUs (37). Higher levels of HIV prevalence and incidence have been observed in communities with laws that restrict the sale of sterile syringes to IDUs compared to communities without these restrictions (118). Successful efforts to repeal laws restricting the sale of sterile needles have improved access to sterile syringes and decreased the sharing of used syringes among IDUs (116,119). The bulk of the research on the effects of syringe exchange programs has demonstrated that these programs are cost effective, reduce needle sharing, and do not lead to increased drug use or initiation of drug injection by non-IDUs (112,120–126). Despite the preponderance of evidence and the recommendations of objective review panels, federal restrictions on the funding of syringe exchange programs remain in place because of questions about the interpretation of the available data and concern that the existence of these programs would condone the use of illicit drugs or lead to other negative consequences (112,125,126). Barriers to the implementation of syringe exchange programs are increasingly being overcome at the local level. From 1994 to 1998, the number of identifiable syringe exchange programs nearly doubled from 68 to 113 (127). In 1998, 110 programs provided more than 19 million syringes to IDUs and almost all provided other prevention services including condoms, bleach for cleaning syringes, voluntary HIV counseling and testing, and referral to drug treatment (127).

Well-informed policies and laws can be powerful interventions that further public health efforts to limit the continued spread of HIV infection (128,129). However, much remains to be learned about the impact of public

policy on HIV risk and the ways in which research and public health practice can inform the development of sound public policy. New policy challenges will emerge as the epidemic, science, and the social and political landscape continue to change over time. Although some battles may be lost, it is essential that researchers and public health officials remain fully engaged in these debates if they hope to win the war against HIV (129).

TECHNICAL ASSISTANCE AND CAPACITY BUILDING

All elements of the public health response to HIV and AIDS are strengthened by a wide range of activities conducted at the local, state, and national levels that provide technical assistance and support capacity building. The goal of capacity building is to enhance and sustain the ability of individuals, organizations, and communities to perform the core public health functions that are essential to HIV prevention (130). These efforts include: (1) technology transfer; (2) technical/capacity building assistance; (3) training; (4) skills building; and (5) information dissemination. Some efforts, such as HIV/AIDS postdoctoral training programs, have the potential to have a broad effect on public health activities by increasing the availability of highly trained and culturally competent professionals. Other efforts directly improve the current ability of individuals and agencies conducting HIV/AIDS surveillance, community planning, behavioral interventions, or program evaluation.

Substantial resources have been dedicated to improving the ability of community-based organizations and health departments to conduct effective HIV prevention programs. Activities supported by these resources have sought to overcome the challenges faced by many of the community-based organizations that were created in response to the HIV epidemic. Many of these agencies are relatively new, have small staffs, experience rapid staff turnover, may not have access to scientific publications, and have limited financial resources (98,131,132). Technical assistance and capacity building to overcome these challenges is provided by CDC, the National Association of State and Territorial AIDS Directors (NASTAD), the National Minority AIDS Council, and other organizations. These activities include:

- The National Technical Assistance Providers Network for Community Planning, which provides technical assistance on management of the community planning process and the development of local HIV prevention plans (133).
- The Capacity Building Assistance Providers program that works to strengthen the efforts of organizations serving racial and ethnic minority populations (98).
- Fact sheets, guidelines, resource manuals, and user-friendly replication packages that provide information on community planning, HIV counseling, testing, and referral, effective behavioral interventions, evaluation, and other topics (134–136).
- A national database of prevention resources and programs, the National Prevention Information Network, that can be accessed by telephone or the internet (137).
- National conferences and televised satellite broadcasts for prevention providers that address emerging issues, disseminate information about effective interventions, and provide opportunities for skill building, networking, and resource sharing (138,139).
- Regional training on behavioral interventions and formative research provided by the STD/HIV Prevention Training Centers (140).
- Local consultation and hands-on assistance provided by behavioral and social scientists who participate in the American Psychological Association's Behavioral and Social Science Volunteers program (141).

ADDITIONAL CHALLENGES AND FUTURE DIRECTIONS

There have been many accomplishments in the fight against HIV and AIDS, and prevention efforts have contributed to substantial declines in HIV- and AIDS-related morbidity and mortality in the United States. Unfortunately, it is clear that HIV will remain a major threat to the health of Americans for many years to come. An effective preventive vaccine remains out of reach, and HIV infection rates continue to be unacceptably high. As we look forward and consider the next steps in the public health response to HIV, we must grapple with difficult questions that continue to have major implications for the planning and delivery of prevention efforts:

- Why do 40,000 people a year continue to contract HIV in the United States despite widespread awareness of HIV and the presence of community-based prevention programs?
- Why does the disproportionate impact of HIV and AIDS on African Americans and Latinos continue to increase?
- Why do one-quarter of people who are infected with HIV not know that they are seropositive?
- How can the effectiveness of public health efforts to prevent HIV transmission be improved, particularly when some communities have been saturated with prevention messages?

Answering these questions and implementing prevention efforts have become increasingly difficult in light of the challenges that public health now faces. The growing number of people living with HIV and the expense of HAART has strained the budgets of drug assistance programs, requiring significant increases in funding and the implementation of strategies to ration access to this life-saving therapy in some communities (14). The

availability of HAART has changed perceptions about the severity and transmissibility of HIV and has caused some people to be more willing to risk infection (142–144). As the number of people living with HIV has increased, so has the probability that uninfected persons will have contact with sex or needle sharing partners who are HIV-seropositive. This growing risk is compounded by the transmission of drug-resistant HIV that may limit the treatment options of people who are newly infected and outbreaks of STDs that facilitate HIV transmission (18,20,145). A comprehensive approach to prevention for people living with HIV infection is needed and should include early diagnosis of HIV infection, linkages to medical care and prevention services, strategies to improve adherence to care, and life-long support for the adoption and maintenance of reduced risk practices (11,146).

Other emerging factors have also presented additional challenges to HIV prevention efforts in communities that had previously demonstrated substantial reductions in HIV risk. The internet has created new opportunities for people to meet sex partners and may facilitate the formation of risky partnerships (147,148). A new generation of MSM that has had little first-hand experience with the effects of HIV is at considerable risk of infection (43,149–151). There is evidence that "safer sex fatigue" that results from years of exposure to prevention messages and long-term efforts to maintain safer sex practices has led to increased risk among older MSM (152). Dissatisfaction with prevention programs in the gay community has contributed to a backlash against prevention and a view that prevention messages for MSM are outdated or overly simplistic (7,20). In particular, the ability of prevention efforts to reach African-American and Latino MSM has been increasingly questioned given that these groups continue to represent a disproportionate and growing percentage of HIV and AIDS cases among MSM (20,153). Addressing the urgent needs of these men will require further examination of the unique cultural experiences of African-American and Latino MSM that may facilitate or hinder prevention efforts and for research leading to the development of effective interventions that are tailored to meet the needs of these populations (154,155).

In some cases, prevention programs do not have the resources or tools they need to limit the further spread of HIV. Spending on prevention efforts represents a small proportion of state and federal governments' expenditures on AIDS. Less than 8% of federal HIV/AIDS funding is spent on prevention (156). Changes in perceptions about the importance of HIV has contributed to a decline in donations to AIDS organizations, which has limited the activities of some prevention programs and jeopardized the existence of others (157). There is an inadequate number of effective interventions for some high-risk populations. There is a surprising lack of rigorously evaluated interventions for MSM; this is particularly true for racial/ethnic minority MSM and young MSM. Although MSM represent more than half of all people living with HIV (158), just six of the 36 effective interventions (17%) in the compendium of effective interventions were developed for MSM (none of which focused specifically on racial/ethnic minority MSM) (74). As the epidemic evolves, the critical need for interventions for racial/ethnic minority youth, at-risk women, people living in rural areas, incarcerated populations, and people living with HIV infection has become increasingly apparent (65,70). There is still much that needs to be learned about the "active ingredients" of behavioral interventions. Most behavioral interventions consist of multiple elements and little is known about which components are critical to their success, the optimal number of intervention sessions, and their duration. Important questions remain unanswered regarding the characteristics of staff and volunteers who make effective interventionists and how community membership, professional training, demographic characteristics, and personal traits of interventionists affect intervention outcomes. Finally, there is a clear need to develop a better understanding of why some intervention participants change their behavior and others do not. This knowledge should lead to the creation of second and third generation intervention strategies that better address the needs of those whose behavior is unaffected by existing interventions.

The HIV epidemic does not exist in isolation. It is intricately intertwined with other health problems that facilitate the spread of HIV and make it more likely that some individuals (and some communities) will be exposed to HIV than others. The interaction between HIV risk and other health issues involves factors that exist within the individual as well as structural and environmental factors in the community. At the individual level, health factors that interact with HIV risk include the effects of substance use, sexual abuse, and other mental health issues (159–162). Structural factors that influence HIV risk at the community level include: (1) differential educational and economic opportunities that contribute to poverty and homelessness; (2) differential access to drug treatment, testing for sexually transmitted infection and HIV, medical care, and antiretroviral therapies; and (3) the effects of racism, sexism, homophobia and HIV-related stigma (128,163,164). In order to move prevention efforts to the next level, public health must better understand and address the influences of these factors that limit the effectiveness of prevention programs and sustain the epidemic within vulnerable populations.

Improving efforts to prevent HIV transmission and AIDS not only requires that we develop a better understanding of factors that contribute to individuals' risk but we also critically examine the performance of the public health system. There are substantial gaps in the delivery of medical care and prevention services that limit the effectiveness of the public health response to HIV. Health care providers are not always aware of current public health guidelines and may fail to provide recommended

treatments or appropriate counseling to their patients (165–167). Racial/ethnic disparities, fear of discrimination, and stigma can affect the health care system and limit the ability and willingness of some people to access appropriate care (164,168,169). Furthermore, the effectiveness of community-based HIV prevention programs may be decreased by barriers to the adoption of rigorously evaluated interventions that significantly reduce HIV risk (132,170). In the years to come, we must devote more effort to addressing these and other problems that diminish the ability of public health agencies and their partners to function effectively.

As public health struggles to deal with disparities in HIV treatment and care in this country, there is a clear consensus that America needs to provide additional leadership and resources to combat HIV and AIDS in developing countries. The burden of HIV-related disease in these countries is overwhelming. Globally, there are 40 million people living with HIV, the vast majority of whom live in sub-Saharan Africa and Southeast Asia (171). In sub-Saharan Africa, HIV prevalence is estimated to be 8.8% among adults, and exceeds 20% in seven southern countries (172). The effects of HIV have been devastating in these countries, causing infant mortality to double and overall life expectancy to decline by 10 or more years (172,173). The magnitude of the global HIV pandemic is sobering and makes the role of structural factors that concentrate the virus within marginalized populations even more apparent (174). Strengthening our response to the global HIV crisis will require a substantial increase in human and financial resources to ameliorate these long-standing disparities. Doing so will require that we look back on the hard-earned lessons of the past 20 years, while at the same time looking ahead to the new and recurring challenges that HIV will continue to present for many years to come.

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Chapter 41

The Global Impact of HIV and AIDS

Marjorie Opuni and Stefano Bertozzi

The human immunodeÞciency virus (HIV) and its most severe clinical manifestation, the acquired immunodeÞciency syndrome (AIDS), have produced a devastating pandemic of enormous proportions. As of the end of 2002, the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) estimated there were 42 million persons worldwide living with HIV or AIDS (Fig. 41.1) (1). In addition, more than 20 million people have already died making a cumulative of more than 60 million HIV infections since the beginning of the epidemic (2).

The spread of HIV/AIDS has far exceeded expert predictions made earlier in the epidemic (4). And the epidemicÕs demographic, social and economic impacts have been much greater than expected. This is especially true in developing countries where 95% of people living with HIV live (1). It is most particularly true in sub-Saharan Africa the continent with over 70% of people living with HIV.

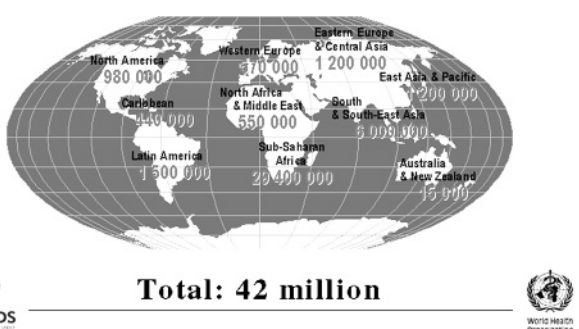

FIG. 41.1. Adults and children estimated to be living with HIV/AIDS as of end 2001.
Source: UNAIDS/WHO, AIDS epidemic update: December 2001. Geneva: UNAIDS, 2001.

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Despite the rapid ascertainment of the epidemiology of HIV and AIDS, the discovery of the etiologic agent itself and ways to prevent its spread, and the development of diagnostic tests and antiretroviral therapies, it is clear that HTV will continue to be a global problem well into the 21st century. There were Þve million new HIV infections globally in 2002 and there is every reason to believe that the epidemic will evolve much further (1). The epidemic is spreading rapidly in regions that had previously kept HIV at bay, such as Eastern Europe. And though prevalence in the worldÕs most populated countries in Asia remains low, in many of them, the preconditions exist for increasing spread.

This chapter summarizes the global impact of HIV/AIDS outside of the United States, with a focus on the developing world, including overviews of the epidemiology, demographic impact and socioeconomic impact of the epidemic. The monitoring of these trends is essential to the planning and implementation of a global response to HIV/AIDS. It is also crucial to evaluating the effectiveness of HIV/AIDS prevention, care and impact itigation interventions around the world.

EPIDEMIOLOGIC PATTERNS OF HIV INFECTION AND TRANSMISSION

The pandemic of HIV/AIDS reßects many coexisting sub-epidemics in different regions and populations (6). As described elsewhere in this book (Chapter **00**), HIV-1 is the predominant type of HIV throughout the world while HIV-2 is found primarily among persons from West Africa. Many epidemiologic, sociodemographic, behavioral, and biologic factors contribute to the differential spread of HIV. The character of each sub-epidemic remains largely determined by the extent to which people are exposed to HIV by one or more of the three primary modes of transmission: (1) sexual (vaginal, anal, and oral); (2) parenteral, i.e. by injection (including the sharing of drug-injecting equipment), transfusion, or transplantation

of HIV-infected blood, blood components, tissues, or organs; and (3) maternal-infant, from woman to her fetus or infant.

The vast majority of HIV infections in the world are transmitted sexually, most often through heterosexual intercourse (7). Many factors influence the risk of sexual transmission, including specific sexual practices, presence of other sexually transmitted infections, and stage of HIV infection in the source partner (8).

The transfusion of HIV-infected blood, notably to children with malaria-associated anemia, accounted for up to 10% of new HIV infections earlier in the epidemic (9,10). While screening for HIV in transfused blood is generally available, the window period during which recent infection is not detectable, as well as interruptions in the supply of HIV test kits and laboratory errors continue to contribute to an HIV risk of variable and undetermined importance associated with this medical procedure. The transmission risk posed by clinical injections is difficult to quantify but is thought to account for some infections (11–13), and has been associated with significant outbreaks (14,15).

Mother-infant transmission has become common in areas with high rates of HIV infection among women of childbearing age. In the absence of interventions, approximately one-quarter to one-third of infants born to HIV-I-infected women become infected, regardless of the subtype of HIV-I involved (16–20). In contrast mother-infant transmission of HIV-2 is much less efficient, with closer to 1% of infected mothers transmitting to their infants (21–23). While most transmission occurs during pregnancy or at delivery, breast-feeding has also contributed to overall mother-to-child transmission, at least of HIV-I (24–26).

In a relatively short time, the epidemic has evolved quickly and differently in the various regions of the world. As the epidemic emerges in a given area, there have been common attributes regardless of the predominant modes of transmission. The emerging stage is characterized by limited numbers of detected infections that increase rapidly though silently over a few years in groups at highest risk (e.g. sex workers, patients at sexually transmitted infection clinics, injection drug users), particularly in urban settings, well before the clinical impact of advanced HIV disease is recognized. After rising quickly, HIV infection prevalence in higher-risk groups then begins to level, while spreading more gradually to lower-risk and rural populations.

EPIDEMIOLOGIC PATTERNS OF THE CLINICAL MANIFESTATIONS OF HIV/AIDS

Although the clinical and immunologic manifestations of HIV infection are addressed in other chapters of this book, it is important to realize that the wide spectrum of clinical conditions described in North America does not necessarily reflect the typical clinical picture of HIV disease in other parts of the world (27-29). With the rapid global spread of HIV and AIDS, a plethora of opportunistic pathogens— viruses, bacteria, fungi, protozoa, helminths, and arthropods— that are more frequent or severe in HIV-infected persons have been reported from various areas (30). Some of these organisms are only locally important, while relatively few pathogens cause the majority of morbidity and mortality in HIV-infected persons.

Although the reasons for the different clinical manifestations of HIV-related disease are not completely understood, they are likely to include the differing prevalence of opportunistic organisms in the environment and the behaviors and environmental conditions that expose persons to these pathogens. In addition, the differences in recognized disease patterns reflect varying capabilities to diagnose reliably specific conditions (29). Determining the spectrum of opportunistic infections and conditions in a given region requires well-functioning surveillance systems and diagnostic services; both are often limited in developing countries.

Tuberculosis (TB) is probably the most common and serious opportunistic infection in persons infected with HIV in Africa, Asia and Latin America (29,31–33). In many developing countries, half or more of the adult population have been infected with *Mycobacterium tuberculosis* and are at greatly increased risk for reactivation of tuberculosis when cell-mediated immunity declines following HIV infection (34). Beyond the TB-related morbidity and mortality in the AIDS patients themselves, the increased frequency of clinical tuberculosis due to HIV infection enlarges the reservoir and the risk of TB transmission to the public at large. The HIV epidemic has had a devastating impact on tuberculosis control efforts in many countries and given the magnitude of the burden of tuberculosis infection in the world compounded by the emergence of drug-resistant *M. tuberculosis* strains, tuberculosis will remain a major opportunistic infection globally (31).

