# The Vaccine Book

## Second Edition

*Edited by*

**Barry R. Bloom**
The Harvard-T.H.Chan School of Public Health
Boston, Massachusetts

**Paul-Henri Lambert**
Centre of Vaccinology
Department of Pathology and Immunology
University of Geneva, Switzerland

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*This book is dedicated*
*to the scientists who create and develop vaccines,*
*to the courageous volunteers who enable us all to*
*learn how safe and effective they are,*
*to those who deliver vaccines to save the lives*
*of children and adults everywhere, often under*
*difficult circumstances.*

# Contributors

**Rafi Ahmed**, Emory University School of Medicine, Emory Vaccine Center and Department of Microbiology and Immunology, Atlanta, GA, United States

**Roy M. Anderson**, Department of Infectious Disease Epidemiology, School of Public Health, Faculty of Medicine, Imperial College London, London, United Kingdom

**Daniel Bakken**, Strategy, Global Marketing, and Commercial Development, Pfizer Vaccines, Pfizer Inc, Collegeville, PA, United States

**Jeffrey M. Bethony**, George Washington University Medical Center, Microbiology, Immunology, and Tropical Medicine, Washington, DC, United States

**Barry R. Bloom**, Department of Immunology and Infectious Diseases, Harvard-TH Chan School of Public Health, Boston, MA, United States

**Christoph J. Blomke**, University of Oxford, Oxford Vaccine Group, Centre for Clinical Vaccinology and Tropical Medicine, Department of Paediatrics, Oxford, United Kingdom

**Matthew J. Bottomley**, GSK VaccinesSrl, Via Fiorentina, Siena, Italy

**Donna Boyce**, Global Regulatory Affairs, Worldwide Safety and Regulatory, Pfizer Vaccines, Pfizer Inc, Collegeville, PA, United States

**Michael J. Carter**, University of Oxford, Oxford Vaccine Group, Centre for Clinical Vaccinology and Tropical Medicine, Department of Paediatrics, Oxford, United Kingdom

**Wilbur H. Chen**, Adult Clinical Studies Section, Center for Vaccine Development and Medicine, University of Maryland School of Medicine, Baltimore, MD, United States

**Rhea N. Coler**, Infectious Disease Research Institute, Seattle, WA, United States

**Adam DeZure**, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

**Gordon Dougan**, The Wellcome Trust Sanger Institute, The Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom

**Eve Dubé**, Institut national de santé publique du Québec and Centre de recherche du CHU de Québec – Université Laval, Québec (QC), Canada

**Ali H. Ellebedy**, Emory University School of Medicine, Emory Vaccine Center and Department of Microbiology and Immunology, Atlanta, GA, United States

**Janet A. Englund**, University of Washington, Department of Pediatrics and Seattle Children's Hospital, Division of Pediatric Infectious Diseases, Seattle, WA, United States

**Oretta Finco**, GSK VaccinesSrl, Via Fiorentina, Siena, Italy

**Adam Finn**, University of Bristol, Bristol, United Kingdom

**Michel Goldman**, Institute for Interdisciplinary Innovation in Healthcare, Université Libre de Bruxelles, Belgium

**Barney S. Graham**, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

**Scott A. Halperin**, Dalhousie University, Department of Pediatrics; Department of Microbiology and Immunology; Canadian Center for Vaccinology, IWK Health Centre, and Nova Scotia Health Authority, Halifax, Nova Scotia, Canada

**Neal A. Halsey**, Institute for Vaccine Safety, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

**Willem A. Hanekom**, Initiative Lead for TB Vaccines, TB Team Division of Global Health, Bill & Melinda Gates Foundation, Seattle, WA, United States

**Tasuku Honjo**, Graduate School of Medicine, Kyoto University, Department of Immunology and Cell Biology and Department of Genomic Medicine, Kyoto, Japan

**Luis Jodar**, Global Medicines Development Group, Medical and Scientific Affairs, Pfizer Vaccines, Pfizer Inc, Collegeville, PA, United States

**Carl H. June**, Center for Cellular Immunotherapies, Abramson Cancer Center; University of Pennsylvania, Department of Pathology and Laboratory Medicine, Perelman School of Medicine, Philadelphia, PA, United States

**Paul-Henri Lambert**, University of Geneva, Centre of Vaccinology, Department of Pathology and Immunology, Geneva, Switzerland

**Heidi J. Larson**, Department of Infectious Disease Epidemiology, London School of Hygiene & Tropical Medicine, London, United Kingdom

**Ramanan Laxminarayan**, Public Health Foundation of India, New Delhi, Delhi, India; Center for Disease Dynamics, Economics & Policy, Washington, DC; Princeton University, NJ, United States

**Myron M. Levine**, Global Health, Vaccinology and Infectious Diseases, University of Maryland School of Medicine, Baltimore, MD, United States

**Stephen S. Lim**, Institute for Health Metrics and Evaluation, Seattle, WA, United States

**Noni E. MacDonald**, Department of Paediatrics, Dalhousie University and Canadian Center for Vaccinology, Halifax (NB), Canada

**Calman A. MacLennan**, University of Oxford, The Jenner Institute, Nuffield Department of Medicine, Oxford, United Kingdom

**Kathleen Maletic Neuzil**, University of Maryland School of Medicine, Professor of Medicine and Pediatrics, Director, Center for Vaccine Development, Deputy Director, Institute for Global Health, Baltimore, MD, United States

**Richard Malley**, Kenneth Macintosh Chair in Pediatric Infectious Diseases, Division of Infectious Diseases, Boston Children's Hospital; Harvard Medical School, Boston, MA, United States

**Mark A. Miller**, Fogarty International Center, National Institutes of Health, Bethesda, MD, United States

**Nagahiro Minato**, Graduate School of Medicine, Kyoto University, Department of Immunology and Cell Biology and Department of Genomic Medicine, Kyoto, Japan

**Seth Mnookin**, Graduate Program in Science Writing and Comparative Media Studies/ Writing Program, Massachusetts Institute of Technology, Cambridge, MA, United States

**Christopher J.L. Murray**, Institute for Health Metrics and Evaluation, Seattle, WA, United States

**Ankur Mutreja**, The Hilleman Laboratories, New Delhi, Delhi, India

**Katherine L. O'Brien**, International Vaccine Access Center, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States

**Mark T. Orr**, Infectious Disease Research Institute, Seattle, WA, United States

**Justin R. Ortiz**, World Health Organization, Medical Officer, Initiative for Vaccine Research (IVR), Immunization, Vaccines and Biologicals (IVB), Family, Women's and Children's Health (FWC) Cluster, Geneva, Switzerland

**Umesh D. Parashar**, National Center for Immunizations and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States

**Nathan C. Peters**, University of Calgary, Snyder Institute for Chronic Diseases, Department of Microbiology Immunology and Infectious Diseases, Cumming School of Medicine, Alberta, Canada

**Andrew J. Pollard**, University of Oxford, Oxford Vaccine Group, Centre for Clinical Vaccinology and Tropical Medicine, Department of Paediatrics, Oxford, United Kingdom

**Meena Ramakrishnan**, Consultant, Philadelphia, PA, United States

**Rino Rappuoli**, GSK VaccinesSrl, Via Fiorentina, Siena, Italy

**Steven G. Reed**, Infectious Disease Research Institute, Seattle, WA, United States

**James M. Robinson**, James Robinson Biologics Consulting, Merck & Co Inc, New Jersey, United States

**David L. Sacks**, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

**Daniel A. Salmon**, Institute for Vaccine Safety, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

**Claire-Anne Siegrist**, University of Geneva, Centre of Vaccinology, Department of Pathology and Immunology, Geneva, Switzerland

**Catherine M. Slack**, University of KwaZulu-Natal (UKZN), HIV AIDS Vaccines Ethics Group (HAVEG), School of Applied Human Sciences, College of Humanities, KwaZulu-Natal, South Africa

**Margaret Stanley**, University of Cambridge, Department of Pathology, Cambridge, United Kingdom

**Johan Vekemans**, GSK Vaccines, Brussels, Belgium

**Bruce D. Walker**, Ragon Institute of MGH, MIT and Harvard; Howard Hughes Medical Institute, Cambridge, MA, United States

# Preface

Vaccines represent the most cost-effective medical intervention known to prevent death and disease. From the creation of the first vaccine by Edward Jenner in 1796, the first human disease, smallpox, was declared eradicated from the face of the earth in 1977 by a global immunization campaign. Yet in 1974, only 5% of the world's children received the six childhood vaccines recommended by WHO. Since then, through extraordinary international public and private collaborations, the number of children receiving these basic vaccines has risen to more than 80%, and in the ensuing years more than 10 million children's lives have been saved. Through creative basic and applied research a number of new vaccines have been developed, and many more are in progress.

Over the past century the world has experienced a *demographic transition*, with people living longer, an increase in the aging population in most societies, and more people living in cities. That has been paralleled by an *epidemiological transition*, in which the diseases that have taken their toll on humankind have changed dramatically. Vaccines have contributed in a significant way to this epidemiological transition by reducing the number of children dying before the age of 6. That has profoundly extended life expectancy, with a concomitant increase in chronic and degenerative diseases. It is a remarkable achievement that infectious diseases no longer represent the largest cause of death in the world, although they still remain the major cause of death in many developing countries, particularly in Africa because there are major barriers to providing children and adults with vaccines.

The aims of this book are to share some of the knowledge acquired over the past quarter century and excitement about the future potential of vaccines to prevent infectious diseases with a wide audience—students, health professionals, and anyone interested in the field of vaccines. We have sought to engage readers who are nonexperts as well as scientists with a specific interest in immunization by presenting a very broad view of vaccines and immunization. We have received the generous support of many contributors who have summarized here the best current knowledge and experience in vaccines around the world. The book is purposely not designed to be comprehensive, but rather to be selective in presenting innovative approaches and problems that are scientifically and practically challenging.

Vaccines are relatively unique among medical interventions in that they are given to healthy individuals, rather than most that are given to people already

ill. Their importance to health is in preventing disease, thus saving costs in both human and financial terms. That circumstance requires the highest level of safety and quality, and some of the chapters will indicate how vaccines are produced and monitored to assured the highest level of safety possible.

Most vaccines are currently given to children. Currently in the United States it is recommended that children be immunized against 14 childhood diseases. In many cases the effectiveness of vaccines wanes with time and it is recommended that booster shots be given, which leads to the challenge of immunization of adolescents and adults. Several existing vaccines, for example, HPV, and other vaccines under development, for example, HIV/AIDS and tuberculosis will be targeted at these groups. Their potential impact would be greatly increased if there were an infrastructure for introducing them in populations. Another major area of enormous promise is the development of specific monoclonal antibodies or T-cell therapies, which have already proven to be the most dramatic new intervention to reduce the spread of certain cancers. Finally there are the challenges such as when people age their immune systems decline, and an increasingly important issue is how to protect the elderly against infectious killers such as influenza and pneumonia. These represent new areas in vaccines that are included in this edition of the book.

Beyond the challenges of these and other specific vaccines, we believe it is important to understand immunization in a broader context. There are chapters that evaluate the impact of current vaccines on world health and make projections of future impacts if new vaccines could be developed against some of the major killers of mankind. We have included a broad overview, beyond the challenges of the laboratory, of issues critical to the success of any vaccine. For example, chapters deal with the somewhat unique economics of the vaccine industry, critical issues of vaccine safety, and concerns about risks and the regulatory environment. Other chapters address how vaccine clinical trials are designed, the infrastructures required to introduce and deliver vaccines effectively at scale, and the special ethical issues posed by vaccines.

The success of vaccines in reducing childhood mortality and morbidity from polio, measles, diphtheria, tetanus has, in many places, created a sense that infectious diseases no longer pose a risk or of complacency. The recent Ebola epidemic in W. Africa, which infected 26,000 people, with 11,000 deaths should remind us of the epidemic potential of infectious diseases in the absence of vaccines. A new chapter presents the state of efforts to develop vaccines against Ebola. Despite enormous efforts to assure the safety of all vaccines and the enormous amount of scientific data establishing that there is no causal association of vaccines and a variety of illness, including the fraudulent claims regarding autism, the problem of public acceptance of vaccines has become a major problem in many countries. In 2007, France reported 40 measles cases; in 2011, there were 15,000 cases with 6 deaths. In 2013 the United States had the largest number of measles and pertussis outbreaks and cases in 20 years. There are two new chapters devoted to understanding issues relating to vaccine hesitancy

and acceptance and to the more general issue of public trust in science and how scientists can listen to public concerns and communicate more effectively the value of vaccines.

We hope this book conveys some of the power of vaccines and immunization to prevent disease and the challenges yet to be overcome. By striving to make its contents accessible, we hope that this book has its own impact in stimulating readers to contribute in various ways to realizing the potential of vaccines to save millions of lives in future.

**Barry R. Bloom**
**Paul-Henri Lambert**

# Introduction-Global Burden of Disease addressed by Current Vaccines and Vaccines in the Development Pipeline

**Stephen S. Lim, PhD, Christopher J.L. Murray, MD, DPhil**
*Institute for Health Metrics and Evaluation, Seattle, WA, United States*

## 1 INTRODUCTION

Estimates of disease burden provide critical information to guide the research, development and delivery of vaccines. In this chapter we present findings from the Global Burden of Disease (GBD) 2013 study to describe across countries, time, age, and sex, the disease burden attributable to conditions for which there are vaccines currently available as well as vaccines that are in the development pipeline.

## 2 THE GLOBAL BURDEN OF DISEASE STUDY

The GBD study is a powerful platform for understanding the main drivers of poor health at international, national, and local levels. Coordinated by the Institute for Health Metrics and Evaluation (IHME), GBD measures all of the years lost when people die prematurely or suffer from disability. It estimates healthy years lost from over 300 diseases, injuries, and risk factors from 1990 to 2013. The GBD findings are available for 188 countries. GBD results allow decision-makers to compare healthy years lost from fatal conditions, such as cancer, to those lost from nonfatal conditions, such as low back and neck pain. The study provides more policy-relevant information than cause of death data by shedding light on conditions that cut lives short, not just those that kill people primarily in old age. The GBD study also provides insight on potentially preventable causes of disease and injuries, known as risk factors which range from poor diets and high blood sugar to unsafe water and micronutrient deficiencies. Examining the ranking of diseases, injuries, and risk factors in a country, province, or county can help policymakers decide where to invest scarce resources to maximize health gains.

## 3 GLOBAL BURDEN OF DISEASE METHODS

GBD uses more than 50,000 data sources from around the world to estimate disease burden. Years of life lost (YLLs) due to premature death from different causes are calculated using data from vital registration with medical certification of causes of death. Years lived with disability (YLDs) are estimated using sources such as published studies on disease and injuries occurrence, cancer registries, data from outpatient and inpatient facilities, and direct measurements of hearing, vision, and lung function. Disability-adjusted life years (DALYs) are the sum of YLLs and YLDs. Estimates are generated using advanced statistical modeling. For more information about GBD methods, see the papers referenced at the end of this chapter.[1–4]

## 4 VACCINE-PREVENTABLE DISEASE BURDEN FROM GBD 2013

In the remainder of this chapter we briefly describe the disease burden associated with presently available vaccines and vaccines in development and highlight the use of GBD results to identify important variations in disease burden patterns. We encourage readers to use the publicly accessible visualization tools available for the GBD (http://www.healthdata.org/results/data-visualizations) to further explore disease burden patterns for specific conditions mentioned throughout this book.

Tables 1 and 2 show presently available vaccines and vaccines that are in development as denoted by the World Health Organization (WHO)[5] along with the corresponding GBD cause category. It should be noted that this is not a precise mapping of vaccines against disease burden for several reasons. Firstly, for some vaccines such as polio, GBD does not presently estimate the corresponding disease burden. Secondly, the listed vaccines may only address part of the corresponding GBD cause; for example, presently available pneumococcal vaccines address only a selected number of subtypes included under the GBD cause category of pneumococcal pneumonia and meningitis. Finally, the estimates provided in this chapter do not take into account the efficacy of the vaccine; for example, the protective efficacy of Bacillus Calmette-Guérin (BCG) vaccine in reducing tuberculosis disease burden is low as is the more recently developed malaria vaccine. In other words, we present here the full disease burden attributable to a cause, not only the fraction of the cause that is preventable by available vaccines or those under development.

### 4.1 Disease Burden Associated With Current Vaccines

In 2013, 4.9 million deaths globally (8.9% of all global deaths) were attributable to causes corresponding to presently available vaccines. This represents

**TABLE 1** Vaccine Preventable Diseases—Current Vaccines

| WHO current vaccines[a] | GBD cause name |
|---|---|
| Cholera | Cholera |
| Dengue | Dengue |
| Diphtheria | Diphtheria |
| Hepatitis A | Hepatitis A |
| Hepatitis B | Hepatitis B |
| | Liver cancer due to hepatitis B |
| | Cirrhosis due to hepatitis B |
| Hepatitis E | Hepatitis E |
| *Haemophilus influenzae* type b (Hib) | *H. influenzae* type b pneumonia |
| | *H. influenzae* type b meningitis |
| Human papillomavirus (HPV) | Cervical cancer |
| Influenza | Influenza |
| Japanese encephalitis | Encephalitis |
| Tick-borne encephalitis | |
| Malaria | Malaria |
| Measles | Measles |
| Meningococcal meningitis | Meningococcal meningitis |
| Pertussis | Whooping cough |
| Pneumococcal disease | Pneumococcal pneumonia |
| | Pneumococcal meningitis |
| Rabies | Rabies |
| Rotavirus | Rotaviral enteritis |
| Tetanus | Tetanus |
| Tuberculosis | Tuberculosis |
| Typhoid | Typhoid fever |
| Varicella | Varicella and herpes zoster |
| Yellow fever | Yellow fever |

[a]*Mumps, rubella are included as part of the GBD other infectious disease category and not included here. Poliomyelitis is not estimated as part of GBD.*

an annual decline of 3.0% from 1990 levels (Fig. 1 for deaths, Fig. 2 for DALYs); in 1990, 7.2 million deaths globally (15.1% of all global deaths) were attributable to these causes. These declines represent improvements in the original Expanded Program Immunization (EPI) vaccines included in the standardized vaccination schedule established by WHO in 1984 [BCG,

**TABLE 2** Vaccine Preventable Diseases—Pipeline Vaccines

| WHO pipeline vaccines* | GBD cause name |
|---|---|
| Campylobacter | Campylobacter enteritis |
| Chagas disease | Chagas disease |
| Enterotoxigenic *E. coli* | Enterotoxigenic *E. coli* infection |
| HIV | HIV/AIDS |
| Herpes simplex virus | Genital herpes |
| Human hookwork infection | Hookworm disease |
| Leishmaniasis | Leishmaniasis |
| Nontyphoidal salmonelloses | Other salmonella infections |
| Norovirus | Norovirus |
| Paratyphoid fever | Paratyphoid fever |
| Schistosomiasis | Schistosomiasis |
| Shigella | Shigellosis |
| RSV (Respiratory syncytial virus) | Respiratory syncytial virus pneumonia |

**Streptococcus pyogenes are not estimated as part of GBD. Rotavirus vaccines (next generation), streptococcus pneumoniae (pediatric vaccines), tuberculosis (new vaccines), and universal Influenza vaccine are included in the corresponding GBD categories under currently available vaccines.*

diphtheria-tetanus-pertussis (DPT), oral polio and measles] but also the introduction of new vaccines such as Haemophilus influenzae type b (Hib), pneumoccocal conjugate vaccine, and rotavirus vaccine. Declines also reflect other health, for example, treatment of pneumonia, and non-health interventions, for example, improvements in maternal education, that affect these conditions.

The remaining disease burden linked to presently available vaccines is primarily concentrated among children under the age of 5 years as seen in Fig. 3 which describes the number of deaths attributable by cause and age. The concentration of disease burden in the young is further accentuated when using DALYs (Fig. 4), which take into account remaining life-expectancy at the time of death and nonfatal outcomes. Disease burden associated with presently available vaccines in children under 5 years of age is accounted for primarily by causes such as pneumococcal pneumonia and meningitis, Hib pneumonia and meningitis, measles, whooping cough (pertussis), rotaviral enteritis, influenza, and malaria. Among those aged 5 years of age and over, presently available vaccines primarily address disease burden attributable to hepatitis A, B, and E, and HPV (cervical cancer). For these causes, the expected impact of these vaccines on disease burden will not be seen for many years given the more recent

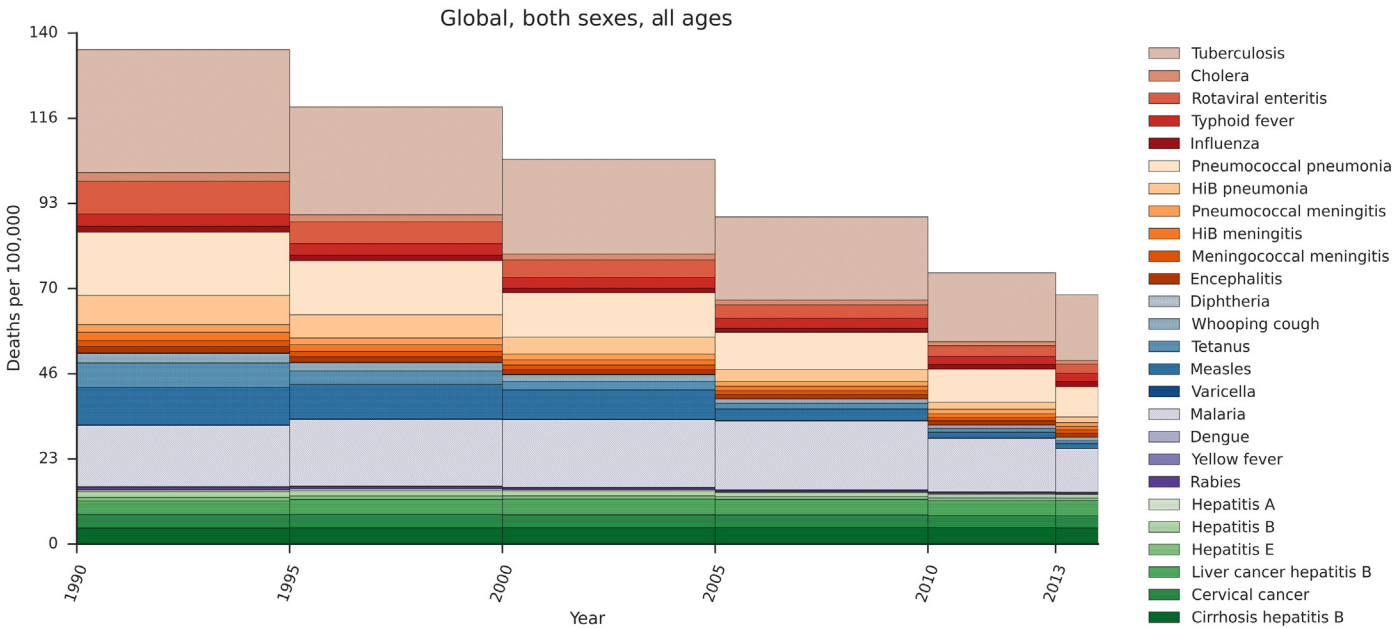


**FIGURE 1** **Global all ages death rate (per 100,000) for VPD current causes for both sexes combined in 1990, 1995, 2000, 2005, 2010, and 2013.**

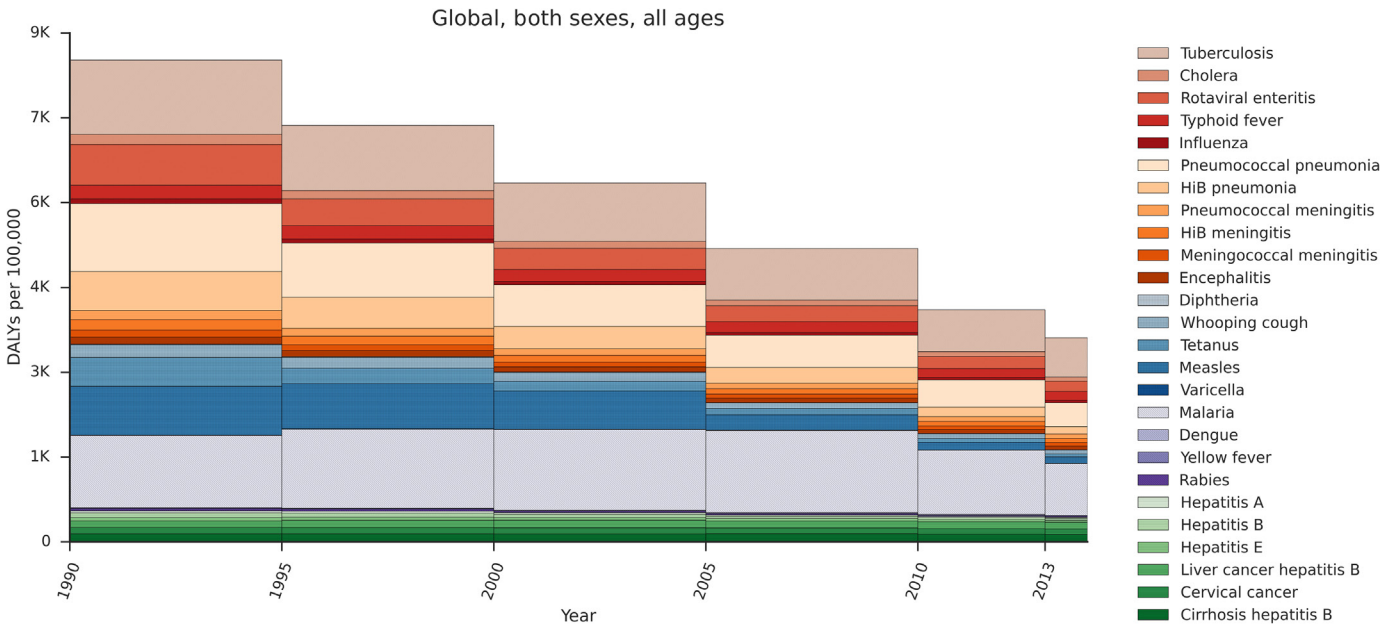


**FIGURE 2** **Global all ages DALYs rate (per 100,000) for VPD current causes for both sexes combined in 1990, 1995, 2000, 2005, 2010, and 2013.**

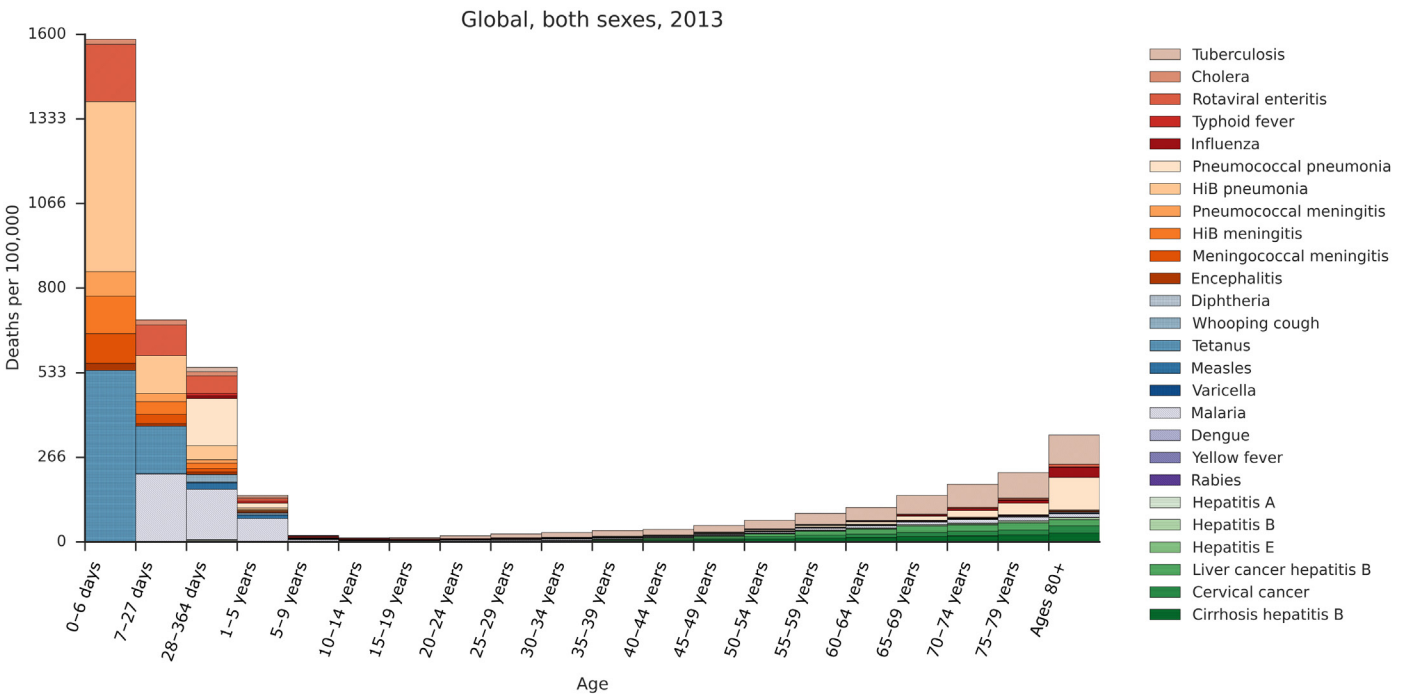


**FIGURE 3** **Global all ages death rate (per 100,000) for VPD current causes by age for both sexes combined in 2013.**

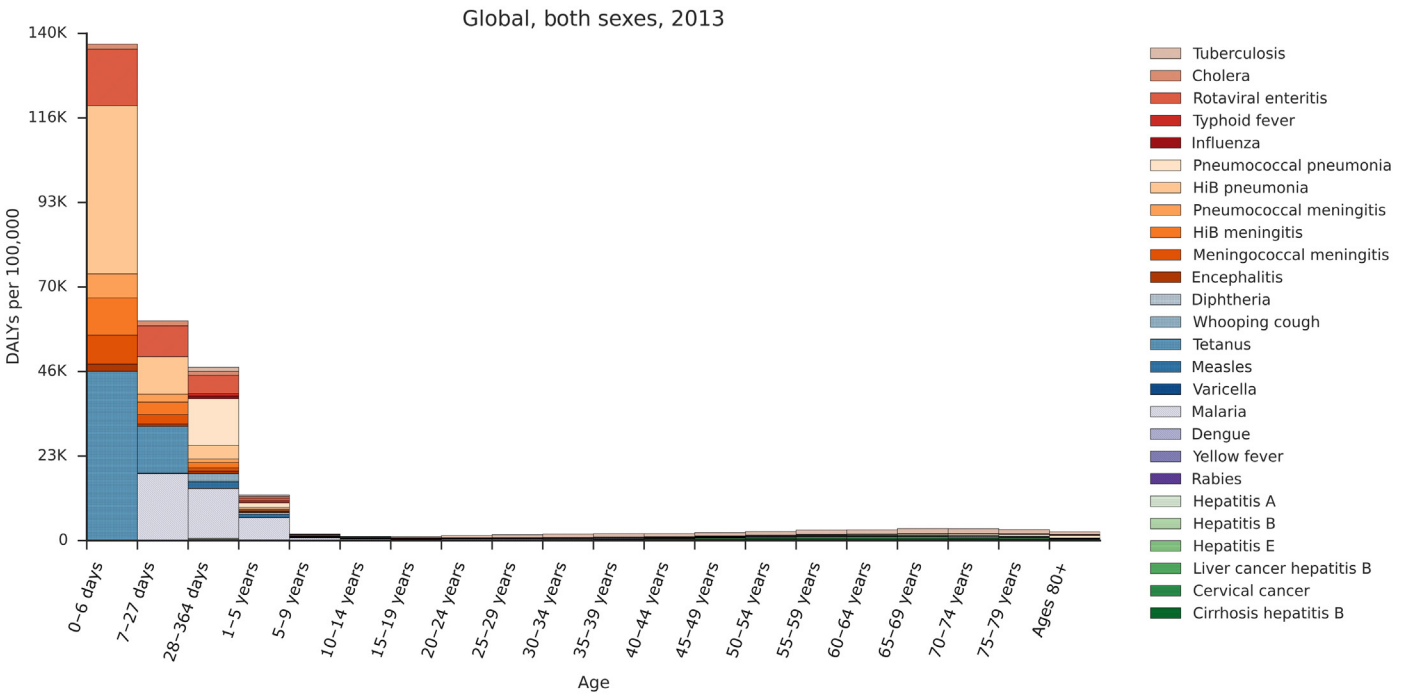


**FIGURE 4** **Global all ages DALYs rate (per 100,000) for VPD current causes by age for both sexes combined in 2013.**

introductions in the majority of countries of hepatitis B (as part of pentavalent vaccine targeting infants) and HPV (targeting adolescents) and the low present use of hepatitis A and E vaccines. Figs. 3 and 4 also highlight the potential for the use of Pneumococcal Conjugate Vaccine (PCV) in older age adults based on the recent CAPITA trial[6].

The disease burden associated with presently available vaccines differs tremendously by geography as shown in Fig. 5. Disease burden is highest in the sub-Saharan African region followed by south Asia, south-east Asia and Oceania. High-income regions (North America, Western Europe, Australasia, southern Latin America, and the high-income countries of the Asia-Pacific) as expected have the lowest burden of disease. This reflects both differences in the composition of causes linked to presently available vaccines, the corresponding underlying risk of disease as well as variability in the coverage of vaccines, particularly new vaccines such as PCV and rotavirus vaccine. These findings with disease burden skewed towards less developed regions highlights the potential of vaccines to address geographical inequalities in health.

Variability in the contribution of associated causes is shown in a heat map of the rank of the different causes associated with currently available vaccines (Fig. 6). In high-income regions, disease burden is skewed more towards vaccines such as hepatitis B and HPV for adult conditions. HPV vaccine (cervical cancer) also figures prominently throughout Latin America. In sub-Saharan Africa, malaria is often the leading cause and measles remains a leading cause in sub-Saharan Africa and south-east Asia. Notably pneumococcal pneumonia is a leading cause of disease burden associated with current vaccines across all regions.

## 4.2 Pipeline Vaccines

In 2013, disease burden associated vaccines in the development pipeline were responsible for 1.7 million deaths globally (3.2%) and 98.0 million DALYs (4.0%). In contrast to existing vaccines, pipeline vaccines have the potential to affect a more diverse set of ages, particularly young and middle-age adults. This is primarily driven by an HIV vaccine as shown in Fig. 7. Other pipeline vaccines target pathogens causing diarrheal disease (eg, salmonella, shigellosis, enterotoxigenic *Escherichia coli*), RSV pneumonia and neglected tropical diseases which primarily affect the very young and the very old. Given the nature of the conditions targeted by pipeline vaccines, it is not surprising that disease burden associated with these vaccines is concentrated in less developed regions such as sub-Saharan Africa (Fig. 8), highlighting the potential for these vaccines to address geographical inequalities in health.

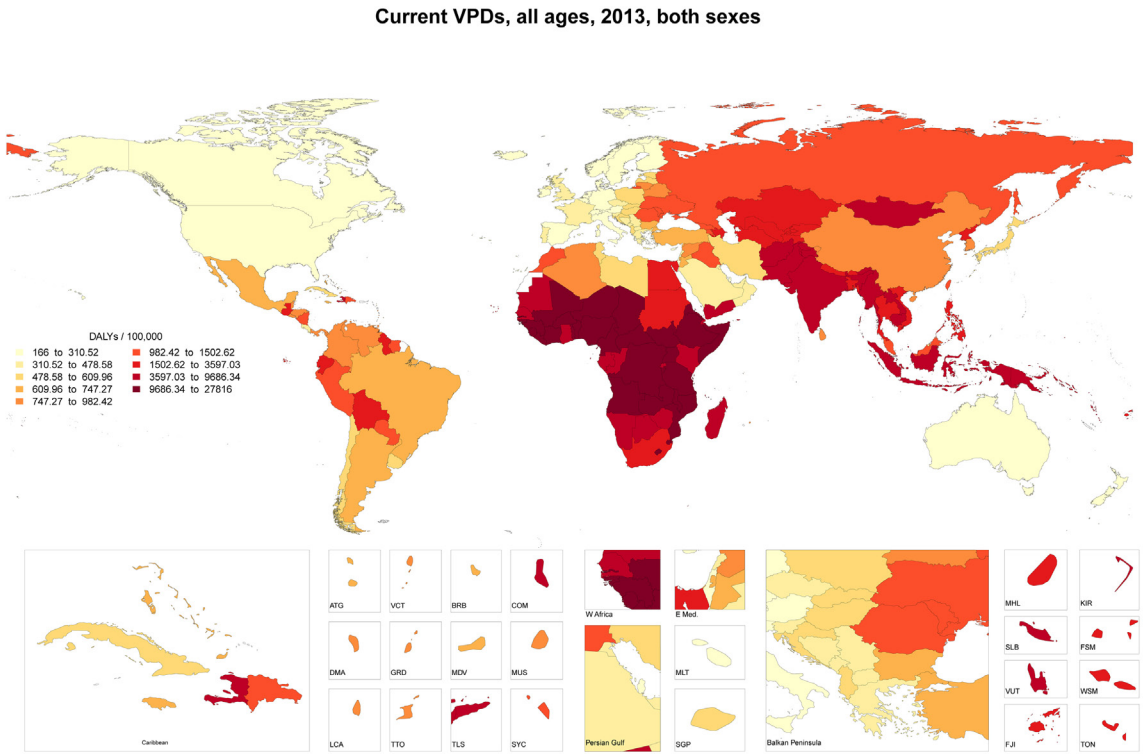


**FIGURE 5** **All ages DALYs rate (per 100,000) for combined VPD current causes for both sexes combined for 188 countries in 2013.**

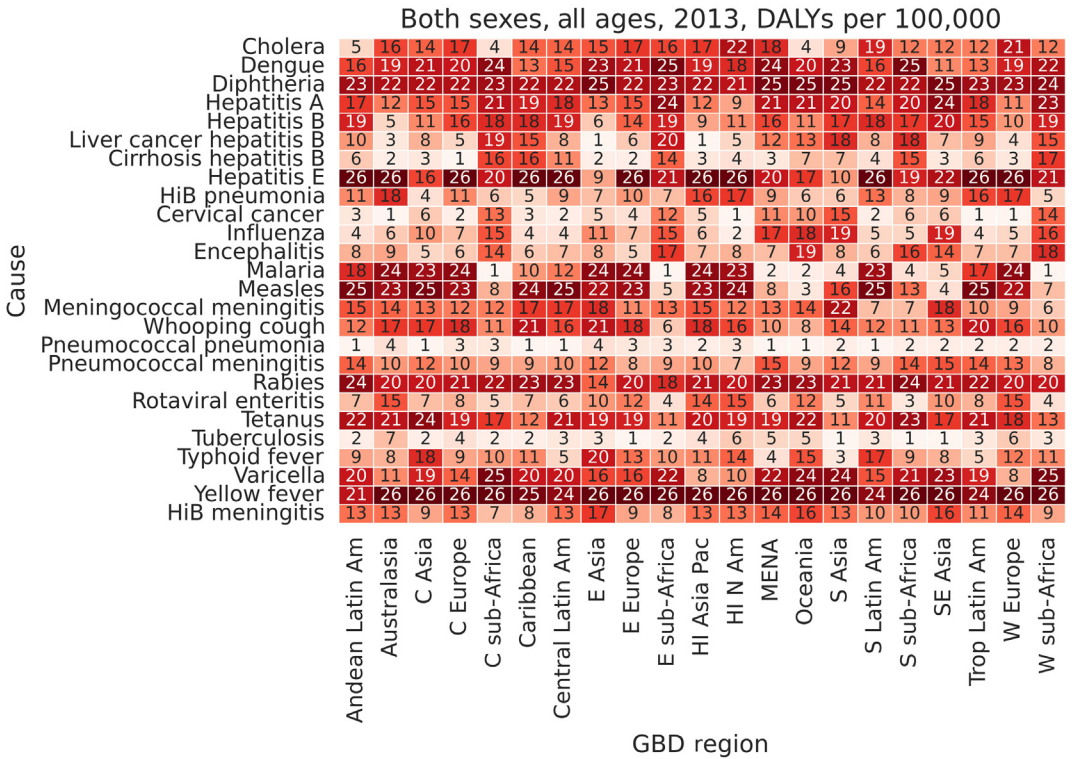


**FIGURE 6** **Heat map of leading causes associated with current vaccines by GBD region for both sexes combined in 2013.**

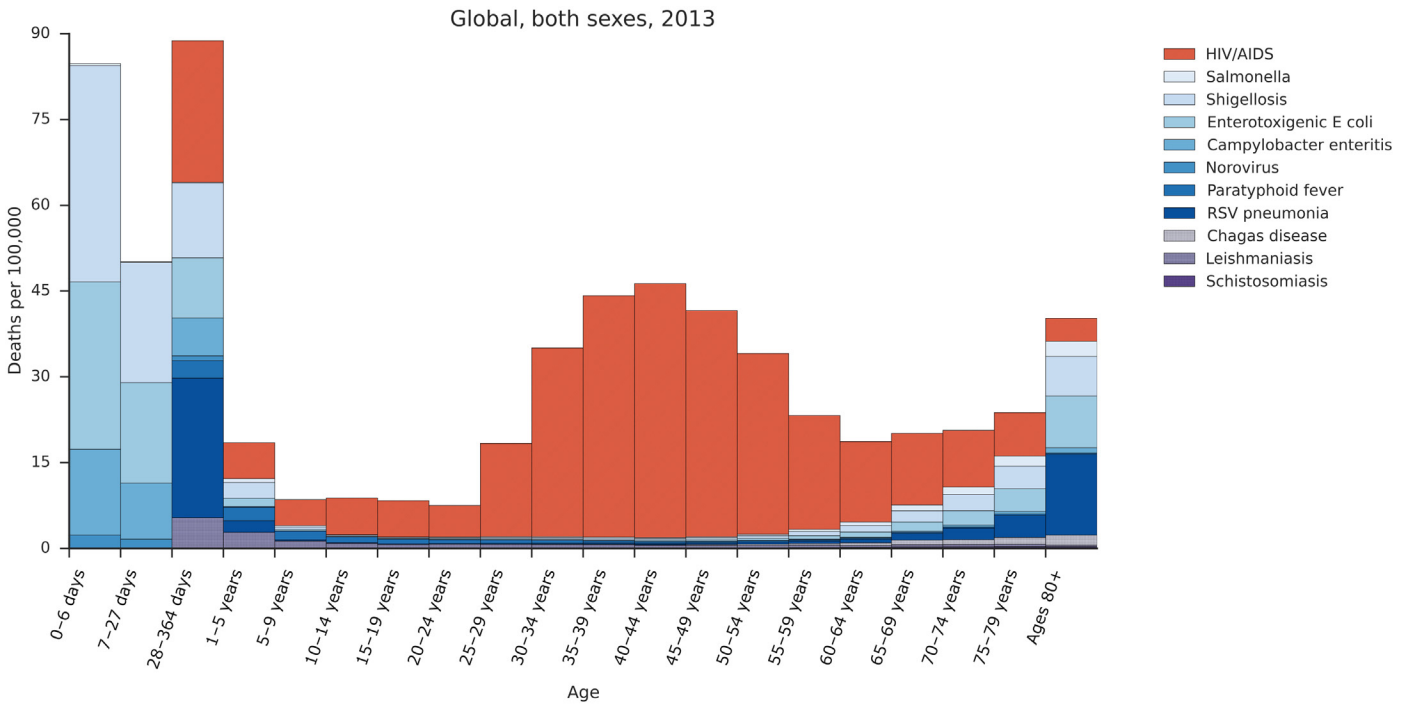


**FIGURE 7** **Global all ages death rate (per 100,000) for VPD pipeline causes by age for both sexes combined in 2013.**

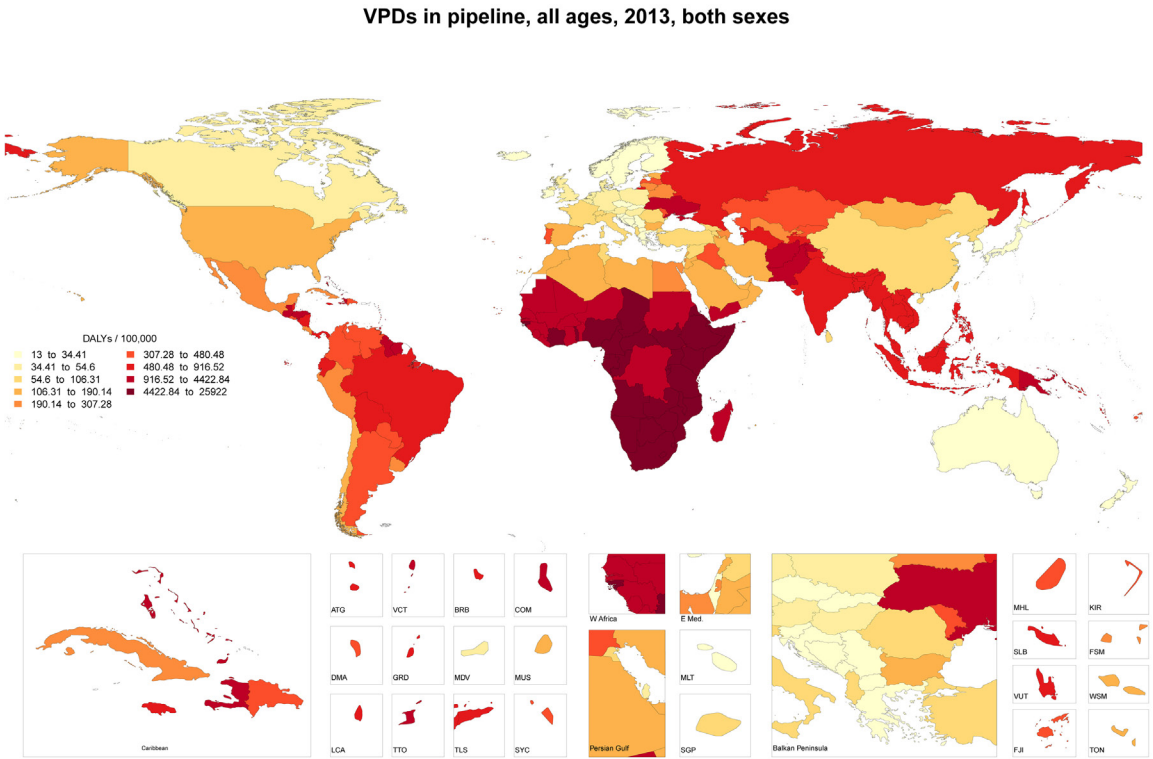


**FIGURE 8** **All ages DALYs rate (per 100,000) for combined VPD pipeline causes for both sexes combined for 188 countries in 2013.**

## 5 CONCLUSIONS

Findings from the GBD Study 2013 highlight the potential for currently available vaccines and vaccines in the development pipeline to address disease burden across multiple age groups and particularly in the poorest, less developed regions of the world.

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Chapter 1

# The Impact of Vaccination on the Epidemiology of Infectious Diseases

Roy M. Anderson
*Department of Infectious Disease Epidemiology, School of Public Health, Faculty of Medicine, Imperial College London, London, United Kingdom*

## Chapter Outline



## 1 INTRODUCTION

Looking back at the past 100 years of medical advances in the prevention and treatment of disease, vaccination is the miracle of modern medicine. In the past 50 years, evidence suggests it has saved more lives worldwide than any other medical product or procedure (Fig. 1.1). Vaccination has a long history, dating back to the work of the British physician Edward Jenner in 1796 on variolation to protect against smallpox,[1] and advancing in complexity in recent times to the

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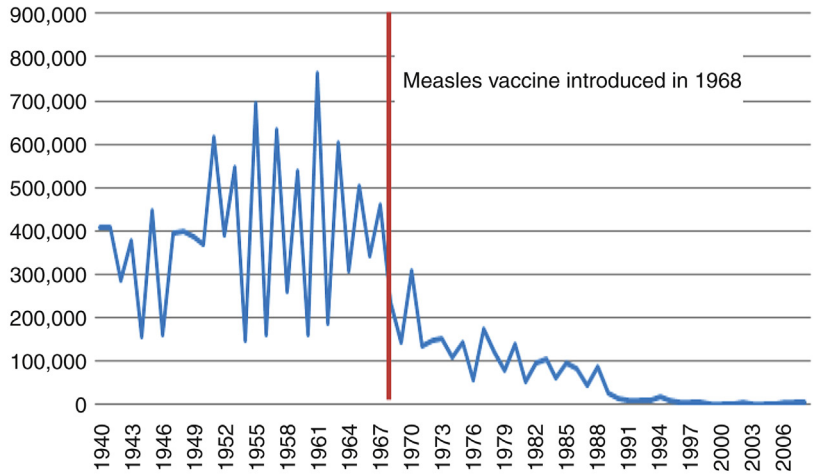


**FIGURE 1.1 Reported measles case in the United Kingdom from 1940 to 2006.** *(Source: Public Health England.)*

production of multivalent vaccines to protect against infections such as pneumonia and human papilloma virus (HPV), with many antigenic strains of the pathogen in circulation in human communities.[2,3] An example of the population level impact of mass vaccination over time in the United Kingdom is shown in Fig. 1.2 which records the decline in measles cases post the introduction of vaccination in 1968.

The global vaccine market rose in value from $12 billion in 2005 to $48 billion at the end of 2015. A relatively few multinational pharmaceutical companies conduct much of the innovation, research, and development in this field. They have released a steady flow of new vaccines into the market over the past decade but the number of major companies involved in vaccine research and development is declining. The expanded programme for immunization in low and middle income countries, which has done so much to reduce the burden of vaccine preventable childhood infections, is mainly supplied through purchases by UNICEF from a number of manufacturers in Asia (especially India) and South America, who produce low cost products, but do not contribute significantly to innovation and the development of new products.

Today, however, most of the "low hanging fruits" for vaccine development have been plucked. What remains of infections that cause significant burdens of morbidity and mortality worldwide, are those where the pathogen populations exhibit much antigenic heterogeneity. Antigenic variation within an infectious disease agent population presents many problems for the development of an efficacious vaccine (Fig. 1.3). HIV is a clear example of a pathogen with high genetic variation both within and between patients. The quasi-species of HIV continually evolves under host immune system selection. Today no effective vaccine is in existence, despite 40 years of intensive research.

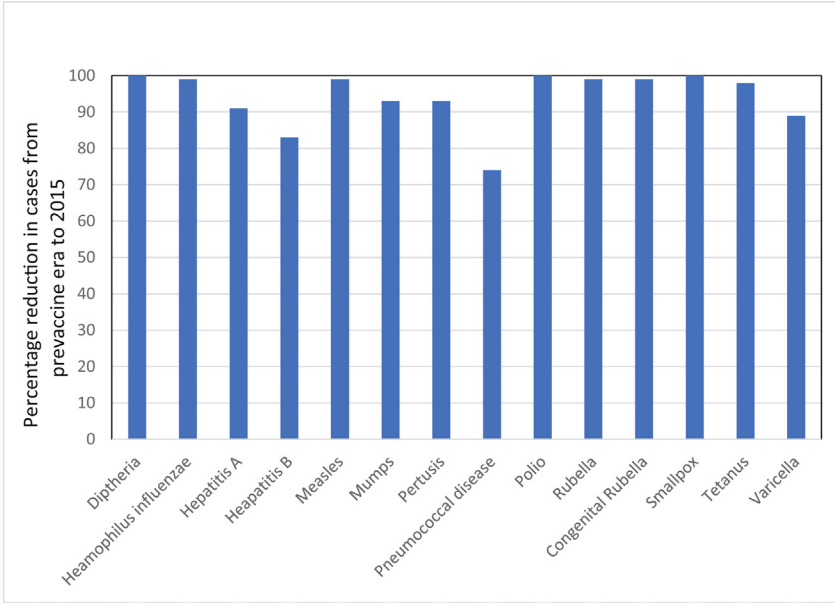


**FIGURE 1.2** **Impact of vaccination in the United States as reflected in the percentage reduction in cases from the prevaccine era to 2015.** *[Source: US Centres for Disease Control data (CDC) 2015.]*

The development of a safe and effective vaccine is only the first step—albeit a vital one—toward the control of an infectious agent. Acceptance of vaccine safety by a population and high uptake are obviously essential, as is an understanding of how best to use the vaccine for community based control. This chapter focuses on the epidemiological impact of vaccination on patterns of infection and morbidity within a vaccinated population. It describes how theory and epidemiological observation help in creating an understanding of how the herd immunity created by vaccination influences patterns of infection and how these change under both different levels of vaccine uptake and different patterns of delivery by age. The key questions examined include the following: What proportion of the population (or a cohort of children) should be immunized to stop transmission? How is this affected by demographic factors such as birth rates? What is the best age to immunize? How does mass vaccination, given a defined degree of herd immunity, affect the age distribution of infection and associated morbidity? How do genetic, social, and spatial sources of heterogeneity influence the design and impact of vaccination programmes? How cost effective is a given vaccine in preventing infection and associated disease?

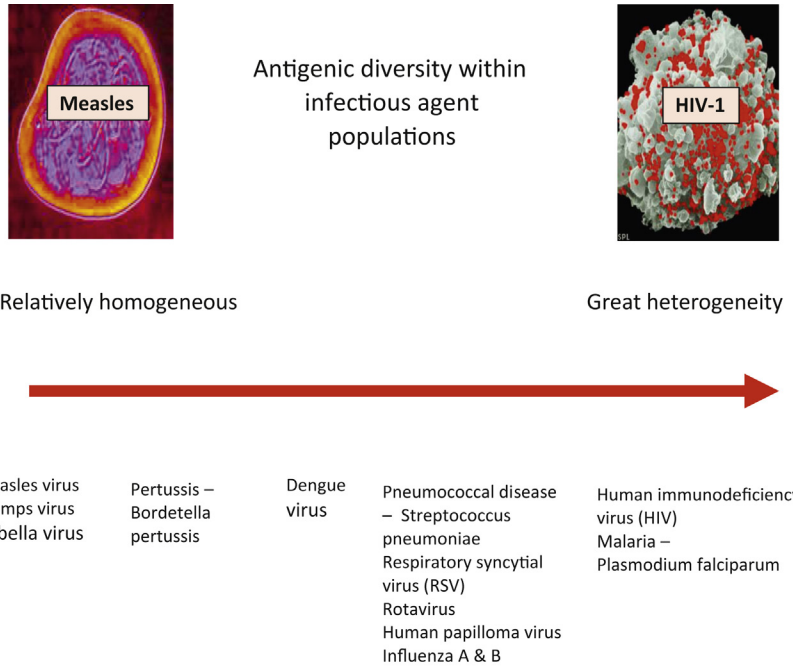


**FIGURE 1.3 Schematic representation of common infectious agents and the degree of antigenic variation with their populations.**

## 2 CHANGING WORLD

Our modern world is changing in ways that influence the spread and evolution of many infectious agents, especially those viruses and bacteria that are directly transmitted between hosts. Three factors are of major importance.

First, population growth worldwide, where predictions suggest that our current population size of 7.3 billion will reach 9.6 billion by 2050. Population size influences directly transmitted infectious diseases in two ways. Increased population density tends to enhance the rate of contact between people and hence transmission. Concomitantly, each transmission event is an opportunity for evolution. As such, population growth enhances the rate of spread and the rate of evolution.

Second, our world is becoming more urbanized with a huge growth in megacities, defined as those above 10 million population size, especially in Asia (Figs. 1.4 and 1.5). These major urban centres will be hotspots for pathogen spread and evolution in the coming decades. Over our past history as a species, new infections in humans are typically acquired from livestock or wild animals.[4] To feed the populations of megacities, livestock are bought into peri-urban or urban areas, and the intimacy of contact with humans is increased. At the same time, to house the growing population of the world, human habitation

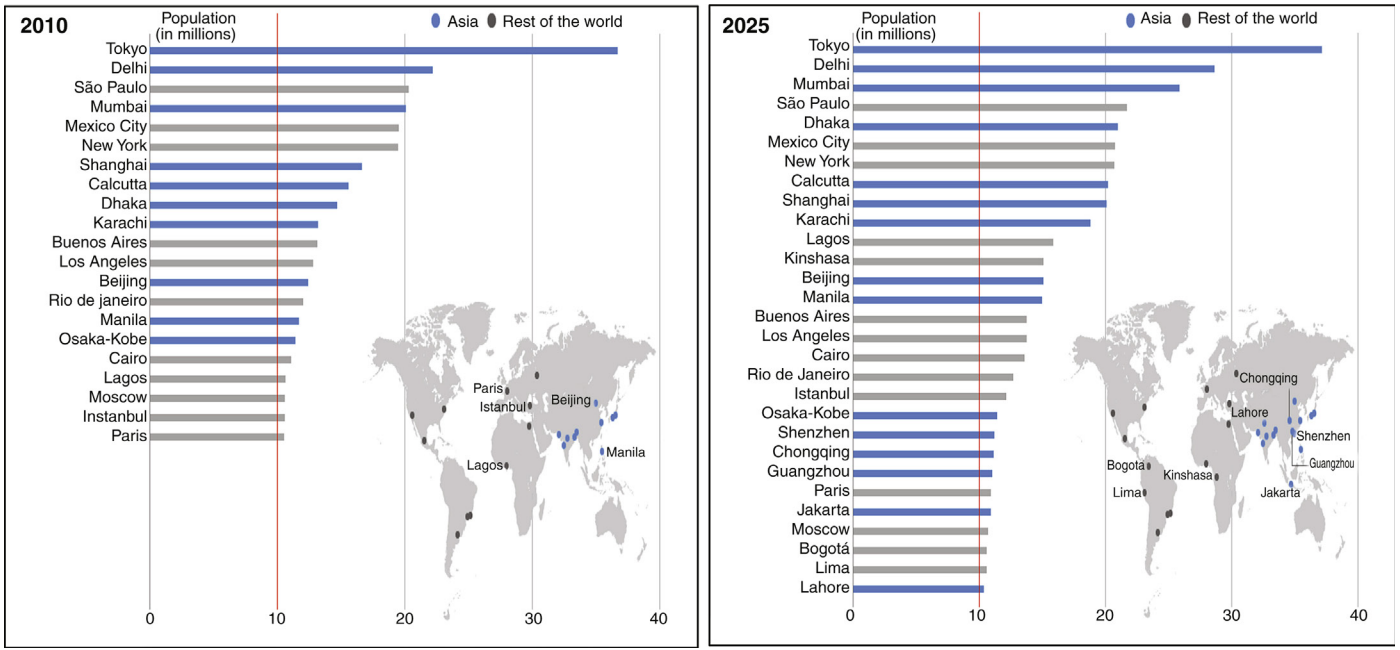


**FIGURE 1.4** **Predicted growth in megacities 2010–25—where a megacity is defined as having a population over 10 million people.** *(Source: UN World Urbanization Prospects: The 2009 Revision.)*

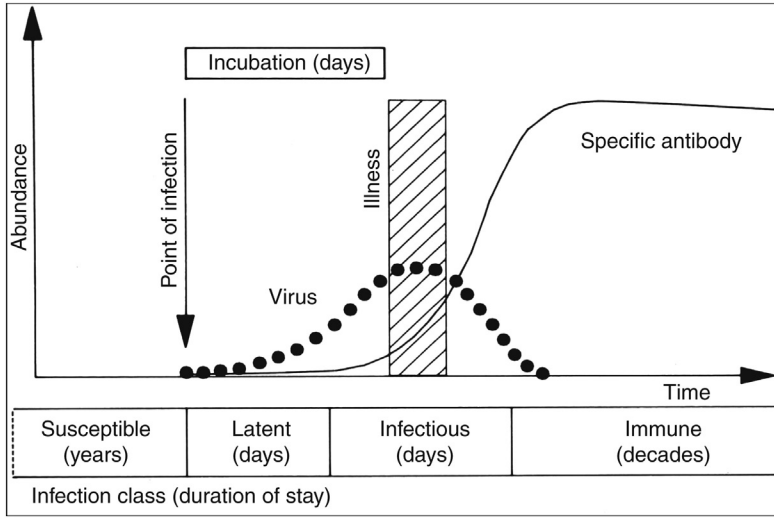


**FIGURE 1.5** **Schematic of the typical course of infection within the host of an acute viral infections such as influenza A.**

increasingly invades the habitats of wild animals and hence increases the intimacy of contact for transmission and evolution of infection to occur.

The third factor is the movement patterns of people in our modern era. Air travel has greatly increased our ability to move large distances within the incubation and infectious periods of many common infections. Our life time tracks have moved from local travel to air travel between continents within four generations of our species.[5] As such, today an infectious agent that emerges in one continent will spread rapidly worldwide within a few weeks to months. This pattern is well illustrated by the spread of a new strain of influenza A, such as the H1N1 strain that emerged in rural Mexico in 2009, and spread world-wide within a few months via air travel.[6,7]

## 3 BASIC EPIDEMIOLOGICAL PRINCIPLES

### 3.1 Basic Reproductive Number $R_0$

A central concept in the epidemiological study of infectious disease and the impact of vaccination is that of the basic reproductive number $R_0$.[8] For directly transmitted microparasites (viruses and bacteria), $R_0$, is the average number of secondary cases of infection generated by one primary case in a wholly susceptible population. Clearly, an infection cannot maintain itself or spread unless $R_0$ is larger than unity in value. In a steady endemic state, each primary case produces on average, one secondary case. The effective reproductive number, $R$, is $R = 1$ at this endemic state. This happens because the basic reproductive number has to be discounted, since at endemic infection many of the contacts

have already experienced infection and are now recovered and immune (for infections that induce lasting immunity). Roughly speaking, if mixing is fairly homogeneous in a defined community, $R_0$ is discounted in proportion to the fraction remaining susceptible, $x$, such that $R = R_0x = 1$. Thus very crudely $R_0$ can be estimated by $1/x$, where in principle the fraction susceptible, $x$, can be estimated from serological studies that determine who has and has not been infected by the presence of infectious agent specific antibodies. The fraction $1-1/x$ measures as a proportion the immunity of the herd or population. The phrase herd immunity (or population immunity) therefore defines the fraction who have either experienced infection and recovered and are immune, or the fraction vaccinated plus the fraction who have been infected.

Simple compartmental mathematical models (susceptibles, infected, and immunes) of the transmission dynamics of directly transmitted viral and bacterial infections that induce lasting immunity yield the following definition of $R_0$:

$$R_0 = \beta X L_I \tag{1.1}$$

Here $\beta$ is the per capita transmission probability from contact between a susceptible and infectious person, $X$ is population size or density and $L_I$ is the average duration of infectiousness (the average infectious period). It is clear from this expression that $R_0$ for a directly transmitted infection will usually increase as host population density rises (this is not true for sexually transmitted infections where $R_0$ depends on the rate of sexual partner acquisition). The criterion that $R_0 > 1$ for the infection to persist translates into a requirement that population density exceeds some threshold value. Bartlett and Black, for example, have shown that populations of 400,000–500,000 or more are needed within island or city communities for the endemic persistence of measles.[9,10] The notion of a threshold density for persistence derives from a deterministic model. Stochastic effects do play an important role in setting these critical community sizes, as do birth rates that determine the inflow of new susceptibles. Most directly transmitted viral and bacterial infections have high threshold densities and hence probably appeared in human populations some 10,000 years ago when agriculture and associated settled communities became more common.[11]

Two of the quantities in Eq. 1.1 are easily measured; namely, population size or density and the average duration of infectiousness based on house hold studies of person to person transmission. Fig. 1.6 records a schematic for acute viral infections showing how changes over time in viral abundance (viral load can be measured in a patient using quantitative PCR methods) are related to some common terms in clinical epidemiology—the latent period (not infectious), the incubation period (delay before symptoms appear), an illness period usually associated with high viraemia, the infectious period, and recovery plus immunity.