Compared to data from industrialized countries, relatively little information is available on HIV disease progression in resource-poor settings, including the chronological order and the stages of immune deficiency at which different opportunistic diseases occur. Apparent differences in disease progression may reflect late initial diagnosis of HIV/AIDS, lack of access to medical care, or death from prevalent opportunistic infections such as tuberculosis that occur relatively early in the course of HIV infection, rather than any appreciable differences in the rate of decline of immunologic function.

EPIDEMIOLOGY BY REGION

Sub-Saharan Africa

Africa has been the region most severely affected by the HIV/AIDS epidemic. At the end of 2002, there were an

estimated 29.4 million people living with HIV/AIDS in sub-Saharan Africa (Fig. 41.1) (1). And the region accounts for three-quarters of the 20 million people that have already died (5). The high prevalence of HIV in many parts of Africa, the wide distribution of HIV-I and HIV-2 subtypes, and the genetic variation within subtypes, suggest that HIV has been on that continent for a relatively long time compared to other regions (6,35,36).

The observed prevalence of HIV infection in Africa varies greatly. Southern Africa has the highest HIV infection rates in the world. The sub-region includes seven countries with adult HIV prevalence rates above 20%: Botswana, Lesotho, Namibia, South Africa, Swaziland, Zambia and Zimbabwe (2). Botswana has the highest prevalence rate with 38.8%, followed by Lesotho, Swaziland and Zimbabwe, which have prevalence rates between 31.0% and 33.7% (2). Prevalence rates in East Africa are slightly less high, although they range from 5–15% with the highest rate found in Kenya (2). In most countries of West and Central Africa prevalence rates remain under 5% in the general population (2). However there are countries in the region where infection rates are higher. In Côte dÕIvoire, Cameroon and the Central African Republic between 9.7 and 12.9% for example, adult HIV prevalence is estimated (2). In Nigeria, a country with a population of almost 110 million, prevalence is estimated to be almost 6% and steadily increasing (2). Burkina Faso and Togo are two other countries in the region with prevalence rates above 5% (3). Extensive demographic changes with shifts from rural to urban areas and numerous population displacements from migrant labor and civil disturbances over the recent decades may have facilitated the spread of HIV in many areas. In the early years of the epidemic, groups at especially high risk for HIV-I infection in Africa included female sex workers, sexually transmitted infection (STI) clinic patients, and men with occupations that involved traveling or being away from family for long time periods such as truck drivers; with urban residents being more at risk than rural populations. Today, the epidemic in most of the region has shifted from those at highest risk to a generalized epidemic with rural populations impacted almost as much as urban populations (4).

Heterosexual HIV transmission is the major mode of spread among adults, with very little transmission recognized from male-to-male sexual exposure or injection drug use. As a result, this transmission pattern in Africa has resulted in male-to-female ratios of HIV-I infection and AIDS cases approaching unity (38). However, age-speciÞc rates of infection and disease vary by sex. For example, among persons with AIDS in Uganda, women outnumber men in the 15 to 24-year age group, whereas men outnumber women in the 30-year and older age group (39). In many African countries, the highest HIV-I seroprevalence rates are among women aged 20–25 years and men aged 25–35 years, reßecting sexual contact between older men and younger women (40). Dominant heterosexual transmission also means that there is a signiÞcant level of mother-to-child transmission. By the end of 2001, an estimated 2.6 million children under 15 were living with HIV/AIDS in sub-Saharan Africa with almost all of them acquiring the virus from their mother (2).

A number of explanations have been proposed for the relative efÞciency of heterosexual transmission and the rapid spread of HIV-1 in sub-Saharan Africa. HIV-1 infection in Africa has been associated with increased numbers of heterosexual partners, a history of prostitution among women, and sexual contact with prostitutes among men (41–43). The role of sexually transmitted infection (STI) in augmenting the transmission of HIV-1 has received considerable attention (44). A number of STIs, especially genital ulcer disease, have been associated with HIV-1 infection in both men and women in various studies (45,46). For men in eastern and southern Africa, lack of circumcision also appears to contribute as a risk factor for HIV infection, either independently or perhaps due to an association between genital ulcer disease and an intact foreskin (47,48). HIV-1 subtypes other than B (relatively little subtype B has been recognized in Africa) have been suggested as contibuting to the heterosexually-transmitted epidemics in Africa (and Asia) based on ecologic patterns of infection, but documentation that the non-B subtypes are actually transmitted more rapidly than subtype B *in vivo* is lacking (49).

Asia and the PaciÞc

By the end of 2002, it was estimated that 1.2 million people were living with HIV/AIDS in East Asia and the PaciÞc while 6 million people were living with HIV/AIDS in South and Southeast Asia (Fig. 41.1) (1). This represents prevalence rates of 0.1% in East Asia and the PaciÞc and 0.6% in South and Southeast Asia (2).

Though these rates remain low, factors such as cross-border opiate traÞc between countries, drug use invloving the sharing of injection equipment and the sex trade pose a substantial risk for further HIV-1 spread in the region.

Studies in China have shown rapid spread of HIV-1 among injecting heroin users in western Yunnan province, a region that shares a border with Myanmar (Burma) (50). Increases in STIs are another important risk factor for HIV in China. In 1999, there were 863,000 reported cases of STI compared to 5,800 reported cases in 1985 (51).

In India, a country with a population larger than all of Africa, the epidemic is very different from state to state. While in some states, there are almost no reports of HIV infection, others such as Tamil Nadu, have reached adult HIV prevalence rates of 2% (37). In southern states epidemics are driven by heterosexual sex while in the northeastern states, there are signiÞcant epidemics among injecting drug users (4).

Commercial sex is also responsible for a high proportion of cases in the most affected countries in Southeast

Asia, with adult prevalence close to 2%: Cambodia and Thailand (1,52). The epidemic in Thailand has been very well documented. Extensive HIV transmission in Thailand began around 1988 with rapid increases in HIV-1 seroprevalence among injecting drug users (IDUs), as evidenced by the prevalence among persons attending drug treatment centers in Bangkok increasing from I% to approximately 40% by the end of 1988 (53). The second wave of the epidemic, which was later shown by molecular epidemiologic studies to be distinct from the Þrst (53Ð56), occurred primarily among female sex workers (53,57). Between June 1989 and June 1990, the median province-speciÞc HIV-I seroprevalence among brothel prostitutes increased from 3.5% to 9.5% with more than 60% among some groups of prostitutes in northern Thailand found to be infected (58,59). Commercial sex was quickly recognized as being the major source of both heterosexual and homosexual HIV transmission. The increased infectiousness that occurs in the primary phase of HIV infection and the large number of clients served by prostitutes, compounded by prevalent sexually transmitted diseases, are likely to have contributed greatly to extensive transmission and the rapidly expanding HIV epidemic (60). The many men who were infected by sexual contact with prostitutes, transmitted HIV in turn to their other female sex partners (61,62) resulting in a subsequent increase in maternal-infant HIV transmission (63,64). Additionally, a substantial number of HIV infections have occurred among men who have sex with men (MSM) (65). Thailand has made considerable progress in the Þght against HIV/AIDS and its HIV prevention campaigns have been well documented. As condom use has increased and visits to female sex workers have decreased, sexual behavior has changed and as a result, HIV prevalence has decreased (52).

In contrast to some of the developing Asian countries mentioned previously, the number of HIV infections appears to be relatively low in industrialized countries in the region. By the end of 2002, an estimated 15,000 adults and children were living with HIV/AIDS in Australia and New Zealand (Fig. 41.1) (1). The pattern in these two countries and other high-income countries in the region such as Japan is similar to that in North America and Western Europe, with homosexual men and injecting drug users predominantly affected.

Latin America and the Caribbean

By the end of 2002, it was estimated that 1.5 million adults and children were living with HIV/AIDS in Latin America and the Caribbean (Fig. 41.1) (1). HIV prevalence rates are especially high in the Caribbean and Central America. Six of the seven countries in the Caribbean have HIV prevalence rates above 1% with the prevalence rate in Haiti estimated at 6.19% at the end of 2001 (2). In Central America, Belize, Guatemala, Guyana, Honduras, Panama and Suriname all had estimated prevalence rates above 1% at the end of 2001 (2).

The geographic distribution of HIV/AIDS in Latin America and the Caribbean is far from homogenous either among or within countries. The great diversity of the countries and populations in the region has led to substantial variations in the patterns and rates of HIV-infection and AIDS.

As in North America, most Latin American and Caribbean countries experienced rapid spread of HIV-1 among homosexual and bisexual men early in the epidemic (66Ð69). High rates of infection among bisexual men and, in some countries (notably Brazil), heterosexual male IDUs have led to an increase in heterosexual transmission of HIV-1 to women (70,71). Transmission of HIV-1 among IDUs has been substantial, at least focally, in many countries in the Americas (72,73). Parenteral transmission of HIV-1 from unscreened blood and the improper use of needles and syringes has also been a problem in some countries (68,73,74).

The proportion of reported AIDS cases resulting from heterosexual transmission has increased dramatically since the early 1980s in most Latin American countries (and heterosexual transmission appears now to be the predominant pattern in a number of countries) (66). Increases in perinatal transmission of HIV-I and pediatric AIDS cases have paralleled the increase in HIV-I infection in women in several countries. The prevalence of infection in antenatal women, although considerably lower than in Africa, nevertheless is appreciable in some areas.

Europe and Central Asia

By the end of 2002, there were an estimated 570,000 people living with HIV/AIDS in Western Europe (Fig. 41.1) (1). As in North America, groups predominantly affected in Western Europe are homosexual and bisexual men and IDUs with very low rates among heterosexuals in the general population (5). Recent information suggests that like in the United States, although risk behavior among homosexuals dropped signiÞcantly during the mid- and late 1980s, there has been a rise in unsafe practices in some communities (4). Among IDUs, the situation varies across countries. Some countries have been successful in containing HIV prevalence in this group at low levels with aggressive prevention efforts and needle-exchange programs (37). In other countries, prevalence rates among IDUs are very high. In Spain, for example, a recent study in Barcelona found a prevalence rate of 51% among injecting drug users (37).

Also like in the United Sates, ethnic minority and poorer populations within Western European countries are at higher risk (4). Several countries, including Belgium, France, the United Kingdom, and Portugal, have had appreciable numbers of HIV infections in persons who came from or were infected in developing countries or

who have had heterosexual sex with a person from a high-prevalence country (4). The HIV strains involved extend well beyond the well-characterized subtype B of Europe and North America and include non-B HIV-I subtypes and HIV-2 (6,75,76).

Until 1995, countries in Eastern Europe and Central Asia had reported few HIV cases, mostly among homosexual men (52). But in the past few years, the rates have changed substantially. Sociodemographic changes due to economic difficulties and ethnic and religious conflicts have led to important increases in high-risk behavior and subsequent HIV spread. By the end of 2002 there were an estimated 1.2 million people living with HIV/AIDS in Eastern Europe and Central Asia (1). Since 1995, HIV has spread quickly among IDUs in the region with prevalence rates in this group ranging from low estimates of 0.2% to a high of 65% in Odessa Ukraine (4). In the Russian Federation, there were more new infections in 2000 than there were for all previous years of the epidemic combined (4).

There are also signs that a sexually transmitted HIV epidemic could evolve quickly in Eastern Europe and Central Asia (52). Rates of sexually transmitted infections, most notably syphilis, have increased significantly. In 1998, WHO found a 50-fold increase in syphilis cases in Russia (77). From these data, they estimated that 1 in 400 Russians were infected with syphilis.

North Africa and the Middle East

Relatively few cases of AIDS have been reported from the countries of the North Africa and the Middle East and surveillance continues to be poor (Fig. 41.1) (1). By the end of 2002, there were an estimated 550,000 people living with HIV/AIDS in the Middle East and North Africa (1).

In areas where data exist, transmission can be attributable to injection drug use, heterosexual contact and contaminated blood transfusion (78). Recent evidence does indicate that new infections are on the rise in this region (42). Studies in one area of Algeria, for example, found prevalence rates around 1% in pregnant women attending antenatal clinics (42). HIV prevalence among STI patients has increased in Sudan, Yemen, and the Syrian Arab Republic (52). And there are signs that HIV-infection rates among tuberculosis patients are increasing. By mid-2001, these rates had reached 8% in the Sudan, 4.8% in Oman, 4.2% in the Islamic Republic of Iran and 2.1% in Pakistan (1).

CHANGING DEMOGRAPHIC PATTERNS DUE TO HIV/AIDS

Mortality

Since the virus eventually causes the death of virtually all infected adults and children, the HIV/AIDS epidemic has caused mortality rates to increase dramatically in highly affected countries especially in sub-Saharan Africa (79). It has changed the age and sex patterns of mortality, increasing the death rates for adults in their most productive ages as well as increasing death rates for children.

Since HIV is primarily spread through sexual transmission, most of the people becoming infected with the virus are adults and youths. HIV survival time in developing countries for incident cases is estimated to be approximately 8–11 years (80). This means that HIV is not only increasing mortality; it is increasing it in age groups that have historically had the lowest mortality rates. It also means that it takes some time before the long-term effects of the epidemic on country mortality rates become evident.

Data from national-level surveys and censuses provide information on mortality levels and trends, though these data do not specify cause of death. Between the late 1980s and mid 1990s, national surveys in Uganda, Zambia and Zimbabwe record huge increases in adult mortality concentrated in early adulthood, at the ages at which one would expect most AIDS deaths to occur (81). In just six years men's death rates in Uganda almost doubled while women's death rates more than doubled. In Zambia, over a six-year period, women's death rates almost doubled while men's death rates more than doubled. Similarly, national data from Zimbabwe show that within a decade, one of the countries in Africa with the lowest mortality rates became one of those with the highest mortality rates. The probability of dying between ages 15 and 60 years in the mid 1980s was less than 20%, while 10 years later it was more than 40%.

Several community studies have also identified HIV/AIDS as a major cause of death providing further insight into these national surveys. In studies in Tanzania and Uganda with adult HIV prevalence rates of 7% and 8% respectively, 47% and 40% of adult deaths were attributable to HIV (82,83). In another Ugandan cohort with an adult HIV prevalence of 16%, 73.5% of adult deaths were attributable to HIV infection (84). Even in a cohort in rural Tanzania with a relatively low HIV prevalence of 4%, HIV increased overall adult mortality by more than 50% (85).

HIV-related deaths among children are harder to measure than among adults (80). In low-income countries, background mortality rates for young children are much higher than for young adults. Also, children who die of AIDS are more likely than adults to die without an HIV-related diagnosis. As mentioned above, it is estimated that in the absence of interventions, approximately one-quarter to one-third of infants born to HIV-I-infected women become infected and this is reduced by close to 50% with antiretroviral therapy (86). Since most infected children will develop AIDS and die within a few years of birth, infant and child mortality will increase in high HIV-prevalence countries as a result of AIDS. The impact of the epidemic on child mortality is likely to be even larger

since the probability of death for HIV-negative children born to HIV-positive mothers may be greater than children born to HIV-negative mothers given the quality of care that they are able to provide (87).

Life expectancy at birth, which is determined by the proportion of the population dying at each age, is particularly sensitive to HIV/AIDS as deaths among young adults and young children result in many years of life lost (79). According to country models compiled by the United States Bureau of the Census, life expectancy in many sub-Saharan African countries has declined drastically because of HIV/AIDS. In two of the most highly affected countries, Botswana and Zimbabwe, it is estimated that life expectancy in 2000 was 39 and 38 years respectively compared to the 70 years estimated in the non-AIDS scenario in both countries (88).

Fertility

There is increasing evidence that HIV/AIDS also has major impacts on fertility in highly affected countries (89). The epidemic impacts fertility in several ways. Biological effects of the epidemic have an impact on women with HIV. In addition, the epidemic may cause behavior changes in the general population.

HIV lowers a woman's fecundity and ability to bear a pregnancy to term (89). Studies in a number of countries in sub-Saharan Africa have shown significant reductions in fertility with estimated reductions in the risk of pregnancy in HIV-infected women ranging from 16% to 26% (90–94).

Throughout sub-Saharan Africa, the lower fertility in HIV-infected women is unlikely to be explained by an increased use of contraception since the use of modern contraceptive methods is low and most women are unaware of their HIV-serostatus (89). Instead, these lower fertility rates are likely to be due in part to lower rates of conception. This is due in part to the fact that there is an association between HIV infection and infection with other STIs. Because serological tests for syphilis are quite easy to undertake, several studies have shown that higher proportions of HIV-positive women have been infected with syphilis at some time in the past (95,96). Other STIs that are less easily detected including gonorrhea and chlamydia independently lead to reduced fertility and it is probable that these are also having an impact on the fertility of HIV-positive women (89). The fecundity of HIV-positive women may also be reduced for several other reasons. Women with HIV are more likely to have amenorrhea or irregular menses than are HIV-negative women; there may be repercussions for women's fertility from the decreased production of spermatozoa in their HIV-infected male partners; and there may be reductions in the frequency of sexual intercourse due to illness (92,97–99). Another factor causing lower fertility rates among women with HIV are the increased rates of pregnancy loss (part of the increased loss may also be explained by the association between HIV infection and infection with other STI) (92,95–97,100). Finally, it should be noted that pregnancies may accelerate disease progression and development of AIDS, causing HIV-positive women who remain fecund to have shorter mean survival times than those who do not (89).