The transmission probability $\beta$ is difficult to measure and alternative expressions for $R_0$ have more practical use in infectious disease epidemiology. For example, it is possible to express $R_0$ in terms of an easily measurable quantity

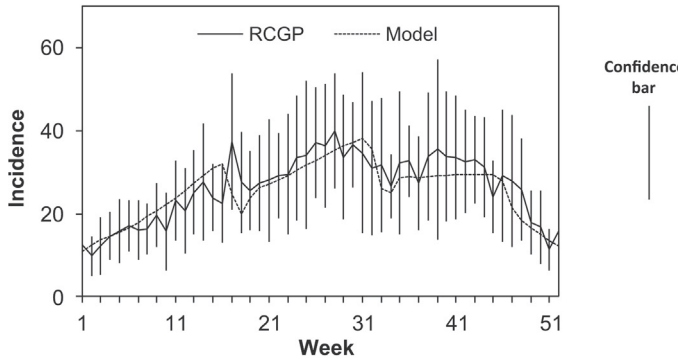


**FIGURE 1.6 Patterns of seasonal measles incidence in England and Wales as reported by the Royal College of General Practitioners (RGCP).** The model line reflects a fit to the observed data. Note the impact of school terms and holidays. The confidence bar reflects variation by year—with the solid line representing an average from many years.

derived from age stratified serological surveys; namely, the average age at infection A.[12] Ignoring sources of heterogeneity (eg, spatial or age related exposure), we can express $R_0$ as follows:

$$R_0 \cong (L - M)/(A - M) \tag{1.2}$$

Here, $A$ is the average age at infection, $M$ is the average duration of protection arising from maternal antibodies post the birth of a child and $L$ is life expectancy. The value of $M$ for most viral infections is of the order of 6 months since maternally derived antibodies decay with a half-life of 6 months, although low concentrations may be detectable up to 1 year postbirth. Note that as $R_0$ increases in value (high transmission) the average age at infection decreases. This equation is based on the assumption that the human population is stable in size. In growing populations the equivalent expression is:

$$R_0 \sim B/(A - M) \tag{1.3}$$

The term $B$ is the reciprocal of the intrinsic birth rate of the population.

The magnitude of $R_0$ for a particular infection in a defined population will determine the difficulty of control by mass vaccination. The higher the transmission potential as measured by $R_0$, the greater the fraction that will have to be immunized to slow transmission. This will be dealt with in a later section, but first we consider some other epidemiological parameters or measures that are important in the interpretation of observed epidemiological pattern.

## 3.2 Fluctuations in the Incidence of Infection Over Time

Many acute viral and bacterial infections show marked periodicity in incidences—both on an annual time scale and longer multiyear time scales. The

annual cycles are the consequence of seasonality in exposure to infection, and are very common among all infectious diseases. For the vaccine preventable childhood viral and bacterial infections, seasonality is in part due to climatic and human behavioral changes (in cold weather, more time is spent inside, often in close proximity to others thus enhancing rates of transmission). However, it has become apparent recently that school terms and holidays, which control assembly and disassembly of school aged children, play a very important role in transmission of many directly transmitted infections including influenza A.[13] Classrooms and playgrounds are very important settings for effective transmission! The seasonal patterns in measles incidence in England and Wales prior to wide scale vaccination are portrayed in Fig. 1.6.

Aside from the seasonal trends in incidence, much more interesting longer term fluctuations are apparent for many vaccine preventable viral and bacterial infections that induce lasting immunity on recovery. For example, a 2 year cycle can be seen in measles incidence in the United Kingdom in Fig. 1.1 between 1949 and 1967. Intuition suggests that an epidemic in a susceptible population will eventually fade as the supply of susceptibles is exhausted (the effective reproductive number falls below unity in value), and will only grow again as this supply is replaced by new births and $R$ rises above unity in value. This is exactly what happens for many infections—they show boom and bust patterns with multiyear cycles, the length of which is determined by a number of epidemiological and infection specific parameters.[12,14] Theory shows that the interepidemic period $\tau$ is defined as:

$$\tau \cong 2\pi \left[ \frac{L(L_I + L_L)}{R_0 - 1} \right]^{\frac{1}{2}} \tag{1.4}$$

Here $L$ is human life expectancy, $L_I$ is the average infectious period and $L_L$ is the latent period before an infected host becomes infectious. This can be expressed in terms of the average age at infection (which is related to the magnitude of $R_0$—see Eq. 1.3):

$$\tau = 2\pi \left[ A(L_I + L_L) \right]^{1/2} \tag{1.5}$$

Interestingly, in the dynamics of the system in the epidemic phase, far from the steady state, there is a decoupling of the time-scales of the spread of infection and the replenishment by births (or immigration) of the susceptibles. It is the latter that dominates in the observed cycles in incidence since in cities such as Lagos in Nigeria, prior to mass vaccination the high birth rate essentially gave annual cycles in measles incidence. This is to be compared with the 2 year cycles in the United Kingdom where the birth rate was less than half that in Lagos.

It is to be expected that mass vaccination will impact the pattern of multiyear cycles in incidence. By essential reducing the magnitude of the effective

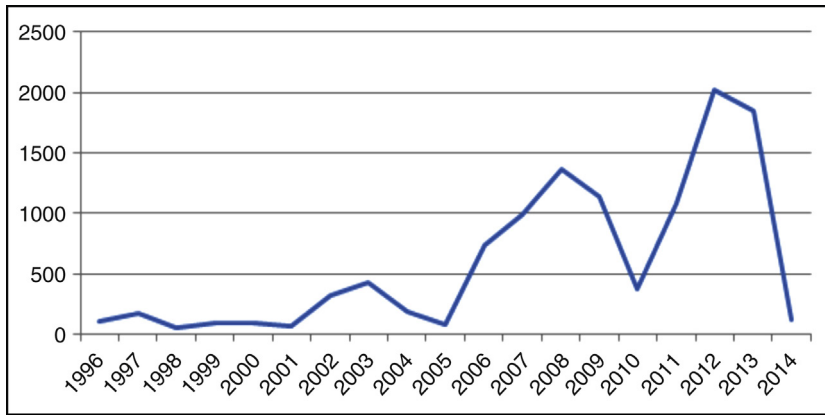


**FIGURE 1.7 The impact of changes in vaccine coverage for measles on the interepidemic period in the United Kingdom over the period 1996–2014.** The vertical axis records reported cases and the horizontal one records the year.

reproductive number, $R$, Eq. 1.4 suggests it will lengthen the interepidemic period. This is exactly what is observed as shown in Fig. 1.7 which records the incidence of measles in the United Kingdom. After many years of high vaccine coverage in children, uptake declined after it was suggested that vaccination, particularly with the measles–mumps and rubella (MMR) vaccine, was related to the development of autism in children in 1998. This association was subsequentially shown to be false[15] but vaccine coverage declined to just 80% in 2003–2004, but slowly recovered thereafter (Fig. 1.8). As shown in Fig. 1.7, the interepidemic period lengthened under good vaccine coverage from 2 years prior to the stat of vaccination in 1968, to around 4 years from 2001 to 2014.

## 3.3 Age Specific Serology

As noted in Eqs. 1.2 and 1.3, estimates of $R_0$ can be derived from a knowledge of the average age of infection. This can be determined from age stratified reports of cases of infection, but a more reliable source is age stratified serology to detect antibodies to defined infectious agent antigens. Cross-sectional by age and gender surveys are the most commonly performed but, in an ideal world, the surveys would be cohort based and longitudinal.

A schematic example of such a cross-sectional survey from which the average age at infection can be estimated is presented in Fig. 1.9, which records the decay in maternally derived antibodies and the rise in seropositivity with age resulting from recovery from infection with measles. Note that the peak in susceptibility (relevant for the ideal age at which to vaccinate) is around 1–1.5 years of age. Vaccination when high titres of maternally derived antibodies are present results in lower vaccine efficacy.[16] The rapidity in the rise in seropositivity resulting from infection is positively associated with the magnitude of $R_0$.

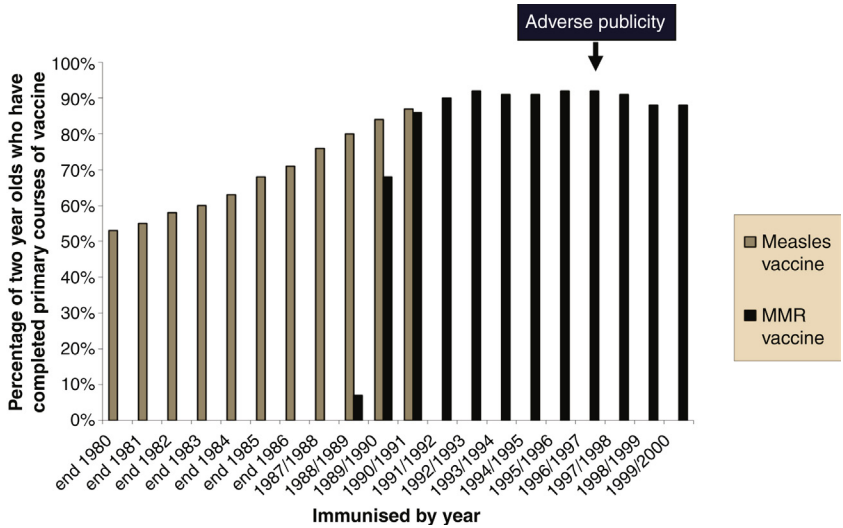


**FIGURE 1.8** **Percentage of children who had completed the primary course of measles or MMR vaccine at 2 years of age, Great Britain, 1980–2000.** Adverse publicity in February 1998, created by a publication suggesting a link between MMR, autism plus inflammatory bowel disease, which was sub sequentially proved to be a false assertion.[15] *(Source: Department of Health, Statistics Division, United Kingdom.)*

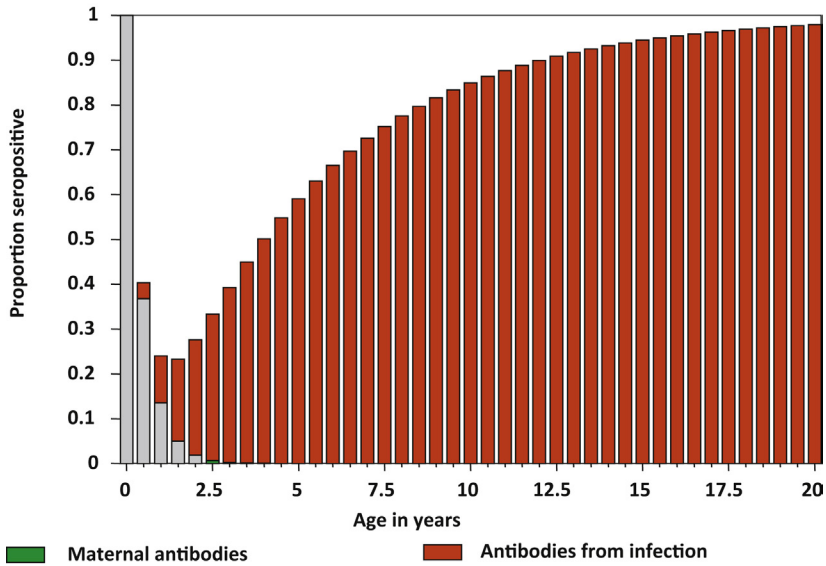


**FIGURE 1.9** **Schematic of a cross-sectional age stratified serological survey for antibodies for the measles virus.** The decay in maternally derived antibodies is recorded as is the rise in seropositivity resulting from infection. The average age at infection is 5 years.

One possible consequence of effective mass vaccination over the longer term is the effect vaccination, as opposed to natural infection, may have on the duration of protection provided by a mother's maternal antibodies to the new born child. Recent work by Waaijenborg and coworkers[17] in The Netherlands, suggests that children of mothers vaccinated against measles and, possibly, rubella have lower concentrations of maternal antibodies and lose protection by maternal antibodies at an earlier age than children of mothers in communities that oppose vaccination. This potentially increases the risk of disease transmission in highly vaccinated populations. However, as suggested in Fig. 1.9, most infection occurs much later than the wane of maternal antibodies so the effect is likely to be small.

## 3.4 Generation and Doubling Times

An alternative way to measure $R_0$ is via estimating the distribution of the generation time of an infectious disease. The generation time is defined as the time interval between infection of a primary case and infection of a secondary case caused by the primary case.[18] In the early stages of an epidemic, say of a new strain of influenza A, understanding the time intervals between successive generations of infected individuals is of importance to estimating the transmission potential of an infectious agent as measured by $R_0$. The serial interval, is the time interval between onset of a primary case and onset of a secondary case generated by the primary case. It is usually difficult directly to observe the actual time of infection for directly transmitted viral or bacterial disease. The distributions of the serial interval and generation times can be measured in practice based on contact tracing and molecular epidemiological genome sequencing techniques which identify who acquired infection from whom with calendar times of onset among traced cases or on the time intervals between the onset of the first and of the subsequent cases in households.

The generation–time distribution is related to the magnitude of $R_0$ via the following equation:

$$\frac{1}{R_0} = \int_0^\infty \exp(-r\sigma) g(\sigma) d\sigma \tag{1.6}$$

Here $r$ is the initial intrinsic exponential growth rate parameter of the rise in case numbers, $\sigma$ is the generation time and $g(\sigma)$ is the probability density function of the generation time $\sigma$.

In the early stages of the growth of case numbers or the beginnings of a new wave of cases for a recurrent epidemic cycle this equation can be simplified to give an expression for the initial intrinsic growth rate $r$;

$$r \approx (R_0 - 1)/\sigma_a \tag{1.7}$$

where $\sigma_a$ is the average generation time. The parameter, $r$, can be estimated from data on case number rise over time by fitting an exponential model to give $R_0$ in the early phase of the epidemic.

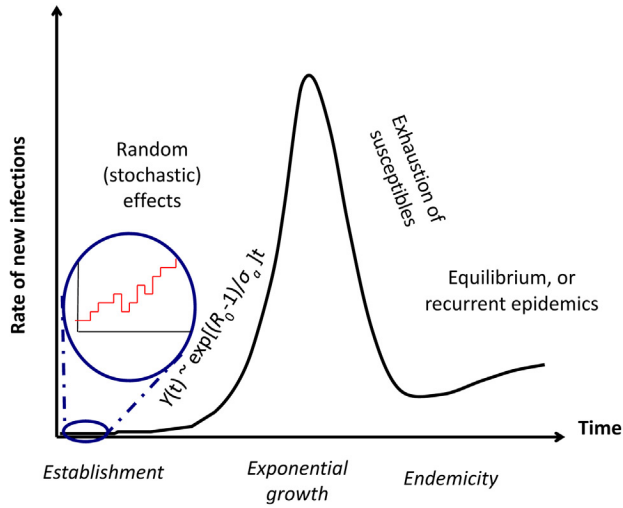


**FIGURE 1.10** **Schematic of an epidemic with the growth rate in case reports over time giving a method for estimating the magnitude of $R_0$ from the intrinsic growth rate of the early stages of the epidemic (Eq 1.8).**

Fig. 1.10 presents a schematic of this growth rate of cases numbers and the expression relating $r$ to $R_0$.

## 4 VACCINE COVERAGE REQUIRED TO INTERRUPT TRANSMISSION

The concepts outlined in the previous section create a template for exploring how vaccination influences observed epidemiological pattern and, most importantly, what level of vaccine coverage is required to block transmission and eradicate the infection. Many sources of heterogeneity can influence such calculations, but the concepts derived from simple models of transmission and mass vaccination are informative in settings broad policy objectives on the desired level of coverage required.[12]

To eradicate transmission, the effective reproductive number $R$, where $R = R_0x$ and $x$ is the fraction susceptible in the population, must be brought below unity in value. If a proportion $p$ are successfully immunized ($p$ equals the vaccination coverage proportion $q$ times the vaccine efficacy $\varepsilon$ measured as a proportion effectively protected by the vaccine, $p = q\varepsilon$), then $x = 1-p$. This gives the following crude guide to the level of coverage required:

$$p > q\varepsilon > [1 - 1/R_0] \tag{1.8}$$

To give a simple example, if $R_0$ is around 15 in value, as it was for measles transmission in the United Kingdom prior to vaccination (average age of infection of around 5 years of age), then the fraction that must be successfully

**TABLE 1.1** Key Epidemiological Parameters for Some Childhood Vaccine Preventable Infections[12]

| Infection/ infectious agent | Average age at infection in years | Interepidemic period in years | $R_0$ | Critical level of effective immunization required to block transmission |
|---|---|---|---|---|
| Measles | 4–5 | 2 | 15–17 | 92–95 |
| Pertussis | 4–5 | 3–4 | 15–17 | 92–95 |
| Mumps | 6–7 | 3 | 10–12 | 90–92 |
| Rubella | 9–10 | 3–5 | 7–8 | 85–87 |
| Diphtheria | 11–14 | 4–6 | 5–6 | 80–85 |
| Polio virus | 12–15 | 3–5 | 5–6 | 80–85 |

immunized is roughly 94% of each cohort. Table 1.1 lists some key epidemiological properties of some childhood vaccine preventable viral and bacterial infections.

The previous calculations assume that vaccination takes place immediately post the wane of maternally derived antibodies. This is rarely the case, and in practice vaccine is delivered to a cohort at some average age $V_a$. In this case, the critical coverage level of effective vaccination is given by[12];

$$P > [1 - (1/R_0)]/[1 - (V_a/L)] \tag{1.9}$$

where $L$ is human life expectancy. It is clear from this equation that higher levels of coverage are required as the average age at vaccination increases. In particular, these calculations highlight the need to vaccinate as soon as programmatically possible after the decay in maternal antibodies (ie, after 6–12 months of age). Delaying delivery to 3–4 years of age greatly reduces the impact of the accumulated herd immunity arising from cohort vaccination, and creates pockets of susceptibility in infants in which the viral or bacterial infection can persist. Parents delaying the vaccination of their infants often arises after scare stories in the press concerning vaccine safety, as arose for the MMR vaccine in 1998 in the United Kingdom. The result of this, some years later in 2012–15, was minor epidemics of measles created by pockets of susceptibility in young children in some regions of the United Kingdom.[19,20].

## 5 A SHIFTING AVERAGE AGE AT INFECTION

Cohort based vaccination acts to reduce the effective reproductive number post the introduction of mass vaccination as the level of herd immunity builds up over time. Concomitantly, this acts to change the observed epidemiology of the

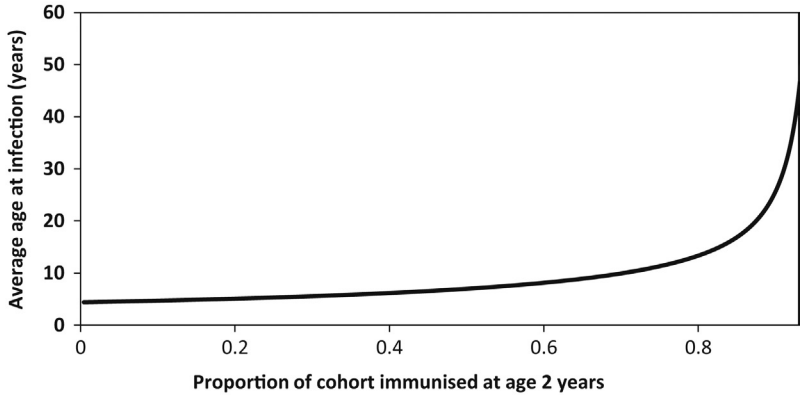


**FIGURE 1.11** **The impact of mass vaccination at age 2 years on the average age at infection A for an infectious disease where A was 5 years of age prior to the start of vaccination.**[12] A rough approximation of the relationship if vaccination is close to birth is given by $A' = A/(1-p)$, where $A'$ is the average age at infection when vaccination is occurring, p is the proportion effectively immunized and $A$ is the average age at infection prior to vaccination.[12]

infection within the population. It has already been mentioned that mass vaccination acts to lengthen the interepidemic period. It also acts to increase the average age of infection A in those still unvaccinated, as the effective reproductive number $R$ declines in value from its pristine value of $R_0$. Simple theory predicts the relationship displayed in Fig. 1.11, with the value of $A$ only increasing significantly once coverage at age 2 years rises above 80%. However, if this impact of mass immunization is not understood it can lead to false claims concerning, for example, vaccine efficacy decaying with age postimmunization.

This has arisen in the United States where clusters of cases in university students have been reported during the 1980s and 1990s. For example, in early 1988 an outbreak of 84 measles cases occurred at a college in Colorado in which over 98% of students had documentation of adequate measles immunity (physician diagnosed measles, receipt of live measles vaccine on or after the first birthday, or serologic evidence of immunity) due to an immunization requirement in effect since 1986.[21] This was interpreted as a failure of one dose of the measles vaccine to protect against infection and, as such, the authors recommended two doses of measles vaccine for college entrants to reduce measles outbreaks in college populations. Part of the explanation is likely to be a significant shift in the average age at infection in those still susceptible to infection at college entry.

## 6 PERVERSE EFFECTS OF VACCINATION

One consequence of shifting the average age at infection by mass vaccination is to shift the pattern of morbidity caused by infection if such morbidity is age related, as is often the case. Many texts on paediatric infections state that disease arising from infection is more common in the young as opposed to adults. This

is true in terms of numbers of cases but may not be the case once translated into cases of serious morbidity per case of infection, given that most infections occur in the young for diseases such as measles.

Typically, the case complication rate in terms of morbidity rises with age as well illustrated by measles, mumps, and rubella. In the former, the number of cases of measles encephalitis per case of infection rises linearly with age.[22] In the case of mumps, the risk of serious complications is most acute in the 20–40 year olds.[23] For rubella, it is the hazard of significant congenital abnormalities (congenital rubella syndrome, CRS) in offspring of women who acquire rubella during pregnancy, especially during the first trimester.[24] CRS typically occurs in roughly 80% of infants born to mothers who contract rubella in the first trimester of pregnancy.

Analyses of the likelihood of mass vaccination moving more people into the high risk age classes than was the case before mass vaccination is a complex problem and not one where intuition alone will lead to the best vaccination policy. Calculations are required based on the functional form of the precise age-related risk of serious disease and the level of vaccine coverage. In most cases, such calculations give encouraging results. Provided the risk of serious complications from infection does not rise faster than linearly with age, no perverse impacts are predicted. This is not the case for mumps and rubella. Detailed studies have been conducted on both[23,25] and the conclusion is that all programmes for mumps, independent of vaccination coverage, are very unlikely to make matters worse. For rubella the situation is different since some vaccine coverage levels can create more cases of CRS than was the case prior to mass vaccination.

A summary of these effects is presented in Fig. 1.12 for rubella vaccination involving girls at age 12 years and boys and girls at age 2 years. The graph records the ratio of CRS cases after vaccination divided by cases before, as a function of the proportion of the 12 year old girls vaccinated and the 2 year old boys and girls vaccinated, assuming an average age at infection of 6 years prior to the start of mass vaccination. In an unvaccinated population most women acquire infection before the pregnancy age classes and hence the risk of CRS in the infant is very low since most mothers are immune and therefore do not acquire infection. Vaccination reduces the exposure to infection of those not vaccinated and raises the average age at infection such that some women enter the pregnancy age classes still susceptible to infection. As shown in Fig. 1.12 this risk arises for low to moderate levels of coverage but does not materialize at high coverage over 85–90%. This risk can be greatly reduced by immunising boys and girls both at high coverage to significantly impacting the circulation of the virus.[26]

Note that the relative benefits of such rubella immunization programmes will be influenced by the prevailing pattern of age dependent fertility. As shown in Fig. 1.13 this can change over time in given populations as illustrated by data from the United Kingdom in years 1981 and 1996. Such changes must be taken into account when reviewing rubella vaccination policies.

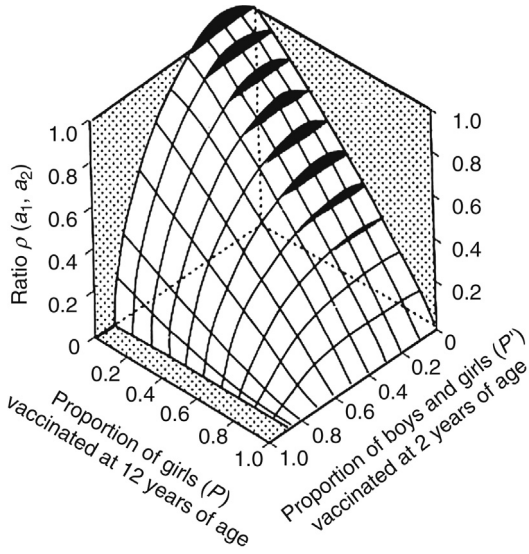


**FIGURE 1.12** **Mass vaccination can increase the incidence of serious disease per case of infection if the likelihood rises with age.** Average age of infection prior to vaccination was set as 6 years. In the shaded region a two stage policy rubella vaccination programme of vaccinating boys and girls at age 2 years and girls at age 12 years is predicted to create more CRS cases than was the case before vaccination for certain levels of coverage *(the black regions)*[26]. The vertical axis records cases CRS cases after vaccination divided by the numbers before.

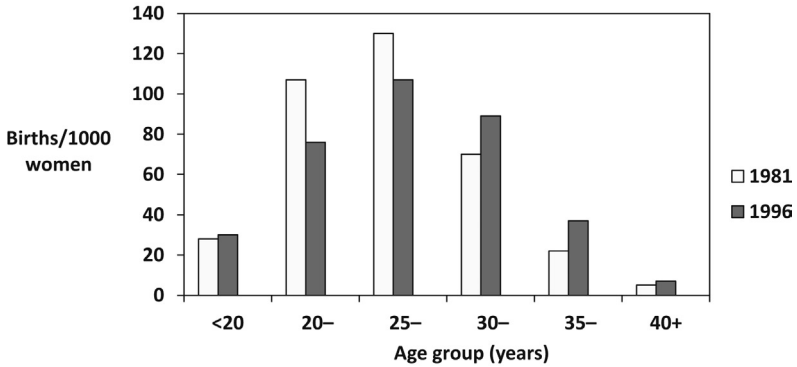


**FIGURE 1.13** **Demography–age specific birth by age group in years in the United Kingdom in 1981 and 1996.**

## 7 TROUGHS IN HERD IMMUNITY AS A CONSEQUENCE OF THE INTRODUCTION OF COHORT BASED VACCINATION

Previous sections have highlighted the impact of vaccination on the average age at infection in those still susceptible. When cohort immunization begins for a defined set of age classes such as 2 and 3 year olds, those just older who are

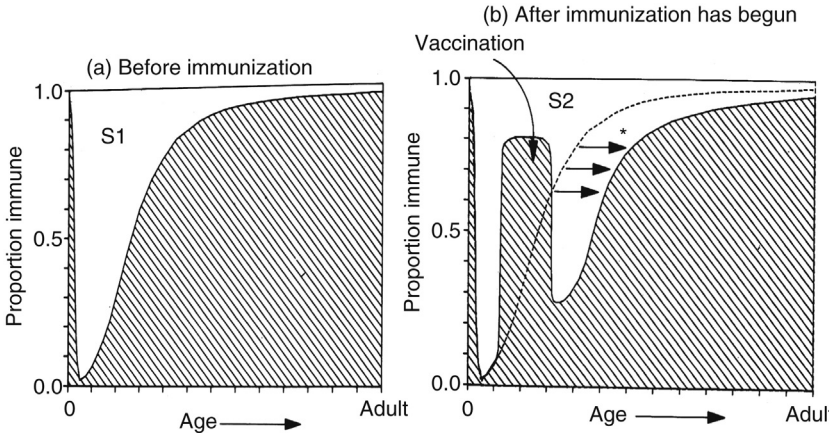


**FIGURE 1.14 Schematic representation of the impact of cohort vaccination on the cross-sectional age serology profile.** In graph (a) the profile is before vaccination and shows maternal antibody protection in infants and then serpositivity due to the recovery from infection. In graph (b) a small set of child age classes are immunized and this acts to reduce overall transmission which implies that those older than the vaccinated classes are exposed to a lower rate of infection due to the herd immunity generated by vaccination.

still susceptible will experience a reduced rate of infection due to the impact of herd immunity created by vaccinating the younger age group on virus circulation. This is illustrated schematically in Fig. 1.14 by reference to the impact on a cross-sectional serological profile stratified by age.

Few countries carry out regular population-based serological screening of immunity to various common infectious agents. The first to do so was Finland who started sampling children and adults in 1980. Serological studies for rubella antibodies from 1979 to 1991 reveal the pattern predicted by theoretical studies of the transmission dynamics of the virus and the impact of cohort vaccination[27] as shown in Fig. 1.15. In the period 1980–82 vaccination was targeted at girls age 12–13 years of age, but in Nov. 1982, MMR vaccine was administered to boys and girls aged 14–18 months and 6 years of age.[28] The impact of the programme in reducing exposure to infection in those just older than the vaccination age is shown by a trough of susceptibility that moves across the three dimensional profile as the cohort programme progresses over time. This trough is most apparent in the female population at the start of the programme of immunisation that only targeted girls.

## 8 INDIRECT AND DIRECT EFFECTS OF VACCINATION—BENEFITS OF HERD IMMUNITY

Vaccination benefits directly those who are successfully immunized, and indirectly those who are not, by the creation of herd or population immunity in the vaccinated that reduces exposure to infection in the remainder of the

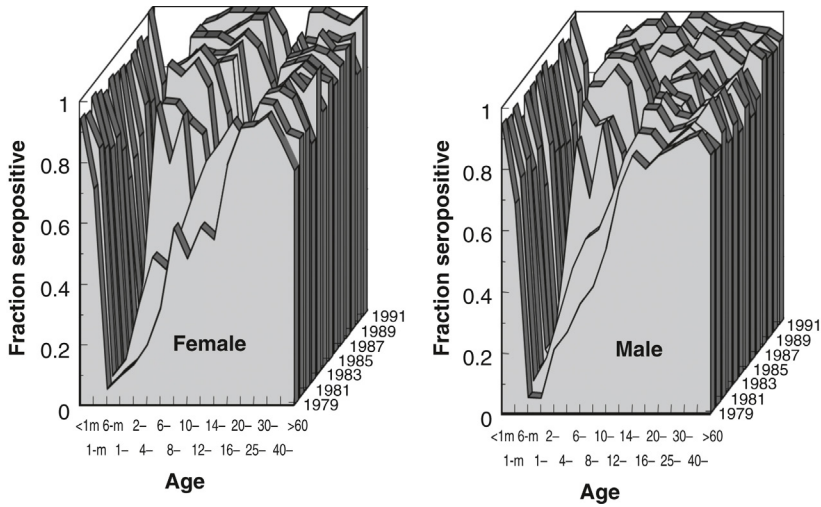


**FIGURE 1.15** **Cross-sectional by age (months and years), and longitudinal over time (year), serological survey in Finland for antibodies to the rubella virus in males and females.**[28]

population. The magnitude of the indirect effects will depend on the proportion of the population immunized rising as this increases. Calculations can be made of the relative magnitude of the direct effects that increase linearly with the proportion immunized, and the indirect effects that increase in a very nonlinear manner as immunization rises. This pattern is displayed in Fig. 1.16 from which it can be seen that the magnitude of the indirect effects only begin to rise steeply when vaccine coverage reaches high levels of over 75%.

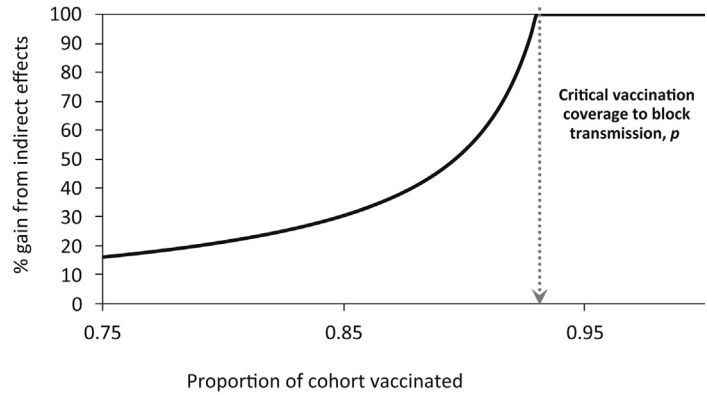


**FIGURE 1.16** **The indirect effects of vaccination as a function of the proportion immunized up to the critical level of vaccination, *p*, required to eradicate infection.** The indirect effects are represented as a % gain over the direct effects (for those immunized). The indirect effects represent the benefits of herd immunity for those not immunized in reducing their chance of acquiring infection.

## 9 HEALTH ECONOMICS—COSTS AND BENEFITS OF VACCINATION

Any evaluation of the costs and benefits of vaccination programmes must take account of both the direct and indirect benefits of vaccination. If this is not done, any health economic evaluation that solely depends on calculations of the direct costs will underestimate the true benefit, especially at high vaccine coverage levels. Surprisingly, many health economic evaluations of the costs and benefits of introducing a programme of vaccination (eg, when a new vaccine enters the market) do not take account of the indirect impact of herd immunity. The reason for this relates to the simplicity of calculating the direct costs and the complexity of using mathematical models of the transmission dynamics of the infectious agent to calculate the indirect herd immunity related benefits. Such models with full age structure and other complexities have only entered the literature over the past 30 years and their use by policy makers in the field of vaccination has been limited to date.

Aside from early studies on rubella,[26] which did not address costs but did factor in indirect benefits, the first major study of indirect benefits and costs was in the evaluation of a varicella vaccine in the United States[29] in the 1990s. The study by Halloran and coworkers concluded that, although implementation of a vaccination programme resulted in a shift in the age distribution of the remaining varicella cases toward older ages with higher complication rates (as demonstrated in Fig. 1.11), the overall reduction in cases resulted in decreased morbidity as measured by overall number of hospitalizations and number of primary cases. They also argued that routine immunization with live-virus varicella vaccine would probably result in a substantial reduction in the number of uncomplicated primary cases of chickenpox, as well as a decreased number of complicated cases requiring hospitalization. This led to the introduction of this vaccine in the US national programme of immunization.

More recently, the uses of transmission models on which to base cost benefit calculations has expanded to cover a range of new and widely used vaccines. A recent example is that of the evaluation of influenza A immunization for all age groups in England, and not just for the very young and elderly who are at greatest risk of serious morbidity from infection.[30] Annual seasonal influenza vaccination is recommended for people most at risk of infection and its complications in many high-income countries. However, the age and clinical risk groups considered most at risk of infection, and hence targeted by vaccination, differ widely between countries.[31] The authors of the influenza A study noted that, despite the limitations of the available data, their study was one of the very few cost-effectiveness evaluations of seasonal influenza vaccination that used a transmission dynamic model to factor in the indirect effects of immunization.

They concluded that a well-matched vaccine to the strains in circulation would reduce the incidence of laboratory-confirmed influenza illness from 8.2% (95% range 4.3–13%) to 5.9% (95% range 2.9–9.7%), with 56–73% of this due

to indirect protection. They stressed that influenza A immunization is likely to be cost-effective, unless both low severity of the dominant viral strain, and poor vaccine matching to that strain, occurs. Their main conclusion was that the current seasonal influenza vaccination programme in England appears to substantially reduce disease burden and provides good value for money.

The lessons from both the varicella and the influenza A studies, is that accurate cost-benefit evaluations must be based on direct and indirect benefits. Additional considerations of the broader benefits of immunization include their impact on increased education and cognitive attainment, greater productivity in the work force, increased wealth by savings and investment, and a "demographic dividend", with fewer births and greater investments of parents in fewer children[32]

## 10 PARTIALLY EFFECTIVE VACCINES—EFFICACY VERSUS DURATION OF PROTECTION

As noted in the introduction, most of the "low hanging fruit" for vaccine development have been plucked, and the infections targeted by the majority of the currently available vaccines target pathogens where little antigenic variation exists. Influenza A is an exception, with drift and shift in antigenic composition of the circulating strains resulting in the vaccine being modified annually to match the appropriate viral strains.

Those pathogens that continue to cause a great deal of morbidity and mortality worldwide, for which we do not currently have effective vaccines, tend to be ones in which genetic variation in surface antigens is high, either resulting in a constantly moving antigenic landscape or a large number of strains with different antigenic compositions to target in any potential vaccine. To some extent, this problem has been addressed by the production of polyvalent vaccines for pneumococcal disease, HPV and rotavirus, but this raises the question of how natural selection might favor those stains not targeted in the current vaccines. This issue is addressed in a later section.

For the constantly moving antigenic targets presented by, for example, HIV-1 and *Plasmodium falciparum,* success in vaccine development has been limited to date. Recent results for a malaria vaccine look promising,[33] but the vaccine is partially efficacious in protecting against both infection and associated morbidity, and the long term duration of protection afforded to those immunized is uncertain at present. The first malaria vaccine candidate (RTS,S/AS01) to reach phase 3 clinical testing is partially effective against clinical disease in young African children up to 4 years after vaccination.[33] The results suggest that the vaccine could prevent a substantial number of cases of clinical malaria, especially in areas of high transmission. Such progress is encouraging, given the ability of the malaria parasites to generate antigenic variation in three different ways; namely, by mutation, recombination, and multiple but slightly different copies

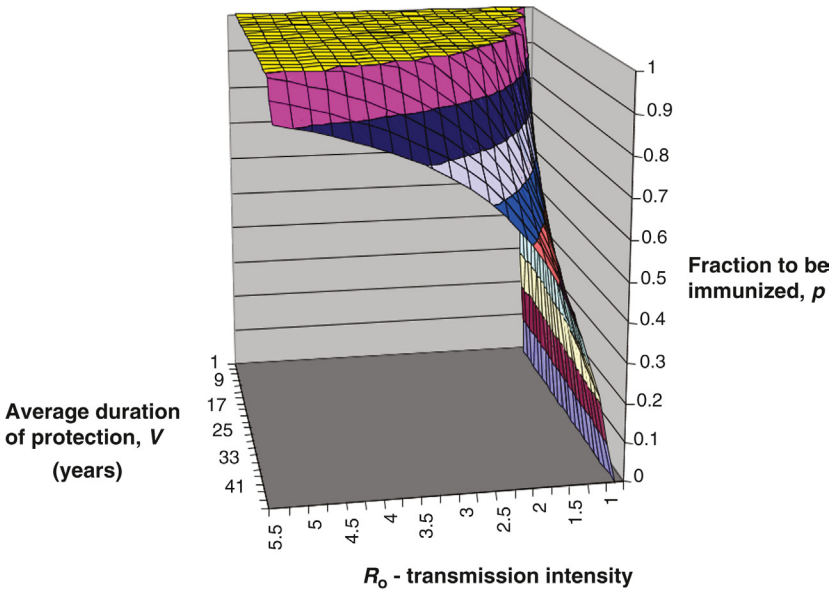


**FIGURE 1.17** **Protective vaccine with limited life of efficacy.** Critical proportion to be immunized, $p$, to block transmission as a function of $R_o$ and the duration of protection, $V$ (efficacy, $\varepsilon = 1$).

of the genes that encode for surface antigens (the so called var genes numbering approximately 60) whose expression can be switched on and off.[34]

Vaccines that are partially efficacious, and do not reach the target protection of 80–90% plus that most widely used childhood vaccines possess, and which vaccine developers aim for, may still be powerful public health tools in preventing infection and associated morbidity and mortality. It is not just efficacy that determines impact. The duration of protection is equally important. For a vaccine that provides protection for an average of $V$ years where life expectancy is $L$ years, the eradication criterion defined earlier by Eq. 1.8 must be modified to mirror the properties of the vaccine[34]

A relationship is plotted in Fig. 1.17 for various values of $R_0$ and the duration of vaccine protection $V$, from which it can be seen that short durations of protection makes blocking transmission difficult. However, a low efficacy vaccine can be very effective if the duration of protection it offers is long.[35]

In the case of HIV-1 vaccines (none available at present), the situation could be very complicated if immunization does not protect against infection but acts to reduced viral load. In this sense it would be acting as an immunotherapy. In trial studies of such products, if or when they become available, many epidemiological parameters must be measured. At a bare minimum, the following should be measured: (1) of those receiving the vaccine (a single or short course of injections), the fraction who seroconvert and seem to be immunized (the apparent efficacy); (2) average duration of protection relative to average lifespan

of sexual activity; (3) fraction of vaccinated individuals who, when exposed to the virus, become infected (vaccine failure rate); (4) ratio $q$ of the infectiousness of infected vaccinated individuals relative to that of unvaccinated people; and (5) ratio of the length of the average incubation period of AIDS in infected vaccinated individuals relative to that in unvaccinated persons.[35] Population outcomes from treating individuals with such immunotherapeutic products are many and varied, and will of course include perverse outcomes where immunotherapy may be beneficial to the individual but not to the community if it leads to continued low infectiousness and sustained risk behaviors.

The general issue surrounding the development of partially efficacious vaccines relates to the need in clinical trials, for potential vaccines against the more antigenically heterogeneous infectious agents, not only to measure efficacy (the fraction protected), but other properties as well. Most importantly, these include the prevention of morbidity as opposed to infection (as for the malaria vaccine RTS,S/AS01) and the duration of protection induced (Fig. 1.17).

## 11 SPATIAL AND OTHER HETEROGENEITIES

The real world is replete with complexity relating to the many factors that control the likelihood of transmission of an infectious agent between individuals. These heterogeneities can affect the simple concepts outlined earlier, on how mass vaccination influences observed epidemiological patterns, in many different ways. Perhaps the most important relates to space and the prevailing networks of contact between individuals that result in transmission. Genome sequencing of pathogens has increasingly offered a way of defining "who infects whom", and is being used to define networks of transmission events. A good recent example is that of the study of networks of HIV-1 transmission in Amsterdam, since the near the beginning of the epidemic in 1987–2007, based on specific gene or whole genome sequencing.[36]

Mathematical and computational tools are now available to facilitate incorporating the details of such social or transmission networks and the diffusion of infection, not only through time, but also across space. Such individual-based spatially structured stochastic models are computationally intensive, and also require many parameters to be measured or estimated.[37,38] They also do not necessarily permit the derivation of general insights into the controlling factors of observed pattern. However, they do permit much greater flexibility in determining how known heterogeneities influence the impact of a defined vaccination programme.

A schematic diagram of the level of detail that can be examined is presented in Fig. 1.18, showing three different scales for the study of transmission and the impact of control measures; namely, the household, the local network in which a person lives, and the larger spatial scale of people movements between home, work, and beyond. Increasingly, new tools are being used to populate the data demands of such models, including mobile phone tracking of an individual's movements. An illustration of this technique is presented in Fig. 1.19, which

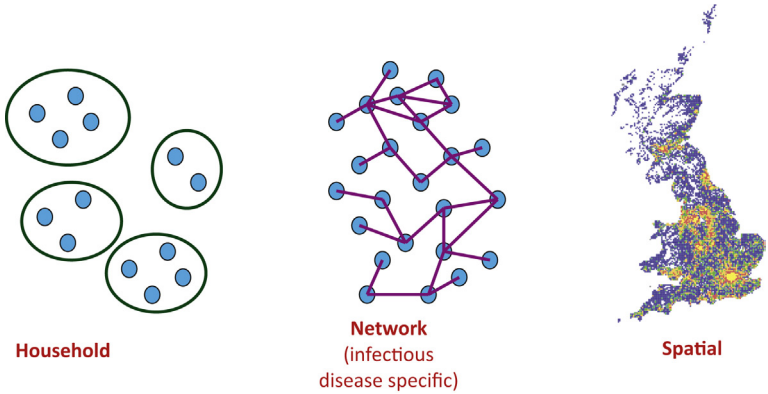


**FIGURE 1.18 Three different social and spatial scales commonly used to structure individual based stochastic simulations models of infectious disease spread.**

records data from a sample of 10,000 mobile phones in the United Kingdom (data anonymized), recording the distance moved per unit of time. The graph presents a frequency distribution of these recorded movements, and shows that most people move locally while a few move long distances. These long movements will be responsible for the jump of infection from one town to the next, or between cities and from country to country.

These simulations models of infectious disease spread and how vaccination impacts the epidemiology of a given disease, incorporating movements in space and different scales of people interactions, have been used in recent years to

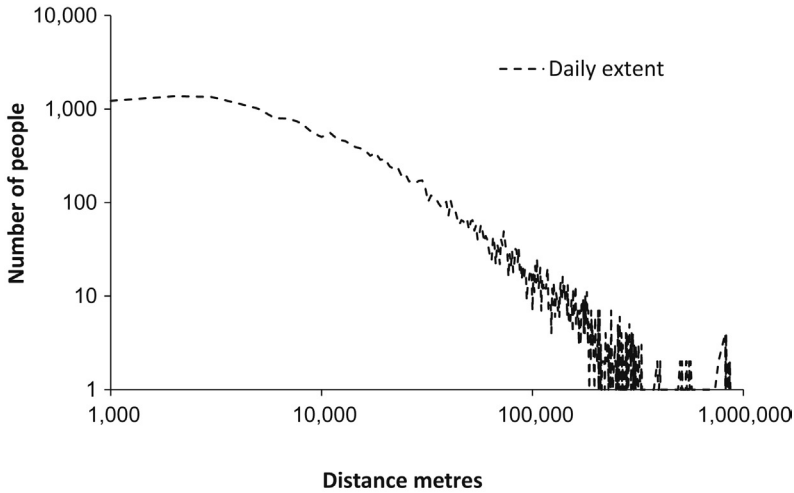


**FIGURE 1.19 Spatial kernels of people movement in the United Kingdom.** Anonymized mobile phone data from a sample of 10,000 people giving the frequency versus distanced moved per time unit of 1 day.

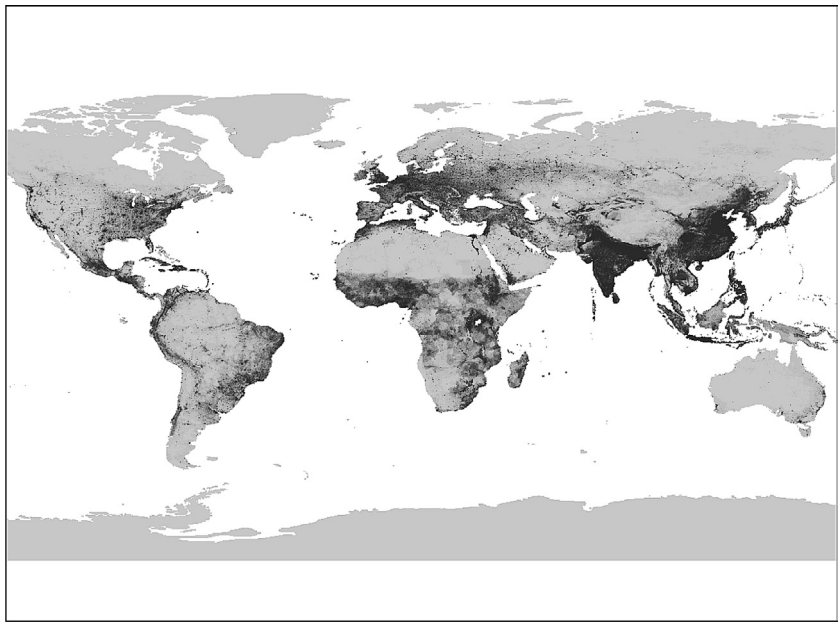

**FIGURE 1.20** **Global map of human population density in 2008 based on satellite imagery (from Oakridge National Laboratory, United States).** The darker the area the higher the density. *Light grey* denotes minimal human occupancy.

explore what might be optimal control strategies.[39,40] Some generalities emerge, which also come from more complex deterministic models that include crude representations of various forms of heterogeneity.[41] Spatial structure is important, and for many directly transmitted vaccine-preventable infections, cities with high population density serve to seed infection into smaller populations. Seeking to achieve very high vaccine coverage in these high density communities is clearly important to protect the population as a whole. This is of particular importance in many developing countries, where logistics dictate that getting vaccine to remote communities is difficult. In these circumstances high coverage in cities acts to protect remote areas.

Maps of the world-wide distribution of human population density, as shown in Fig. 1.20, reveal the densely crowded regions of the world, especially in Asia. These are the areas where the highest net rates of transmission of directly transmitted infectious agents pertain. Concomitantly, these are the areas where evolution of these infectious agents will probably be most rapid.

## 12 NATURAL SELECTION AND MASS VACCINATION

The advent of polyvalent vaccines for HPV, pneumococcal infection and rotavirus, for example, targeted at some subsection of the antigenic strains of the infectious agent circulating in a population, raises the question of whether or

not natural selection will drive the nontargeted stains to become prevalent. With the advent of a potential dengue vaccine, its variable efficacy against the four different serotypes also raise the possibility that when it is introduced it may act as a selective agent.[42]

Herd immunity will act as a very strong selective force and strain replacement is a very likely outcome. Epidemiological monitoring is most advanced for the pneumococcal vaccines and HPV, given the duration of time they have been in use. In the case of the pneumococcal vaccine, the products available have moved from a heptavalent vaccine (containing antigens to the six most common serotypes in children) to a 13 valent one from 2000 to 2012. Epidemiological evidence of strain replacement is clear cut—the vaccine imposes a strong selective pressure and once rare stains slowly become dominant under the pressure of herd immunity to their competitors.[43] To combat this trend, antigens from more and more serotypes are planned to be incorporated in future pneumococcal vaccines.

In the case of HPV vaccination, although concern was expressed about the selection of strains that then become oncogenic, to date the evidence for strain replacement leading to the circulation of newly recognized pathogenic strains is limited.[44] However, in general, where multivalent vaccines are employed, good molecular epidemiological surveillance is essential. This applies also to the potential wide scale use of future dengue, RSV (respiratory syncytial virus) and malaria vaccines.

## 13 DISCUSSION

Theory and epidemiological observation reveal a number of simple concepts that pertain in all cases where mass or cohort vaccination is used to control the spread of an infectious agent. First, eradication by mass vaccination will be difficult for infections that have very large basic reproductive numbers (the average number of secondary cases generated by one primary case in a susceptible population), especially when vaccine efficacy is less than 80–85%, in communities with high birth rates (constant renewal of the supply of susceptibles), or when vaccine coverage is heterogeneous especially in densely populated urban centres. Few infections fall in this category, but some such as measles, mumps, rubella, malaria (in hyper endemic areas), and RSV are certainly at the high end of recorded $R_0$ values. Interestingly, influenza A has low $R_0$ values (between 1.5 and 2.5), and hence if a universal vaccine became available, pandemics would be easy to control with moderate vaccine coverage.

Heterogeneity in vaccine coverage will continue to thwart achieving transmission eradication of most common childhood infections in many regions of the world. Poor coverage levels may be due to many factors, including simple logistics of reaching remote human communities, poor education of parents leading to a failure to appreciate the value of immunization and scare stories about vaccine safety which, although typically unfounded on epidemiological

evidence, continue to cause problems even in highly educated communities such as California in the United States.

Given that smallpox is the only directly transmitted infection to have been eradicated from humans, high levels of coverage against the childhood infections will have to be maintained while pockets of infection (eg, polio) remain in some countries, due to the high mobility of some people in our modern era.

Theory and observation show that mass vaccination acts to increase the interepidemic period, increase the average age at infection, and may in some circumstances create troughs in susceptibility in cohort immunization programmes, which may need to be filled by extending vaccination to a broader range of age classes. It is important that policy makers and public health workers are aware of these effects.

The future, in terms of the development of new vaccines, and vaccines for rare but lethal infections such as the Ebola virus, looks bright, given advances in basic plus applied research and manufacturing. The issue of adverse events, however, will not go away since all vaccines cause some morbidity in a very few individuals for reasons that are probably associated with genetic background. In the genes that matter in developing an immune response [HLA and the immune response (Ir) genes], humans show great genetic diversity (perhaps showing how important an ability to combat infections has been in the past evolution of *Homo sapiens*). As such, we all respond in different ways when exposed to foreign antigens. Modern societies demand safer and safer medicines, and although current vaccines are very safe, this will not prevent public reaction when adverse events in a very few vaccinated children occur. Indemnification of manufacturers by governments against these events will have to continue, if we are to maintain a vaccine producing industry. At present, there is a need to encourage more manufacturers into this field, to cope with world demand, especially in a crisis such as an influenza A pandemic

The future will undoubtedly see the production of some partially efficacious vaccines for the antigenically variable infectious agents. Indeed, this era is already with us, given new vaccines for falciparum malaria and dengue. However, care must be taken in the design of clinical trials for such products, to ensure measurement of not only efficacy against infection, but also changes in morbidity and duration of protection. In addition, sufficient funds must be set aside to ensure long-term phase IV monitoring of their impact on, for example, strain replacement patterns.

It is to be hoped that the remaining major cause of childhood infections can all be addressed by vaccination in the coming decades. It is also to be hoped that vaccines will be developed for some of the neglected tropical diseases in developing regions of the world, such as the soil transmitted helminths, schistosome parasites, and the filarial infections, that cause such a high burden of morbidity. The ability to develop effective vaccines against helminths in the veterinary field, argues that this should be possible for humans. Continued scientific progress in understanding both epidemiology and immunology will help to meet these aims.

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Chapter 2

# How Vaccines Work

**Claire-Anne Siegrist, MD, Paul-Henri Lambert, MD**
*University of Geneva, Centre of Vaccinology, Department of Pathology and Immunology, Geneva, Switzerland*

## Chapter Outline



## 1 INTRODUCTION

Most of the current vaccines are delivered through intramuscular or subcutaneous injection. What happens between the injection, the early reaction, and the induction of protective mechanisms is explained in this chapter. Vaccine responses depend on their interaction with the immune system and understanding the main features of this interaction may help designing vaccines and defining optimal vaccination strategies. Five steps are involved: (1) initial events at the site of injection and the draining lymph nodes (dLNs); (2) recognition of antigenic specificities at B- and T-cell level; (3) cell proliferation, maturation, and differentiation; (4) effector stage with production of antibodies and effector T cells; and (5) building up of immunological memory that allows later responses at the time of exposure to the specific pathogen. We will briefly review each of these steps and consider the importance of the vaccine types and vaccine formulations in the outcome of induced responses.

More emphasis will be given to vaccine-induced antibody responses. Indeed most of the current vaccines essentially work through effects of antibodies.[1] Antibodies can protect by a number of effector mechanisms.[2] They can bind to the enzymatic active sites of microbial toxins and prevent their action and

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diffusion. They can neutralize viral replication through preventing virus entry into their target cells. They can activate the complement cascade and promote opsonization and phagocytosis of bacteria by macrophages and neutrophils. These effects are essential to rapidly limit the microbial load and to help clearing extracellular pathogens from the body. However, critical factors influence vaccine-induced antibody responses.

## 2 WHAT FOLLOWS A VACCINE INJECTION? BASIS OF ANTIBODY RESPONSE TO PROTEIN VACCINES

When one injects a classical subunit vaccine (eg, influenza or tetanus toxoid) intramuscularly, the first reaction is local pain, followed by varying levels of swelling and redness. This reaction reflects an inflammation at the injection site, characterized by increased vascular permeability and local recruitment of inflammatory cells from circulating blood.[3]

The Lymph that may contain antigens and antigen-transporting cells arrives from the injection site through lymphatic channels. These channels open on the outer part of the dLN, in the subcapsular (or marginal) sinus. Antigens and antigen-containing cells are distributed through small conduits within the lymph node to the outer cortex and to the inner medulla. The cortex is filled with lymphocytes and the outer cortex contains aggregates of cells called follicles (B-cell zones). T cells are densely located around the cortical follicles and also extend to the medulla (Fig. 2.1).

## 3 VACCINE ANTIGEN RECOGNITION

Antibody responses to protein vaccines depend on their recognition by B-cell receptors and interactions between B- and T cells within lymph nodes. The immune system can recognize $10^7$–$10^9$ different antigenic moieties: B- and T cells carry a highly diverse set of antigen receptors that are generated in naïve cells through gene rearrangement.[4,5] Soluble antigens, such as those present in a classical influenza vaccine, which are drained from the site of injection to the marginal sinus of the local lymph nodes are translocated by specific subcapsular macrophages into the B-cell zone. If the vaccine forms a depot at the site of injection, for example, tetanus toxoid adsorbed to an aluminum salt, antigens are also captured by attracted monocytes/dendritic cells (DCs) which then migrate to the T–B cell zone border of the dLNs. There, antigen transported by activated DCs recruit and activate antigen-specific CD4 T helper cells that provide appropriate cofactors for the stimulation of antigen-specific B cells. This leads to the first step of the antibody response, called “extrafollicular” (Fig. 2.2). It is associated with B-cell proliferation and differentiation into plasma cells but the resulting antibodies are of low affinity and the response is short-lived.[6] It should be noted that replicating live vaccines which are usually injected subcutaneously, are more widely distributed than subunit vaccines. They can induce

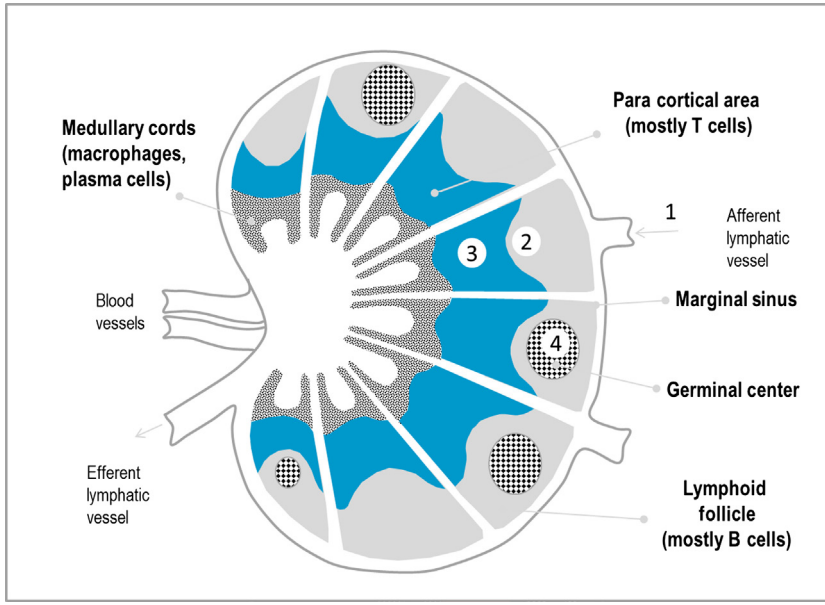


**FIGURE 2.1** **Lymph node architecture and vaccine antigens pathway.** First, vaccine antigens migrate from the injection site to the dLNs through afferent lymphatic channels (Step 1). They are then picked up by subcapsular macrophages in the marginal sinus and delivered to B cells in the B-cell zone (Step 2). These B cells benefit from a helper effect from T cells located at the T–B cell border (Step 3) and are essential elements of the germinal center (GC) that develops within lymphoid follicles (Step 4). The process will eventually lead to the differentiation of plasma cells and memory cells.

responses in multiple lymphoid sites largely dependant on the viral vaccine dynamics and tropism.

## 4 GERMINAL CENTER REACTION

The second step of the immune response is essential. It is the GC reaction. Activated antigen-specific B cells and CD4 follicular helper T cells (Tfh) are attracted by antigen-bearing follicular dendritic cells (FDCs) and form specialized units, GCs, within lymphoid follicles (Fig. 2.2). A GC can be considered as a B-cell factory. It is providing an optimal environment where within a few days, B-cell clones actively proliferate. This proliferation is associated with an extensive somatic hypermutation process that affects the variable-region segments of immunoglobulin. In some B cells, this results in higher affinity B-cell receptors. Such B cells compete efficiently for binding to the vaccine antigen that persists at the surface of FDCs. After antigen processing, they benefit from helper signals and further proliferate.[7] B cells also switch from IgM to IgA, IgG, or IgE antibody production. Finally, they mature either into antibody-producing plasma cells or into memory B cells. Plasma cells become detectable in blood

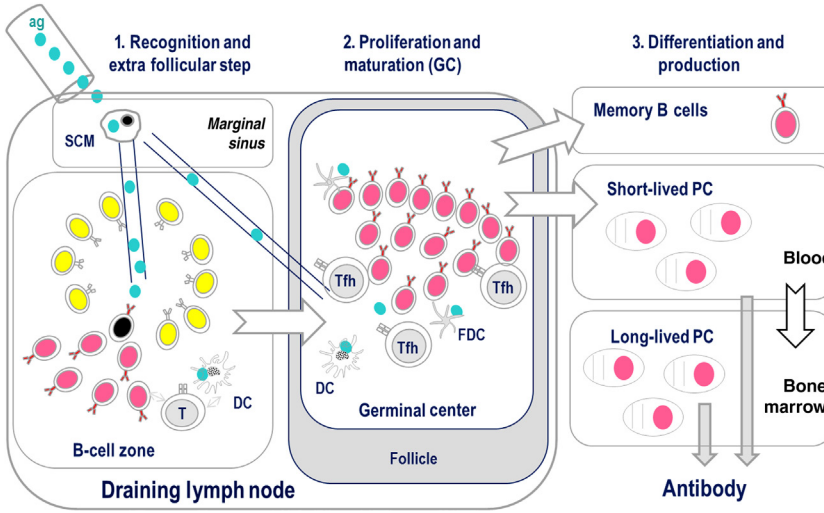


**FIGURE 2.2 Vaccine-induced B-cell responses.** Sequence of events leading from inflammation at the site of injection to lymph node localization, B-cell recognition, T-cell help, GC reaction and final differentiation into memory cells or antibody-producing plasma cells that may home into bone marrow niches. *FDC*, follicular dendritic cell; *DC*, dendritic cell; *Tfh*, follicular helper T cell; *PC*, plasma cell; and *SCM*, subcapsular macrophage.

after 10–14 days, reaching their peak at 4 weeks after immunization. Most plasma cells die after a few weeks. However, some home to "survival niches" in the bone marrow where they are rescued from apoptosis, become long-lived plasma cells, and are responsible for the prolonged persistence of antibody production.[8]

Some essential factors control the initial peak antibody responses: (1) antigen characteristics such as the epitopic structure (2) the administered dose, (3) B-/T-cell repertoires, largely influenced by genetics, and (4) the activation status of antigen-presenting cells and the generation of Tfh (GC control) that are dependent on the triggering of innate immunity by adjuvants and pathogen-associated molecular patterns. Adjuvants can optimize antigen delivery to B cells and activate DCs, follicular helper T cells and B cells.

The duration of the antibody response is largely dependent on the number of long-lived plasma cells that have been induced. For example, the persistence of HBsAg vaccine antibodies may be predicted on the basis of initial antibody titers.[9]

## 5 BUILDING B-CELL MEMORY

Sustaining protection using a protein vaccine is usually dependent on the administration of a booster dose of the same vaccine several months or years after the priming series. Following a booster dose, antibody levels rise rapidly with a peak around day 7. Antibody titers are higher than after the priming dose and the quality of these antibodies, for example, neutralizing capacity, is also better than in the

initial stages. This reflects a higher affinity. This antibody response (so-called secondary response) lasts longer than the primary one. In fact, secondary responses reflect the restimulation of persisting memory B cells that were induced during the primary immunization.[1] All protein vaccines induce memory. As previously described, memory B cells are produced within GCs in parallel with the induction of antibody-producing plasma cells. However, after migrating in the blood, they localize in the B-cell zone of all lymph nodes and remain quiescent until a new antigen encounter. Their reactivation can result from a natural microbial exposure (colonization, infection), exposure to cross-reacting microbial antigens, or booster vaccine doses. This leads to a very rapid cell multiplication and differentiation, explaining the rapid increase of antibody level (IgG) within 4–7 days. The improved quality of these secondary antibodies reflects the affinity maturation that takes place during the first phase of the immune response. During the GC reaction, random somatic mutations occur in B-cell immunoglobulin genes. Some of them confer a better capacity to bind to the vaccine antigen and these cells get a definite competing advantage in the context of limited availability of antigen. Within the GC, only those B cells that strongly bind antigen on FDCs receive the appropriate survival signals. Memory B cells continue their affinity maturation during several months and this leads to the persistence of cells that have at their surface immunoglobulin receptors of higher affinity than the antibodies produced as a result of the primary response. Thus, when reactivated, memory B cells produce antibodies of higher affinity.