As or more importantly, the epidemic is also likely to cause behavior changes in the general population. The HIV/AIDS epidemic appears to cause important changes in contraceptive use with an increase in the use of condoms and the switching to condom use from other forms of contraception (89). If condoms are used by couples who did not practice contraception previously, this will result in an overall decline in fertility. However, if they replace more effective methods of contraception with condoms, this will cause slight increases in fertility. The epidemic is also having an impact on fertility by increasing women's age of first sex (101–103). Finally, changes in post-partum behavior including reduction in breastfeeding or shortening of abstinence after the birth of a child to discourage a partner from seeking other partners that may be HIV-infected would tend to increase fertility (89).

The Combined Impact of HIV on Mortality and Fertility

The combined impact of the HIV/AIDS epidemic on mortality and fertility is causing the population structure in highly affected countries to change. Graphs of population structure depict populations according to age groups with men on one side of a central axis and women on the other. When both mortality and fertility rates are high, the graph has a wide base and tapers off steadily with increasing age. In most developing countries, therefore, the structure of the population by age and gender usually has the shape of a pyramid, while in developed countries the pyramid becomes more like a column.

As illustrated by the projected population structure of Botswana for 2020 in Fig. 41.2, the HIV/AIDS epidemic is introducing a completely newly shaped population structure in highly affected countries that the UN HIV/AIDS program refers to as the "population chimney" (37). Fewer babies are born since many HIV-infected women die or become infertile before the end of their reproductive years and up to a third of the infants born to HIV-positive mothers acquire HIV/AIDS. This causes the base of the pyramid to be less broad. In addition, the center of the pyramid shrinks radically as the populations of women above their early 20s and men above their early 30s die 10 or 15 years after the age at which they become sexually active. Since only those who have not been infected survive to older ages, the pyramid becomes a chimney.

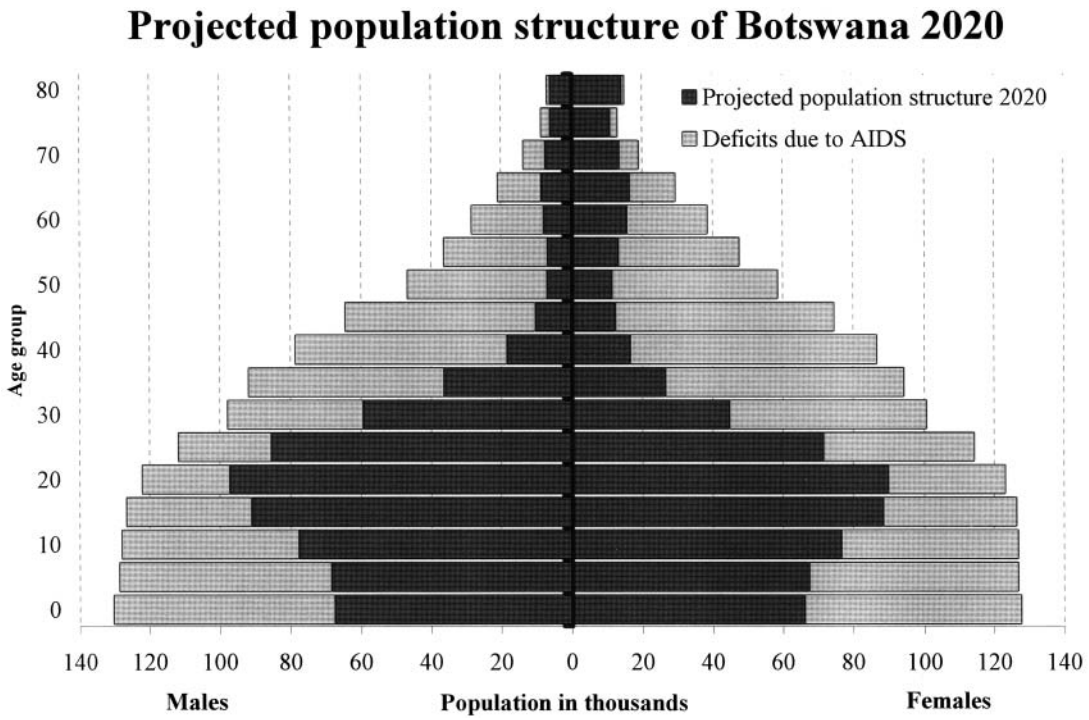

FIG. 41.2. Projected population structure of Botswana 2020.
Source: UNAIDS. Report on the global HIV/AIDS epidemic. Geneva: UNAIDS, 2000.

Orphanhood

DeÞned by UNAIDS and UNICEF as children who lose their mother to AIDS before reaching the age of 15, AIDS orphans are an important consequence of the epidemic in highly affected countries (104). Many of these children also lose their fathers to AIDS. However, because reliable data on the number of paternal orphans are not available in many countries, UNAIDS/UNICEF orphan statistics do not include children who have only lost their fathers. It is estimated that by the end of 2001, globally, a cumulative total of 13 million will have lost their mother or both parents to AIDS and 10.4 million of them will be under the age of 15 (104).

Given the severity of the epidemic in sub-Saharan Africa, the proportion of children orphaned by HIV/AIDS in Africa is much higher than in other regions. Cohort studies in rural populations with levels of HIV prevalence of around 10% estimate that between 7% and 15% of children have lost at least one parent to HIV/AIDS (105). A limited number of community studies have been conducted on the impact of AIDS orphanhood on the health and development of these children (94,106Ð111). These studies from sub-Saharan Africa, show that most children are taken care of by extended family members. As death rates continue to increase, however, the consequences for the health and development of AIDS orphans can be severe as extended family absorption capacities are stretched to their limit (87).

SOCIOECONOMIC IMPACT OF HIV/AIDS

Macroeconomic Impact

Determination of the macroeconomic impact of HIV/AIDS is extremely difÞcult. Most economists agree that in high prevalence countries, the epidemic has had a major impact on national economies but not necessarily a major impact on per capita gross domestic product (GDP). The epidemic stimulates higher demand for health care as well as a reduction in income among those directly affected. This leads to increases in health spending with associated reductions in savings and in nonhealth spending. It also causes cost pressures for companies in terms of higher turnover due to AIDS mortality especially when that mortality includes skilled workers or workers with employer-based health insurance or disability and mortality beneÞts. It may also reduce business markets. Nonetheless, predictions of the net macroeconomic impact of AIDS have varied widely and economists continue to debate this issue. Numerous factors affect economic performance and attributing changes in performance to one particular cause is difÞcult.

During the early years of the epidemic, several economists projected that the macroeconomic impact of HIV/AIDS would be catastrophic leading to a major reduction in the economic output of certain countries (112Ð114). SigniÞcantly less alarmist, later analyses claimed that in high prevalence countries, growth rates of

national income may decrease by two-thirds of a percentage point and annual growth rate of national income per person may decrease by as much as one-third of a percentage point through the year 2010 (115–117). More recent studies using similar methodologies concluded that by 2015, the economies of Swaziland and Botswana will grow 1.1 and 2.5 percentage points less than they would have because of the epidemic (118,119).

On the other hand, cross-country regressions involving 51 countries in 1997, suggested that there was "little support for the widespread claim that the AIDS epidemic will slow the growth rate of income per capita (120)." The authors argued that even though the epidemic was advanced in some countries, after they controlled for other influences on economic growth, they found no evidence that economies in higher HIV prevalence countries grew at significantly slower rates than countries with lower HIV prevalence rates. This view has also been supported by the World Bank which argues that the macroeconomic impact of HIV/AIDS is likely to be small in most countries as declines in population growth due to HIV/AIDS will tend to offset declines in economic growth causing the net impact on gross domestic product (GDP) growth per capita to be small (121).

Two recent modeling exercises in South Africa, one of the hardest-hit countries in the world, further illustrate the uncertainty of macroeconomic analyzes due in large measure to the assumptions underlying the model. At the same time these results do indicate that in highly affected countries like South Africa, even the most optimistic scenarios project negative impact on the macroeconomy. The first conducted by ING Barrings forecasts that over the next 15 years, South Africa's economy is likely to grow 0.3 to 0.4 percentage points less per year than it would have because of HIV/AIDS (122). The second study concluded that between 1997 and 2010, GDP growth rates in the AIDS and non-AIDS scenarios diverge steadily, reaching a maximum differential of 2.6 percentage points in 2008 (123). The result is a GDP level in 2010 that is 17% lower in the AIDS scenario than it would have been without AIDS.

Impact on Health

In all countries, the HIV/AIDS epidemic has meant additional pressure on the health sector, as countries have invested resources in HIV prevention interventions. In highly affected countries, the epidemic has also had an important impact on both the demand and supply of health care services. On the one hand, HIV/AIDS increases the number of people demanding health care services and causes significant increases in national health spending. On the other hand, it reduces the supply of care available at a given quality and price due to illness and death among health care workers.

Two decades into the epidemic, there is increasing consensus on the components of an effective HIV prevention response within countries (124,125). In Thailand and Uganda, where there is evidence that prevention interventions have had an impact on decreasing HIV incidence rates, it is estimated that US$ 78.5 million and US$ 37.5 million was spent on prevention respectively (126). The cost of the "successful" prevention programs in Uganda and Senegal, the latter another comparatively well-funded effort that has been successful in maintaining low prevalence rates, was extrapolated to the rest of sub-Saharan Africa correcting for a number of factors expected to affect the cost of large-scale prevention programs. This exercise suggested that in 1996 the continent would have had to spend approximately US$1 billion in national and donor resources combined (126,127). A more recent exercise performed for the UN General Assembly on AIDS in June, 2001 estimated that by 2005, approximately US$ 4.8 billion will be needed to implement globally a comprehensive package of HIV/AIDS prevention interventions in middle and low-income countries (128).

The health care sector does not bear all of the costs of HIV prevention efforts as some of these interventions are multisectoral or even coordinated by other sectors. Nonetheless, the health sector does bear large proportions of these costs as many interventions, including STI prevention and care, voluntary counseling and testing and prevention of mother-to-child transmission are health sector specific. In countries where per capita health expenditure can be as low as US$ 5.00, HIV prevention interventions alone constitute a significant burden (129). However, as prevalence rises, the impact of prevention efforts on the health sector is quickly overtaken by the increased demand for care by people living with HIV/AIDS and by illness and death among health care workers. And this impact is even worse because it is concentrated in low-income countries.

As described above, the natural history of HIV illness and the pattern of opportunistic illnesses differ across regions. This means that the palliative care, treatment of opportunistic infections and prophylaxis for opportunistic infections required by an HIV-infected person also varies from region to region (130). Regardless, HIV/AIDS treatment costs are high. The World Bank estimated lifetime cost per patient for palliative care and prevention of the more common opportunistic illnesses to range between US$ 300 and US$ 1,000 (1996 dollars) (121). When including care costs for rarer opportunistic illnesses, the figures were estimated to range between US$ 500 and US$ 1,700. And these costs have increased dramatically in the age of highly active antiretroviral therapy (HAART). An estimated US$ 4.4 billion will be needed in middle and low-income countries to provide medical care to those people who have access to health services and to provide support to orphans (128).

Data from studies in Eastern and Southern Africa show that there has been a steady increase in the number of HIV-infected patients in hospitals. In a Tanzanian hospital, the

prevalence of HIV-I infection among hospitalized patients was 32.8% indicating that HIV infection was the major cause of illness leading to admission to the hospital (131). In Zimbabwe, 50% of all in-patients in wards studied were infected-with HIV (132).

Tuberculosis and other clinical conditions become more difficult to diagnose when associated with HIV/AIDS. This and the chronic nature of HIV/AIDS translate into increased cost of care to both the health care service and the users (37). A recent study in Zimbabwe showed that costs per in-patient stay were twice as high for HIV/AIDS patients compared with other patients (132).

Not surprisingly, these increases have led to overcrowding in the medical establishments of high prevalence countries. Total hospital admissions in a rural South African hospital increased by 81% between 1991 and 1998 (133). In Kenyatta National Hospital in Nairobi, Kenya, there was severe overcrowding in 1997, with bed occupancy estimated at 190%Ñ doubled from 1988/1989 (134).

It has been argued that the increased demand for health care services by people with HIV would also lead to the crowding out of non-HIV/AIDS related patients in high prevalence countries (121). Data from several studies have suggested that the epidemic was having a negative impact on the overall quality of care provided (135). However, a longitudinal study in Kenya from 1988/1989 through 1992 to 1997, revealed that the hospital Òdoes not appear to have been overtaken by the chronic care demands of the terminally ill, in particular those with clinical AIDSÓ (134). In 1997, fewer sick patients with advanced HIV/AIDS disease and more HlV-negative patients were admitted. Compared with 1992, HIV seroprevalence among patients stabilized. And though there was overcrowding, fewer patients died compared with 1992.

Just as the epidemic affects the demand for health care, it also has an important effect on the supply of health care in highly affected countries. As in other sectors of the economy, rising rates of HIV infection in health care workers will increase rates of absenteeism, reduce productivity, and lead to higher levels of spending for treatment, death benefits, additional staff recruitment and training of new health personnel (37). In Zambia, deaths among health care workers in one hospital increased 13-fold between 1980 and 1990, largely because of HIV (136). Furthermore, the risk of HIV infection is likely to have a negative impact on the supply of health care workers by dissuading some individuals from these occupations (121). HIV/AIDS is also affecting the supply of health care by increasing the cost of maintaining safety levels for medical procedures (121).

One of the alternatives to hospital care that has been advocated as a solution in highly affected countries is home-based care including both hospital-initiated home care and care provided by nongovernmental organizations (NGO) working on HIV/AIDS (137). Both of these models have important limitations. Most NGO home care initiatives have focused on providing counseling and spiritual support. At the same time, hospital outreach services have not necessarily been less expensive. A study in Zimbabwe estimated that each home visit is equivalent to one to three hospital days (138). In addition, the study found that coverage was low and the care often inefficient with a large proportion of the resources not directly benefiting the patient or family. Gradually, community-rooted home care initiatives are emerging; often organized by people living with HIV/AIDS themselves (137). Ideally, these initiatives are well integrated within a larger continuum of care including health facilities and NGO service providers. Experts believe that community-based initiatives integrated within the larger continuum of care available in a country will reduce the cost of institutional health care and improve the quality of life of people living with HIV/AIDS and their families (137).

Impact on Education

The epidemic can have significant impacts on both the demand and the supply of education in high prevalence countries. On the one hand, it has an impact on the number of children in the school system as well as on the ability of these children to learn. On the other hand, the epidemic also affects the provision of education given the frequency of HIV infections among teachers and other professionals in the education sector.

On the demand side, as discussed above, the demographic effects of the epidemic in high prevalence countries will result in an overall reduction in the absolute number of school-age children. Likewise, changes that occur before and after an AlDS-related death in a household tend to reduce the demand for education. The extended illness that infected parents experience places an increased demand on the time of children for providing care and support (139). The ability of families to pay for school fees and other education costs are also compromised in HIV/AIDS affected households. It may become necessary for children to be withdrawn from school in order to work to subsidize the family income. There is also some indication that the expected returns of investment in childrenÕs education may be reduced where prime-age adult mortality is high (121).

As children are orphaned and are taken in by extended families, considerable financial burden is placed on families who may not be able to afford to keep all children in school. In other cases, as they lose their primary source of financial support with the death of one or both parents, orphans are forced to find work to support themselves and their younger siblings, thereby causing them to drop out of school. A study in Zambia found that in urban areas 32% of orphans were not enrolled in school compared to 25% of non-orphans. In rural areas 68% of orphans were not enrolled compared to 48% of non-orphans (139). In a study in Zimbabwe, 48% of orphans of primary age had

dropped out of school and no orphans of secondary school age was still in school (37).

On the supply side, the most important impact of the epidemic is the increased rates of morbidity and mortality among teachers. In the absence of better data, and given recent experience in Africa with teacher attrition, it can reasonably be assumed that HIV prevalence is as high among teachers as it is in the general population. Teacher attrition due to HIV/AIDS is especially devastating in the highly affected countries in Africa where there are only one or two teachers per school and the loss of a teacher can completely deprive students in that area of access (139). In the Central African Republic, for example, a country that has only a third as many primary school teachers as it needs, almost as many teachers died as retired between 1996 and 1998 with 85% of them being HIV-infected (37).

Even among teachers who are not infected, morale can be highly compromised. Rates of absenteeism are increasing as teachers take time off to attend funerals and mourn lost friends and family members or to moonlight to subsidize family income (139). In addition, with rising AIDS mortality the education sector will also be faced with attrition among planners and administrators at all levels. Even if these personnel are replaced there would be a considerable loss of experience, which can be expected to have an effect on the functioning of the system.

Impact on Agriculture

Data on the impact of HIV/AIDS on the agriculture sector in highly affected countries are limited. Most of the arguments presented on this issue are based on conventional wisdom supported by a few small-scale studies, most of which were conducted by the UNÕs Food and Agriculture Organization (FAO) (140Ð145). Yet agriculture is the most important sector in most developing countries both in terms of production and employment. In some countries, it might provide employment to as much as 80% of the countryÕs population (37). The mere size of this sector therefore implies that in highly affected countries, the epidemic will have a signiÞcant impact.

The Food and Agricultural Organization has estimated that in the 27 most affected countries in Africa, seven million agricultural workers have died from HIV-related causes since 1985, and 16 million more deaths are likely in the next two decades. In the ten most affected African countries (Namibia, Botswana, Zimbabwe, Mozambique, South Africa, Kenya, Malawi, Uganda, Tanzania, Central African Republic, Ivory Coast, Cameroon), labor force decreases ranging from 10Ð26% are anticipated (146).