In addition to the essential factors that control primary responses, a critical determinant of the quality of secondary responses is the timing of vaccine boosters. The interval between priming and boosting should be sufficient to allow for affinity maturation. A too short interval would result in additional primary dose effect. For example, in adolescents receiving two doses of 10 μg of hepatitis B vaccine, it was found that antibody responses measured 1 month after the second immunization were much higher after a 6-months interval than a 4-months interval.[10] Similarly, it was observed in young adolescents that superior responses were induced after a 0–6-months than a 0–2-months schedule with a bivalent adjuvanted HPV vaccine.[11]

Therefore, on the basis of immunological data, it appears now logical to consider as an ideal protein vaccine schedule, the following sequence: priming with one or two doses (at 1-month interval), then rest for 4 or 5 months to allow for B-cell response maturation, and then boosting at 6 months. Obviously, adjustments have often to be made in relation to programmatic limitations or epidemiologic considerations.

## 6 RESPONSE TO POLYSACCHARIDE VACCINES

Antibody responses to polysaccharides are independent from T-cell help and do not involve a GC reaction. Polysaccharides are presented to the immune system during bacterial infections or after vaccination (eg, pneumococcal

polysaccharide vaccines). This leads to the rapid induction of a wave of short-lived antibody-producing plasma cells. This is the end result of a sequence of events.[6] First, injected polysaccharides diffuse through lymphatic vessels to the local dLN. They enter the dLN through the marginal sinus and are captured by subcapsular sinus macrophages that translocate them to the marginal B-cell zone (Fig. 2.1). In the B-cell zone, the PS antigens are exposed to a large number of B cells and bind to the few cells that carry surface immunoglobulins able to recognize this particular PS antigen with a sufficient strength. The cross-linking of B-cell receptors results in the activation and proliferation of these B-cell clones which differentiate into short-lived antibody-producing plasma cells. The absence of GC reaction explains why vaccination with plain polysaccharide vaccines induces antibody responses that are relatively modest and do not last for very long. A characteristic of these responses is the poor induction of memory.[12] Usually a second injection of the same vaccine after a few months induces again a primary response similar to the first injection. However, the second administration may result in a decreased response to the PS vaccine or to a corresponding PS-conjugate. This hyporesponsiveness was first reported for meningococcal C polysaccharide vaccine and subsequently for pneumococcal vaccines and is probably reflecting an exhaustion of the antigen-specific B-cell reservoir.[13]

Responses to polysaccharides can be markedly improved through the use of protein-conjugated glycoconjugates.[14] When capsular PS are conjugated to a protein carrier, there is an effective GC response due to the differentiation of carrier-specific Tfh cells and PS-specific B cells which differentiate into high-affinity antibody-producing cells, long-lived plasma cells, and memory B cells.

The assessment of antibody responses is initially based on measurement of the amount of antigen-binding immunoglobulin (eg, ELISA assays) or the number of antigen-specific B cells (ELISPOT assays) before or after their in vitro reactivation by antigen. However, functional assays for example, viral or toxin neutralization assays, measurement of serum bactericidal or opsonophagocytic activity, and influenza hemagglutination inhibition may better correlate with vaccine efficacy.

## 7 VACCINE-INDUCED T-CELL RESPONSES

All protein vaccines induce T-cell responses. They are essential to support the induction of antibodies (helper effects). They also participate in effector mechanisms that contribute to reducing the microbial load and clearing pathogens in infections by viruses and intracellular pathogens and they play a major role in controlling immune responses and limiting the risk of concomitant autoimmune manifestations.

The production of T-cell precursors takes place in bone marrow where, like B cells, their initial repertoire are being developed through receptor gene

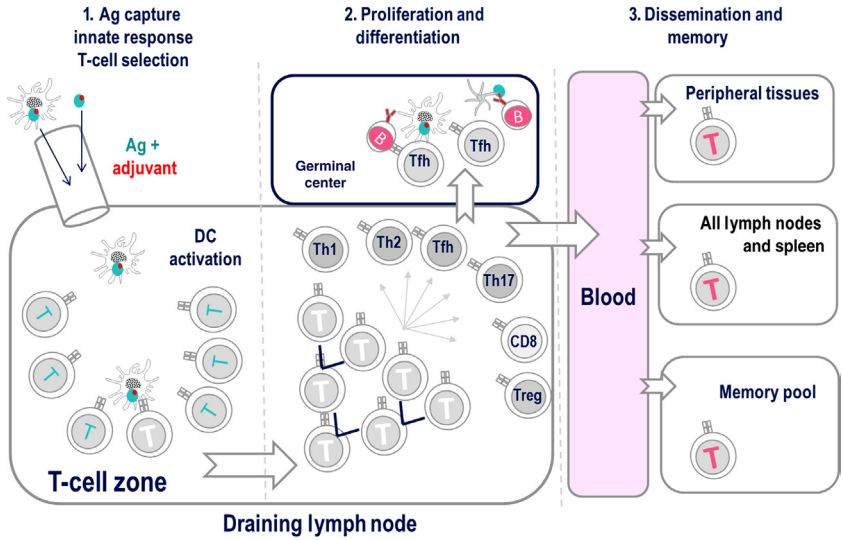


**FIGURE 2.3** **Vaccine-induced primary T-cell response.** Sequence of events leading from inflammation at the site of injection to lymph node localization, T-cell recognition, T-cell help, and final T-cell differentiation. *Ag*, antigen; *DC*, dendritic cell; *T*, T cell; and *Tfh*, follicular helper T cell.

rearrangements. This results in a hugely diverse capacity of recognition of antigen moieties. However, two major characteristics dominate the recognition pattern. First, T-cell receptors only see small peptides bound in the groove of HLA surface molecules. Second, T cells have to undergo a severe selection process in the thymus while maturing into potential effector cells (CD4, CD8). Paradoxically, the T-cell repertoire that participates in defense mechanisms against foreign invaders is initially selected on its capacity to bind self-peptide–HLA complexes on thymic cortical epithelial cells.[15] However, a subsequent negative selection takes place in the thymus medulla and only low affinity self-reactors leave the thymus. Medium-affinity cells form a pool of regulatory T cells. Interestingly, the protective function of vaccine-induced T cells is relying on cross-reactivity between self and foreign peptide moieties.[16] After vaccination, initial contacts between specific T cells and vaccine antigens primarily take place in the dLNs (Fig. 2.3). T cells recognize peptide–HLA complexes presented on DCs, in the T-cell zone of the dLN. T-cell activation and proliferation require a number of cofactors, including cytokines and other chemokines as well as cell-to-cell contact. As previously indicated, $CD4^+$ helper T cells and particularly Tfh strongly contribute to T-dependent B-cell responses. Some of the CD4 progeny cells primarily release Th1-type cytokines (IFN-γ, TNF-α/β, IL-2) and others Th2-type cytokines (IL-4, IL-5, IL-14, IL-6, IL-10), whereas Th17 cells produce IL-17, IL-21, IL-22. Conversely to B cells, T cells do not undergo extensive somatic mutations or affinity maturation in peripheral lymphoid organs after immune activation.

## 8 INNATE IMMUNITY AND T-CELL DIFFERENTIATION

How do vaccines induce more or less specific T cells of one or another cell type?

A key step is the activation of innate immunity. At the site of vaccine injection, the formulated antigens induce a local inflammation which favors the recruitment and activation of DCs as professional antigen-presenting cells. These cells capture the antigens as well as the accompanying adjuvant or microbial DC activator which makes them express activation markers while migrating toward the local dLNs. DCs, monocytes, and neutrophils can be activated by "danger signals" of microbial origin. They have receptors—including the so-called Toll-like receptors (TLR) which can recognize evolutionarily conserved pathogen patterns that differ from self-antigens.[17] Their activation results in the increased expression of critical surface molecules and the production of proinflammatory cytokines and chemokines.[18–20]

In the absence of activation signals, DC may present processed antigenic peptides to specific T cells in a tolerogenic mode. These DC remain immature and, on contact with naïve T cells, trigger their differentiation into regulatory $CD4^+$ T cells that maintain immune tolerance.[21]

A moderate level of DC activation, for example, when using aluminum salts as adjuvant, may favor Th2-type responses. Strong inflammatory signals favor the differentiation of naïve $CD4^+$ T cells toward the Th1 type. With some adjuvants, there is an induction of Th17 cells, which play a major role in tissue inflammation, particularly at mucosal level.

$CD4^+$ T cells may also differentiate into regulatory T cells (Treg), which inhibit T-cell proliferation/activation and help terminate a T-cell response. They also play an essential role in avoiding excessive cross-reactions against autologous tissues during responses to microbial aggression.[22]

Live viral vaccines introduce antigens within the cell cytosol, ensuring their access to MHC class I molecules. As a result, they also induce $CD8^+$ T cells which after activation can produce high amounts of IFN-γ and become potent killer cells. $CD8^+$ T cells recognize peptides associated with MHC-type 1 whereas $CD4^+$ T cells recognize peptides associated with MHC-type 2 molecules.[23]

## 9 VACCINE-INDUCED T-CELL MEMORY

T-cell memory is a critical component of immune responses to intracellular pathogens. Following the antigen-driven expansion and the death of effector cells after antigen clearance, some of the remaining T cells differentiate into memory T cells of two different types: central memory and effector memory T cells.[24] The first ones are located in lymphoid organs and bone marrow and have a high proliferative potential whereas the second ones stay in peripheral tissues in a preactivated form that enables them with immediate action on pathogen recognition. A third type of memory T cells (resident memory cells) was recently

recognized as memory T cells which remain settled within specific organs such as the intestine, the lungs, and the skin. They appear important for the protection against mucosal infections.[25]

It is useful to know that the establishment of T-cell memory requires some time after the initial priming. Secondary T-cell responses are lower if vaccine boosters are given too early. Through homeostatic proliferation, memory T cells may persist lifelong, even without antigen exposure.[26]

A number of T-cell parameters can be measured during vaccine studies. Some are quantitative for example, measurement of T-cell proliferation following antigen stimulation with a dye and quantification of T-cell frequencies by ELISPOT or flow cytometry. Some assays add a functional component, for example, assessment of the production of cytokines by ELISPOT or flow cytometry, or cytotoxic assays.

## 10 CONCLUSIONS

There is now a better understanding of the different factors that affect vaccine-induced responses. This allows for a more rational development of new vaccines and building more appropriate vaccination strategies. A particular attention is required for the selection of an appropriate formulation. Vaccine characteristics have a significant impact on the initial steps of immune responses, extrafollicular responses, and the generation of GCs. A critical aspect of vaccine efficacy is the duration of protection. In most cases, it depends on the quality of memory induced by priming doses. Both B- and T-cell memory are slowly maturing, which implies the need for a sufficient delay before giving a vaccine booster dose. Responses to live viral vaccines are more disseminated and the exposure to vaccine antigens is often prolonged, resulting into stronger- and longer-lasting responses. The understanding of how adjuvants influence the T- and B-cell machinery should help to define optimal pathways toward protective vaccine responses.

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Chapter 3

# Vaccine Design in the 21st Century

**Matthew J. Bottomley, MA, PhD, Rino Rappuoli, PhD, Oretta Finco, PhD**
*GSK VaccinesSrl, Via Fiorentina, Siena, Italy*

## Chapter Outline



## 1 INTRODUCTION

Vaccines are well-established medical interventions capable of preventing infectious disease. There are many notable vaccine success stories, starting more than 200 years ago with the earliest work by Jenner that led to a cowpox-based immunization to prevent smallpox disease. Subsequent work by Pasteur during the 19th century refined and consolidated the basis of vaccinology through the principles of isolation, inactivation, and administration of key components from disease-causing pathogens. Relatively soon, this basis had enabled the development of several "first generation" vaccines that afforded protection against rabies, typhoid, cholera, and plague (within the 19th century), followed by tuberculosis, yellow fever, and pertussis by the first half of the 20th century. Breakthroughs in mammalian cell culture technology in the second half of the 20th century led to the development of "second generation" vaccines, protecting against polio, measles, rubella mumps, and varicella (as reviewed previously[1]). In the late 20th century the first polysaccharide and glycoconjugate vaccines were developed, some of which have been refined and are implemented on a global scale.

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Despite estimates that vaccines have saved several hundred million cases of disease and more than 100 million deaths, there are still numerous pathogens causing globally significant morbidity and mortality, for which effective vaccines are not yet available. Here, we describe the existing and emerging technologies and strategies that we believe will be crucial for design of next generation vaccines to address unmet medical needs relevant across the world in the 21st century.

## 2 STRATEGIES FOR MODERN VACCINE DESIGN

### 2.1 Glycoconjugate Vaccines

In the mid-20th century, plain polysaccharide vaccines were developed to protect against pneumococcal, meningococcal, and *Haemophilus influenzae* type B (Hib) infection and disease. Such vaccines were based on the use of capsular polysaccharide (CPS) preparations derived from the surface of these bacteria. The high abundance and surface-exposure of CPS make them readily accessible to antibodies and thus susceptible to opsonophagocytosis and complement-mediated bactericidal killing, the two main processes underlying polysaccharide vaccine-induced immunity. However, plain polysaccharide vaccines were effective in adults but not in infants and young children, and therefore improvements were required.

A major breakthrough in the 1980–90s was the development and implementation of glycoconjugate vaccines, using CPS components chemically conjugated to carrier proteins,[2] such as the chemically detoxified diphtheria or tetanus toxoids (DT or TT), or CRM197 a nontoxic mutant of diphtheria toxin.[3] Covalent coupling of CPS to a carrier protein enables recruitment of T-cell help, resulting in the generation of an affinity-matured and protective immune response in all age groups. The first glycoconjugate vaccine targeted Hib and dramatically reduced Hib meningitis and patient mortality following introduction in North America.[4]

While the great majority of Hib disease was caused by one serotype, more complex epidemiology exists for many other pathogens, for which several immunologically distinct serogroups (or serotypes) circulate and cause disease. For such pathogens, broadly protective glycoconjugate vaccines can be designed by including multiple CPS serogroups in a "multivalent" formulation. For example, a highly successful 7-valent glycoconjugate vaccine against *Streptococcus pneumoniae* conferred large reductions in pneumococcal meningitis and invasive pneumococcal disease in all age groups, between 1998 and 2007.[5] However, while such multivalent vaccines are broadly protective, there are now more than 90 distinct disease-causing pneumococcal serotypes, suggesting that an alternative pneumococcal vaccine based on one or a few highly conserved protein antigens, rather than a complex formulation of many different CPS components, would increase breadth of protection and ease of manufacturing.[6]

Glycoconjugate vaccines have also been developed and implemented to protect against *Neisseria meningitidis*. In 1999 a monovalent formulation was introduced in the United Kingdom to control the hyperendemic *N. meningitidis* serogroup C (MenC). Routine nationwide implementation directly reduced MenC disease, acquisition and carriage, and conferred a herd protection effect.[7] Subsequently, tetravalent glycoconjugate vaccines have been licensed to protect against *N. meningitidis* serogroups A, C, W, and Y.[8] Perhaps most remarkably of all has been the rapid development and broad deployment of the monovalent glycoconjugate vaccine (MenAfriVac™) to protect against MenA in sub-Saharan Africa, a region that experiences annual meningococcal outbreaks and devastating epidemics. The MenAfriVac vaccine was pioneered by the "Meningitis Vaccine Project" (MVP)[9] and within a decade it was administered on a large public health scale in several neighboring African countries with excellent results both in preventing MenA disease and in eliminating carriage, likely aided by strong herd protection.[10,11] Building on the success of the MVP, a similar pentavalent glycoconjugate vaccine to protect against MenACWYX is now under preclinical development. Promising preclinical studies have also shown that glycoconjugate vaccines of MenX CPS combined with CRM197 could be developed to protect against MenX, currently emerging in Africa.[12] However, a glycoconjugate vaccine against MenB is generally not considered viable because the MenB CPS resembles a neuraminic acid moiety present on human tissues, shows poor immunogenicity in humans, and generated debate regarding the risk of undesirable autoimmune responses.[13,14]

Glycoconjugate vaccines are also under clinical development to combat Group B streptococcus (GBS)[15] and *Salmonella* Typhi.[16] Further, while an early small-scale clinical trial using a glycoconjugate vaccine against *Staphylococcus aureus* was promising,[17] subsequent *S. aureus* trials have failed, as discussed recently.[18] Nevertheless, new trials are ongoing for a multivalent staphylococcal vaccine containing both protein and glycoconjugate antigens.[19]

Recent research has continued to build on the great achievements of the glycoconjugate vaccine field, especially by attempting to improve CPS production and conjugation methodologies. Standard glycoconjugates are prepared by CPS purification from the cultured pathogenic bacteria, followed by CPS fragmentation to generate poly- or oligosaccharides of specific composition and size. A recently developed alternative that avoids pathogen manipulation is the use of purified recombinant polymerases directing capsule biosynthesis to enable safer production of the CPS in vitro.[20] Alternatively, the impurities and batch variability associated with bacterial CPS generation could be eliminated by using chemically synthesized oligosaccharides.[21] Indeed, a synthetic oligosaccharide Hib vaccine showed clinical results comparable to those obtained using standard Hib vaccines.[22] In any case, following CPS/oligosaccharide preparation, conjugation to the carrier protein is typically a chemical reaction that covalently attaches the oligosaccharide to one sort of amino acid (usually lysine, aspartic/glutamic acid, or cysteine), available on the surface of the carrier protein.

The latter is therefore not a precisely defined site-specific conjugation, such that some variability in the glycoconjugate product is obtained. To reduce variability, a variety of chemistry-driven methods have been developed to enable controlled site-specific glycoconjugation, in principle offering to deliver glycoconjugate vaccines with better-defined labeling sites and stoichiometry.[21] Alternative genetic-based approaches are also conceivable, where rare codons could direct the incorporation of nonnatural amino acids into a recombinant carrier protein to enable its site-specific labeling with defined oligosaccharides.[21] Both these examples open the possibility to add saccharide units in selected well-exposed regions of a carrier protein without masking its beneficial protective epitopes (Fig. 3.1).

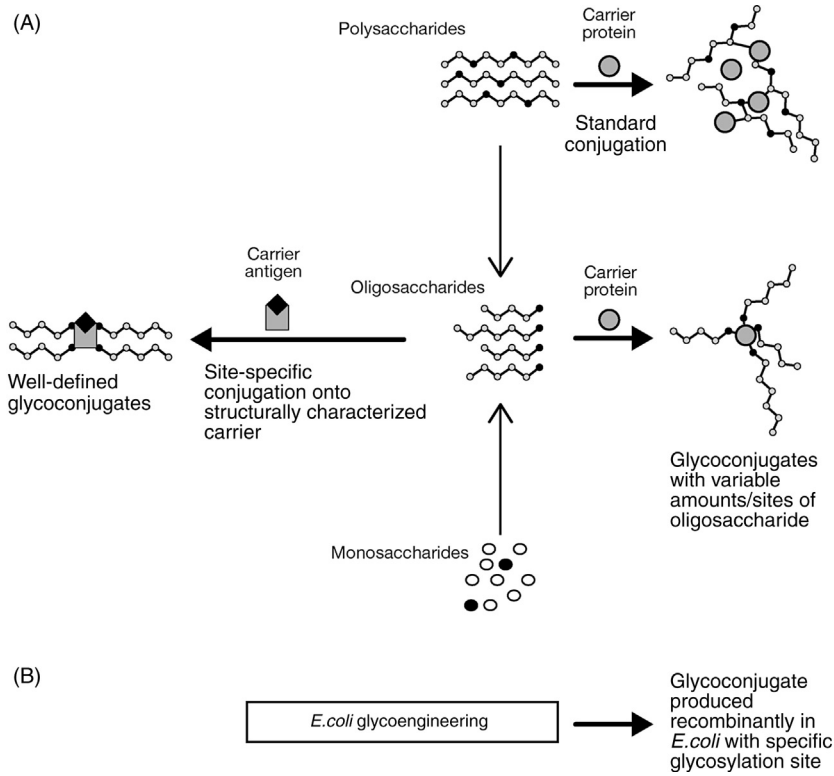


**FIGURE 3.1 Glycoconjugate vaccines.** (A) Progression from plain polysaccharide antigens to polysaccharide-carrier glycoconjugates (right) enabled the development of more efficacious vaccines against Hib, *S. pneumoniae*, and *N. meningitidis*. Ongoing refinements in oligosaccharide production processes, site-specific conjugation strategies, and the use of new carrier antigens that simultaneously present oligosaccharides and protective protein epitopes (♦) is expected to potentiate the development of novel well-defined glycoconjugates with enhanced safety and efficacy profiles (left). (B) An important new alternative approach to generate glycoconjugate vaccines is represented by the use of genetically modified *Escherichia coli* to directly produce recombinant glycoprotein antigens.

Novel site-specific chemical glycoconjugation approaches have shown initial promise yet appear to be hampered by relatively low yields. One study demonstrating improved conjugation yields used a copper-free reaction mechanism for tyrosine-specific labeling.[23] Further, advances in the ability to directly produce protein antigens with posttranslational addition of specific polysaccharides in *E. coli* has opened new possibilities to generate "bio-conjugate" vaccines.[24] In preclinical studies, the approach was successful in generating antigens protective against *S. aureus*.[25] Moreover, a promising bio-conjugate vaccine against *Shigella dysenteriae* was made using the polysaccharide component of the *Shigella* O1 lipopolysaccharide conjugated to exotoxin protein A of *Pseudomonas aeruginosa*, and both the CPS and carrier components were immunogenic,[26] suggesting that the bio-conjugate approach may be broadly applicable. Similarly, attempts to make hybrid antigens by combining CPS and protein carrier components that both target the same pathogen (rather than simply coupling the CPS to an unrelated carrier) have shown promise in preclinical studies targeting *Clostridium difficile*, by chemically conjugating the clostridial PSII polysaccharide and the TcdA and TcdB toxin proteins.[27] Indeed, evidence that a protein can act both as CPS carrier and as an immunogen emerged from clinical studies using pneumococcal polysaccharides conjugated to protein D from nontypable *H. influenzae*.[28] In such cases the design of conjugation sites should include structural considerations, such that CPS moieties do not perturb beneficial conformational epitopes on the carrier protein. Continued efforts in this arena will accelerate the journey toward "precision" bio/glycoconjugate vaccines safely produced in vitro with scalable production processes.

## 2.2 Protein Subunit Vaccines and Structure-Based Antigen Design

While glycoconjugate vaccines have been highly effective, they are not the only modern vaccine strategy available. Indeed, several early protein-based vaccines have been very successful. Notably, by purification from host pathogens followed by chemical inactivation, toxoid protein vaccines were developed in the early 20th century against diphtheria and tetanus; and, soon after, inactivated influenza virus vaccines were developed, using viral hemagglutinin purified from infected eggs as the main antigen. Later, in the 1980–90s, several bacterially produced protein subunit vaccines were licensed to protect against pertussis, and led to the proposal of a genetically detoxified form of the pertussis toxin (PT) that showed superior immunogenicity over chemically detoxified PT.[29] Finally, late in the 20th century, efforts to develop a vaccine against hepatitis B virus (HBV) led to the first widely implemented vaccines composed purely of a recombinant protein subunit (the HBV surface antigen, HBsAg).[30]

Subsequently, efforts to generate new recombinant protein-based vaccines were initiated for many other disease targets that had been difficult to address via previous technologies. One interesting example is the case of *N. meningitidis*

serogroup B (MenB), where a CPS-based vaccine was not feasible (mentioned earlier). Consequently, a protein-based subunit vaccine against MenB was sought. This challenge was greatly facilitated by the dawn of the genomic era at the turn of the 21st century, which accelerated the computational identification and selection of potential meningococcal protein antigens, an approach now termed "reverse vaccinology."[31] Extensive computational and experimental screening led to the identification of three main protective protein antigens[32] and later the development and licensure of the first genome-derived recombinant protein-based vaccine (Bexsero®) against MenB, approved by the European Medicines Agency in 2013, and subsequently in over 35 countries.[33] Reverse vaccinology has been applied to several other vaccine research programs, with promising results in the quest for protective antigens against GBS,[34] extraintestinal pathogenic *E. coli*,[35] and *S. aureus*[36] to name a few examples.

Reverse vaccinology indeed presents a speedy route to candidate identification, yet frequently reveals antigens for which prior biological information is unavailable. Given the high attention focused on vaccine safety, it is desirable that the antigenic composition of any formulation is extremely well-characterized and understood when proceeding with clinical trials, in order to ensure safety, antigen formulation stability, and reproducible vaccine efficacy. Detailed biochemical, biophysical, and structural biology investigations can combine effectively with functional studies to provide the high degree of antigen characterization required to support the vaccine development process.

In addition to providing exquisitely detailed antigen characterization, it has also been demonstrated over the last decade that structural biology, powered by X-ray crystallography, electron cryomicroscopy (cryoEM), nuclear magnetic resonance spectroscopy, and computational studies, can make a very significant contribution to the design and optimization of vaccine antigens.[37] A number of key studies demonstrating the combination of computational and structural biology in vaccine antigen design (an approach termed "structural vaccinology") have been reported, as reviewed recently.[38]

Structural vaccinology is a multidisciplinary strategy that combines the insights gained through high-resolution structural and computational biology studies with neighboring fields such as formulation science, immunology, animal studies, and serology, in order to design, evaluate, optimize, and deliver leading candidate vaccine antigens. There are at least three key ways in which structural biology can support vaccine research. First, structural biology can highlight potential weaknesses in an antigen, such that issues of poor biochemical behavior can be resolved; as exemplified by studies leading to the design of a novel form of the respiratory syncytial virus (RSV) glycoprotein F antigen in a highly stable nonaggregating postfusion conformation capable of raising high titers of neutralizing antibodies in preclinical studies.[39] Second, structural studies can reveal conformational heterogeneity in an antigen, which may suggest routes to engineer mutated forms of the antigen that adopt only the preferred conformation most likely to elicit the desired immune response. For example,

the crystal structure determination of the RSV F protein in complex with the Fab fragment of the human antibody D25 (specific for an antigenic site targeted by potently neutralizing antibodies) provided the first detailed insights into the atomic structure of the prefusion F conformation.[40] Moreover, that structure enabled the design of site-directed mutations that locked the F protein in the prefusion conformation, via the introduction of stabilizing intramolecular disulfide bonds and hydrophobic cavity-filling residues, yielding an immunogen capable of eliciting high-titers of RSV-specific neutralizing activity in mice and macaques.[41] Third, when combined with epitope mapping studies that identify the regions of an antigen that are crucial for raising protective or neutralizing antibody responses, structural information can be used to generate novel immunogenic protein surfaces with enhanced breadth of coverage due to the introduction of epitopes from multiple pathogenic variants onto a single vaccine antigen. This strategy of epitope grafting was demonstrated by engineering the meningococcal factor H binding protein variant 1 to display more than 20 surface-exposed residues from variants 2 and 3, thus generating a novel hybrid surface that conferred broader strain protection and overcame the issue of high sequence variability on meningococcal surface antigens.[42]

Structural vaccinology has been applied extensively in research toward a vaccine against human immunodeficiency virus (HIV). Efforts have focused on designing immunogens that raise protective antibody responses targeting the gp120 or gp41 components of the HIV envelope glycoprotein (Env) trimer, the only target for neutralizing antibodies. For example, the structure of CD4-bound gp120 was used for the rational design of a gp120 construct with mutations that lock it in the receptor-bound state, thus eliciting a greater proportion of antibodies focused on conserved CD4 and coreceptor binding sites.[43] Recently, cryoEM and crystal structures of HIV Env (in genetically engineered soluble and stabilized mutant forms) have been determined in complexes with broadly neutralizing Fab fragments.[44–46] These structures have provided the molecular basis for the design of novel immunogens capable of eliciting broadly neutralizing antibodies against HIV Env, and it is now a major ongoing challenge to develop such research into efficacious vaccines.

In an even more creative fashion, structural vaccinology has been combined with nanobiology, via the design of self-assembling protein nanoparticles presenting multiple copies of an antigen in an ordered array. For example, in seeking to design a broadly protective influenza vaccine, a single genetic construct was used to encode an influenza hemagglutinin (HA) antigen followed by a C-terminal bacterial ferritin protein, thus generating nanoparticles composed of 24 ferritin protomers that self-assembled to display 8 copies of the trimeric HA in a native-like conformation, with the HA head projecting outward. In preclinical studies, this antigen-nanoparticle was successful in raising anti-HA antibodies targeting both the stem and the receptor-binding site in the head, and provided broader and more potent immunity than standard influenza vaccines.[47] More recently, the same authors also performed iterative structure-based design

to obtain a stable HA stem-only fragment displayed on ferritin nanoparticles. This novel HA stem-only nanoparticle lacked the immunodominant sequence-variable head domain, focused the immune response onto the immunogenically subdominant highly conserved stem region of HA, and conferred heterosubtypic protection in preclinical studies.[48]

The benefits of combining structural vaccinology and nanobiology are manifold. The considerably larger antigen-nanoparticle is more immunogenic than the individual recombinant proteins, the multiple copies in ordered arrays enhance B-cell receptor avidity and mimic the surface of the natural pathogenic organism, and the ability to genetically encode antigen display on a nanoparticle means that a precisely controlled number and orientation of antigenic constructs can be achieved, potentially allowing focusing of the immune response against a carefully selected region of the antigen identified previously by epitope mapping. It emerges from these pioneering studies that structural vaccinology has the potential to drive the design of promising new vaccine candidates, and this ability is inextricably linked to obtaining high-quality structural information, which is somewhat unpredictable and a potential hurdle, but which is becoming easier to overcome due to continuous improvements in protein crystallography[38] and major breakthroughs in cryoEM.[49] These purely structural techniques can be effectively combined with the complementary ability to reliably perform mapping of conformational epitopes in solution via hydrogen–deuterium exchange mass spectrometry (HDX-MS).[50] Because structural vaccinology is also dependent on the ability to perform epitope mapping using antibody reagents, several recent technological advances in human B-cell cloning and antibody production have potentiated structure-based antigen design enormously, and these breakthroughs are discussed later.

## 2.3 B-Cell Repertoires, Antibody Discovery, and the Human Immune Response

For more than 30 years it has been known that antibody-mediated immune responses are crucial for preventing infection, while T-cell-mediated effector mechanisms are important in controlling the clearance of virus-infected cells. Antibodies are the primary elements of adaptive immunity, and the induction and maintenance of protective levels of antibodies underlie the basis of the immune response to vaccination. The B-cell response is initiated by the cognate interaction between activated antigen-specific T cells and B cells that have captured and processed the antigen through the B-cell receptor (BCR). The cognate T–B interaction leads to the expansion of antigen-specific B cells and to their differentiation into short-lived plasma cells, which represent the first line of defense through the production of unmutated antibodies, usually of the IgM isotype.

The extra-follicular aforementioned response is followed by formation of the germinal center (GC) in the lymphoid organs. The GC reaction is driven by

the presence of the antigen on the surface of the follicular dendritic cells (FDCs) in the form of immune complexes, and the antigen:antibody immune complexes continuously stimulate resident antigen-specific B cells.[51] The interaction of B cells with follicular helper T cells ($T_{FH}$) within the GC drives proliferation, isotype switching, somatic hypermutation, and affinity maturation of the BCR leading to the generation of memory B cells and long-lived plasma cells that produce high-affinity somatically mutated antibodies of switched isotypes (typically IgG).[52] Plasma cells with higher affinity for the antigen that emerge from GCs can migrate to the bone marrow, where they persist in specialized survival niches.[53] This pool of long-lived plasma cells continuously secretes antibodies, and is therefore responsible for sustained serum antibody levels even in the absence of antigen.[51] Memory B cells generated by a GC reaction recirculate in secondary lymphoid organs and peripheral blood, are highly capable of capturing the antigen due to their high affinity BCR, and can be triggered to proliferate and differentiate into antibody-secreting plasma cells once they reencounter the antigen. Typically, the newly generated plasma cells reach a peak level in the blood on day 7 after antigenic boost and antibody titers concomitantly increase in the serum.[54]

Not all antibody responses are equally effective. T-cell-independent antibody responses to free polysaccharides are known to be short-lived, whereas T-cell-dependent antigens can elicit immunity lasting for decades or a lifetime. The continued dissection of the basic mechanisms defining the dynamics of the immune response to vaccination and a deeper knowledge on the correlates of vaccine-induced protection or biological signatures of responsiveness are fundamental aspects in the development of novel vaccines in the 21st century.