The epidemicÕs impact on agricultural labor in turn causes cost pressures on agricultural estates and diminishes disposable cash income available for household agricultural production (147,148). A study of six agricultural estates in Kenya found that rates of HIV prevalence were high with one estate having a rate of 25% (147). Illness and death primarily due to HIV/AIDS was the most important reason for employee exits from the estates. All estates experienced substantial cost increases with medical expenses increasing ten-fold between 1989 and 1997 and funeral expenses increasing Þve-fold over the same period. HIV/AIDS also resulted in psychological and economic difÞculties among employees and increased illness and funeral related absenteeism and loss of skilled labor. The authors of this study concluded that the epidemic has led to a decline in quantity and quality of processed products, increased costs of production, and a decline in company proÞtability.

A study of the impact of HIV/AIDS on rural Ugandan families in subsistence agriculture illustrates that these households are particularly vulnerable to the impact of the epidemic (144). Household members had longer working hours to make up for labor shortages and loss of income from ill persons; remote Þelds tended to be left fallow and the total output of the agricultural unit declined to accommodate for labor shortages. Some agricultural yields declined as a result of delays in essential farming operations and lack of resources to purchase agricultural inputs. Labor-intensive crops were abandoned in favor of less demanding ones reducing the variety of crops that may lead to less varied food, with a negative impact on nutrition. This study found that the burden of HIV/AIDS on rural households was born disproportionately by women. Single men generally remarried and most single headed households were headed by women with limited economic opportunities and limited rights to land and property.

Impact on Business

The epidemic impacts the business sector in two different ways. As discussed above in the agriculture impact section, companies face cost pressures given the impact of HIV/AIDS on business productivity and company costs. In addition, it also affects business markets given its impact on the general population in high prevalence countries.

Conventional wisdom suggests that the epidemic affects business productivity and costs through its effects on company employees, although the effect of HIV/AIDS on the workforce is disputed (149). Conventional wisdom also suggests that HIV/AIDS-related mortality reduces the supply of available productive and skilled labor (150). Absenteeism rates increase as a result of employees becoming ill due to HIV and its associated opportunistic infections, the demands of caring for family members who are ill, and the need to attend funerals. Absenteeism rates have risen signiÞcantly in highly affected countries. This disrupts the production cycle, causes under-utilization of equipment and increases the use of temporary staff. In short, high rates of HIV morbidity and mortality lead to

increases in staff turnover, loss of skills and knowledge and declining employee morale.

The most important analysis of African Þrms to date including almost 1,000 companies from 1992Ð1994 concluded that the impact of HIV/AIDS on staff turnover was minimal, although it recognized the situation could change with the evolution of the epidemic (151). This result may be due to the fact that the analysis was conducted relatively early on in the epidemic. It may also reßect that the workforce in many of these Þrms was relatively unskilled with high rates of turnover, even in the absence of AIDS. A number of smaller studies and anecdotal evidence indicate that the epidemic has an important impact on the workforce which in turn lowers productivity of Þrms and increases company costs (150). In Kenya, Zambia and Malawi, it is estimated that HIV/AIDS-related absenteeism accounts for 25% and 54% of company costs (152). In Kenya, a survey of Þve companies found that AIDS was costing companies 3% of their proÞts in the mid-1990s and could cost as much as 8% of proÞts by 2005 (153). In one transport company in Zimbabwe, the total cost to the company arising from HIV/AIDS was assumed to equal 20% of company proÞts (154).

The demand for recruitment and training rises as a result of increased staff turnover and loss of skills (150,154). Company life insurance premiums and pension fund commitments rise as a result of early retirement or death. In Zimbabwe, for example, life insurance premiums quadrupled as a result of HIV/AIDS (155). Finally, considerable costs can be added where businesses provide for the funeral costs of employees.

The impact of the epidemic on the customer base is only now beginning to be studied. But Òwith HIV/AIDS beginning to have a substantial impact on the demographic proÞle of the most-affected countries, markets are changingÓ(149). Young adults are the group that is most affected by HIV/AIDS and are also the major source of demand for goods and services. Those who are ill will redirect spending towards caring costs and since they are less likely to work, they are likely to have less money to spend. Moreover, as mentioned above in the hardest hit countries, governments are likely to redirect spending towards health and away from items favorable to growth including education and infrastructure investment.

Impact on Households

As economists have expanded their HIV/AIDS impact analyses to include studies of the epidemicÕs impact on particular sectors in addition to macroeconomic impact analyses, they have also increasingly focused on the impact of the epidemic on individual households. It is at this level that the epidemic has its most signiÞcant impact. The epidemic usually strikes prime-age adults who are at the peak of their economically productive years incapacitathlg them for signiÞcant periods of time before killing them. These adults are often parents, as well as workers providing for their households. And unlike other illnesses, HIV/AIDS-related illness and death tend to be clustered within selected households with two or more adults affected as one member infects his or her partner(s).

Prime-age adult deaths affect households both directly through medical expenses and funeral costs and indirectly because of the foregone earnings of the deceased (156). The World BankÕs publication entitled *Confronting AIDS* provides a detailed overview of data from studies in Abidjan, CÔte dÕIvoire, Kagera, Tanzania, Rakai Uganda, and Chiang Mai, Thailand on the impact of prime-age adult deaths (121). These studies conÞrm that the impact of any prime-age adult death in a household is signiÞcant and the direct impact of HIV-related deaths is slightly greater because of the higher medical expenditures of people with HIV. Household income usually decreases as the main breadwinner ceases to work for wages and other household members reduce the number of hours they work for wages in order to care for the sick person. These households devote increasing shares of their expenditure on medical and funeral costs, decreasing their consumption of basic necessities including food, energy, accommodation, hygiene and maintenance.

These studies also show that affected households have developed a variety of strategies to cope with the impact of an HIV-related death (121). The composition of households is altered to cushion the shock of the death as dependent children are sent to live with relatives or relatives join the household to assist in household or farming tasks. Savings decrease as households sell available assets such as land, livestock, or durable goods such as bicycles and radios to pay for medical treatment or burial costs. Finally, affected households often Þnd external assistance in the form of cash transfers or loans from extended family members and the local community, as well as nongovermnental organizations, to help pay for medical and burial costs.

Given these coping strategies, it follows that in general the poorer the household, the greater and more persistent the impact of a prime-age adult death from AIDS. Poorer households do not have the assets to cushion the impact of the death and have greater difÞculties accessing credit as they are unable to guarantee repayment. This greater impact on poorer households is illustrated by the 15% drop in food consumption during the year that followed an adult death in Tanzania (121). Though there are no data available on the impact of an adult death on child malnutrition, it is likely that this decrease in total food consumption has had a signiÞcant impact on child malnutrition in these households. In addition, as was discussed above under the impact on the educational sector, one of the responses to a prime-age adult death in poorer households is to remove children from schools as school fees become unaffordable and their labor is required in the household. In short, the epidemic not only

has a greater impact on poorer households, it also facilitates the intergenerational transmission of poverty.

CONCLUSION

As the HIV/AIDS pandemic enters the twenty-Þrst century, the pace of HIV transmission is increasing in sizeable parts of the world. Given the existing numbers of persons with HIV infection, the number of AIDS cases would continue to increase even if there were no further HIV transmission.

In contrast to most industrialized countries where HIV incidence and prevalence have stabilized, for the developing countries of sub-Saharan Africa, Asia and the PaciÞc, Latin America and the Caribbean, Eastern Europe and Central Asia, North Africa and the Middle East, available epidemiologic data predict continued high rates of transmission and increases in HIV seroprevalence. Large parts of Africa have already been devastated by the epidemic and the coming years will bring an increased burden of AIDS-related death. Asia, home to half of the worldÕs population, will be an important region in the AIDS epidemic in this next millennium. India alone has more people than all of Africa and extensive HIV transmission has begun in south and Southeast Asia. In many countries, AIDS has become or will likely become the leading cause of death in adults and one of the leading causes of infant and child mortality.

Data from several countries have shown that it is possible to reduce the spread of HIV with existing technology and interventions. Prevention successes in Senegal, Thailand and Uganda demonstrate the importance of aggressive national intervention programs that include well-deÞned components. The experience of these countries shows that sound programs and political commitment make a difference.

The challenge of HIV and AIDS underscores the importance of well-coordinated research to support strong prevention and treatment efforts. Epidemiological and surveillance data have been essential in tracking the HIV epidemic, directing prevention activities, and guiding research. What continues to be lacking is information on the patterns of risk behavior. Work in this area with increasing emphasis on behavioral research and surveillance will be needed to target prevention interventions better. Second generation surveillance systems that compare biological and behavioral data for maximum explanatory power and adapt to the changing needs of epidemics will be essential to producing quality data that increase and improve the national responses.

Important progress has been made in the area of research on the demographic impact of HIV/AIDS in the last 10 years. Data on the demographic impact of the HIV/AIDS epidemic has highlighted the devastating effects of HIV on mortality and fertility. Likewise, advances have been made in measuring the socio-economic impact of the epidemic. And these data together with epidemiological data have aroused global awareness on the importance of action.

However, there remain signiÞcant gaps in our understanding of the socioeconomic impact of the epidemic. Important areas of uncertainty exist on the macroeconomic impact of the epidemic as well as on the impact of HIV/AIDS on certain speciÞc sectors including agriculture and business. Research efforts in these areas are essential to ensure data-driven policy development and decision-making.

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Chapter 42

Nursing Perspectives in Care of Persons with HIV Infection

Richard L. Sowell, Troy Spicer and Arlene J. Lowenstein

Human immunodeÞciency virus (HIV) infection and the resultant acquired immunodeÞciency syndrome (AIDS) Þrst thought, in the U.S., to be a disease of urban homosexual males, is growing at a rapid pace among other populations. HIV infection is increasingly associated with drug use and heterosexual transmission, and is occurring in both urban and rural communities. Women currently represent one of the fastest growing groups being diagnosed with HIV/AIDS with African-American women being disproportionately affected by this disease (1). Providing high-quality care to such a diverse population with HIV infection is a complex and challenging task.

THE NURSING RESPONSIBILITY MODEL

HIV infection is a care-intensive disease across the continuum of the illness and involves patient contact with many health care and social service disciplines. Nurses play a major role in direct clinical care of HIV-infected patients, and have a responsibility to help patients adapt socially, psychologically, and physically to the illness. Figure 42.1 depicts a model of nursing responsibility in HIV/AIDS that provides a comprehensive overview of the nurseÕs role in caring for persons with HIV infection (2).

Nursing involvement actually begins before diagnosis, through the provision of health education about prevention strategies and wellness care in the community. From the original HIV-antibody testing to the end stage of AIDS, it is nurses who handle client assessment and offer direct patient care, which also includes symptom management, counseling support, health education, and bereavement support for families and friends. Care problems are complex and unique to each patient, because each patient exhibits a different constellation of symptoms as the disease progresses. Nursing care follows the patient, in homes and ambulatory care settings, as well as in hospitals and in all types of urban and rural communities.

Nurses must take the responsibility to coordinate interdisciplinary resources, linking the HIV-infected individual to appropriate services, including primary and acute care facilities and social and community support services that may be needed during the course of illness (3,4). Nurses can provide education about the importance of recognizing both families of biologic origin and families of choice, including signiÞcant others and partners, as participants in care and decision-making, if that is the clientÕs choice. Financial, housing, nutrition, and transportation problems frequently grow worse as the disease progresses. Clients must be linked to services that will help them make decisions in these areas, and participate in treatment planning. Nurses must understand and navigate through the legal and ethical issues involved in Þnding the necessary linkages and services for their clients. In many locations, nurses take full responsibility as case managers (5), or work within interdisciplinary case management systems that are based on client need for type of services (6,7).

Nurses are well positioned for involvement in the sort of community advocacy that encourages communities with inadequate services to develop programs and linkages, and the political awareness to develop programs and linkages, along with Þnancial resources to carry out programs (8,9). Nurses also are a prime resource for funneling information to the community at large and to other health care professionals that can reduce fear and discriminatory action toward persons with HIV infection (10Ð13).

Recent advances in treatment using highly active antiretroviral therapy (HAART) have resulted in a

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FIG. 42.1. The nursing responsibilities model (© Richard Sewell, with permission).

decrease in the number of deaths from AIDS (1,14). As a result of HAART, HIV infection has become a chronic disease for many individuals. However, the number of new HIV infections is estimated to have remained constant. This situation has resulted in a growing number of persons with HIV infection who require care. Nursing administrators will need to encourage their staffs to develop and implement HIV/AIDS patient care program-planning in the community and in hospitals, in both urban and rural settings (2,9,15). Nursing care standards and quality improvement programs should be developed and monitored for effectiveness. Nursing administrators, as part of the senior management team, must work to ensure the presence of a safe workplace through development and implementation of appropriate employee orientation, work-place safety programs, and supportive human resource policies.

Nurse educators must be well-educated about HIV infection, so that their students can benefit from accurate, up-to-date information and develop a supportive rather than a judgmental attitude toward HIV-infected clients. Students and beginning practitioners will work with diverse populations of persons with HIV infection, diagnosed and undiagnosed, and they must learn to confront their own fears and attitudinal barriers (16). Students will need guidance and emotional support from nurse educators as they learn to elicit, understand, and negotiate their own and their clientsÕ multicultural health beliefs (17). Students need support as they deal with occupational hazards and become aware of the need for self-protection, through the use of universal precautions and personal safe health care practices. HIV-related research is needed to provide the research base for future nursing practice; nurses in academic settings have the responsibility to develop and facilitate research programs and studies that will add to the current knowledge base.

MAJOR CONCEPTS ACROSS THE CONTINUUM OF ILLNESS

When structuring care for persons with HIV infection, there are underlying concepts that providers must consider throughout the illness, regardless of stage. These concepts may be emphasized more strongly at certain points or developed differently during the course of illness, but they shape the assessment and structuring of care.

Holistic nursing practice concepts can provide the framework for care. The focus of holistic practice, according to one group (18), is Òaimed not at ameliorating symptoms but at improving clientÕs ability to live, to be well, to live a high quality of life, and to focus their lives in meaningful and useful directions.Ó They view the essence of nursing practice as helping clients to develop and fully use their sense of self as they cope with changes in their physical, psychological, spiritual, and social worlds. This client-nurse relationship must also be expanded to the patientsÕ families, both families of origin and families of choice.

The chronic disease model of Corbin and Strauss (19) holds that persons with any chronic disease must Òwork hardÓ to manage their illnesses. There are whole sets of decisions to be made and tasks to be carried out by the individual, or parents, in the case of children. These decisions and tasks may be carried out by the client alone, or in conjunction with others. Each person fashions his or her own unique response to the illness and to the health care providerÕs recommendations for treatment. Required tasks for the affected individual and/or their families are: to become informed about the disease and its progression; to meet personal resistance to staying in treatment; to prepare for pain management; and to face the issues of needing to be cared for, coping with body concept changes and body deterioration, and as McKusick (20) suggests, to make friends with the notion of dying.

Relationships with supportive practitioners can allow the patient and family members to incorporate and expand their senses of self, while making the myriad decisions that will be required as they work to manage the illness. Client and family involvement in decision-making must continue through the duration of the illness and is essential to the death-relating tasks and, in the end stage, dissolution of the relationship. Investigators have found that clients with HIV infection who participated in illness management activities improved their quality of life (21). Others have found that bereaved partners of gay men who died from AIDS were better able to work through the loss when they and their partners were involved in those activities (22). Nurses are in a pivotal position to foster client and family participation in decision-making and to assist in gathering information on which to base those decisions.

Besides provision of care, nurses work to prevent further transmission of HIV in the community at large and within health care settings. Prevention of transmission can be fostered through practice of universal precautions and by extending health education and information to clients, their families, and to a wider community.

Many HIV-infected clients have a history of health behavior that places them at risk for poor health. These behaviors may be related to cultural norms within their ethnic backgrounds or chosen life styles. These behaviors and attitudes may be difficult, if not impossible, to change (23). According to one study (24), the more symptoms persons with AIDS reported, the greater the tendency toward unhealthy behaviors.

The Mersey Harm Reduction model (25) was developed to deal with the harmful consequences of the growing substance-abusing population in the Mersey area of Liverpool, England. This model can be applied to other populations with protracted illness. The first principle is that total avoidance of unhealthy behaviors should not be the only objective of services to this population, since that would exclude a substantial proportion of people who are committed to their current life styles. For example, many

clients are well aware that HIV can be transmitted through sexual activity, but they will not or cannot practice abstinence or even protected sexual activity. Nurses and other providers who are committed to changing unhealthy behaviors in their clients will face frustration and burnout when those clients ignore the information and education that they have worked hard to provide.

The second principle of the adapted Harm Reduction Model holds that behavior change should be conceptualized as the Þnal goal in a series of harm-reduction objectives. Rather than anticipating total change in behavior, nurses and health care providers need to recognize that small changes may be easier for clients to adopt. When a series of health-promoting behaviors are developed, the clients may choose to select those behaviors that they feel they can accomplish, while ignoring those they have no intention of changing. For example, clients who are not willing to abstain from sexual activity, may accept information about types of sexual activities that provide sexual gratiÞcation other than direct, unprotected, intercourse.

The third principle is to provide user-friendly services. Nurses should help establish a supportive rather than judgmental milieu; they should avoid overwhelming clients with excessive information and instead should emphasize information and explanations that the clients feel they can handle. If trust can be established, clients will be more likely to maintain contact. Continuing contact provides opportunities to provide reinforcement and support for behavior changes that have been accomplished. In addition, it will enable clients to obtain assistance for further behavior change, and for other problems as they arise.