Nearly all licensed vaccines confer protection against infectious diseases by stimulating the production of antibodies by B cells, but the nature of a successful antibody response has been difficult to capture. The isolation and characterization of the antibodies produced by the antigen-specific B-cell repertoire has therefore acquired importance in the last decades, to dissect the response to vaccine antigens. Antibodies consist of heavy (μ, α, γ, δ, ε) and light chains (κ, λ), are linked by disulfide bonds, and each chain contains variable and constant domains. Antigen binding occurs in the variable domain, which is generated by recombination of a finite set of tandemly arranged variable (V), diversity (D), and joining (J) germline gene segments. This process, called VDJ recombination, assures a high diversity of the antibody repertoire and allows antibodies to recognize an extraordinary variety of antigens. Diversity in the antibody repertoire is mainly concentrated at the variable site of the heavy chain (IgH VDJ gene segment), also known as the IgH complementarity-determining region 3 (CDR-H3), the most diverse component in terms of length and sequence and the principal determinant of antibody specificity.[55]

A milestone in the understanding of antibody responses has been the development of technologies for the production of human monoclonal antibodies (mAbs) by using Epstein–Barr (EB) virus transformation,[56] by phage display,[57]

in genetically modified mice,[58,59] by stimulation with TLR agonists,[54] or by producing human hybridomas[60] for immortalization of antibody-producing B cells. Since 2008, advances in sequencing technologies have enabled the amplification and cloning into expression vectors of both the heavy and light chain immunoglobulin (Ig) genes from single B cells,[61] allowing isolation and synthetic production of human mAbs by transfection of producer cells in vitro. To date, this technology has been mainly applied to identify high-affinity influenza-specific antibodies[62] and to isolate broadly neutralizing antibodies (bnAbs) against HIV.[63] These first examples of the isolation and characterization of bnAbs induced by infection have highlighted that understanding the mechanisms leading to the elicitation of neutralizing antibodies can aid the design of more effective vaccines. Such methods have been used to investigate mAbs generated against a variety of antigens, and have allowed characterization of "key" antibodies with a protective role in response to vaccines against influenza, tetanus, Hib, and some serotypes of *S. pneumoniae* as well as to natural infection (reviewed in[64]). Nonetheless, one key limitation is the low-throughput of single B-cell cloning technology used to isolate mAbs, such that we can only interrogate a miniscule slice of the full antibody repertoire.

Recent advances in next-generation sequencing (NGS) technology have enabled the sequencing of antibody genes from millions of cells simultaneously, giving a high-resolution characterization of the antibody sequence repertoire, and of the changes that occur following vaccination.[65] These approaches have yielded important insights into the B-cell response and have raised the possibility of using specific antibody sequences as measures of vaccine immunogenicity. The antibody repertoire has been examined using NGS after vaccination with influenza and tetanus.[66,67] These studies revealed minor changes in the VDJ segment usage, and the size and diversity of the different B-cell lineages after vaccination, but they have opened up the possibility, through the analysis of the B-cell repertoire of different individuals, to identify "antibody signatures" (common Ig VDJ sequences) providing insights into the adaptive immune responses elicited by vaccination. The majority of published studies are consistent with the notion that while the VH gene repertoire is highly private (unique to an individual) a small number of CDR-H3 appear to be shared among different individuals (ie, are stereotypical or public). Boyd and coworkers[68] observed convergent antibody signatures (stereotyped CDR-H3 sequences) in patients experiencing acute dengue infection, suggesting that Ig-sequencing aimed at detecting stereotypical responses could be used as a tool for identifying common sequences induced by vaccine antigens or pathogens in different individuals.

Further, analyses of the human antibody repertoire offer the novel possibility of tracing the evolutionary paths that lead to the generation of broadly neutralizing Abs (bnAbs) targeting conserved antigenic epitopes. The availability of new techniques to isolate human mAbs, combined with the ability to determine protein structures in atomic detail, allows to finely describe

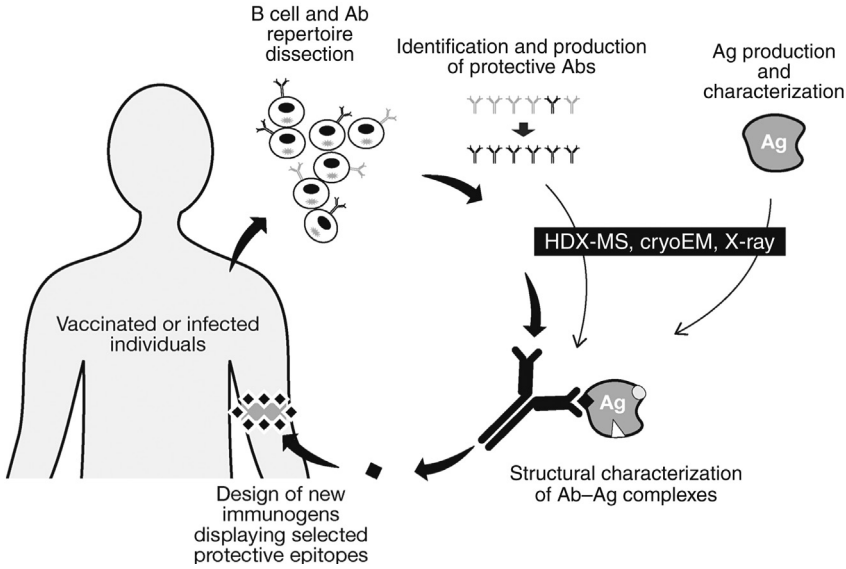


**FIGURE 3.2** Starting center-left, a schematic flow-path representation of how human B-cell repertoire analyses, the selection of protective antibodies, antibody and antigen (Ag) production can be combined with the structural characterization of a protective epitope (♦), followed by its selection over nonprotective epitopes (○ and Δ within Ag, not targeted by protective Ab) in order to allow the rational design of novel immunogens.

antigen:antibody interactions. Such high-resolution epitope mapping enables the design of novel immunogens and vaccination schedules that will elicit an immune response driven by a B-cell clonal selection that leads to the production of the best bnAbs[69,70] (Fig. 3.2). Many advances in this field have been driven by the quest for a vaccine to prevent HIV infection, but should be applicable to combat other pathogens like pandemic influenza and RSV.

Finally, in recent studies, the analysis of the Ig gene repertoire has been combined with the mining of the antigen-specific mAb repertoire that comprises the human serum polyclonal response.[71] The new perspective offered by combining the analysis of the B cell and antibody repertoire induced by tetanus toxoid (TT) vaccination has highlighted that the anti-TT serum IgG repertoire is composed of a limited number of antibody clonotypes (80–100) while the B-cell BCR repertoire diversity in the memory and plasmablasts compartments is orders of magnitude greater than that of the serological repertoire.[72] This suggests that most peripheral B-cell-encoded antibodies are unlikely to be present in detectable amounts as soluble proteins in blood or secretions and thus are unlikely to contribute to humoral immunity, leaving unanswered questions regarding the nature and dynamics that regulate the serological memory. Collectively, these examples of our growing understanding of the immune response highlight a new era in which a detailed understanding of pathogenic antigen-specific

human immunology can drive vaccine development in the design of more efficacious vaccine antigens to prevent current and future pathogenic threats.

## 2.4 Nucleic Acid Vector Vaccine Delivery Systems

Most licensed vaccines used to date are based on immunizations that elicit a protective antibody response and indeed the correlates (or surrogates) of protection established are typically based on the functional antibody levels induced.[73] However, the immune system has evolved to be redundant, and nonantibody-based cellular immune mechanisms, which can act alone or in synergy with antibodies, can provide a major contribution to protection. With this in mind, significant efforts have been made to design novel vaccines focused on induction of cellular responses able to promote clearance of some of the most challenging pathogens, which have so far proved recalcitrant to traditional vaccine design strategies, such as malaria, tuberculosis, HIV, hepatitis C virus, and Ebola virus.

In particular, $CD8^+$ T-cell responses have been demonstrated to contribute to protection in both preclinical and clinical experiments.[74] One way to elicit such $CD8^+$ responses (which are poorly induced by conventional protein subunit antigens) is via the delivery of DNA vectors harboring genes encoding intracellular antigen expression. Several approaches have been explored to achieve this aim, including the use of naked DNA fragments or virally derived systems based on alphavirus, poxvirus, vaccinia virus, or lentivirus. Replication-defective human adenovirus 5 (Ad5) vectors have been used for gene delivery in a number of vaccine development studies and showed promising immunological performance in preclinical and clinical trials, most importantly including the ability to induce relatively potent antigen-specific $CD8^+$ T-cell responses in humans, for example, against HIV[75] and Ebola.[76] However, most humans have been previously exposed to Ad5 and thus present high titers of anti-Ad5-neutralizing antibodies, which limit the immunological potency of these vector delivery systems.

To circumvent the limitations of human adenoviral vaccine vectors, an alternative approach has been developed using related naturally occurring simian adenoviral vectors isolated from chimpanzees and against which most humans do not display neutralizing antibody titers. From thousands of adenoviral strains, a library containing numerous replication-defective chimpanzee adenovirus (ChAd) vectors able to grow in human cell lines was developed and several were demonstrated to potently induce $CD8^+$ T-cell responses in mice and macaques, and some were shown to be safe and immunogenic in humans.[77] The many noncross-reactive ChAd strains appear to be suitable candidates as vaccine delivery vectors, such that preexisting neutralizing antibodies should not be an issue for broad application of this strategy, which may enable a versatile "one vector—one disease" approach. Indeed, a number of studies have now demonstrated that ChAd vectors have the essential properties required for human vaccine development, including immunogenicity, safety and ease of large-scale

manufacturing.[78] Further recent developments in viral-based delivery of genetic vaccines include a heterologous prime-boost strategy based on the combination of a ChAd vector followed by a modified vaccinia Ankara (MVA) vector.[79] Promising results were obtained by generation of very high levels of both $CD8^+$ and $CD4^+$ T-cells specific for the hepatitis C virus antigens delivered genetically, suggesting that this approach may be suitable as a prophylactic HCV vaccine. The clinical efficacy of ChAd vectors is still to be fully demonstrated. However, in rapid response to the recent West African outbreak of Ebola virus that caused more than 8500 deaths, an expedited vaccine development program enabled a clinical trial to assess performance of a monovalent ChAd3 vaccine encoding the surface glycoprotein of *Zaire ebolavirus*. In Phase I trials, the vaccine was safe and immunogenic,[80] further supporting the optimism surrounding ChAd technology.

For over 2 decades it has been known that RNA molecules can be used to express proteins in vivo,[81,82] suggesting opportunities for RNA-based vaccines as an alternative strategy to elicit immune responses (reviewed elsewhere[83]). RNA vaccines display several advantages compared to DNA vaccines. RNA avoids the issue of possible integration of plasmid DNA into the genome of an immunized host, and it is translated directly in the cytoplasm. Finally, the kinetics of antigen expression following RNA injection appear to peak and decay rapidly, while DNA administration can induce antigen expression persisting for many weeks.[84] Overall, RNA-based vaccines better mimic antigen expression occurring during an acute infection, which could induce stronger antigen-specific immune responses. The effectiveness of RNA vaccines may also be related to the fact that RNA is known to be a potent stimulator of innate immunity. Hence, the functionality of RNA vaccines involves at least two components: (1) local expression of antigen to facilitate presentation by MHC molecules and (2) engagement of pattern recognition receptors to stimulate innate immunity leading to potentiation of antigen-specific immune responses.

Although studies in animal models seemed to be very promising, the feasibility of using RNA as a new nucleic acid vaccine was initially challenged, due to the instability of naked RNA in the presence of tissue fluids and the uncertainty of developing reasonable manufacturing processes yielding a stable formulation. Nevertheless, several efforts have been made to increase the efficiency and stability of RNA-vaccines, focusing the research on delivery systems, adjuvants, and engineering of the RNA molecule. Encapsulation in liposomes[81] and complexation with cationic polymers[85,86] can protect RNA from degradation and enhance cellular uptake. Moreover, self-amplifying replicons have the potential of capturing the advantages of both DNA vaccines and viral delivery while overcoming the drawbacks of each technology. Recently a self-amplifying RNA was encapsulated in lipid nanoparticles (LNPs) to implement the self-amplifying mRNA (SAM®) vaccine technology as a platform for multiple disease targets, showing promising results in animal models.[87] These favorable observations led RNA-vaccines to move into human clinical trials as

immunotherapeutics in the "cancer-vaccine" field, taking advantage of the expression of specific markers by cancer cells to direct the immune response and attack the tumor. RNA vaccines against proteins produced in excess in tumor cells were used to formulate a vaccine against lung cancer, designing a vaccine with different antigens which is consequently better at targeting the tumor cells.[88] Clinical studies in metastatic melanoma and renal cell carcinoma patients have shown the elicitation of antigen-specific immune responses (both antibodies and T cells).[89] RNA-vaccines against prostate cancer and melanoma are currently in clinical trials. The use of RNA-vaccines for the prevention of infectious diseases is also under evaluation. Clinical trials have been performed with RNA replicon vaccines packaged in viral particles encoding for cytomegalovirus (CMV) gB and pp65/IE1 proteins. The vaccine has shown to be well tolerated and immunogenic in healthy CMV seronegative volunteers, with the added value of inducing $CD8^+$ T-cell responses.[90] A vaccine against rabies is currently in a clinical trial (https://clinicaltrials.gov/ct2/show/NCT02241135) while vaccines against influenza, HIV, or tuberculosis are still at the research stage.

The future of the RNA vaccines will rely on the formulation with new synthetic delivery systems to combine the effectiveness of live attenuated vaccines, an equal or better safety profile than plasmid DNA vaccines, and completely synthetic methods of manufacture.

## 2.5 Synthetic Viral Seeds for Rapid Generation of Influenza Vaccines

Because new influenza variants emerge and spread globally through human populations so rapidly, it is not always possible with current health organizations and manufacturing capabilities to provide new, well-matched influenza vaccines in a timely manner. In pandemic scenarios, little if any vaccine has been available during the initial waves of virus spread.[91] Recent efforts to improve vaccine responses to the emergence of new influenza variants have included research into universal influenza vaccines, increasing the number of strains in each vaccine, and increasing the speed of vaccine production. Indeed, synthetic biology now enables the rapid conversion of digitally transmitted sequences into genes that encode new influenza variants,[92] thus providing a unique tool to rapidly respond to the need of pandemic vaccine availability.

The synthetic approach to generate vaccine viruses from sequence data has proven to be feasible, starting from the available hemagglutinin (HA) and neuraminidase (NA) gene sequences, and applying cell-free gene assembly techniques for rapid and accurate gene synthesis. Viral RNA expression constructs encoding HA and NA and plasmid DNAs encoding viral backbone genes were then used to transfect Madin–Darby canine kidney (MDCK) cells, qualified for vaccine manufacture. Viruses for use in vaccines were rescued from MDCK cells with increased yield of the essential vaccine antigen, HA. The

implementation of synthetic vaccine seeds has demonstrated the capability of accelerating the response to influenza pandemics reducing the time required for vaccine manufacturing from months to weeks. In a recent emergency to respond to a potential influenza pandemic, the use of a synthetic seed virus, containing the HA and NA genes from a supplied A/H7N9 virus sequence, was investigated in conjunction with the MDCK cell culture technology. Together, these approaches resulted in impressively rapid vaccine production rates, much faster than currently possible with standard methods. Synthetic technology has been used to respond to the H7N9 influenza outbreak by producing a synthetic virus that was used to make a vaccine. In a Phase I trial the cell culture–derived H7N9 vaccine was safe and immunogenic, with significant and potentially protective immune responses after two doses in most subjects with no preexisting immunity to the H7N9 virus.[93] This particular vaccine was stockpiled by the US Government before the second wave of the outbreak, and overall these observations have provided a strong rationale for further clinical development of synthetic vaccine reagents.

## 3 CONCLUSIONS AND FUTURE OUTLOOK

The development of partially effective plain CPS vaccines led to the development of the first highly effective glycoconjugate vaccines around the end of the 20th century. Several glycoconjugate vaccines are now available to protect against many strains of pneumococcus, meningococcus, and *H. influenza* type B. In the first decades of the 21st century, further refinements in glycoconjugation technologies, and large clinical trials, are expected to deliver new glycoconjugate vaccines broadly protective against several additional globally important pathogens.

Nevertheless, glycoconjugate vaccines are not suitable to protect against many other important pathogens, where instead protein subunit vaccines containing protective immunogens may be effective. Recombinant protein vaccines against hepatitis B virus and serogroup B meningococcus are now widely available. The biochemical, biophysical, and three-dimensional structural characterization of protein antigens can play a major role in enabling the design and optimization of protein immunogens. The application of this strategy, termed structural vaccinology, coupled with immunological insights that can now be obtained via analyses of B-cell repertoires from infected or immunized humans, and antibody discovery and production technologies, is likely to be a key driver in vaccine development in the 21st century, and is already starting to deliver strong candidate vaccine antigens to protect against HIV, RSV, and influenza.

While most licensed vaccines are based on antibody-mediated protection, novel nucleic acid vaccine strategies capable of inducing potent cellular responses are under development to combat pathogens such as malaria, HCV, ebola, and HIV, which have so far resisted standard protein-based vaccine strategies. Notably, several replication-defective simian adenovirus nucleic

acid vectors have been shown to induce strong T-cell responses and are safe and immunogenic in humans, underlining the potential of this genetic vaccine approach. Similarly, RNA vaccines are emerging; they offer several benefits over DNA vaccines and, with improved synthetic delivery systems and manufacturability, appear to be applicable to protect against cancer or infectious disease.

In a distinct arena of vaccine technology, in order to be ready to meet the future demands of possible influenza pandemics, notable progress has been made in using cell culture technology to produce the virus, potentially from a rapidly generated synthetic nucleic acid seed, such that vaccine production can be expedited at large scale.

Collectively, all the advances outlined here demonstrate that the future is bright for the design and development of novel vaccines. Considering the additional possibilities presented by their formulation and delivery using next-generation technologies, including an increasing array of potent adjuvants (see chapter: Vaccine Adjuvants), these novel 21st-century vaccines have great promise to further reduce morbidity and mortality on a global scale.

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Chapter 4

# Vaccine Adjuvants[a]

**Steven G. Reed, PhD, Mark T. Orr, PhD, Rhea N. Coler, MSc, PhD**
*Infectious Disease Research Institute, Seattle, WA, United States*

## Chapter Outline



The widespread use of effective vaccines against infectious diseases has been one of the most important public health advances in the 20th and 21st centuries. Early vaccines consisting of attenuated or inactivated pathogens or toxins may elicit robust, protective immune responses, but this approach cannot always be used because it is impractical to culture large numbers of organisms, lack of efficacy, or because of safety concerns. In such cases subunits (eg, microbial proteins or carbohydrates) are being promoted as vaccine antigens. Subunit antigens are often poorly immunogenic on their own as they do not properly stimulate innate immunity. This is likely the cause of the reduced efficacy of the acellular pertussis vaccine.[1] Adjuvants are molecules, compounds, or supramolecular complexes that boost the potency and longevity of specific immune response to antigens, but cause minimal toxicity or long-lasting immune effects on their own.[2–4] Adjuvants can be used to enhance immunogenicity, modulate the type of immune response, reduce the amount of antigen or the number of immunizations required for efficacy, and/or improve the efficacy of vaccines in specific populations (eg, newborns or elderly). To be maximally effective, adjuvants must be selected judiciously and formulated appropriately based on the desired immune response.[5–7]

First generation adjuvants were empirically developed to augment the immune response to insufficiently immunogenic antigens. Of these, only aluminum salts—including aluminum oxyhydroxide and aluminum phosphate (collectively, alum)—and squalene based oil-in-water (o/w) emulsions (eg,

a. How do vaccines work: understanding immune responses to vaccines.

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AS03 or MF59™) have been included as part of FDA-licensed vaccines[8] However, the number of adjuvants with acceptable efficacy and safety profiles is limited, and these proprietary molecules/compounds are in the hands of a few companies, as is most of the formulation expertise. Lack of access to appropriate adjuvants, and lack of know-how regarding the formulation and use of adjuvants is one of the primary barriers to the development of new effective vaccines and immune therapeutics.

Critical to the early emergence of effective immune responses is the engagement of the innate immune system, characterized by the involvement of innate pattern recognition receptors (PRR) such as the toll-like receptors (TLRs) or the RIG-I-like receptors (RLRs) that recognize pathogen associated molecular patterns (PAMPs), leading to the production of cytokines and chemokines. In turn, these activate antigen presenting cells (APC) in particular dendritic cells (DCs) that initiate a cascade of signals to cells of the adaptive immune response, preparing them for the development of antigen-specific immunity. Thus one key strategy for improving vaccine performance involves the stimulation of innate immunity that facilitate antigen uptake, stimulation of antigen presenting cells, and downstream adaptive immunity. However, many adjuvants fail during product development owing to factors such as manufacturability, stability, lack of efficacy, unacceptable levels of tolerability, or safety concerns.

This chapter outlines the potential benefits of adjuvants in current and future vaccines and describes the importance of formulation and mechanisms of action of adjuvants. Moreover, we emphasize safety considerations and other crucial aspects in the clinical development of effective adjuvants that will help facilitate effective next-generation vaccines against tuberculosis (TB) and other global vaccine challenges.

## 1 BRIEF ADJUVANT HISTORY

More than a century ago, key findings of an inducible immune response after immunization with inactivated cowpox resulted in the development of vaccines.[9,10] Vaccination is now considered the best strategy available to efficiently control infectious diseases and thus lower morbidity and mortality rates. Among the most promising vaccination strategies are the protein subunit vaccines that present desirable qualities for a vaccine, which are specificity, efficacy, safety, and ease of production.[11] In 1926 alum was the first adjuvant that was used in a vaccine against diphtheria.[12,13] For several decades after this initial adjuvant use, o/w emulsion components were the only formulations available to adjuvant vaccines (Table 4.1). Alum and emulsion adjuvants proved safe and substantially increase the efficacy of numerous vaccines, yet until the last two decades little work was done to determine the mechanisms of action of these adjuvants or develop next generation adjuvants. The development and FDA licensure of the Cervarix vaccine for human papilloma virus (HPV) which includes a combination adjuvant comprised of alum and the TLR4 agonist monophosphoryl lipid

**TABLE 4.1** Adjuvant Formulation Platform Characteristics

| Formulation | Composition | Manufacturing method | Size | Surface charge | Delivery routes |
|---|---|---|---|---|---|
| Aqueous/ micellar suspension | Buffer, phospholipid, or surfactant | High pressure homogenization | ~20–100 nm | Neutral, cationic, or anionic | i.m., s.c., i.d., i.n., oral |
| Alum | Aluminum oxyhydroxide or Aluminum phosphate | Gentle mixing | ~1–10 μm | Cationic | i.m., s.c. |
| Emulsion | Metabolizable oil, phospholipid or surfactant, antioxidant | High pressure homogenization | ~100 nm | Neutral | i.m., s.c. |
| Liposome | Phospholipid, cholesterol with or without saponin | High pressure homogenization | ~100 nm | Neutral, cationic, or anionic | i.m., s.c., i.d., i.n., oral |

(MPL®, collectively termed AS04) in 2009 marked a key turning point in clinical adjuvant development.[14] AS04 is the first FDA-licensed adjuvant to include a known PAMP. This licensure hinged on the demonstration that AS04 induced a more effective immune response against the HPV antigen than alum alone.

## 2 ADJUVANTS IN CURRENT APPROVED VACCINES

Aluminum salts (Aluminum phosphate and aluminum hydroxide; alum), o/w emulsions ( MF59 and AS03$^{TM}$), and monophosphoryl lipid A (MPL), a natural glycolipid derived from *Salmonella* cell membranes, are all components of approved preventative vaccines against infectious disease.[15] AS03 and MPL are owned by GlaxoSmithKline[16] and MF59 is owned by Novartis. Alum is a component of several licensed human vaccines, including diphtheria–pertussis–tetanus (DPT), diphtheria–tetanus (DT), DT combined with hepatitis B virus (HBV), Haemophilus influenza B or inactivated polio virus (IPV), hepatitis A (HAV), Streptococcus pneumonia, meningococcal, and HPV.[17]

## 3 ADJUVANT DEVELOPMENT

Since vaccines are often employed prophylactically in populations of very young people, it is important that medical risks to the subject (ie, safety) and other adverse effects (ie, tolerability) are addressed. Vaccine adjuvants designed for therapeutic uses, such as in cancer, may have a different risk–benefit profile.

Adjuvant development is based on enhancing and shaping vaccine-induced responses without compromising safety by selectively adding well-defined molecules, formulations or both.[8,15] They thus offer the potential to compensate for a lack of innate immune stimulation, enhance the longevity of the antigen-specific immune response and can improve protection in a pathogen-specific manner.[18] Addition of adjuvants to some vaccines may substantially reduce the amount of antigen and/or number of immunizations required to achieve the desired immune responses.[15,19]

Adjuvants are most often developed in conjunction with specific vaccine candidates with a focus on optimizing the immune responses needed to protect against a specific infection. With the exception of Bacille Calmette Guerin (BCG) for TB and the shingles vaccine, licensed vaccines primarily work by eliciting protective antibody responses. Therefore adjuvant development has largely focused on boosting the humoral immune system, which both alum and emulsion adjuvants achieve while maintaining the favorable safety profile needed for prophylactic administration. Alum and emulsion adjuvants chiefly augment humoral immunity by driving production of endogenous danger-associated molecular pattern (DAMP) molecules which activate inflammasome cascades to produce IL-1β.[17]

Next generation vaccines against challenging infections such as TB, human immunodeficiency virus (HIV), and malaria will likely require induction of effective cellular immunity to augment the humoral immune response. Development of adjuvants to boost cellular immunity including Th1 and CTL immunity represents one of the greatest unmet needs in vaccine adjuvant development. An emerging strategy to meet this need is to incorporate PAMPs into vaccine adjuvants as there is a robust network of crosstalk between the innate and adaptive immune system. A number of PAMPs have been tested as vaccine candidates, with the TLR ligand class of molecules being the most clinically advanced, including the TLR4 ligand MPL incorporated into several vaccines. Signaling through TLR can result in two signaling cascades: the first is dependent on the molecule MyD88 (for myeloid differentiation Factor 88) downstream of TLR1, TLR2, TLR4, TLR5, TLR6, TLR7, TLR8, and TLR9; the second is dependent on TRIF (TIR-domain-containing adaptor-inducing interferon-b) and is associated with TLR3 and TLR4.[20,21] The recognition of PAMPs by TLRs can result in the expression of costimulatory molecules such as CD40, CD80, and CD86 as well as the expression of proinflammatory cytokines (IL-1, IL-6, IL-8, IL-12, TNF-α, COX-2, and type1 interferons) that collectively shape the development of an adaptive immune response by both B and T lymphocytes.[22] As shown by the development of AS04, PAMPs including TLR4 agonists can also boost the efficacy of the humoral immune response over first generation adjuvants including alum and o/w emulsions. This may be due to greater induction of cellular immunity, in particular T follicular helper cells, which shape the humoral immune response.

## 4 MECHANISMS OF ACTION

Alum was originally thought to boost the immune response by slowing the release of the antigen from the immunization site thus prolonging antigen presentation (depot effect). However this hypothesis has recently been disproven, at least in animal models, as excision of the immunization site within hours of injection did not impair the adaptive immune response. Rather alum's adjuvant activity depends on the production of IL-1β downstream of the release of DAMPs such as uric acid and host DNA which are released by damaged cells and recognized by specific receptors.[23–26]

MF59 consists of an oil (squalene)-in-water nanoemulsion composed of <250 nm droplet; it is licensed Europe in influenza vaccines.[27] MF59 formulation has also been tested with herpes simplex virus (HSV), HBV, and HIV vaccine candidates. Overall, MF59 has an excellent safety profile, and with several antigens significant increase in antibody titers with reportedly more balanced Th1/Th2 responses than those obtained with alum. MF59 causes a local increase in extracellular ATP in the muscle which leads to recruitment of monocytes, macrophages, and granulocytes and production of cytokine and chemokine which shape the adaptive immune response.[28] Injection of apyrase to hydrolyze the ATP reduces the T cell and antibody responses to MF59 adjuvanted vaccination.[29]

## 5 FORMULATION

Vaccine formulation is a critical component of development. Vaccine antigens and adjuvants can be made more effective by employing appropriate particulate delivery systems to increase cell uptake and provide sustained release of antigen and the active pharmaceutical ingredients (Fig. 4.1). Particulate formulations such as liposomes, aluminum gels, and micellar suspensions are more amenable to uptake by APCs, which specialize in the phagocytosis of invading particulate pathogens. The most high profile example of the importance of adjuvant formulation comes from the early development of the malaria vaccine RTS,S. Using a human

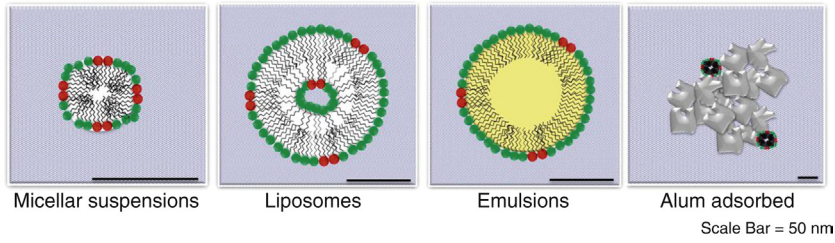


**FIGURE 4.1 Adjuvant formulations.** *Green* indicates polar phospholipids, *red* indicates TLR4 agonists such as MPL or GLA, *yellow* indicates an oily core.

challenge model of malaria infection it was shown that unformulated MPL did not improve the efficacy of RTS,S, whereas formulating MPL in an o/w emulsion or liposome dramatically increased the protective efficacy of the vaccine.[30]

*Aqueous/Micellar Formulations*: Amphiphilic molecules such as some TLR4 ligands self-assemble into aggregates upon exposure to aqueous medium. High energy input with sonication or microfluidization in the presence of small amounts of surfactant enables particle size reduction until a nanosuspension is achieved. Such "aqueous formulations," in some cases, have shown equivalent immunogenic activity compared to more complex formulations, such as those described later. A minimum of excipients and a simple manufacturing procedure involved in the production of micellar formulations indicate good product potential. In other cases, adjuvants (eg, CpG ODN) are water-soluble so the simplest formulation is a buffered solution. Aqueous formulations are excellent candidates for multiple routes of administration including indtradermal.

*Alum-adsorbed Formulations*: In this formulation, agonists are adsorbed onto aluminum oxyhydroxide (alum) particles. Some TLR agonists (MPL or CpG ODN) strongly adsorb to alum. The alum particles consist of nanometer crystals that assemble into aggregates of several micrometers and provide a stable particulate formulation for the sustained release of adjuvant. Given that the FDA recently approved GSK's cervical cancer vaccine Cervarix® containing a MPL-alum adjuvant formulation these formulations have a proven commercial track record.[31,32] Alum-based formulations are designed for intramuscular or subcutaneous administration.

*Emulsions*: o/w emulsions consist of metabolizable oils emulsified with biocompatible surfactants in an aqueous bulk phase. Emulsion droplets are ~100 nm in diameter and are stable for years. MF59 and other emulsions have been shown to effectively and safely induce immune responses to influenza antigens, including enabling dose sparing.[33–35] Emulsion formulations are generally administered intramuscularly or subcutaneously.

*Liposomes*: Liposomes consist of vesicles formed by the assembly of phospholipid bilayers. These vesicles can be made at ~100 nm diameters and have good stability. Liposomes are versatile and biocompatible vehicles for adjuvant formulation. Amphiphilic molecules can be localized within the lipid bilayer, while more hydrophilic agonists such as TLR7/8 or TLR9 agonists can be encapsulated in the aqueous interior of the liposomes, electrostatically associated to the liposome surface, or solubilized in the aqueous bulk phase.[36–38] Liposomes may be administered intranasally, orally, intradermally, intramuscularly, or subcutaneously.