The harm reduction concept is gaining growing acceptance as a realistic approach in the areas of care delivery, education, and prevention. Initial evidence of effectiveness has been encouraging (26). Harm reduction considers the user's wants along with the provider's wants, so that consensus and realistic goal setting can occur. Harm reduction is future-oriented; it is not concentrated on past practices. Information that can lead to positive behavior changes can be supplied without negative judgments about past practices. Outreach is required; social and health services need to be brought to people on their own turf and on their own terms. User-friendly, rather than judgmental and punishment-based services, make it more likely that less-harmful options will be considered as acceptable in lieu of abstinence or total avoidance of harmful behaviors. Harm reduction considers providers as well as clients and is focused on future-oriented community health rather than on institutional agendas. The harm reduction model provides a useful framework within which nurses can work effectively to integrate the complexity of issues associated with HIV infection. It can be implemented successfully with diverse populations and across the continuum of illness. Nurses can take leadership in interdisciplinary planning to introduce and support these concepts.

NURSING RESPONSIBILITIES IN PREVENTION AND TESTING

The various physical, social, and psychological consequences of HIV infection may result in severe stress for clients and their families (27–31). Many people are aware that their behavior and life-style decisions have put them into a high-risk category for HIV infection. Fear of stigmatization and/or discrimination and real or perceived lack of access to services may keep them from conÞrming their fears of infection. Diagnosis and medical intervention may be delayed until symptoms can no longer be ignored, and when they may be less effective (32).

The seemingly sudden advent of the HIV/AIDS epidemic had a profound impact on both health care professionals and the overall health care system. In the early 1980s, a generation of health care providers were faced with an inßux of patients with an incurable, life-threatening, infectious disease; they were ill prepared to respond. The response of health care workers has mirrored that of society in general. This response has often been characterized by fear of contagion and avoidance of individuals infected with or thought to be at risk for AIDS (10).

Today, more than 20 years into the AIDS epidemic, there has been rapid advancement in knowledge related to the causative agent of AIDS, modes of transmission of HIV and the course of the disease (33,34). Such information should provide a rational basis for the health care professional's response to caring for people with this illness. Yet the contrary attitude prevails. Research examining the attitudes and knowledge of health professionals (including nurses) about HIV infection indicates that fear, misinformation, and prejudice remain pervasive (35–38).

Nurses have been in the forefront of responding to the HIV/AIDS epidemic both in the provision of direct care and in organization of care delivery systems (6,39). Yet many nurses lack knowledge and experience related to the care of patients with HIV infection. This lack of experience has resulted in part from nurses avoiding care of these patients or from working in communities with a low incidence of HIV infection.

The rapid increase of reported cases of AIDS in diverse segments of the population, as well as in all geographic regions, including rural areas, strongly suggests that at some point all nurses will be involved. For nurses having limited experience with this disease, ongoing education is necessary to provide a knowledge base that supports quality nursing practice. However, education does not always change attitudes or behavior (35,40,41). Additionally some clinically based health care providers continue to mistrust the experts and question exactly how much is really known about transmission of HIV in the patient care setting (42).

Fear of HIV infection by health care professionals can still result in refusal to provide care and in unnecessary isolation practices (6,43). The more likely scenario, however, is the exhibiting of behaviors by health care providers that make the HIV-infected person feel stigmatized or devalued. In a focus group study of HIV-infected women (44), it was reported that fear and insensitive behavior by health care providers affected the patientsÕ quality of care, and their willingness to seek health care services. Other studies of low-income and gay populations have shown similar results (45).

Nurse practitioners are providing an increasing amount of care for vulnerable populations and may be in a primary position to provide education and encourage appropriate testing and early intervention for populations at high risk for HIV infection (46). Other nurses, who are involved in community activities, including school and community health education programs, should also be a major resource for information regarding available services. Nurses can provide a non-threatening atmosphere, in which questions can be raised, thus encouraging earlier diagnosis and treatment, and prevention of further transmission of the infection. School health programs provide an ideal opportunity to instruct appropriate, healthful behaviors. Risk-related sexual and drug practices may begin in adolescence, and this group needs to be a prime target for health education and risk reducing programs (32). Studies of school-based AIDS education have been shown to have impact on risk-related behaviors (47). However, adolescents are not all alike, and effective health education and sex education programs must be tailored to their culture and lifestyle. Investigators (48) found that different interventions were needed for sexually active and high-risk teenage populations than were effective with lower-risk groups.

Making the decision to undergo antibody testing requires the courage to face the possibility of HIV infection and the courage to take the chance that the provider will exhibit a professional, supportive attitude and maintain conÞdentiality. Regardless of whether the test results are positive or negative, post-test counseling is essential (32). Figure 42.2 models a comprehensive approach to HIV/AIDS education and prevention. The person who tests negative may still be at risk for HIV infection and needs continuing health education and ongoing counseling and support for behavioral changes, to reduce the likelihood of infection. Also, follow-up testing may be needed if the incubation period has not been long enough to reveal a positive test. Knowledge of the disease may be Òstreet knowledge,Ó learned from friends, Þlled with many untruths, half-truths, and inappropriate emotional overtones. This is an opportune time for nursing intervention, to help correct some of those misconceptions.

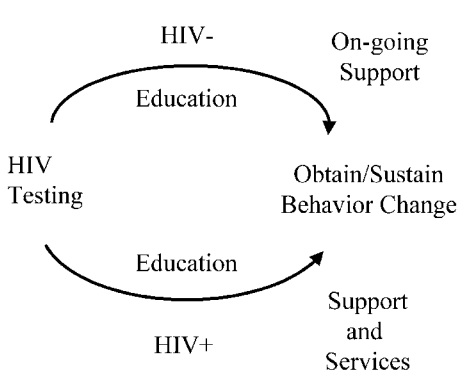

FIG. 42.2. Comprehensive approach to HIV/AIDS education and prevention (© Richard Sewell, with permission).

Persons testing positive have had their worst fears conÞrmed. Counseling support is crucial at this point and must be delivered with sensitivity to the gender and cultural and ethnic background of the client (32,49). Clients need to know what the test results actually mean, and unless Western blotting was done, about the need for conÞrmation to avoid false positives. Persons who test positive will need to learn a whole new vocabulary, as providers talk about T cells and CD4s. They must be linked to services that may be needed over the course of the illness. Even though the person testing positive may be asymptomatic, this is an appropriate time to begin case management activities. A case manager may assist the client to develop a plan of care. Case managers may provide education about the natural history of the disease and information on access to health services, so the early symptoms may be reported and treated appropriately. Case managers must assess the psychosocial, spiritual, and educational needs and prepare for linkages to services that will be needed in the future (6).

EARLY INTERVENTION

Increasingly, testing and counseling are being viewed as early intervention activities. Individuals who are identiÞed will then have the opportunity to implement behavior changes and health promotion activities that can, potentially, delay deterioration of immune status. Early identiÞcation allows a thorough medical and psychosocial assessment to deÞne immediate and long-term needs, that can maximize the length and quality of life (32).

The goal of early intervention is to keep people well as long as possible. The tasks required in early intervention for both client and provider are many. A client seeking early intervention may require treatment for many common illnesses not associated with HIV infection. The health assessment can identify additional infections and health care problems that need treatment. The nursing assessment should incorporate the health assessment into the psychosocial and spiritual context of the individualÕs life in devising a comprehensive client plan of care.

Education and information may be the greatest needs at this point, to permit the client to take part in the shared decision-making that will be necessary. Decisions such as

when or if to start antiretroviral therapy, prophylaxis for opportunistic infections, and the appropriate course of management and treatment, need to be jointly made by a well-informed client and provider. (50) When decisions are made unilaterally by the provider, the client may not Þnd them relevant or acceptable, decreasing chances of adherence to the prescribed treatment or regimen. It is important to acknowledge the use of alternative therapies by many individuals with HIV infection (51). When possible, incorporation of such therapies promotes adherence to the treatment plan, and allows providers to be knowledgeable about all the medications and approaches being used by clients.

To be effective, education needs to be ongoing and interactive. A trusting relationship needs to be developed between client and provider. The provider must acknowledge and respect the clientÕs competence in making decisions about his or her own life. In addition, some clients with HIV infection are better informed about their condition and its treatment than many providers. We have found that when providers were not forthright with information, some clients interpreted this as a lack of knowledge by the provider, diminishing their willingness to adhere to treatment.

The nurse has a critical part to play in explaining a treatment program to the client. Medications currently in use to treat HIV infection and related opportunistic infections can have many unpleasant side effects. Clients must be given explanations about the reasons for taking the medications, and their potential side effects, and information that will allow them to make an informed choice about whether the expected beneÞt outweighs the potential harm due to side effects (52Ð54). Nurses can be particularly helpful in providing information to the client and family, as appropriate, to manage those side effects so that discomfort can be reduced to a tolerable level. Support provided by nurses can be an important factor in assisting patients in adhering to drug treatment regimes that include multiple drugs and require rigorous dosing schedules (55,56).

Certain studies indicate that good nutrition, exercise, and stress management may have positive impacts on the bodyÕs ability to Þght infection and maintain or improve functioning (57Ð59). Nurses can provide information on these and other health promoting behaviors, to both the client and family. Family members may make more concrete contributions to these health promotional behaviors when they are well informed (22). Such information may also beneÞt the family members by enhancing their own general health behaviors.

Whenever possible, a comprehensive plan of care, that includes both medical and psychosocial issues, needs to be developed early. Tables 42.1 and 42.2 list frequent nursing diagnoses and selected interventions across the stages of HIV infection. The plan will differ for each client, and nursing diagnoses and interventions other than the ones illustrated will also be needed.

Decisions such as advanced medical directives, medical power of attorney, and guardianship of children need to be considered before the potential neurologic and emotional complications associated with AIDS places legal decision-making at risk. Many HIV-infected patients are not ready to deal with the prospect of severe illness, disability, or death, when they do not yet feel sick. Because of their role in direct care and education with clients and clientsÕ families, nurses are in a good position to develop a trusting relationship. This relationship will be necessary so that clients can be assisted in confronting these issues in a timely manner, though they may still be in denial or unprepared to consider such sensitive and distressing subjects. However, one of the most important issues to be considered is how to balance the need to plan for future illness with the desire to maintain hope and a focus on living.

Psychological assessment needs to be done to identify levels of depression, anxiety, and suicide risk. Anxiety and depression are two of the most common emotional responses to a diagnosis of HIV infection (27,31). Such emotional responses can be linked to the uncertainty and stigma associated with this illness (60Ð62). For some people, peer support groups can be an effective method to help cope with fear, anxiety, and feelings of isolation. Nurses can provide information and linkages about available resources. IdentiÞcation of problems with drug and alcohol use is also an important part of psychosocial assessment. Many people living in poor social conditions have used drugs and alcohol as a means of coping, and the option of substance abuse treatment needs to be made available. Behavior change related to substance abuse may require long-term solutions and incorporate principles of the harm-reduction model previously discussed.

Because Þnancial, housing, nutrition, and transportation problems frequently grow worse as the disease progresses, these areas must be included in assessment. Problems in these areas may be pre-existing or begin emerging after the diagnosis of HIV infection, especially if clients lose their jobs because their HIV status becomes known. The usual debilitating course of HIV infection mandates that issues of long-term housing, medical care Þnancing, and general Þnancial management be regularly addressed in psychosocial care planning. Practical concerns such as mobility and wheelchair access should be considered. Case management can provide a framework in which medical and psychosocial aspects can be integrated into a comprehensive care plan.

Case management models are found in both inpatient and community settings (6,7,63,64). Models in both settings have the overall objective of coordinating and linking clients to an appropriate level of service based on need. Nurses have the expertise needed to be effective case managers. Nurse care managers can be particularly beneÞcial to clients with early HIV infection, when information and health promotion is a critical need, and in later stages when physical assessment and symptom

management become prime considerations. An interdisciplinary approach is critical and the case management role may be to link to available services and provide a conduit for appropriate information to reach service providers. In instances where services are not available, the nurse case manager, in collaboration with social service providers, can take the initiative to develop services or community networks that support the needed

TABLE 42.1. *Physiologic challenges: Selected nursing interventions*

Nursing Diagnosis	Potential Source/ Cause	Early Interventions	Late Interventions
Impaired gas exchange	Pneumocystis arinii pneumonia (PCP) Anemia Cytomegalovirus (CMV) pneumonia Pulmonary Kaposi's Sarcoma	• Educate populations at risk of bene ts of early intervention and treatment • Encourage and provide early HIV screening to identify at risk persons • Monitor compliance with PCP prophylaxisexaminations • Monitor hemoglobin and hematocrit	• Monitor mental status • Monitor vital signs, blood gasses • Position for comfort, elevate head of bed • Educate regarding treatments and examinations • Implement actions to reduce fear and anxiety • Monitor effects of assisted ventilation
Diarrhea	Cryptosporidium Isospora Mycobacterium avium-intracellulare complex (MAC) Salmonella Giardia	• Educate regarding dietary risks, e.g., raw chicken, eggs, meat • Educate regarding good hygiene and handwashing • Educate regarding refraining from risky oral-anal sex practices	• Monitor for weight loss, malnutrition • Implement measures to ensure adequate hydration • Educate regarding good hygiene and handwashing • Diet counseling • Educate regarding treatments and examinations
Potential for infection due	IV devices Herpes Zoster Psoriasis Herpes simplex virus Immobility Poor nutritional status Diarrhea	• Assess skin for lesions, pressure areas, turgor • Monitor for signs and symptoms of secondary infection • Monitor for disruption of skin integrity • Educate regarding good hygiene	• Ensure good hygiene • Use pressure-relieving devices • Turn and position frequently • Meticulous IV device care • Infection control measures • Work with physicians to eliminate/treat underlying conditions
Pain	Peripheral neuropathy Tumor Reiter's syndrome Herpes zoster Herpes simplex	• Monitor for pain, assess location, duration, type, etc. of pain • Identify contributing and/or risk factors for pain • Monitor and educate regarding drug therapy and reporting symptoms	• Explore alternative techniques to cope with pain, e.g. relaxation, visualization, distraction • Encourage client verbalize pain • Comfort measures such as egg-crate mattresses, positioning and supporting limbs • Administer and monitor the effectiveness of analgesics
Altered Nutrition	Anorexia Nausea/vomiting Malabsorption In ammation/lesions of GI tract Diarrhea	• Educate and monitor regarding measures to promot lean body mass • Educate and assess regarding sound nutritional practices • Early detection and treatment of conditions such as in ammation/ lesions of GI tract and diarrhea	• Assess and monitor nutrition parameters (e.g. weight, serum albumin, food intake) • Treat underlying conditions • Dietary change as appropriate (high calorie—high protein, bland) • Offer dietary supplements • Implement and monitor parenteral nutrition and assess effectiveness and related complications or side effects; begin nursing measures to respond to complications

TABLE 42.2. *Physiologic challenges: Selected nursing interventions*

Nursing Diagnosis	Potential Source/ Cause	Early Interventions	Late Interventions
Knowledge de cits	New diagnosis Impaired or dereased learning disability Lack of awareness of need for education Development of new conditions	• Increase awareness of need for education • Assess level and readiness to learn • Develop an individualized teaching plan • Evaluate and revise education approaches • Referral of client to other services such as peer or professional counseling services • Include family/signi cant others in education process • Provide suf cient, accurate, timely information (e.g. treatment options, informed consent, prevention of transmission, health promotion)	• Refocus education of information from general to speci c with regard to each client's infections and conditions • Provide speci c teaching regarding treatments and procedures • Educate regarding realistic self-care goals; teach speci c delegated nursing tasks such as IV maintenance • Referral to resources/agencies providing supportive care
Social isolation	Stigmatizing disease Fear of contagion by others Alterations of body image Decreased ability to socialize Alterations in life-style due to chronic illness (related to fatigue, medications, and changes in physical appearance, hospitalizations) Avoidance and/or pity by family and friends of clients	• Establish open and therapeutic communication • Referral to peer and professional counseling • Assist client in inventorying sources of social support • Assist client in identifying need and timing for disclosure of HIV status to signi cant others • Referral of family and signi cant others to sources of social support across the potential circumstances of disease progression • Identify HIV sensitive health and social services providers as potential referral sources	• Develop supportive, therapeutic environment in clinical setting • Establish open communication across the continuum, including talking and explaining procedures during nursing care, even with clients with limited ability to respond • Appropriate but not excessive infection control measures • Establish liberal visitation with incorporation of family and signi cant others in the plan • Provision of safe space and opportunity for socialization for clients and signi cant others • Use radio and television as source of stimulation • Appropriate use of touch
Altered body image	Stigma of disease Past experiences with others with disease Urinating Skin lesions Fatigue Decreased mobility Reaction to responses of others	• Assess for early problems of altered body image, e.g. ruminating about imagined alterations, issues of contagion • Assess clients' understanding and acceptance with physical manifestations of disease progression • Provide accurate information and assist client in establishing realistic plans to respond to physical changes in appearance • Assist client in exploring measures to control or disguise manifestations (e.g. skin lesions, eruptions)	• Work with client to maximize functional abilities • Work with client to minimize physical manifestations • Refer to peer and professional counseling • Establish open, accepting communication with client • Establish and support a therapeutic, caring, clinical environment • Assist client and signiticant others in establishing an accepting and supportive home environment • Identify and implement nursing measures to decrease effects of physical problems such as incontinence, nausea, vomiting, fatigue

TABLE 42.2. *Continued*

Nursing Diagnosis	Potential Source/ Cause	Early Interventions	Late Interventions
		• Referral to peer support and counseling • Provide information and education to family and signi cant others related to client's need for acceptance and emotional support in the face of physical manifestations of disease	
Anxiety	Stigmatizing disease Uncertainty Perceived lack of control New diagnoses Fear of rejection/ descrimination related to societal attitudes Fear of pain Fear of dis gurement Fear of death	• Provide accurate information • Open communication • Assist client in articulating source of anxiety • Assist client in developing a plan to address anxieties • Refer to peer and protessional counseling • Spiritual/pastoral support • Informed consent regarding power of attorney, living will, shared decision making	• Facilitate return of control to patient • Facilitate open communication • Provide accurate information regarding treatments and procedures • Spiritual/pastoral support • Implement nursing measures to control pain • Maximize functional abilities • Inclusion of family and signi cant others in plan of care • Obtain informed consent regarding treatments and procedures • Facilitate shared decision making
Altered role performance	Fatigue Physical debilities Pain Emotional Distress/fear Alteration of body image Lack of information	• Assess client's emotional status, physical status, and knowledge level regarding transmission of virus and legal rights • Educate and advocate for client regarding his rights • Identify HIV-sensitive services and make appropriate referrals • Job/career counseling as appropriate reterrals • Family counseling • Referral to community-based case management	• Explore and assist negotiating of role expectations and obligations with client and family • Identify and assist in obtaining entitlements • Referral to community-based case management • Maximize tunctional abilities • Support maintenance of control

services. Where formal case management services have not been established, both hospital and community nurses need to provide health care networks that promote the continuity of services for people with HIV infection, across the continuum of their illness.

ACUTE CARE

Persons with HIV infection can be hospitalized for a wide range of infections and symptoms related to immune suppression. For the individual patient, hospitalization can represent the initial acute disease, or one in a series related to debilitating opportunistic infections. Regardless of the personÕs stage of illness, a primary reason for admission to the hospital is the need for supportive nursing care.

An important objective for the professional nurse providing care to HIV-infected patients is to establish a therapeutic environment. The overall nursing goal is to maximize the quality of life for the patient, while responding to the individualÕs current manifestations of HIV disease. A challenge for nurses is to help to maintain the clientÕs functional ability even when the person is experiencing rapid disease progression.

Hospitalization may exacerbate emotional distress and be viewed by the individual and family members as representing a deterioration of health status. The HIV-infected patient often enters the hospital uncertain of the future, concerned about loss of functional ability and

afraid that this will be the infection that he or she will be unable to overcome. Universally, the health care professional who has the most frequent contact with the patients is the nurse. It is the nurse who is most responsible for establishing open communications that acknowledge the fears and concerns of the patient. There is a valid need for health care workers to take appropriate precautions to prevent transmission of disease within the hospital setting. However, the practice of unnecessary or exaggerated precautions with the HIV-infected patient can limit patient-nurse interactions and increase the patientÕs sense of isolation, abandonment, and anger (65). There is a growing understanding that emotional stress can negatively affect an individualÕs physical status (66,67). For the patient entering the hospital in a state of immune suppression, unnecessary stress encountered within the health care setting may, potentially, compromise further the patientÕs ability to respond to treatment.

While respecting the patientsÕ conÞdentiality, inclusion of family and signiÞcant others in the daily hospital routine can serve to decrease the sense of isolation. As part of the nursing function, nurses need to establish a ßexible schedule with their patients and families that promotes a balance between the patientÕs need to rest and receive treatment and to interact with signiÞcant other individuals who provide ongoing emotional support. It is essential that the nursing plan of care be developed with the patient and that it integrates the medical treatment required into a holistic approach.

Nurses can serve appropriately as coordinators within the interdisciplinary therapeutic inpatient care team. An important nursing responsibility is to ensure that the hygiene, nutrition, emotional, and spiritual needs of a patient are adequately considered in overall delivery of treatment and care. The nursesÕ central role in providing information/education to patients and family members, along with an understanding of treatment options, care procedures, and relevant infection control precautions by the patient and family, can facilitate the holistic treatment of the patient.

Provision of Care

Persons with HIV infection are often admitted to the hospital in acute crisis, or with the potential for their conditions to deteriorate rapidly. This requires that nurses fully utilize their assessment skills in establishing baseline measurements from which future changes in condition can be evaluated. Nursing assessment should focus acutely on respiratory, neurologic, and gastrointestinal status, because opportunistic infections affecting these systems can be life-threatening (68Ð70).

The nurse as the direct care member of the treatment/care team should take responsibility for frequent, ongoing, monitoring of a patient who is potentially unstable or in distress. Changes in the patientÕs condition should be noted as early as possible and the nurse prepared to respond knowledgeably, as well as to communicate the necessary information to the physician and other team members. Once an initial assessment of the patient is complete, the nursing process (assessment, planning, intervention, and evaluation/reassessment) provides a framework for comprehensive nursing care to the patient.

Special Problems

Frequently associated with HIV-related gastrointestinal opportunistic infections (such as those caused by cryptosporidium, cytomegalovirus, or *Mycobacterium avium* complex) are alterations in hygiene and nutritional needs. Diarrhea, due to these and other causes, is a common condition encountered in HIV-infected patients. Episodes of diarrhea can be explosive and last for days or weeks. Profuse diarrhea can affect the individualÕs willingness to eat, affect his or her electrolyte balance, and may be associated with severe abdominal pain. Skin breakdown in the perianal area may result, providing a new avenue of infection. Diarrhea also adds to the psychological burden of the patient, with fear of loss of control and embarrassment. Monitoring response to antidiarrheal therapy, and communications with the physician regarding the treatment plan, are essential.

Debility and weight loss that typically accompany HIV infection over time also carry enormous psychological implications for the patient. The physical and emotional complications of HIV infection are quite complex and often interrelated. Maintaining or assisting the patient who has this challenging illness, with its special problems, will test the nursing skills of even the most experienced nurse.

Alteration of Nutrition Status

In addition to diarrhea, prolonged fevers and poor nutritional intake contribute to generalized wasting in many individuals with HIV infection (71,72). Nutritional assessment for each patient and early intervention by the nurse are crucial in identifying and modifying the risk of this complication. Assessment should include a review of pertinent laboratory values, patient weights, diet recall, and direct observation of nutritional intake (73). Once patients at risk are identiÞed, the nurse must institute early nutritional intervention, such as frequent weights, dietary intake monitoring, offering foods of choice, and teaching dietary strategies. Consultation with a dietitian or nutritionist may help to individualize a dietary program. Appropriate strategies to avoid weight loss include assisting patients in selecting high-calorie, high-protein foods, avoidance of lactose in the intolerant, and offering frequent small meals. Nurses must also be alert to factors that contribute to poor dietary intake such as stomatitis, early Þlling, or a disagreeable taste in the mouth from

medications, and must then take appropriate action to ameliorate these conditions.

Early assessment of those at risk for weight loss and wasting, initiating appropriate interventions, and identiÞcation and communication of factors associated with poor dietary intake to other members of the health care team, offer the patient the hope of avoiding enteral and parenteral interventions. Early nutritional intervention and preservation of normal dietary routines as much as possible are more palatable to patients than the inconvenient, expensive, and potentially harmful invasive interventions such as long-term intravenous lines and parenteral nutrition. Prompt recognition of correctable problems, combined with appropriate nutritional interventions by the nurse, can delay and possibly diminish the impact of intractable wasting.

Alteration of Mental Status

Alteration of mental status, including cognitive, motor, affective, and behavioral changes, is common in patients with HIV infection, especially those with advanced immune deÞciency. An estimated 20Ð30% of persons with very late stage HIV infection will suffer profound cognitive and motor impairment (74,75). The difÞculty experienced in performing a health assessment of any acutely ill patient is often made even more complicated by such severe alterations in mental status in HIV-infected individuals. This difÞculty in assessment can complicate the formulation of an appropriate plan of intervention for the patient. Maintaining meaningful and effective patient-nurse communication is a challenge under such circumstances and can make evaluation of signiÞcant changes in physical and mental condition even more difÞcult.

Mental status alterations can present the nurse with another dilemma. The nurse is often faced with making decisions related to preserving the safety of a mentally compromised patient. Decisions about physical restraint or chemical sedation of a patient are most often made by the nurse. The nurse must weigh the patientÕs need for safety against his or her right of self-determination and need for self-care.

Dual Infection with Tuberculosis

The development of tuberculosis in the HIV-infected individual represents a serious situation with pressing implications for the nurse. First, it may be the nursesÕ responsibility to ensure initiation of respiratory precautions for any patient with HIV infection and suspicious pulmonary symptoms (76). Further, the nurse must be vigilant in maintaining appropriate respiratory precautions by staff and patient families (77). The importance of adherence to proper respiratory precautions goes beyond the nurseÕs responsibility to advocate for the patient and their families. Although HIV is rather difÞcult to transmit from patient to nurse, pulmonary tuberculosis represents a real occupational hazard for the nurse and others in health care. Therefore, the nurse is obligated to ensure that the workplace is made as safe as possible.

In addition to supplies and teaching about isolation, the patient and family must receive from the nurse timely and accurate information about treatments, procedures, and examinations. It is essential that the nurse establish continuity with community agencies, particularly with public health nurses and epidemiologists, for follow-up upon discharge.

Discharge Planning

An ideal example of the importance of discharge planning is tuberculosis follow-up after hospital discharge. Effective discharge planning for a patient with tuberculosis has public health implications because of the requirement for compliance with long-term treatment. As discharge planner, the nurse identiÞes potential needs of the patients as they return to their communities and also identiÞes the resources in the community to meet those needs (78). Communication and feedback between hospital nurses and nurses and other health care providers in the community (hospice, the public health departments, and home health) are critical for continuity of care. Nurses should work to provide discharge planning and encourage that communication. Communication information systems need to be developed to permit hospital nurses to be kept up-to-date on community resources and to allow for those necessary linkages outside of the hospital.

END STAGE

ÒI am not afraid of death; I am afraid of dyingÓ(79) is a major concern for patients as the disease takes its course. The psychological work of coping with the fear of disability is replaced by the specter of going through the process of dying. A major issue for persons in the Þnal stage of the disease is the need to stay in control of their lives and their deaths. There are several important issues to be addressed in this area. Considerations of quantity versus quality of life will require decisions about life support and advance directives, aggressive treatment versus supportive care options, and institutional or home care.

Choices of where dying will take place need to be made. Patients and families have the task of deciding whether dying at home is an option (80). Home care is not easy, given the physical, mental, and Þnancial problems to be faced, but may be an option in those families with the necessary resources. Hospice support may be available, at home or in an institution. Nurses have the responsibility to

ensure that individuals and families understand their available options and support them in their choices.

Fear of pain may be a major component in facing the process of dying; pain management takes on special importance in the end stage (81). Pain is common in people with AIDS (82). Common types of pain include abdominal, neuropathic, esophageal, head, and pain related to Kaposi's sarcoma. Pain may make it impossible to maintain quality of life. Negative attitudes about the need for pain relief have been documented among health care providers for their patients who were chemically dependent, and these attitudes have affected the treatment provided (83). This is especially important because substance abusers are the second most common risk group for HIV infection in the United States. Pain control needs to be highly individualized. The client has the right to deÞne when pain exists and to expect adequate interventions to provide relief when possible.

Issues centering on family relationships often come to a head at this time. Special problems may arise for HIV-infected parents with regard to their ability to maintain relationships with their children. This can be especially difÞcult for women, who have traditionally been the caretakers within families. as parents approach the end stage of their illnesses, the care and well being of children after the parents die must be faced. A growing number of children who are orphaned by parents with AIDS are requiring foster care. Many of these children are also HIV-infected (84,85). In 1991, 1,149 HIV-antibody positive children were already in the foster care system (86). Conßicts may arise in decisions to be made about families of choice and families of origin and how family members will be integrated into the Þnal stage of life. Responsibilities for making Þnal decisions need to be clearly spelled out. Nurses need to support clients' decisions about who is most important to them and who will play integral roles in the Þnal stages of life. Finally, surviving families cannot be forgotten once death has occurred; bereavement counseling may be needed to resolve issues of loss. Some family members or signiÞcant others will face their own deaths from AIDS, and may require support to deal not only with loss but also with their future prospects (87).

NURSING ADMINISTRATION

Nurse managers may be found in hospital or outpatient settings, and are responsible for establishing speciÞc policies that outline clinical standards, and procedures that ensure a high quality of care for patients with HIV infection. Clearly linked to standards of care is the need to establish personnel performance criteria. Consumerism in health care demands that high quality care measures focus on patient care outcomes. This does not suggest that the "end result" outcome for HIV-infected patients will be cure, but rather that incremental process outcomes are achieved. Patient satisfaction with care can be an important outcome for patients with terminal disease (88,89). Nurse managers can support the nursing staff in delivering quality care by establishing consistent organizational policies and procedures that guide staff in making controversial decisions encountered in the care of HIV-infected patients. Issues such as self-determination, use of alternative therapies, or referral to sources that may offer speciÞc medical treatments, can produce controversial patient care situations in which nurse managers need to support the patient's rights, as well as those of the nursing staff.

A nurse's refusal to care for an HIV-infected patient presents an important challenge for the nurse manger. Institution-speciÞc policies are needed to guide supervisory actions in such situations. Often, existing personnel and grievance procedures can be used if disciplinary measures are required (90). The optimal approach to responding to staff fear or concerns associated with the care of persons with HIV infection is establishment of an ongoing in-service program focusing on updated information related to this disease. Education should be reinforced by clearly articulated clinical guidelines that promote a safe working environment for all staff. The low risk of infection with HIV when following the Centers for Disease Control (CDC) guidelines for handling blood and other bodily ßuids (91) make it critical that staff be taught universal precautions and safe needle disposal practices.

Approximately 80% of signiÞcant exposures to HIV among health care workers involve needle sticks (92). Of those health care workers most likely to sustain a needle stick, nurses constitute 60Ð70% of them (93,94). However, even among nurses who have a good understanding of HIV-related issues, implementation of universal precautions is not consistent (95,96).

Universal precautions guidelines need to be mandated as part of institutional policy. Employees unwilling to incorporate these precautions into routine practice need to expect corrective or disciplinary action. It is no longer acceptable to isolate or avoid the admission of persons with HIV infection because nurses or other hospital staff do not take advantage of available safety precautions when caring for patients.

HEALTH CARE WORKERSÑCONCERNS AND EMPLOYEE ASSISTANCE

Persons with HIV infection require a large physical and emotional investment from nurses. Hospitalized patients are often young and exhibit a wide range of disÞguring signs. No direct care provider should be expected to withstand repeated encounters with stress without support. Nurse managers need to work creatively to develop strategies that support their staff. While support efforts need to be individualized to speciÞc agencies, the inclusion of psychologists and pastoral care workers in the interdisciplinary care team may be valuable to patients and staff. Clinical support groups for nurses that care for

patients with HIV infection have been successful in a teaching hospital in New York City (97).

The AIDS epidemic has raised questions about whether the HIV-infected health care worker may safely continue his or her patient care activities. Universal precautions should be adequate to ensure staff and patient safety in most clinical settings. Nurses who develop symptomatic HIV infection often require support and assistance. Nurse managers need to take the lead in establishing clear employment assistance procedures for all HIV-infected health care workers, where they do not already exist. When appropriate procedures are in place, the nurse manager is responsible for helping the employee to implement appropriate procedures.

NURSING EDUCATION

The AIDS epidemic has resulted in a compelling need in nursing education to reach students early in the educational and socialization process (98). Faculty in schools of nursing must develop a comprehensive approach to HIV infection within the curriculum.

Issues associated with HIV infection span the continuum of care and affect the delivery of nursing care at a variety of levels. Educational content in schools of nursing will need to reßect this, if future nurses are to be prepared adequately for the realities of the work environment. HIV/AIDS care issues should not be limited to advanced adult health nursing courses. These issues are also relevant content for those studying parent-child, mental health, community health, and nursing administration, as well as in courses dealing with ethical and professional issues. A necessary Þrst step within educational institutions may be the need to update faculty knowledge in the area of HIV infection. If faculty are not knowledgeable or comfortable in provision of care to HIV-infected persons, students will sense their discomfort. The value of positive faculty role modeling in developing positive attitudes and behaviors toward AIDS care in nursing students cannot be overemphasized.

Without a vaccine or a curative treatment, the need to care for persons with HIV infection can be expected to increase for many years to come. Nurse educators have a unique opportunity and responsibility to take a leadership role in responding meaningfully to the AIDS epidemic. Research studies need to be designed to examine current interventions and to develop and test creative approaches to care. The National Center for Nursing Research has acknowledged the need for nurses to investigate both the physiological and psychosocial aspects of HIV infection (99). Research needs to be based on the clinical realities of caring for HIV-infected persons and to focus on the evaluation of nursing interventions as they relate to client outcomes. Student involvement in research activities can set the stage for further work and interest in the area. The future of nursing lies with the next generation of nurses. It is that future that nurse educators have the chance to inßuence positively to the beneÞt of society, the nursing profession, and especially to those persons and their families who are at risk for HIV infection.

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Chapter 43

Ethical Challenges of the Global AIDS Epidemic

Ronald Bayer

In a quite fundamental sense the AIDS epidemic has provided the occasion for the effort to articulate an ethics of public health. Although such an effort drew upon the insights and perspectives of bioethics, it was clear that more was needed. Bioethics had taken root in the United States largely in an effort to redress the balance of power between clinicians and patients and researchers and subjects. Central to that effort was an explicit focus on the rights of the individual. Hence the protection of autonomy became the core commitment of bioethics. Important as was the protection of the individual, it could not serve as the only or even preeminent principal in the context of public health, where the focus was, by deÞnition, on the protection of the community. Nevertheless, as the ethics of public health took shape in the context of the AIDS epidemic, the critical importance of individual rightsÑ claims of privacy, bodily integrity, freedom from unwarranted restraintsÑw as clear. Only in later stages of the epidemic when therapeutic advances made access to care a critical matter did questions of justice take on greater salience. Finally, it was the emerging demand for access to care on the part of the poorest peoples in nations which bore the brunt of the global epidemic that set the stage for vital questions about the moral duties of the world's richest nations to the poorest.