## 6 ADJUVANTS FOR UNMET NEEDS; HIV AND TUBERCULOSIS VACCINES

The only licensed vaccine against TB, BCG, is effective in limiting the severity of childhood TB, but does not prevent infection or cases of adult pulmonary TB. Therefore there is an urgent need to develop new vaccines to augment or

replace BCG. The need for new TB vaccines, along with vaccines for malaria has largely driven the development of new adjuvants to boost cellular immunity against infectious diseases. For TB the dominant concept is to drive Th1 CD4+ T-cell responses, the response most associated with protection against TB, to prevent the transition from latency to disease (or to prevent primary disease progression). Such a response is characterized by the production of cytokines such as gamma interferon (IFN-γ), which is responsible for macrophage activation; tumor necrosis factor (TNF), which is important for granuloma development and maintenance; and interleukin 2 (IL-2), which is responsible for the clonal expansion of T lymphocytes and is thus involved in maintaining the memory immune response.[39–41] There are several TB vaccine candidates in clinical studies that include recombinant proteins and a number of different adjuvants.[42–45] These include AS01 and AS02, MPL formulated in liposomes or an o/w emulsion developed by GSK; GLA-SE, a synthetic TLR4 agonist in an o/w emulsion developed by the Infectious Disease Research Institute; CAF01, consisting of trehalose dibehenate (a ligand for the C-type lectin receptor Mincle) formulated in liposomes and developed by Statens Serum Institut; and IC31, a cationic peptide and TLR9 agonist oligodeoxynucleotide. In each of these adjuvants both the immunostimulatory PRR agonist and formulation have been extensively studied and optimized to drive Th1 responses to specific vaccine antigens.

Surprisingly the field of adjuvant development for HIV has lagged behind those of TB and malaria, with most candidate vaccines being either unadjuvanted or adjuvanted with alum or o/w emulsions. For HIV vaccine development, it is becoming increasingly apparent that antibody durability is an important consideration, and, as with influenza vaccines, breadth of antibody responses covering more than one serotype of virus is important also. The development of broadly neutralizing antibodies against HIV often requires extremely long CDR regions, requiring extensive germinal center interactions between HIV-specific B and T cells. Thus adjuvants that promote cellular immunity, especially T follicular helper cells needed to sustain germinal centers, may be critical to the development of an effective HIV vaccine.

Adjuvants are increasingly reaching advanced development and licensing stages, providing new tools to fill previously unmet clinical needs. In addition to regulating the breadth of protective immunity adjuvants can significantly improve vaccine manufacturing capacity by increasing vaccine dose- and dosage-sparing. This may be particularly important for vaccines for diseases for which the economic rationale for vaccine development is weak (eg, diseases primarily affecting developing, rather than developed, nations) or in the case of pandemics such as newly emerging influenza strains such as H5 or H7 avian influenza. As these new adjuvants are developed they will need to demonstrate the ability to fill a clear unmet need and provide superior benefit over an unadjuvanted vaccine or existing adjuvants which have a long track record of safety in humans. Despite these regulatory hurdles the development

of new classes of vaccine adjuvants and new insights into the mechanisms of adjuvants, both new and old, in the last 10 years signal the start of a new age of vaccine adjuvant development.

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Chapter 5

# Vaccine Production: Main Steps and Considerations

**James M. Robinson, PE**
*James Robinson Biologics Consulting, Merck & Co Inc, New Jersey, United States*

## Chapter Outline



The vast majority of the more than 1 billion doses of vaccines manufactured worldwide each year are given to perfectly healthy people.[1–4] It is this fact that drives the requirements for vaccines to be among the most rigorously designed, monitored, and compliant products manufactured today.[5]

This chapter provides a high-level overview of typical manufacturing processes for major vaccine types, outlines important considerations in the development and maintenance of vaccine manufacturing processes, highlights some key challenges faced by manufacturers of vaccine products, and outlines some of the dilemmas faced by the vaccine manufacturer.

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## 1 MANUFACTURING BASICS

The dictionary definition of a vaccine is "a biological preparation that provides active acquired immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing microorganism and is often made from weakened or killed forms of the microbe, its toxins or one of its surface proteins. The agent stimulates the body's immune system to recognize the agent as a threat, destroy it, and keep a record of it, so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters." The manufacturing basics for vaccines are the steps necessary to make the agent noted in a manner that is safe, effective, and consistent over the life cycle of a vaccine. Those steps for a number of vaccine types are described in this section. The descriptions do not represent any specific brand of vaccine as each manufacturer must define and validate its methods to obtain license approval and to produce and release batches of product (see Regulatory Considerations). These approaches vary between companies, and hence, generic descriptions are provided to solely provide a general understanding of the production systems.

### 1.1 Bacterial Antigen Vaccines

The production of a traditional bacterial antigen vaccine provides a good foundation for understanding other vaccine types. For example, the production of tetanus toxoid vaccine starts with growth of the organism *Clostridium tetani*. A specific culture of the organism is obtained, expanded, and frozen to create a master seed for all future production. This master seed is typically further expanded to make working seeds, which are then used to start individual batches of product for release for use. The frozen working seeds are recovered on solid agar, then liquid culture allowing several days to a week between transfers for the bacteria to adapt to media and grow. The temperature and culture conditions are closely controlled; the transfers are executed in controlled environments to prevent culture contamination from the production environment. Ultimately, the culture has sufficient viable cell density to inoculate the production bioreactor. After the organism expands in the bioreactor, the culture is harvested and cells are removed via centrifugation and/or filtration, allowing the secreted toxin to be recovered. The toxin is treated with a chemical agent such as formaldehyde, which causes the toxin molecules to cross-link eliminating the toxicity, but retaining the protein structure needed to elicit a protective immune response. The resulting molecule is called a toxoid. The toxoid is purified by a variety of methods which may include precipitation (addition of a salt to cause the toxoid or impurities to selectively precipitate and to be removed from the solution), ultrafiltration (separation of the toxoid from impurities based on size differences), and/or chromatography (separation of toxoid from impurities based on differences of charge and/or size). The toxoid is tested for purity, lack of toxicity, and potency prior to formulation into the final vaccine. Tetanus toxoid may

be mixed with an adjuvant to increase the immune response. Traditionally, tetanus toxoid is adjuvated with aluminum salts (aluminum hydroxide, aluminum phosphate, etc.). It can be administered as a monovalent vaccine or mixed with diphtheria and/or pertussis toxoids, as well as other antigens, in a combination vaccine. The tetanus toxoid is generally stable in this form without the additional of stabilizers or special processing (lyophilization), and hence represents a fairly simple, but not trivial, manufacturing process example. Diphtheria and pertussis toxoid vaccines are made in a similar fashion.

There are a number of bacterial-based antigen vaccines which follow similar production approaches but do not require the "toxoiding." In some cases the antigen of interest is secreted as in the aforementioned tetanus example, in other cases, the antigen needs to be extracted from the cell paste following the bioreactor harvest (polysaccharide-based vaccine processes for *Haemophilus influenzae* type B; meningitis types A, C, W135, and Y; and pneumococcal vaccines, recover the polysaccharide from the cell wall). In some cases the purified product is not stable and needs to be lyophilized. Lyophilization, also known as freeze-drying, is a process that allows the removal of water at low temperatures to maintain potency during the manufacturing process and providing greater stability of the final drug product during storage and distribution to the end user. Table 5.1 shows examples of a variety of vaccines, the cultivation and purification approaches, and the stabilization requirements.

## 1.2 Live Virus Vaccine

Perhaps the most effective means of developing a robust and protective response, often with a low vaccine dose, is through the use of a live virus vaccine (LVV). The viruses used in production are altered from wild-type viruses to weaken, or "attenuate" them such that a robust protective response is obtained without severe disease. In some cases the virus may not replicate in the human host (cowpox used to protect from smallpox) or be altered genetically such that it does not replicate. Similarly, the live virus may be innocuous but used as a viral vector vaccine to deliver other antigens (an approach being tested for Ebola vaccine).

The production of viral vaccines adds a complexity to the bacterial antigen production processes in that viruses need a living organism to amplify and so in order to make virus, you must first expand a cell culture system for the viral expansion. Many traditional viral vaccines are grown in fertile chicken eggs such that the target virus is injected into the egg and then infects the embryo; after several days in controlled temperature and humidity conditions, the virus is harvested from the chicken embryo (yellow fever vaccine) or allantoic fluid (live-attenuated influenza vaccine) and is further purified and processed to make the final vaccine. Although the chicken embryo-based production has been a reliable method for making many vaccines today and for many decades, it is at significant biosecurity effort (vaccination, quarantine practices, limited access,

**TABLE 5.1** Vaccine Manufacturing Process Information from U.S. Package Inserts

| Disease | Trade name | Generic name | Cell culture/ fermentation | Isolation | Purification | Formulation |
|---|---|---|---|---|---|---|
| Anthrax | BIOTHRAX | Anthrax vaccine adsorbed | Defined media, avirulent, *Bacillus anthracis* | ND | Sterile filtrate of culture medium | Aluminum hydroxide |
| Typhoid fever | Vivotif | Live Oral Ty21a | Fermentation, complex media | Centrifugation | ND | Lyophilized product |
| Influenza | Fluzone® | Inactivated influenza virus vaccine | Propagation on embryonated chicken eggs | Low speed centrifugation and filtration | Purification on linear sucrose density gradient followed by additional purification by chemical means | Phosphate buffered saline with gelatin as stabilizer |
| Japanese encephalitis | JE-VAX | Japanese encephalitis virus vaccine inactivated | Intracerebral inoculation of mice. | Harvest of brain tissue/ homogenized | Centrifugation, followed by inactivation. Further purification by ultracentrifugation through 40% sucrose. | Lyophilized |
| Hepatitis B | Recombivax HB | Hepatitis B vaccine (recombinant) | Recombinant hepatitis B surface antigen (HBsAg) produced in yeast cells grown in a complex media | Released from yeast by cell disruption | Series of chemical and physical methods (ND) followed by treatment with formaldehyde. | Amorphous aluminum hydroxyphosphate sulfate |
| Polio | Poliovirus Vaccine Inactivated | IPOL | Type 1, 2, 3 poliovirus individually grown in Vero cells on microcarriers | Clarification (method ND) and concentration | Purification by chromatography; inactivation by formalin | Medium M-199 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Haemophilus influenza | ActHIB | Haemophilus b conjugate vaccine (tetanus toxoid conjugate) | Grown in a semisynthetic medium | Centrifugation | Phenol extraction and alcohol precipitation; Hib polysaccharide conjugated to tetanus toxiod | Lyophilized |
| Hepatitis A | HAVRIX | Hepatitis A vaccine, inactivated | Hepatitis A (strain HM175) propagated in MRC-5 human diploid cells | Cells lysis | Purification by ultrafiltration and chromatography followed by formalin inactivation | Adsorbed onto aluminum hydroxide |
| Yellow fever | YF-VAX | Yellow fever vaccine | Cultured on living avian leukosis virus-free chicken embryos | Homogenization | Centrifugation | Lyophilized |
| Measles, mumps, rubella, and varicella | ProQuad | Measles, mumps, rubella, and varicella (Oka/Merck) virus vaccine live | Measles and mumps viruses propagated separately in chick embryo cell culture; rubella virus propagated in WI-38; varicella virus propagated on MRC-5 | ND | ND | Lyophilized |
| Rabies | RabAvert | Rabies vaccine | Rabies virus grown in primary culture on chicken fibroblasts | Inactivated with beta-propiolactone | Purification by zonal centrifugation in a sucrose density-gradient | Lyophilized |

extensive testing of flocks) that the chicken flocks be protected from disease that would reduce availability of eggs (avian influenza) or that could infect the manufacturing process. These LVVs use eggs that are certified to be free of avian viruses with extensive testing of the flocks and monitoring of bird health.

Many LVVs use an immortalized cell line which has been thoroughly tested and certified to be free of adventitious agents that would have a deleterious effect on the manufacturing process or vaccine safety. These cell lines, similar to the master seeds for the bacterial products, are specific for each product and are frozen into master and working cell banks allowing long-term availability and viability of the cells and manufacturing processes they support. Many cell lines require an attached surface to multiply and to be viable through the manufacturing process (eg, Vero cells, MRC-5 cells); this requires special equipment and processing to support the virus expansion. The most popular options for this production are roller bottles (bottles slowly turning to allow nutrients to wash over growing cells, while controlling temperature and dissolved gas concentration), flat plate reactors (which have multiple parallel plates for cell culture attachment and growth, pumping nutrients through the device), or microcarriers (small beads in suspension in a bioreactor allowing a surface for growth and bioreactor mixing for nutrient replacement). In each case, as the cells expand and need to be transferred to a large-scale device, they must be detached, typically with addition of a enzyme like trypsin, then reattached to the new surface (by removal/dilution of trypsin and addition of other nutrients). With these processes being done in a sterile environment, the equipment costs and complexity is high, often requiring robotics and clean room operations to reduce risk of failure. Once the expansion of the culture is complete, which could take several weeks, the culture is infected and the viral production is generally fairly fast (several days). When infection is complete, the virus may be collected from the culture media (if secreted) or purified from the disrupted cells. Unit operations in this case are similar to those described in bacterial antigen production.

Because a virus needs a living cell to expand, once the cells are removed, the virus may have limited stability. The processing times are strictly controlled to limit degradation of potency and often the material is frozen to −20 or −70°C to preserve potency between manufacturing steps. Most LVVs are ultimately freeze-dried (MMR, varicella) or may be delivered frozen (live attenuated influenza vaccine). Some need to be frozen until use even after lyophilization to prolong shelf life. There are exceptions like rotavirus vaccine which is stable at 2–8°C for 2 years.

## 1.3 Inactivated Virus Vaccines

For many viral diseases, exposure to the viral proteins, without an active infection, can produce protection against the disease. In these cases, one would produce the viral antigen similar to the processes described for LVVs, but the virus is inactivated by chemical means to render it noninfectious. The inactivation

may take place before or after purification. The best examples of inactivated virus vaccines include inactivated influenza vaccine, largely grown on chicken embryos but also in cell culture, where the virus is inactivated with formaldehyde or BPL (β-propiolactone); inactivated poliovirus vaccine, grown in Vero cells on microcarriers in large bioreactors, inactivated with formaldehyde; and hepatitis A vaccine, grown in MRC-5 cells on flat plate reactors and inactivated with formaldehyde.

## 1.4 Recombinant Vaccines

Advances in genetic engineering have allowed the production of several vaccine antigens without use of the native infectious organism. In this case, a yeast culture, such as *Saccharomyces cerevisiae* can be altered to produce a vaccine antigen such as the hepatitis B surface antigen (HBsAg), which protects against hepatitis B infection. In this case the process resembles the bacterial antigen process. At the end of the fermentation process, the HBsAg is harvested by lysing the yeast cells. It is separated by hydrophobic interaction and size-exclusion chromatography. The resulting HBsAg is assembled into 22-nm diameter lipoprotein particles. The HBsAg is purified to greater than 99% for protein by a series of physical and chemical methods. The purified protein is treated in phosphate buffer with formaldehyde, sterile filtered, and then coprecipitated with alum (potassium aluminum sulfate) to form bulk vaccine adjuvated with amorphous aluminum hydroxyphosphate sulfate. The vaccine contains no detectable yeast DNA but may contain not more than 1% yeast protein.[6–8] Similar approaches are used to make human papillomavirus (HPV) vaccines.

## 1.5 Conjugate Vaccines

The production of *Haemophilus* type b conjugate includes the separate production of capsular polysaccharide from *H. influenzae* type b and a carrier protein such as tetanus protein from *C. tetani* (ie, purified tetanus toxoid), CRM protein from *Corynebacterium diphtheriae*, or outer membrane protein complex of *Neisseria meningitidis*.[5] The production of polysaccharide and tetanus toxoid was described earlier.

The industrial conjugation process was initially developed using tetanus toxoid by the J.B. Robbins team at the National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, Maryland.[9] Conjugate preparation is a two-step process that involves: activation of the Hib capsular polysaccharide and conjugation of activated polysaccharide to tetanus protein through a spacer.

Activation includes chemical fragmentation of the native polysaccharide to a specified molecular weight target and covalent linkage of adipic acid dihydrazide. The activated polysaccharide is then covalently linked to the purified tetanus protein by carbodiimide-mediated condensation using 1-ethyl-3(3-dimethylaminopropyl)carbodiimide. Purification of the conjugated material

is performed to obtain high molecular weight conjugate molecules devoid of chemical residues and free protein and polysaccharide.

Conjugate bulk is then diluted in an appropriate buffer, filled into unit-dose and/or multidose vials, and lyophilized.[5]

### 1.6 Vaccine Formulation and Filling

The focus of this chapter to this point has been the production of drug substance or active ingredient of the vaccine. The drug substance is further processed through formulation and filling, labeling and packaging to become drug product ready for use by the patient.

The formulation of the vaccine is designed to maximize the stability of the vaccine while delivering it in a format that allows efficient distribution and preferred clinical delivery of the product. The formulated vaccine may include an adjuvant to enhance the immune response, stabilizers to prolong shelf life, and/or preservatives to allow multidose vials to be delivered.[5]

After formulation, the product is filled into vials or syringes under strictly controlled conditions to prevent introduction of any viable or nonviable contamination, and sealed to ensure container closure integrity during shelf life. Filled vials may be lyophilized in order to increase stability; in this case, the vials are fitted with special stoppers that are partially inserted during drying to allow moisture to escape, and fully inserted and capped after drying. Quality control (QC) testing at this stage usually consists of safety, potency, purity, sterility, and other assays specific to the product.

## 2 CONSIDERATIONS FOR MANUFACTURING VACCINES

This section outlines some key considerations in developing, licensing, and maintaining a vaccine for safe, consistent, and reliable supply. It should not be considered a complete list of requirements or considerations, but is used to illustrate the complexity and challenges of development and manufacturing vaccines for people not in the vaccine manufacturing industry. The section is divided into methods of manufacturing (including starting materials), manufacturing components, supply chain, supporting systems, facilities, process development and validation, analytical development and validation, characterization, and quality systems.

### 2.1 Methods of Manufacturing

There are two key points worth repeating from the opening of this chapter—vaccines are generally given to healthy people to reduce the future risk of disease, hence, it is important that they do not cause any harm to the patient. Besides the obvious impact to the patient, a vaccine considered to be unsafe would risk low acceptance and compliance with vaccination recommendations

and therefore increase the risk of disease outbreaks in the greater population. Second, the starting materials are a critical resource and need to be fully characterized, shown to be safe, performant, and stable, and made available in sufficient quantities to support long-term supply. The safety database is generated in clinical trials using the starting materials of early development and manufacturing and they are the foundation of the safety profile. Changes to the vaccine starting materials (or any element of the vaccine manufacturing process) may result in unanticipated changes to the vaccine performance and safety and are strictly regulated to ensure safety and effectiveness.

The master cell bank needs to be of a certified source (eg, from a previously licensed cell bank), or fully documented to show the source of the cells and the materials used to produce/expand the cells that are free of risks to the patient. These risks include adventitious viral agents in the cell bank from the original source material or from the media and reagents used to expand the cell bank, tumorigenicity of the cell line, genetic stability of the cell line, and long-term viability of the cell line (ability to freeze, thaw, and use for many years or decades). Points to consider have been published by FDA for characterization and certification of cell lines for use in biological manufacturing and are updated based on growing experience and advancement of analytical methods.

The first step in manufacturing is the establishment of a "master seed." This is a collection of vialed cells which form the starting material for all future production. It is extensively characterized for performance, stability, and the absence of any adventitious agents. For viral production, the master seed includes a "master cell bank" and a "master virus." From this bank, working cell banks are prepared, which are used as the routine starting culture for production lots. The final vaccine is a direct function of its starting materials, and a change in this seed can be as complicated as initiating a new product development altogether. Hence, manufacturers are advised to make sufficient master seed materials to support the full life-cycle of production, which can be several decades for vaccines.

Similar to cell banks, the source of virus for the viral master seed and the culture for the master seed of microbial and recombinant products needs to be carefully documented, reagents used certified to be safe-sourced and/or tested to be free of adventitious agents, and stable for long-term use in manufacturing. For recombinant products, the seeds need to show genetic stability such that the genes inserted to produce the target molecule do not change over time from what was tested in clinical studies used to license the product.

With starting material secured, the raw materials used to expand the seeds, produce, purify, and inactivate the products must be likewise shown to be safe, stable, and readily available for long periods of time without substantial change in composition. A significant challenge in vaccine manufacturing is that many raw materials are of biological origin (extracts of animal, plant, or microbial origin) and are subject to significant, sometimes undetectable, variability in normal raw material release testing. The variability in the raw materials adds

to the inherent variability of the biological manufacturing process creating a challenge for the reliable supply of the product. Ideally, complex, biologically sourced raw materials are replaced with chemically well-defined entities with less variability and higher purity profiles. In many cases, these materials are not readily defined, available, or cost-effective such that the cost of manufacturing can be below what most markets can afford to pay for the vaccine. Typically the variability is accepted, but can cause supply disruptions if careful raw material characterization or screening cannot be developed to support the process control. Further, raw materials of animal origin (eg, calf serum often used in cell culture, enzymes used in cell culture and purification) are subject to considerable testing burden to confirm the source of the material to be safe and/or the viral clearance steps used to process the raw material and/or the vaccine itself are adequate to eliminate or greatly reduce any risk of deleterious effect. Manufacturers must "validate" that the process is reducing these risks to an acceptable level on a consistent basis. Again, it is ideal to develop a medium free of any animal-sourced ingredients to minimize the challenges of testing and sourcing the raw materials. Vendors supplying raw materials for biological production are subject to the same good manufacturing and documentation practices of vaccine manufacturers. Raw materials must be tested and released for use against prescribed specifications. Animal-sourced materials must be certified to be safe sourced. Vendors are subject to audit or qualification of the manufacturer for compliance with good manufacturing practices, including change control documentation and notification. Ultimately, the manufacturer is responsible for the quality of the product, even for steps managed prior to its receipt of the materials and the control of these processes are key for long-term success in manufacturing.

A key element of process control beyond the starting and raw materials are the batch records and standard operating procedures that detail the manufacturing process to ensure consistent production of vaccine relative to what was proven safe and effective in the clinic. Manufacturers are required to manage the "recipe" for making vaccines such that each lot is made following the process prescribed in the license. This includes following detailed standard operating procedures documented such that as new people are hired to make the product, they are able, through adequate training and following the prescribed procedures, to make the product in a way identical to the original batches and/or following changes approved by regulators to those processes or procedures. In addition to following the procedures, analytical tests are completed during and after the production of a batch are need to demonstrate that they are within an allowed variability as specified in the product license. This includes product-specific tests (antigen content, potency, etc.) as well as nonspecific tests (pH, bio-burden, etc.). Trending these data over time is a key element of vaccine manufacturing to be able to identify variability in the process that may not be obvious in individual batch testing with respect to process drift or an undetected change in raw material quality.

## 2.2 Manufacturing Components

Similar to raw material sourcing, the components used in the manufacture of the product, and in particular the components that contact the product during processing, are subject to strict control and are an important element of the overall process control and quality systems governing the production process. In addition to composition testing to confirm appropriate materials of construction, components are testing to confirm they do not alter the product during processing. Whereas many traditional vaccine manufacturing processes used glass and stainless steel equipment, where the product contact equipment is largely inert (ie, not additive to the product), more recent processes use polymer-based components and even disposable equipment (use once and discard) to reduce manufacturing time, improve worker safety (handling glass), reduce risk of cross-contamination (and equipment cleaning requirements), ease sterilization of manufacturing equipment, and to allow closure of manufacturing systems from the external environment. Although there are many benefits of the new approaches, they bring new complexities. Extractables and leachables (E&L) are the elements of the product-contact components that could contaminate a product stream during processing and each polymer/component must be tested in your manufacturing environment to confirm that the components are not additive to the process with your specific product. (Standard testing approaches are being sought to replace testing of every component/product combination explicitly.) There is also a need for strict change control at the manufacturer (and their suppliers) to identify significant changes of source materials or process changes that could alter the E&L profile and require additional confirmatory testing to permit a change. These changes may be process improvements by the vendor or necessary changes due to availability of raw materials. Qualified substitutes for every product contact component are recommended to secure supply performance, but it is difficult due to many proprietary resins and designs that are not interchangeable.

## 2.3 Supply Chain

The supply chain supporting a manufacturing process and delivery is a key to long-term success. The complexity of making a single lot with respect to raw materials and components is outlined previously. A similar complexity exists for raw materials and components used in the analytical release processes (eg, test reagents, test equipment, disposable components) that support the manufacturing process. Further, the raw materials and components have their own supply chains; vendors providing raw materials often purchase starting materials from their suppliers and so on. For components, the manufacturer may purchase from an assembler, who purchases components from multiple suppliers, who purchase the resins from still another supplier. Traceability on changes in manufacturing processes and resins is very challenging in these complex supply chains, yet this is the norm, not the exception.

In addition to sourcing the materials in the appropriate quantities at the right time, within the specified quality and license requirements, one must also allow for increases in demand with short notice, position inventory for supply interruptions, manage contracts for ongoing supply and quality, all while controlling costs. In addition, the materials that are prepared and released must be stored at the appropriate temperature, and delivered while maintaining the cold chain control to many markets around the world. (These markets may have different regulatory requirements and the supply chain needs to ensure that the products made of each market go only to that market.) Backup suppliers for every key raw material and component are recommended and can only really be considered backup if used with some frequency (dual suppliers) and with sufficient capacity to assume all supply if the alternate supplier fails (often with little or no notice). Inventory is a solution to this challenge, but it adds cost and if a quality defect is found, a higher amount of inventory is at risk of discard. The challenges noted are easily as complex and impactful as the technical aspects of manufacturing vaccines and the systems and controls need to be equally rigorous to those noted in the technical production challenges.

## 2.4 Process Development, Analytical Development, Validation, and Product Characterization

The targeted outcome of process development is a fit for use, well-understood manufacturing, and release process for the vaccine. In very simple terms, you want a process that is easy to execute consistently, by multiple people, for a long period of time, with multiple sources of equipment and raw materials, without interruption or failure. To define the true robustness of a process, one must understand the desired outcome and the edges of process control that lead to failure, such that the process and failure modes are understood and can be controlled such to avoid failure. Given enough time and energy, this outcome *is* possible. Using "Quality by Design" (QbD), ideally one identifies the most likely process failure modes and sets specifications for inputs to the process that ensure successful outputs. By controlling the variability of the inputs and executing the standard process, you increase the success rate of the process regardless of people, raw material source, equipment change, and so on, provided you stay within the "design space." Likewise, once this is "validated" or demonstrated over a sustained period, there is the potential added benefit of reduced release testing (parametric release, provided input conditions are met one may be permitted to reduce final product testing). Operating in this mode can provide high first-time quality and low operating costs. The investment in science and technology and understanding is high, but most of all, the investment in time is often the obstacle that prevents this ideal state. In the "race" of getting a new product to market to have impact on a disease that is taking lives or reducing quality of life, one tends to make many risk-based decisions and accepts a less-than-perfect process for the sake

of responsiveness. Unfortunately, once the vaccine is licensed with a "sub-optimal" process, with the high obstacles to change noted throughout this chapter, the willingness to reinvest in the ideal process is generally lower than the need to take on the next vaccine product development challenge. Rigorous monitoring and control, as well as documenting failure investigations and building a database and design space through experience can allow a firm to get nearer to this optimal process leveraging QbD principles in a retrospective manner to build a design space over time.

Analytical procedures capable of confirming that the process has performed as designed and that the product meets requirements established during clinical safety and efficacy testing is a key element of the manufacturing process. The effort to develop these processes can be more challenging than developing the manufacturing process itself as you essentially test to confirm the presence of the "biologically active product" and excipients in the right concentrations, as well as the absence of nearly every component used in the manufacturing process (raw materials, E&L, etc.), particularly during validation. Beyond analytics that support release, methods that "characterize" the product, critical for future change control, are often required. These may include protein sequencing, particle size, isoelectric point, typical residuals profile, among others. Further, the analytics are often required to develop the process in the first place, before the product is truly defined, making this an iterative process as well, as the product and methods are refined in parallel. One complexity of the development process often missed by people outside the industry is that you are always trying to bridge your process data from earliest preclinical lots through all clinical steps, to the current manufacturing process, while the raw materials, process, and analytics are in considerable flux. Ultimately, the analytics must be considered part of the manufacturing process as it is impossible to separate the impact of either individually. They stand as one. To that effect, every challenge noted previously with respect to manufacturing processes also stands for the complex supporting analytical processes.

## 2.5 Validation

Validation is demonstrating that the process performs as expected and that the desired outcome is reliably delivered when the process is executed according to approved procedures (author's definition). The need to validate the process exists with all modern regulators, but the requirements vary. Key performance characteristics that need to be validated, other than product meeting obvious specified attributes (eg, potency, purity, sterility), include viral clearance (product and potentially raw materials), container closure integrity (product not exposed to external environment during manufacturing or shipping/storage), product stable/performing during full range of process hold times or process durations, process performant at extreme of boundary conditions established for process control.

For analytics, rigorous validation requirements are also well known and guidance from FDA (Guidance for Industry Analytical Procedures and Methods Validation for Drugs and Biologics—Feb 2014) is readily available as draft, nonbinding guidance. "Parameters that may be evaluated during method development are specificity, linearity, limits of detection (LOD) and quantitation limits (LOQ), range, accuracy, and precision." Validation is often completed in the final product matrix and revalidation may be required if manufacturing process changes warrant it.

## 2.6 Supporting Systems and Facility Requirements

In addition to the manufacture, release, distribution, and control of vaccine manufacturing noted, one must also consider the rigor of the support systems of any industrial operation. Site and management controls; environmental, health, and safety practices and controls; and waste management (in particular potential hazardous or infectious waste) are all technical systems that need to be managed in addition to the manufacturing and analytics themselves. These systems can be more intense than at a nonbiological facility due to the handling of biological agents and inactivating agents that cannot be released in an uncontrolled manner. Additionally, systems for process automation, process control, material control, material ordering, and movement within the facility are essential complications of biological manufacturing that are not often discussed and will not be discussed in detail in this chapter.

From a facilities perspective, one must focus on protecting the product during manufacture from conditions that can lead to product failure. Temperature control during operation and storage is one example, essential to support product potency and stability. In addition, providing the proper air quality for each process step requires strict control of air supply volume, temperature, humidity, and particle burden, especially if the product is exposed to the processing room environment. For this case, the strictest controls of air quality and people gowning and movement are essential to maintain a high probability of sterility of the process. Excursions to these quality requirements must be investigated and confirmed to have no product impact; otherwise batches of product may be discarded. Likewise, containment of the biological organisms is managed to reduce any risk of environmental contamination or cross-contamination of products or batches. For facilities that produce multiple products, many procedures and engineering controls are necessary to maintain segregation of products and between critical process steps within a batch. Facilities and equipment also need to be validated to show they reliable perform the operations intended, but also to show they can be thoroughly cleaned and sterilized between uses.

## 2.7 Quality Systems and Regulatory Considerations

A foundational element of successful product manufacturing and release are the supporting quality systems that govern material handling, management of

documents, change control, employee training and qualification, process trending, product investigations, and ultimately batch release in conformance with the product license. The quality organization, typically responsible for all analytical testing as well, can be 20–40% of the total operating organization. The focus of this organization is confirmation that required procedures are executed using qualified/release raw materials, components, and batch records; the execution of the batch was successful with respect to the batch meeting all specifications outlined in the analytical release; all equipment and facilities performed as designed and outlined in the product license; and any deviations from the currently approved licensed process are investigated and confirmed to have no impact of potency, safety, stability, or efficacy, and are conformant to the license.