EXCEPTIONALISM AND PUBLIC HEALTH: THE ETHICS OF TESTING, REPORTING AND PARTNER NOTIFICATION

In the early and mid-1980s, at the outset of the American encounter with AIDS, it was necessary to face a set of fundamental questions: Did the history of responses to lethal infectious diseases provide lessons about how best to contain the spread of HIV? Should the policies developed to control sexually transmitted diseases or other communicable conditions be applied to AIDS? If AIDS were not to be so treated, what would justify such differential policies?

To understand the importance of these questions, it is necessary to recall that conventional approaches to public health threats typically provided a warrant, when deemed appropriate, for mandating compulsory examination and screening, breaching the conÞdentiality of the clinical relationship by reporting to public health registries the names of those diagnosed with ÒdangerousÓ diseases, imposing treatment, and in the most extreme cases, conÞning persons through the power of quarantine. To be sure, many aspects of this public health tradition, forged at the outset of the 20th century, had been modulated over the decades, in part because of changes in the patterns of morbidity and mortality.

Nevertheless, it was the specter of the historically coercive aspects of the public health tradition that most concerned proponents of civil liberties and advocates of gay rights and bioethics as they considered the potential direction of public health policy in the presence of AIDS, a disease that so disproportionately affected disfavored groups, gay men, drug users, the poor in minority communities. Although there were some public health traditionalists in the United States who pressed to have AIDS and HIV infection brought under the broad statutory provisions established to control the spread of sexually transmitted and other communicable diseases, they were in the distinct minority. In place of the conventional approach to public health threats, there emerged an alternative viewÑbroadly deÞned as e xceptionalist (1)Ñthat took as its starting point the need to craft policies that were persuasive rather than coercive, that viewed the protection

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of the rights of those who were infected as integral rather than as antagonistic to the goals of disease prevention. For those who advanced this new perspective, privacy and conÞdentiality were to be accorded great importance. In all, the goal was to avoid, at all costs, measures and practices that might be counterproductive, that might Òdrive the epidemic undergroundÓ by inspiring fear and distrust rather than fostering engagement between public health ofÞcials and those most at risk. How the exceptionalist perspective with its commitment to non coercive approaches to HIV affected policy is most clearly illustrated in the debates over HIV testing, reporting of HIV by name to public health registries, and partner notiÞcation efforts.

From the moment of its introduction in 1985, the HIV test became the subject of intense debate. Fear that those identiÞed as having HIV might be subject to discrimination and stigma; concern about how the diagnosis of HIV infection, in the absence of effective therapy, could produce unbearable psychological burdens; and a belief that testing had little to do with behavioral change, led AIDS activists generally, and gay leaders speciÞcally, to adopt a posture of hostility and/or skepticism regarding the test. On the other hand, many public health ofÞcials believed that the identiÞcation of infected persons could play a crucial role in fostering behavioral change. Out of their confrontations emerged a broad consensus that, except in a few well-deÞned circumstances, people should be tested only with their informed, voluntary, and speciÞc consent (2).

Much of the early discussion of HIV testing occurred in the context of extreme therapeutic limits. And indeed in the epidemic's early years the primary function of testing was as an adjunct to prevention efforts. As a result of clinical developmentsÑ the belief that treatment with zidovudine could delay the onset of symptomatic AIDS and the recognition of the importance of primary prophylaxis against *Pneumocystis carinii* pneumoniaÑ by 1990, the medical signiÞcance of identifying those with early HIV disease had become clear. Consequently, the clinical and political contextÑ involving a wide range of constituenciesÑ of the debate about testing underwent a fundamental change (3). Gay organizations began to urge homosexual and bisexual men to have their antibody status determined under conÞdential or anonymous conditions. Physicians pressed for AIDS to be incorporated into the medical mainstream and for the HIV-antibody test to be treated like other blood testsÑ i.e. given with the presumed consent of the patient. In the face of such pressure, those committed to the protection of the rights of the infected continued to insist that testing only occur with speciÞc informed consent. Whatever, in fact, occurred in the context of clinical settings, public policy at the federal and state level continued to reßect a commitment to the voluntaristic premise of the exceptionalist perspective and the enduring inßuence of the ethical underpinnings Þrst enunciated in the mid-1980s when medicine was all but impotent.

Nevertheless, pressure to shift the paradigm of testing away from the exacting standard of informed consent continued to mount. It was especially pronounced in the case of pregnant women and newborns (4). Diagnostic progress was to make it possible to determine whether HIV-positive newborns were truly infected soon after birth, and the improved prospects of clinical management were to make such determinations for infected infants appear all the more critical. So it is not surprising that pediatricians became increasingly impatient with the strict regimen of explicit and speciÞc consent that surrounded the testing of newborns for HIV (5)Ñ all the more so because routine and unconsented testing of newborns for inborn errors of metabolism such as phenylketonuria was mandated in virtually every state, and had provoked little by way of ethical objection.

In late spring 1993, New York State, which together with California had pioneered the enactment of stringent informed consent requirements for HIV testing, seriously considered legislation that would have mandated the screening of newborns for HIV infection. When that legislation ultimately passed in 1996 (6), it provoked a cry of outrage from the advocates of women's rights. Ironically, the passage of a mandatory newborn testing statute occurred when interest had already begun to shift to the question of screening pregnant women. That shift occurred as the result of the 1994 Þnding that the administration of zidovudine during pregnancy could reduce the rate of vertical transmission by two thirds (7).

In the aftermath of that Þnding, pressure mounted to ensure that infected women were identiÞed early in pregnancy. Although advocates on behalf of women's interests fought hard to preserve the right of pregnant women to undergo HIV testing only after speciÞc informed consent, the prospect of saving newborns from HIV infection provided the foundation for a vigorous challenge (8). In June 1996 the American Medical AssociationÕs House of Delegates passed a resolution calling for mandatory testing of pregnant women (9).

Even the Institute of Medicine, which early in the epidemic had opposed testing policies that abrogated the privacy rights of pregnant women, was by the end of the 1990s to endorse routine testing on the basis of an informed right of refusal, a much less exacting standard than speciÞc informed consent (10).

In other contexts as well, the retreat from the exacting standard of speciÞc informed consent with pretest counseling has taken the form of efforts to integrate HIV testing into clinical practice where standards of presumed consent prevail. Strikingly, however, when in 1999 the CDC suggested, as one of several options, a relaxation of the standard of speciÞc informed consent for HIV testing in populations other than pregnant women, it provoked sharp opposition. Ultimately, those who favored a change in course were defeated (11).

A course similar to that which occurred with testing characterized the debate surrounding case reporting for HIV infection. Given the profound stigma that surrounded AIDS in the epidemic's Þrst years, and the extent to which individuals with or at risk for HIV feared the social consequences of having their diagnoses made public, it is not surprising that conÞdentiality of AIDS-related information assumed great salience. From the pragmatic perspective of public health ofÞcials it was crucial to preserve conÞdentiality as a way of assuring that those at risk would come forward for testing and counseling (12). Others objected on grounds of principle. Privacy was a value that should not be lightly set aside.

But, however central were the claims of privacy and the duty to protect conÞdentiality, they were not absolutes. Among the conventionally accepted limits to those claims occurred when individuals with infectious diseases were reported by name to conÞdential public health registries. It was thus not surprising that despite concerns about privacy, little opposition existed in the epidemic's Þrst years to making AIDS cases reportable by name (13). AIDS activists appreciated that such reporting was crucial to the understanding of the epidemiology of the new disease. The acceptance of AIDS case reporting requirements was facilitated by the well-established record of state health departments in protecting such records from unwarranted disclosure.

With the inception of HIV testing, however, debate emerged about whether the names of all infected persons, regardless of whether they had received an AIDS diagnosis, should be reported. Activists who accepted AIDS case reporting opposed HIV reporting because of heightened concerns about privacy, conÞdentiality, and discrimination. For them the potential public health beneÞts of reporting were too limited and the burden on those who would be the subject of reporting too great to justify an abrogation of privacy.

While many public health ofÞcials, especially those who came from states with large AIDS case loads, opposed HIV reporting because of its potential effect on the willingness of people to seek testing and counseling, some public health ofÞcials did become strong advocates of such reporting. Their claims sought to underscore the extent to which the public health beneÞts of HIV reporting would be like those that followed from more broadly conceived reporting requirements, such as those that applied to syphilis, tuberculosis, and AIDS itself (14).

As therapeutic advances began to emerge in the late 1980s, and as the logic of distinguishing between HIV and AIDS became increasingly difÞcult to sustain, Þssures began to appear in the relatively broad and solid alliance against named HIV reporting.

At the end of November 1990, the CDC declared its support for HIV reporting, which it asserted could Òenhance the ability of local, state and national agencies to project the level of required resourcesÓ for care and prevention services (15). The House of Delegates of the American Medical Association also endorsed the reporting of names as well (16). In 1991, New Jersey became the Þrst relatively high-prevalence state to require HIV case reporting by name.

In the following years, the CDC continued to press for name-based reporting of HIV cases, supported by a growing number of public health ofÞcials, including the Council of State and Territorial Epidemiologists (17). Central to their argument was the assertion that AIDS case reporting captured an epidemic that was as much as a decade old and that an accurate picture of the incidence and prevalence of HIV infectionÑ especially in light of the impact of treatmentÑ required a surveillance system based on HIV case reporting. Nothing more tellingly underscores the change that had occurred and the extent to which the claims of public health necessity had trumped the arguments of privacy than an editorial jointly authored by a CDC ofÞcial, an AIDS activist and a lawyer-ethicist long involved in AIDS-related work that appeared in the *New England Journal of Medicine* in 1997.

> We are at a deÞning moment of the epidemic of HIV infection and AIDS. With therapy that delays the progression to AIDS, mental illness, and death, HIV infection or AIDS is becoming a complex clinical disease that does not lend itself to monitoring based on end stage illness. Unless we revise our surveillance system, health authorities will not have reliable information about the prevalence of HIV infection. . . . To correct these deÞciencies, we propose that all states require HIV case reporting (18).

At the end of 1999, in the face of lingering opposition from most AIDS activists, the CDC Þnally proposed that all states put in place an HIV reporting system. And while it left open the possibility of reliance on unique identiÞers that met strict performance criteria, it was clear that the use of names was viewed as preferable (19). Remarkably, of those states that adopted HIV case surveillance after the publication of the CDC's recommendations, virtually all adopted coded systems. By 2002 only a handful of states had not adopted some form of HIV reporting.

In the controversy over partner notiÞcation the limits of privacy were also encountered. What emerged as a source of contention in that period was the extent to which the protection of identiÞable third parties who had been or were currently placed at risk for HIV by already infected individuals provided a warrant for public health interventions. This was not a new issue; it had been confronted in the context of psychiatry in the so-called *Tarasoff* doctrineÑ that physicians who knew that their patients were about to inßict serious harm on other identiÞable individuals had a duty to act to warn or protect (20). While opinions differed about the wisdom of such efforts, there was little principled objection to breaching conÞdentiality under such circumstances.

Thus in the mid to late 1980s, when many AIDS activists argued that the principle of conÞdentiality had to be inviolable, and when public health ofÞcials were loath to endorse legislative mandates *requiring* third party notiÞcation, many ethicists suggested that protection of

unsuspecting sexual partners took precedence over privacy.

> When there are strong clinical grounds for believing that a speciÞc contact has not been informed . . . the prudent course for the physician is to inform the contact of the positive serological status of the patient (21).

When, despite the opposition of most civil libertarians and activists, the American Medical Association's House of Delegates embraced the duty to warn in mid-1988, the AssociationÕs president asserted,

> This is a landmark in the history of medical ethics. We are saying for the Þrst time that, because of the danger to the public health and the danger to unknowing partners who may be contaminated with this lethal disease, the physician may be required to violate patient conÞdentiality. The physician has a responsibility to inform the spouse. This is more than an option. This is a professional responsibility (22).

Some states sought to meet the challenge of endangered third parties by enacting statutes that secured a Òprivilege to disclose.Ó Under such laws physicians could, if they chose, breach conÞdentiality to warn unsuspecting individuals but would not be held liable if they failed to do so.

The depth of antagonism to public health interventions in matters of sexual intimacy was further demonstrated by the deep suspicion of contact tracing programsÑ under which public health ofÞcials would notify those who had been placed at risk without divulging the identity of the individual who had imposed the risk. Such efforts were typically voluntary and relied on the willingness of the index patient to provide the name of his or her contacts.

Despite the four decades of experience with contact tracing, efforts to undertake such public health interventions in the context of AIDS met with Þerce resistance in the Þrst years of the epidemic. Opposition by gay leaders and civil liberties groups had a profound impact on the response of public health ofÞcials, especially in states with relatively large numbers of AIDS cases, where contact tracing efforts remained all but moribund (23). In part, the opposition was fueled by the fact that throughout most of the 1980s, no therapy could be offered to asymptomatic infected individuals. Thus, the role of contact tracing in the context of HIV infection differed radically from its role in the context of other STDs. In the latter case, effective treatments could be offered to notiÞed partners. Once cured, such individuals would no longer pose a threat of transmission. In the case of HIV, nothing could be offered other than information about possible exposure.

For public health ofÞcials, who saw in such information an opportunity to target efforts to foster behavioral changes among individuals still engaging in high-risk behaviorÑ behavior that could place both the individual contacted and future partners at riskÑ that was reason enough to undertake the process. For opponents of contact tracing, the very effort to reach out to such individuals represented a profound intrusion on privacy with little or no compensating beneÞt. The task of behavioral change, they asserted, could be achieved more effectively and efÞciently through community-based HIV prevention efforts (24).

Early misapprehensions about the extent to which public health ofÞcials typically relied on overt coercion in the process of contact tracing, and the degree to which conÞdentiality might be compromised, had by the end of the 1980s all but vanished. With such concerns allayed, many gay leaders had come to recognize that partner notiÞcation, in fact, could be a Òuseful toolÓ in efforts to control AIDS (25). The debate began to shift to one centered on relative efÞcacy (26). That dispute was informed by questions that had already surfaced about the utility of contact tracing in the control of syphilis in populations where individuals had large numbers of sexual partners, many of whom were anonymous (27).

In short, by the early 1990s the exceptionalism of the Þrst years of the AIDS epidemic began to fade and a process of normalization had set in.

THE ETHICS OF RESEARCH

As was true of public health practice, the Þrst years of the epidemic forced a rethinking of the ethics of human subjects research. Such a rethinking extended beyond the U.S. as the debate in the late 1990s broadened to questions of research conducted in poor countries under the sponsorship of wealthy nations.

The ethical analysis of research involving human subjects emerged against a backdrop of torture, abuse and scandal. From the Nuremberg Code, which sought to set out the basic moral principles for research in the wake of the post-war trial of Nazi doctors, to the establishment of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, created in the wake of the revelations about the infamous Tuskegee syphilis experiment, the need to protect potential subjects captured the attention of those appalled by the practice of science. In 1978 the National Commission issued the *Belmont Report*, which codiÞed a set of ethical principles that sought to inform and guide the work of researchers (28). Those principles provided the foundations for regulations subsequently enacted by the Department of Health and Human Services and the Food and Drug Administration. At the core of those guidelines was the radical distinction between research designed to produce socially necessary, generalizable knowledge and therapy designed to beneÞt individuals. Against the former, the *Belmont Report* held, individualsÑ especially those who were socially vulnerableÑ needed protection against conscription. AIDS forced a reconsideration of this formulation.

The HIV epidemic provided the circumstances for the emergence of a broad and potent political movement that sought to reshape radically the conditions under which

research was undertaken. The role of the randomized clinical trial, the importance of placebo controls, the centrality of academic research institutions, the dominance of scientists over subjects, the sharp distinction between research and therapy, and the protectionist ethos of the *Belmont Report* were all brought into question. Although scholars concerned with the methodological demands of sound research and ethicists committed to the protection of research subjects played a crucial role in the ensuing discussions, both as defenders of the received wisdom and as critics, the debate was largely driven by the articulate demands of those most threatened by AIDS (29). Most prominent were groups such as the People with AIDS Coalition and ACT-UP, organizations made up primarily of white, gay men. They were joined by community-based physicians who identiÞed closely with the plight of their patients.

What was so stunning, disconcerting to some, and exciting to others was the rhythm of challenge and response. Rather than the careful exchange of academic arguments, there was the mobilization of disruptive and effective political protest. Most remarkable was the core demand. As Carol Levine noted, ÒThe shortage of proven therapeutic alternatives for AIDS and the belief that trials are, in and of themselves, beneÞcial have led to the claim that people have a right *to be* research subjects. This is the exact opposite of the tradition started with NurembergÑ that people have a right *not to be* research subjectsÓ(30). That striking reversal resulted in a rejection of the model of research conducted at remote academic centers, with restrictive (protective) standards of access, and strict adherence to the Ògold standardÓ of the randomized clinical trial.

Blurring the distinction between research and treatmentÑ expressed forcefully through the slogan ÒA Drug Trial Is Health Care TooÓÑthose insistent on radical reform sought to open wide the points of entry to new Òtherapeutic Ó agents both within and outside of clinical trials; they demanded that the paternalistic ethical warrant for the protection of the vulnerable from research be replaced by an ethical regime informed by respect for the autonomous choice of potential subjects who could weigh, for themselves, the potential risks and beneÞts of new treatments for HIV infection. Moreover, the revisionists demanded a basic reconceptualization of the relationship between researchers and subjects. In place of protocols imposed from above, they proposed a more egalitarian and democratic model in which negotiation would replace a scientiÞc authority. Indeed, research Òsubjects Ó were now thought of as Òparticipants.Ó Furthermore, the role of the carefully controlled clinical trial as providing protection against the wide-scale use of drugs whose safety and efÞcacy had not been proven no longer commanded unquestioned respect (31).

The new perspective did not go without challenge, of course. Some were concerned that the proposed regime would make the conduct of research, so crucial to the needs of those with HIV/AIDS, all but impossible (32), others feared that desperate individuals would, in the absence of the now discredited (paternalistic) ethos, be subject to deception (33).

The AIDS-inspired challenge to the ethics of research was not restricted to issues within the United States. Just as the protective regime surrounding research in the United States was a product of a history of abuse, efforts to enunciate ethical standards for the conduct of research in third world nations was shaped by a history of exploitation, a history characterized by investigations on the poor designed to serve the interests of the privileged. Central to those efforts was the belief that the ethical principles Þrst encountered in industrialized nations, had direct bearing on the norms that should govern research in very different settings (34). Such universalism took as a given the need to assume that insights regarding cultural differences not serve as the basis for moral relativism.

Just as individual informed consent was the Þrst principle of the ethics of research in advanced industrial nations, it was at the heart of the codes designed to guide research in the poorest nations. To preclude exploitation, international consensus also existed on the extent to which it was critical that research be responsive to the health needs and priorities of the community in which it is to be carried out (35). What would remain a matter of uncertainty, however, was whether the needs of the poorest and the requirement of responsiveness could justify research that would be unacceptable in the richest nations, whether the principle of universalism could accommodate research in Burundi that would be prohibited in Brooklyn.

That was the issue that would animate a furious international debate occasioned by the 1994 Þnding that AZT administered to infected women in the second and third trimesters and to their infants for six weeks could reduce by two-thirds the rate of vertical transmission (36). Although superÞcially a conßict over a technical matter involving research designÑ the role of placebosÑ the dispute touched on the deepest questions of what ethical conduct meant in a world characterized by great inequalities and profound inequities.

In 1997, an editorial appeared in the *New England Journal of Medicine* denouncing an international trial designed to determine if it were possible to develop an inexpensive clinical intervention that could provide something approaching what had been attained in wealthy nations in reducing the risk of mother-to-child HIV transmission (37). Those trials assumed that even if successful, the more affordable interventions would be less effective than was standard in Europe and America.

Given the burden of pediatric AIDS in Africa and Asia, it was a matter of some urgency that trials begin to determine whether radically cheaper alternatives to the standard regimen could achieve at least some measure of reduced maternal-fetal HIV transmission. In June 1994, a special consultation of the World Health Organization

considered the challenge and called for the launching of studies to achieve that goal. The consultation made clear its conclusion that placebo-controlled trialsÑ trials in which a comparison is made between an inert substance and the potentially active agentÑÒoffer the best option for obtaining rapid and scientiÞcally valid resultsÓ(38).

There was no question that a placebo-controlled trial would have been considered unethical in the United States or any other advanced industrial nation. No trial that denied access to the effective standard, or to an intervention thought to hold the promise of being at least as effective as, if not more effective than, the prevailing standard of care, would have satisÞed the requirements of ethical review. The question posed by the furious controversy that unfolded was whether it was ethical to conduct such a trial in a poor country. *The New England Journal of Medicine* gave its answer unambiguously:

> Only when there is no known effective treatment is it ethical to compare a potential new treatment with a placebo. When effective treatment exists, a placebo may not be used. Instead, subjects in the control group of the study must receive the best known treatment (37).

Given this premise, the *Journal* rejected as irrelevant the fact that health care available in most Third World countries provided nothing like health care available in industrialized countries. Citing the Declaration of HelsinkiÑ the international code of research ethics adapted by the World Medical Association in 1964Ñ for authority, the editorial noted that control groups had to be provided with the best current therapy, not simply that which was available locally. ÒThe shift in wording between Ôbest CÕ and Ôlocal CÕ may be slight, but the implications are profound. Acceptance of this ethical relativism could result in widespread exploitation of vulnerable Third World populations for research programs that could not be carried out in the sponsor countryÓ(37).

Those who rejected the JournalÕs viewpoint made clear that placebo-controlled trials were dictated by the urgency of the situation. Only placebo-controlled trials could provide Òdef Þnitive,Ó Òclear,Ó ÒÞrmÓ answers about which interventions worked, thus allowing governments to make Òsound judgments about the appropriateness and Þnancial feasibility of providing the intervention.Ó The failure to employ a placebo would have made it difÞcult to clearly determine whether the affordable but less effective intervention was better than no intervention at all. In short, they concluded placebos were crucial to policymakers required to make relatively costly decisions under conditions marked by profound poverty and scarce public health resources (39).

Paralleling the debates over maternal-fetal transmission of HIV were those that surfaced over the ethics of AIDS vaccine trials. In this case the focus was on those research participants who might become infected with HIV during a trial. On the one hand there were those who argued that such individuals be provided with optimal careÑ the retroviral therapy available in the developed countries. On the other hand there were those who asserted that care should reßect that which was consistent with what was available in the host nation (40). So divisive was this controversy that UNAIDS could not come to an agreement on the appropriate ethical norm and indeed had to settle for a procedural rather than substantive solution, a solution that focused on how to reach acceptable agreement rather than one that put forth a standard to guide such deliberations (41).

Thus were the issues joined. So critical were stakes involved in the controversies that they ultimately provoked an international effort to consider ethical standards of research in the Third World. The World Medical Association undertook a series of consultations on the revision of the Declaration of Helsinki; the Council for International Organizations of Medical Sciences (CIOMS) did so as well. Finally, within the United States, which funded much of the international research that had been subject to scrutiny, the National Bioethics Advisory Committee took up the issue of studies in poor nations.

While those who saw in any effort to craft ÒßexibleÓ standards that reßected the uniquely pressing context of international poverty and inequality the treacherous embrace of moral relativism, their opponents persisted in arguing that a failure to consider the context of investigation a failure of moral understanding. Principles could be universal; their application could not be rigid (42).

How profoundly difÞcult the issues of research in the context of global poverty were is best demonstrated by conclusions reached by the World Medical Association, the CIOMS and the National Bioethics Advisory Committee. Despite the fact that each body understood the considerable attention that was focused on their efforts, and the importance of forging a common standard, consensus could not be attained. Only the World Medial Association in its revision of the Declaration of Helsinki adopted the position that once an effective therapy was found it became the standard against which all further interventions had to be measured (43). Regardless of the cost of the intervention, placebo controlled trials were no longer tolerable. The CIOMS recommendations, on the other hand, concluded that there might be sound scientiÞc and ethical reasons to reject the Òbest currentÓ method standard, if the failure to use a placebo would make the results inapplicable in the host country where the search for affordable alternatives was essential (44). That too was the position adopted by the U.S. National Bioethics Advisory Committee (45).

In the end then, the AIDS-inspired debate over research ethics did not end in consensus. Rather it opened wide the question of whether ethical principles could serve as a universal standard in a world characterized by gross inequality, whether that very inequality made adherence to universal standards morally imperative.

SECURING ACCESS TO CARE

In the Þrst years of the epidemic there was little that medicine could offer those with HIV disease. Indeed, that was the context, as noted, within which AIDS activists struggled to increase access to experimental trials. As the prospects for clinical intervention improved, Þrst with the use of prophylactic treatment to prevent Pneumocystis pneumonia and other opportunistic infections and then with AZT, the Þrst widely prescribed antiretroviral agent, it was inevitable that the inequities of the U.S. health care system would be encountered.

Some who needed treatment had private insuranceÑ although they not infrequently faced efforts on the part of their insurers to deny them coverage for their HIV-related conditionsÑ those who were poor or who became impoverished because of their disease could qualify for Medicaid, but many remained unprotected (46). To meet their needs, special programs were developed. The federal government, through the Ryan White Care Act, directed signiÞcant sums to localities to provide medical services. Among the initiatives under the act was the AIDS Drug Assistance Program (ADAP), designed to pay for AIDS-related medicines. But the patchwork effort was never adequate and left many without needed protection (47). Like the End Stage Renal Disease Program that assured access to dialysis and transplantation regardless of the ability to pay, those programs left untouched the basic patterns of medical inequality.

When the protease inhibitors emerged in the mid-1990s and combination antiretroviral therapy became the standard of care, the system was strained to the limits. Medication costs alone for those receiving care could range from $10,000 to $15,000 a year (48). One review of dramatically improved therapeutic prospects added the caveat that the new achievements were important Òat least for those socioeconomically privilegedÓ (49). ADAP experienced persistent shortfalls in funding. When that was the case it was necessary to resort to a host of rationing strategies. At one point, nearly half of the ADAP programs limited access to protease inhibitors (50). In 1996, the coordinator of Oregon's program explained that such coverage Òwould blow our budget out of the waterÓ (51). A 1998 report by the National ADAP Monitoring Project found that 15 states maintained waiting lists for entry to ADAP or for access to protease inhibitors. North Carolina, which had no formal waiting list, had simply stopped authorizing new clients for its ADAP. Only two state programs covered the 14 drugs strongly recommended by the Public Health Service for the prevention of opportunistic infections (47).

The remarkable advances in therapeutics have provided a critical element in the argument that the exceptionalism of the epidemic's early years is no longer appropriate. It is therefore a remarkable paradox that the very same achievements have set the stage for challenging the exceptionalist programs that seek to ensureÑ however inadequatelyÑ access to those same treatments. Thus in one analysis it was argued, Ò The absence of (beneÞts like those in the Ryan White Act) for persons with other stigmatizing diseases is troubling. We believe that this discrepancy leaves AIDS exceptionalism vulnerable to the accusation of injusticeÓ (52). The ÒinjusticeÓ was underscored by one AIDS activist who boldly noted, Ò There are certainly other life-threatening diseases out there. Some of them kill a lot more people than AIDS does. So in one sense, it is almost an advantage to be HIV positive. It makes no senseÓ (53). These expressions of disquiet must be understood, at least in part, as a reßection of concern that in a changing political climate, where the American AIDS epidemic may no longer be seen as immediately threatening, unique services for those with HIV would become vulnerable unless they were embedded in a broader system of a just health care system.

On an international plane the prospect of effective antiretroviral treatment would pose challenges vaster by many orders of magnitude. What justiÞcation was there for a system of pricing that made the cost of drugs beyond the reach of the desperate? Could markets ever respond to need where effective demand was nil? Could the monopoly conÞrmed by patent rights be compatible with a response dictated by claims of the dying? Was the treaty on intellectual property rights, incorporated into the World Trade Organization's international regime, a barrier to survival in context of the AIDS epidemic? What moral obligation did the wealthiest nations have to the poorest to provide the resources necessary to purchase the new life-saving agents and build the medical infrastructure necessary for their appropriate administration? Was there any reason to believe that a global community that permitted millions to die each year from treatable and preventable diseases such as tuberculosis and malaria would respond differently in the face of AIDS?

AIDS activists ultimately seized on this issueÑ Life Over ProÞtsÑ and began an international campaign to confront the pharmaceutical industry. What might have seemed an utterly quixotic undertaking would, however, ultimately take on worldwide dimensions linking protesters in the United States, France, and South Africa, institutional proponents of global health such as the World Health Organization, and a sympathetic public. By the end of the 1990s the pharmaceutical industry was placed on the defensive, perceived as protecting narrow self interest when the lives of millions were at stake. Against the claims that high prices were necessary to fuel the engine of research, that patent protections were crucial to spurring investments in drug investigations, those who sought to turn the terms of discourse asserted that urgency demanded that the barriers to drug access tumble. As a leader of the International AIDS Society said in 2001, Ò This is a war, and when you are in a war as we are worldwide with HIV which will claim more lives than any other infectious disease in history, the rules of the game have got to changeÓ (54).

In the face of continuing criticism, drug companies argued that at most the price of drugs was a small part of the problem. The issue, they said, was the fundamental limits of the medical infrastructure of poor nations and the inability of the poorest to pay for even heavily discounted drugs. But such arguments, whatever their merits, carried little weight, as long as prices remained high or as long as the offer to negotiate price reductions entailed protracted processes. In early 2001 *The Lancet* thus wrote, ÒThe time has come for the pharmaceutical industry and the governments who represent them in trade disputes to acknowledge that the world is facing an extraordinary challengeÓ(55).

> Ultimately, under pressure from generic drug manufacturers, prices began to tumble, and pharmaceutical Þrms began to accept the notion of differential or equity pricing (56).

As drug prices began to fall, it became ever more apparent that challenges posed by the international pharmaceutical industry as it resisted pressure by activists, the generic manufacturers and international public opinion, were not without foundation: Even if drugs were to be provided at cost, even if the principle of equity pricing were to guide sales, even if nations pursued the option of compulsory licensing and parallel imports, the cost of providing antiretroviral therapy was simply beyond the reach of the poorest and most HIV-burdened nations. And even if drugs could be paid for, the necessity of a medical infrastructure that could offer and monitor the use of drugs in a way that was attentive to the needs of individual patients and the risks to public health from drug resistance would require huge investments. This was the context within which a remarkable movement to create a massive funding effort to respond to the threat of AIDS would take shape.

In 2001 a Harvard University group issues a call to the global community. In an era of effective antiretroviral therapy access to care was a moral imperative (57). ÒWe believe that on moral, health, social and economic grounds the international community should provide new scientiÞc and Þnancial leadership for a rapid scaling up of AIDS treatment in the poorest and hardest hit countries of the world.ÓThe goal was to treat one million patients in Africa within three years. It was simply untrue that the infrastructural capacity of African health care systems precluded the provision treatment. There were limits. But they could be overcome with appropriate international assistance.

To those who had asserted that efforts to provide treatment would subvert already fragile prevention programsÑ a claim made by many of the humanitarian foundations that had funded such effortsÑ the Harvard group responded directly: ÒTreatment is necessary to optimize prevention efforts. When treatment is not available less incentive exists for an individual to take an HIV test since HIV positive status not only is associated with social stigmatization but is tantamount to a death sentence. Ultimately, treatment of infected individuals may become a major tool in AIDS prevention.Ó

Finally, it was argued that the provision of treatment was necessary to preserve the social fabric of societies affected with high levels of infectionÑÒIf the current lack of treatment continues a demographic shift is predicted . . . such that teenagers will outnumber their elders by 2020. Further, without treatment, millions of adults in the prime of their working lives will die of AIDS and with them skills and knowledge that are necessary for human and economic development.Ó

In assessing the potential costs of such an effort the Harvard group calculated that one million Africans could be treated for $1.1 billion dollars a year. Were the program to expand to three million individualsÑ a goal achievable in Þve yearsÑ the cost would rise to $3.3 billion a year. (In considering the extension of care to non-Africans who could beneÞt from inaccessible treatment, it was estimated that approximately $1.4 billion would be needed in the Þrst year and $4.2 billion by year Þve.) But even so vast an effort would not cover large numbers of people in need of care. To reach those millions could require signiÞcant investments in medical infrastructure that were not calculated. Finally, in addition to the costs of care it was estimated that for prevention efforts for Africa alone $3 billion was needed annually. Thus, in all, the Þrst yearÕs effort in Africa would require $4 billion. To meet this vast commitment it was proposed that an HIV/AIDS Prevention and Treatment Trust Fund be created. The sum involved, while very large, was not beyond the capacity of the wealthiest nationsÑ 0.01% of an aggregate GNP of $23 trillion.

The moral urgency of AIDS treatment was ampliÞed by UN Secretary General KoÞ Annan, who called for a global trust fund that would spend $7Ð10 billion a year over Òan extended periodÓto face the threat to the worldÕs poorest people (58). Most striking was his assertion that the care which had for so long eluded men, women and children in the less developed nations was a matter of right. Speaking to AfricaÕs lenders he said, ÒEven a year ago few people thought that effective treatment could be brought within reach of poor people in developing countries. . . . There has been a worldwide revolt of public opinion. People no longer accept that the sick are dying simply because they are poor. Everyone who is infected should have access to medicine and medical care. Now we know that that is possible, it is surely an ethical imperative.Ó

Strikingly, the idea of a global trust fund not only won the support of editorialists identiÞed with a liberal or social democratic posture, but by powerful institutions as well. Within days of AnnanÕs speech the International Monetary Fund and the governing committee of the World Bank endorsed the fund, in principle (59). The Gates Foundation pledged $100 million to the effort (60). But between dramatic proposals based on moral principles of solidarity with the most vulnerable and willingness to provide the resources necessary to give life to such

principles and commitments, there is a vast distance. When the G8 met in Genoa in July 2001 they committed $1.2 billion to the global fund that Annan had called for. Declaring the gesture ÒlaudableÓthe UN secretary general was quick to add, ÒIt is not enoughÓ(61).

The gulf between conviction and action has become all the more stark as prevailing and pervasive international inequality has taken on moral signiÞcance: What was the unfortunate has become unfair; inequality has become inequity. In that translation the possibility of human agency, of political action to effect change, has opened wide. It is far from certain how that opportunity will be apprehended.

CONCLUSION

This chapter began with an analysis of ethical and policy issues that emerged in the United States as it confronted the AIDS epidemic. These issues were commonly addressed in other economically advanced nations bounded by the liberal tradition, even when the resolution of the controversies that surfaced may have taken divergent forms. The chapter has concluded with a discussion of the profound moral challenge posed by the AIDS epidemic in the poorest nations, focusing on the question of access to care. It is in those nations that the epidemic will take its toll in tens of millions of lives.

No ethical analysis of the challenges posed by AIDS will ever again be sufÞcient if it is restricted to the challenges faced in wealthy developed nations. Indeed, increasingly the analysis will need to be driven by the complexities of an epidemic in the worldÕs poorest nations. Older concerns rooted in a focus on the need to protect the privacy rights of individuals will inevitably be overshadowed by new concerns about equity. It is not that the older ethics will have no relevance. But with life itself in the balance, unless the material requirements of those who need access to care are given preeminence, little else will matter.

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Subject Index

Page numbers in italics refer to information in figures and tables