The quality organization is required to audit manufacturing and analytical processes routinely to confirm compliance with current good manufacturing practice (cGMP). The quality organization is likewise accountable for the quality of incoming raw materials and audits or qualifies vendors of all critical manufacturing and testing material as well as the organizations responsible for distributing the product to the final user. Trending of internal and external product quality attributes is routine. Quality must investigate any customer complaints and facilitate continuous process improvement based on the root cause assessment of complaints, process failures, and findings during routine and for-cause audits. Whereas this is written such that the quality is accountable for the ultimate product, it should be clearly understood that all members of the organization that are involved in procurement of goods and services, manufacturing and release of product, and distribution to the final customer are accountable for the product quality. Quality systems are designed to assure quality, but the old adage that quality must be built in at every step, by following approved procedures without deviation, and with the full focus and objective of every employee is very true. You cannot test in quality. The FDA Code of Federal Regulations outlines the full requirements of the quality organization.

FDA and other global regulatory agencies routinely audit the facilities and processes of every manufacture on an annual or biennial basis, depending on the products produced. Firms showing the best compliance performance, lowest customer complaints, and consistent continuous improvement can be inspected less frequently by exception.

In the United States, current authority for the regulation of vaccines resides primarily in Section 351 of the Public Health Service Act and specific sections of the Federal Food, Drug and Cosmetic Act.[10,11] Section 351 of the Public Health Service Act gives the federal government the authority to license biologic products and the establishments where they are produced.[6] In the European Union, animal and human vaccines are regulated by the European Medicines Agency (EMA), whose main responsibility is the promotion of public and animal health. The EMA's Committee on Medicinal Products for Human Use through its Vaccine Working Party has oversight for human vaccines. Vaccines are licensed through a centralized procedure that allows for simultaneous

licensure within all countries within the European Union. Harmonization of licensing and regulating procedures for vaccines worldwide has obvious benefits in rapidly delivering safe and effective vaccines to the market. Impediments to harmonization include lack of standardized regulatory procedures and mutual recognition of licenses and inspections between countries and worldwide regulatory agencies. Harmonization of regulation continues to progress as joint FDA–EMA establishment inspections programs have become reality and adherence to harmonized International Conference on Harmonisation (ICH) guidance expected.[5]

ICH Q9, Quality Risk Management, was approved or adopted by the European Union, United States, and Japan in 2005. This guidance provides for a systematic approach to identify and control potential quality issues arising during development and manufacturing of pharmaceuticals, biotechnology products and biologics, improves quality decision making, and provide regulators with a higher degree of confidence in a firm's ability to address potential quality risks.

The guiding principles of quality risk management are that the evaluation of risk to quality is based on scientific knowledge and patient protection and that the level of evaluation is commensurate with the quality risk identified. It is expected that the concepts of quality risk management be embedded within all systems and processes throughout the product lifecycle.

## 3 VACCINE CHALLENGES FROM THE INDUSTRY PERSPECTIVE

This section outlines some challenges specific to the vaccine industry from an informal survey of industry associates across three continents. Many challenges are highlighted previously in this chapter and will not be repeated. Others may provide an interesting perspective to people unfamiliar with the manufacturing experience.

### 3.1 Long Vaccine Life Cycle

Vaccines, unlike many other innovative products, can have long life cycle (compared to novel pharmaceuticals that can be copied and produced generically after patents expire). This is largely due to the complexity and lack of characterization of the relatively variable biological processes and the inability to make a "true copy" or generic version of the product. This attribute makes manufacturing challenging as noted, but also worth the investment of good controls and continuous improvement. The historical approval timing of today's vaccines are as follows (partial list):

- 1950s: Yellow fever, polio vaccines
- 1960s: DTwcP (discontinued in the United States in 2002)
- 1970s: MMR
- 1980s: HIB, Hep B

- 1990s: DTacP, varicella, Pn-Cj (7)
- 2000s: Rotavirus, HPV, zoster, MMRV, Pn-Cj (13)

Many of these products are produced by methods similar to those outlined in the original license (MMR, HIB), others have been replaced by second generation processes using newer technologies (yellow fever, inactivated polio). Regardless, the vaccines have a long life (40+ years), yet facilities useful life is generally 30 years, and equipment useful life is less than 20 years. Over this 40+ year life cycle, the equipment used for the original license is no longer the state of the industry, yet a change in equipment could require new clinical studies and at least full repeat of process validation. While equipment and technology is advancing, so are the regulatory requirements. This is an added challenge to keeping a product "current" when the life cycle is so long. This is clearly a challenge unique to vaccine manufacturing.

## 3.2 High Facility and System Costs

Costs for new facilities for vaccines have been publicly noted and have ranged from 150 to >600M USD, for example, for egg-based and cell culture–based influenza facilities in the United States, respectively, in the last 10 years. The costs are high due high levels of automation and fixed equipment, which must be cleaned and sterilized in place between batches, coupled with low yields and the need for a high number of doses in a short time each year. For higher-yielding processes, single-use equipment has been shown to reduce capital costs as the product contact components are used one time, are available in presterilized ready-to-use format, and do not have to be cleaned as they are disposed after use. In exchange for the lower capital costs, firms may see higher operating costs and the challenges managing the complex component supply chains and E&L validation challenges mentioned earlier. These advances have been largely made in the last 10 years and although progress continues to be made, the investment in large fixed equipment continues as a lower-risk option for many suppliers.

## 3.3 Global Demand Complexity

On the issue of global demand complexity, the industry must tackle the diversity of regulatory requirements (where harmonization is yet to be achieved) for the various markets, competition for the market share within each market, and the practice of many international markets, which operate on a tender-purchase order. On a regular basis, companies compete for business based on cost and either earns all or none of the business from specific countries for 1–3 years (and the product may be specifically made for that market and not useable elsewhere). This is an effective way for governments to manage limited healthcare budgets and increasing buying power and it is good business practice. It also incents companies to continually improve processes and decrease costs

of manufacture, without reducing safety or effectiveness of the vaccines. The challenge comes in the area of planning production. Lead times for vaccines is typically 4–16 months from initial batch start to completion of final drug product released for distribution (longer lead times are associated with more complex or multivalent products). Managing the long lead times and uncertainty in demand makes manufacturing planning a challenge. As vaccines have limited shelf life (18–36 months) and customers generally want 12+ months of shelf life on receipt, the risk of obsolescence of product made and released prior to sale is high and discards of the product result. Higher discards increases average cost of goods and makes a firm less competitive in tender business.

## 4 MANUFACTURING DILEMMAS

The final section of the chapter on manufacturing is intended to outline the dilemmas that vaccine manufacturers face on a regular basis in an effort to help the reader understand that the industry is complex and risk-based decisions need to be taken regularly, but never at the risk of patient safety or product effectiveness.

### 4.1 Purity Versus Cost

In general, one would expect that the purest form of a vaccine antigen would be the safest by means of fewer adverse reactions to residual production process components in the final vaccine. Increased purity generally comes at the cost of lower product yield and hence increases cost of production, cost and size of facilities, and either batch size or batch frequency, increasing the cost risk of any individual batch or the number of batches produced with some finite failure rate. At the same time, the increased safety of purer product may be hypothetical rather than proven through clinical safety evaluation. Evaluating various purity levels to optimize the cost without increasing adverse events is rarely done due to the time and clinical cost required to do so. The ultimate specification on purity is determined based on process capability and confirmation of safety in the clinic at that level versus a true optimization. Lower cost of goods and affordability for more markets could be the outcome of a more targeted approach.

### 4.2 Central Versus Distributed Manufacturing

"Economies of scale" has historically led to the development of a number of large central manufacturing facilities for vaccines around the world. These central facilities have the capability of lower costs of goods by producing a high number of doses from a single, albeit large, investment. However, product distributed from these central facilities are required to meet the regulatory requirements of every market served. As these requirements are not yet fully harmonized, the complexity of the operation and supply chains for the facilities are increased, as

are the number of regulatory audits the facilities receives. Alternatively, smaller, regionally distributed facilities could meet the local regulatory requirements and operate with simpler supply chains and distribution challenges. (Some countries are now requiring some local value-added production commitments in order to adopt the vaccines in their populations. This is positive for the country as it can lead to self-sufficiency during a period of vaccine shortages elsewhere and as it creates a capability.) The challenge for manufacturers is the ability to maintain consistent change control and uniformity of process across such a diverse set of facilities and process scales, the ability to leverage or react to local customer complaints or adverse events when processes may have drifted from the licensed process, and the ability to leverage market supply/demand variability globally instead of "every country for itself." Finally, in order to support transfer of manufacturing processes to the distributed manufacturers, loss of intellectual property is an added challenge of the innovator.

### 4.3 Timing of Investments

The final dilemma of note in this text is that of the timing of investments in manufacturing that is necessary to ensure ample supply on launch for multiple markets against the uncertainty of final approval of the product in all markets. Facility construction, commissioning, demonstration of production, validation of production, and validation of methods typically requires 3–5 years for a large-scale sterile facility. It is ideal to make the consistency lots for the clinical trial in the ultimate manufacturing facility to reduce bridging studies and reduce risk of a process change on scale-up or final facility/process fit that requires additional or prolonged clinical studies to support approval. Yet this timing requires the investment in the production facility during Phase 1 or Phase 2 clinical development when the risk of failure is still rather high. Weighing the risk of product failure against the risk of licensing complexities is a routine dilemma of every manufacturer. The risk can be reduced or diversified if the firm leverages platform processes (a facility that could make product A or product B), so if one fails, the facility is still available for the alternate. Unfortunately, this is rarely done and the risk is often managed through use of launch facilities, followed by scale-up or additional construction after license approval with the modest risk that future facilities may not be identical to the original and additional clinical development could be needed.

## ACKNOWLEDGMENTS

Supply shortages are a key complaint of customers and providers of vaccine manufacturers. This chapter is not meant to be an excuse for vaccine shortages on behalf of manufacturers, but instead it is intended to increase the understanding of the complexity of the manufacturing elements of vaccines, and also to best prepare future manufacturing professionals to develop the processes, analytics, technologies, systems, and manufacturing strategies to make vaccine shortages a historical phenomenon rather than an ongoing challenge.

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Chapter 6

# How are Vaccines Assessed in Clinical Trials?

**Myron M. Levine, MD, DTPH*, Wilbur H. Chen, MD, MSc****

**Global Health, Vaccinology and Infectious Diseases, University of Maryland School of Medicine, Baltimore, MD, United States; **Adult Clinical Studies Section, Center for Vaccine Development and Medicine, University of Maryland School of Medicine, Baltimore, MD, United States*

## Chapter Outline



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## 1 THE VACCINE TESTING PARADIGM

Advances in biotechnology and knowledge of ways to enhance immune responses and immunologic memory have revolutionized the field of human vaccine development, resulting in a vaccine "pipeline" that in recent decades has led to the licensure of many new and improved vaccines. However, a candidate vaccine faces a long, arduous, and expensive road, replete with obstacles, as it commences the journey toward becoming a licensed product that can protect individuals from disease and serve as a public health tool. The step-wise process that involves a series of sequential clinical vaccine studies that must be properly executed to advance a vaccine candidate, incrementally, toward licensure is based on proof of the vaccine's safety, immunogenicity, and efficacy in target populations, and is divided into "Phases". The early phases of the vaccine clinical testing paradigm are associated with the highest risk of failure and consequently the vaccine pipeline rather more resembles a funnel in which many products enter into Phase 1 but the winnowing of candidates results in fewer advancing to Phase 2, even fewer to Phase 3 and only a handful achieving licensure. Thus, the chance of success for a vaccine candidate to become a licensed product and thereupon a potential public health tool increases as each phase in the vaccine development paradigm is successfully achieved. In general, in industrialized countries where mortality from infectious diseases is low and age expectancy is high, safety is the key parameter of selection. For vaccines largely targeted for populations in developing countries where young child mortality remains high and morbidity from infectious diseases remains an important public health burden, a somewhat different risk:benefit ratio operates such that efficacy in preventing the target disease is key and milder forms of adverse reactions can be tolerated if accompanied by high efficacy.

Phase 1 trials undertake the initial careful assessment of the safety and clinical acceptability of the candidate vaccine in small numbers of healthy individuals (usually tens or scores of subjects). Such early dose/response tests can only detect common adverse reactions (some of which may be unacceptable) and provide an initial glimpse of whether relevant immune responses can be generated.

Phase 2 trials evaluate the candidate vaccine in increasingly larger numbers of subjects (typically hundreds) and are usually placebo-controlled to measure better the rate of adverse reactions versus background rates of symptoms and complaints. The level of shedding of a live viral or bacterial vaccine is intensively examined in Phase 2 trials, as is the propensity of the live vaccine to be transmitted to household contacts and to survive in the environment. For vaccines that will ultimately be used in infants and toddlers, Phase 1 and 2 trials must be undertaken in progressively younger subjects (age deescalation) until the target age group is reached. The immunization schedule and dose of vaccine to be used in a Phase 3 trial is identified in Phase 2 trials.

A vaccine candidate that has proven to be well tolerated in Phase 2 trials involving hundreds of persons of the target population group (and often in participants in several different geographic sites to document broad relevance) can progress to evaluations of the vaccine's efficacy in preventing disease. Assessments of efficacy in large-scale Phase 3 trials whenever possible follow a randomized, placebo-controlled (or active agent-controlled), double-blind design. Large clinical trials must also document that different lots of the vaccine have been manufactured in a consistent manner such that the clinical tolerability and immune responses elicited by three different lots of vaccine are similar. These important large safety/immunogenicity studies are termed "lot consistency" trials.

When sufficient evidence of the vaccine's safety, ability to elicit relevant immune responses and efficacy in preventing disease has been assembled and there is documentation of consistent manufacture of the vaccine in an approved manufacturing establishment, a Biologics License Application (BLA) can be submitted for review by a national regulatory agency such as the US Food and Drug Administration (FDA). If approved, the vaccine will become licensed.

The clinical trials that assess the vaccine at each Phase of development are performed according to clinical protocols that must undergo prior review by ethics committees [called Institutional Review Boards (IRBs) in the USA] and particular attention is paid to the informed consent methods and the documentation of informed consent. Moreover, before a clinical trial of a new vaccine can be initiated, a submission must be made to the national regulatory agency (eg, the US FDA) where the clinical protocol and detailed information about the vaccine, its components, method of manufacture, formulation, results of prior animal tests and animal toxicology tests, and other relevant information are included. In the United States such submissions to the Center for Biologics Evaluation and Research of the FDA are in the form of a New Drug Application (IND). The FDA has up to 30 days to review an IND and to request additional information and clarifications or to request modifications. Sometimes the request for modifications or collection of additional information does not delay initiation of the clinical trial. However, should the FDA have substantial concerns about some aspect of the proposed vaccine trial, the FDA can apply a "clinical hold" that prevents the clinical trial from commencing until it receives

satisfactory responses that address the concerns raised; at that point if the concerns have been addressed, approval will be given to initiate the clinical trial. If 30 days pass after the FDA-confirmed date of submission of an IND and no comment has been forthcoming, investigators may initiate the clinical trial.

Certain clinical trials, such as any Phase 2 or Phase 3 vaccine trial in the United States and Phase 1 pediatric vaccine trials within the European Union, must be registered in a clinical trials registry. The International Committee of Medical Journal Editors requests that all clinical trials of products be registered. Examples of clinical trials registries include ClinicalTrials.gov (maintained by the National Library of Medicine, Bethesda, MD, USA),[1] the Pan African Clinical Trials Registry [(www.pactr.org) managed by the South African Cochrane Centre at the South African Medical Research Council],[2] and the European Union Clinical Trials Register.[3] Registration of clinical trials increases transparency for the general public (who can access the websites) as well as for health professionals. It allows interested parties and stakeholders to assess rapidly the landscape of trials ongoing with particular types of vaccines. It also allows the contents of scientific publications about a vaccine to be compared with what was proposed to be studied in the summary of the clinical trial contained in the register.

The stepwise process vaccine development paradigm continues even after a vaccine becomes licensed, as there must be a post-licensure surveillance plan to monitor the safety profile of the newly licensed vaccine and its impact on the target disease once it is in large-scale use. Only post-licensure, when very large numbers of individuals of the target population have received the vaccine in numbers far exceeding the numbers of participants in Phase 3 trials does one have the possibility through Phase 4 surveillance to detect rare but severe adverse events.[4–7] Similarly, Phase 4 post-licensure trials and surveillance methods of different types allow an evaluation of how the vaccine is protecting under real-life conditions and constraints.[8–12]

## 2 ETHICAL ISSUES

Ethical Committees such as the IRBs in the United States are responsible for overseeing the health and satisfactory clinical condition of participants involved in clinical trials. US regulations instruct that the board includes at least five members, at least one who is not a scientist, and one who is not affiliated with the institution. The IRB reviews protocols, investigator's brochures, consent forms, recruiting materials, and additional safety information.

WHO guidance recommends that Ethics Committee members should include individuals with relevant scientific knowledge, expertise in legal matters and/or ethics and lay people whose primary role is to share their insights about the communities from which participants are likely to be drawn. To enhance independence, WHO suggests that the Research Ethics Committee should include members who are not affiliated with organizations that sponsor, fund, or

conduct research reviewed by the Research Ethics Committee. Since committees should be large enough to ensure that multiple perspectives are brought into the discussion, quorum requirements provide that at least five people, including at least one lay member and one nonaffiliated member, be present to make decisions about the proposed research.

## 3 GOOD CLINICAL PRACTICES

Good Clinical Practices, "GCP", refers to the comprehensive regulations and guidelines for conducting clinical trials that must be followed for results of those trials to be contained within an application requesting licensure of the vaccine. GCP covers items such as protocol design, informed consent, record keeping, data reporting, laboratory standard operating procedures (SOPs), adverse event reporting, among others. GCP is intended to assure the integrity and quality of clinical data and to protect the rights and safety of study participants.

## 4 PHASE 1

If the vaccine candidate is based on a technology that has been previously utilized to make other vaccines that ultimately proved to be safe, immunogenic, and efficacious, that generally facilitates the initiation of Phase 1 trials and allows them to be performed at an accelerated pace. For example, conjugate vaccines consisting of polysaccharides from pathogenic bacteria covalently linked to carrier proteins have led to multiple successful vaccines including several *Haemophilus influenzae* type b (Hib) conjugates (Hib capsular polysaccharide linked to tetanus toxoid, $CRM_{197}$ genetically detoxified mutant diphtheria toxin, or outer membrane protein of Group B *Neisseria meningitidis*), multivalent pneumococcal conjugate vaccines (capsular polysaccharides of 10 or 13 serotypes of *Streptococcus pneumoniae* linked to carrier protein), quadrivalent meningococcal conjugate vaccine (capsular polysaccharides of *Neisseria meningitidis* Group A, C, W135, and Y linked to carrier protein) have all proven to be well-tolerated, immunogenic, and efficacious vaccines in children, including young infants. Thus, a new bivalent conjugate vaccine to prevent invasive disease due to nontyphoidal *Salmonella* should be able to enter Phase 1 clinical trials and progress through stepwise age deescalation to infants without generating undue anxiety.[13,14]

Certain target populations and types of vaccines require that they be evaluated in Phase 1 clinical trials of special design and performed with caution. Examples are given later:

### 4.1 Highly Vulnerable Target Populations

Studies of vaccines in infants generally require Phase 1 designs that assess the vaccine in two or three older pediatric age groups before initiating the evaluation

in infants. Pregnant women are regarded as another vulnerable subpopulation, as vaccines will need to be shown to be safe for both the pregnant woman and her developing fetus.

## 4.2 Live Viral and Bacterial Vaccines

The issue with Phase 1 trials of live viral and bacterial vaccines, particularly ones administered via mucosal (oral or nasal) routes is that they may be shed or excreted and may therefore pose a theoretical risk for contacts, including vulnerable hosts such as young infants and pregnant women. As such, the initial Phase 1 trials of live oral enteric vaccines are often carried out under physical containment on research isolation wards where the potential for transmission from vaccinees to contacts (who received placebo) can be evaluated.

## 4.3 Impeded Vaccines

Certain vaccines that are needed to address well recognized public health disease burdens have garnered insufficient support for clinical development because of unexpected severe untoward reactions that occurred in the testing of early candidates of these types of vaccines. Such vaccines can be referred to as impeded vaccines. Two examples are vaccines against respiratory syncytial virus (RSV) and group A *Streptoccoccus pyogenes*. In the 1960s, a formalin-inactivated RSV vaccine tested in randomized controlled trials to assess efficacy was found to cause more severe disease when vaccinees were exposed to RSV than when controls were exposed.[15,16] This phenomenon, which resulted in more hospitalizations for RSV disease and more deaths among vaccinees than among controls, dampened the interest of vaccine industry in supporting clinical trials of new generations of candidate RSV vaccines. A similar situation existed for Group A *S. pyogenes* vaccines and there was even an admonition in the Code of Federal Regulations instructing that vaccines based on products from Group A *S. pyogenes* should not be administered to humans.[17] Thus, subsequent Phase 1 trials with RSV and Group A *S. pyogenes* vaccines have had to be carried out under notably intensive clinical surveillance and regulatory oversight.

## 4.4 Unusual Vaccines

Occasionally investigators seek to undertake a Phase 1 trial of a vaccine that is so unusual that it proves challenging from the regulatory perspective. One example was the first Phase 1 trial of a transgenic plant vaccine in the United States in which a gene encoding a protein (B subunit of *Escherichia coli* heat-labile enterotoxin), considered capable of eliciting a potentially protective immune response, was expressed in an edible plant.[18] Since testing of such a product fell between the remits of two different federal regulatory agencies, the FDA and the Department of Agriculture, a pioneering regulatory path had to be worked out.

### 4.5 Public Health Emergency

Occasionally, an infectious disease emerges that is highly infectious, causes severe or fatal disease and a vaccine is sought because there is no specific therapy. In such a situation compelling pressure is exerted to initiate and complete those vaccine trials as expeditiously as possible. Such was the situation in 2014 with two candidate Ebola vaccines, one of which had previously only been administered to two humans and the other had not as yet been given to any human. Without bypassing any steps, the Phase 1 trials of these vaccines were initiated and completed with historic speed, demonstrating that, as necessary, in the face of a public health emergency the usual time necessary to evaluate a vaccine can be drastically reduced.[19–23] If initial Phase 1 trials of a new vaccine are carried out in an industrialized country and are then repeated in a developing country population, the latter trials are sometimes referred to as Phase 1b trials.[21]

## 5 PHASE 2

The Phase 2 vaccine trials that pave the way for pivotal Phase 3 field trials that assess the efficacy of a vaccine, are typically less visible than the latter. Ideally the sites and populations for Phase 2 trials will be representative of the ultimate target population. However, for various reasons, sometimes Phase 2 and 3 trials are carried out in parallel in other populations. During Phase 2 trials, it is important to select and validate the assays that measure immune response(s) to the vaccine. It is also critical that before the Phase 2 trials begin (or as soon as possible after they begin), the final method of manufacture and the formulation be finalized as this is what must be utilized in the future pivotal Phase 3 efficacy trial and will be commercialized for post-licensure use.

### 5.1 Harmony with Existing Immunization Schedules

Most new vaccines, whether they require administration of only a single dose, or must be given as multiple spaced doses, will have to fit into existing immunization schedules for the target population. This is relevant for infants and toddlers, adolescents, the elderly, and vaccines used in mass immunization campaigns. This key feature of Phase 2 trials addresses the need to harmonize the new vaccine's immunization schedule to be compatible with its being concomitantly administered when other vaccines are already scheduled to be given. Particularly for parenteral vaccines that must be administered to infants and toddlers, the immunization schedules in both industrialized countries and in developing countries are already quite "crowded".[24]

### 5.2 Compatibility with Concomitantly Administered Vaccines

Once an immunization regimen is selected and harmonized to fit within the visits of an existing immunization schedule, Phase 2 trials must also document

that the new vaccine, be it delivered by the parenteral, oral or nasal (or other) route, does not significantly diminish the immune response to any other vaccine administered at the same time, whatever the route, nor does it significantly increase the occurrence of adverse reactions. Similarly, it must be documented that the concomitantly administered, already licensed, routine immunizations do not diminish the immune responses to the candidate new vaccine. Phase 2 trials that address these questions are often complex with multiple study groups, require many participants, and are expensive, particularly for new vaccines that are targeted for an already crowded infant immunization schedule.

For candidate new vaccines that must be administered by parenteral administration, one can readily see the theoretical desirability of creating combination vaccines wherein a new antigen (ie, vaccine) is formulated along with existing vaccines or vaccine combinations. While desirable, many hurdles make this difficult, aside from the complexity of the Phase 2 trials required to test the compatibility of new combinations. For example, the manufacturer of a candidate new vaccine that is keen to incorporate into a combination with other vaccines must either already be the manufacturer of those vaccines or must partner with other manufacturers to try and achieve that goal.

### *5.2.1 Live Vaccines*

For candidate live viral or bacterial vaccines, regulatory authorities pay close attention to the shedding/excretion pattern of the new vaccine and its propensity to be transmitted to family members and other close contacts. Thus, Phase 2 trials of live vaccines must be designed to address these questions and provide quantitative data.

Similarly, regulatory authorities require information on the environmental impact of use of the live vaccines. In some countries and global regions particular attention is paid if the new vaccine constitutes a genetically modified organism (GMO). Whereas many questions can be addressed with data from preclinical experiments, particularly for live bacterial vaccines, it may nevertheless be advantageous for Phase 2 trials to incorporate in their design the gathering of data to address environmental issues. There are precedents for this.

## 5.3 Genetic Stability of Vaccine Isolates

Another concern of regulatory authorities with respect to live vaccines and in particular GMO vaccine strains is that the Phase 2 trials incorporate in their design steps to investigate the genetic stability of shed vaccine organisms and compare shed/excreted isolates to the vaccine strain as it was administered to participants.

## 5.4 Experimental Challenge Studies in Healthy Adult Volunteers to Gather Preliminary Evidence of Vaccine Efficacy

Challenge models of experimental infection with various pathogens in healthy adult volunteers have been developed over the years with various goals including

to establish the pathogenicity of specific putative pathogens, emergent strains, or serotypes, to study pathogenesis and human host-pathogen interaction, to measure in great detail human immune responses, to preliminarily assess the efficacy of candidate vaccines and to identify immunologic correlates of protection. Phase 2 challenge models have been particularly valuable for assessing the efficacy of candidate vaccines to prevent *Plasmodium falciparum* malaria,[25–27] influenza,[28,29] cholera due to classical and *Vibrio cholerae* O1 of El Tor biotypes and Inaba and Ogawa serotypes and serogroup O139,[30–32] typhoid fever,[33] shigellosis caused by *Shigella flexneri* 2a[34,35] and *Shigella sonnei*,[36,37] and diarrhea due to enterotoxigenic *E. coli*.[38]

## 6 PHASE 3

Phase 3 studies are intended to be impeccably designed and executed trials that can demonstrate the efficacy of the candidate vaccine, incorporating into the design all the information accumulated from the Phase 1 and 2 trials. The "gold standard" design, when possible, is a large-scale, adequately powered, randomized, controlled, double-blind trial with allocation at the level of the individual. If the nature of the vaccine candidate and other factors (eg, disease prevalence, incidence, and predictability) allow it, this rigorous design provides evidence of protection of individuals. Nevertheless, there are instances where other Phase 3 designs can or must be utilized to generate convincing evidence of vaccine efficacy, satisfying this key prerequisite for licensure of the new vaccine. Examples of alternative Phase 3 study designs and strategies to achieve licensure are mentioned later.

### 6.1 Trials with Cluster Randomization

In some instances it is necessary or preferable to randomly allocate the candidate vaccine or control preparation not to the individual but to larger units such as classes, schools, families, neighborhoods, or villages. Some of the compelling reasons for cluster rather than individual randomization include:

1. If the live vaccine exhibits or has the potential for person-to-person transmission. Facile person-to-person transmission within families and extended households was observed to be a prominent characteristic of Sabin attenuated vaccine strains in early clinical trials. Consequently, the Sabin oral polio vaccine strains could not be tested using a "gold standard" design.
2. Some vaccines in development are intended to function at the community level not via protection of individuals. For example, transmission-blocking malaria vaccines containing gametocyte antigens will offer no protection to a single vaccinated individual in an otherwise nonvaccinated community. However, if a high level of vaccine coverage with such a vaccine can be achieved, the transmission of malaria can be interrupted as the antibodies directed against the sexual stages of *Plasmodium* will interfere with the development of the parasite within the midgut of the mosquito thereby rendering that

mosquito unable to transmit malaria to other individuals in the community. Thus, the way to evaluate the efficacy of a malaria transmission blocking vaccine is to randomly allocate clusters to receive vaccine or placebo and to achieve a high level of community participation in the clusters. If the vaccine is effective, transmission of malaria will diminish in the vaccinated clusters (thus, significantly fewer new cases) compared to in the placebo clusters.

3. Sometimes logistics and practicality make it easier to immunize clusters rather than individuals, thereby achieving a better-organized, less complex and more economical field trial.
4. If it is critical or otherwise advantageous to investigate the importance of indirect protection (herd immunity) associated with use of a vaccine in a population, a cluster randomized design allows this to be studied. If clusters such as villages are randomly allocated to receive test vaccine or the control preparation (placebo or another vaccine that does not offer protection against the disease of interest), there will be persons in each cluster who consented to participate and are enrolled and persons who decline to participate and are not enrolled. The confirmed attack rate of the disease in the nonparticipants of the vaccine clusters compared to the attack rate in nonparticipants in the placebo clusters provides an estimate of the level of indirect protection offered by the vaccine. The greater the proportion of enrolled subjects, the greater the level of indirect protection.
5. There are occasional instances where the high incidence of a disease is unique in time and geography and it is therefore desirable to evaluate the vaccine using a design that tries to mimic how the vaccine might be utilized by public health authorities in reactive immunization to interrupt transmission of the disease. The ring vaccination trial of Ebola vaccine carried out by the World Health Organization and partners in Guinea in 2015 provides an example.[39] Limitations in the supply of vaccine available, the geographic spottiness of the disease, and the ethical and political need to offer test vaccine to all participants in a timely way during this public health crisis led to a unique trial design. Suspect cases at several Ebola treatment centers in Guinea were expeditiously tested to confirm the disease. Once laboratory confirmation of a case occurred, a ring (cluster) was created consisting of the contacts and the contacts of the contacts of the confirmed case. The ring (cluster) was then randomly allocated (1:1) to become either an "immediate vaccination cluster" or a "21-day delay prior to vaccination cluster".[39] Members of the former clusters, following informed consent, were offered immediate vaccination with the VSZ-ZEBOV vaccine, whereas vaccination of the latter clusters with VSV-ZEBOV began only after a 21-day delay. Outcomes were cases of confirmed Ebola occurring among enrolled participants in the two types of clusters beginning 10 days after onset of vaccination of the cluster. The preliminary data from analysis of this unique trial design indicated 100% efficacy against laboratory-confirmed Ebola disease.

6. If there is a widely accepted immunologic correlate of protection against a pathogen and if other licensed vaccines already exist that confer protection by eliciting such mechanistic immunologic correlates, licensure can be achieved without a controlled field trial of clinical efficacy but rather by large-scale safety/immunogenicity trials that document the immune response stimulated by different lots of the new vaccine and demonstrating noninferiority compared to immune responses elicited by the already licensed vaccine. This is an example of licensure by serological noninferiority.

   Perhaps the best example of licensure of a new vaccine demonstrating serological noninferiority versus an already licensed vaccine was the licensure of the PRP-TT Hib conjugate [the capsular polysaccharide of Hib (polyribosyl ribose phosphate) linked to tetanus toxoid] based on its ability to induce serum anti-PRP antibody responses that were noninferior to the responses elicited by licensed PRP-$CRM_{197}$ (PRP conjugate to mutant diphtheria toxin).[40] PRP-$CRM_{197}$ and another Hib conjugate PRP-OMP (PRP linked to outer membrane protein of group B *N. meningitidis*) were licensed by the FDA based on the results of gold-standard randomized, placebo-controlled, double-blind Phase 3 vaccine efficacy trials.[41,42] During these two field trials of Hib conjugate vaccines and an earlier field trial that showed the efficacy in toddlers (above age 18 months) and preschool children of an unconjugated PRP polysaccharide vaccine,[43] attaining a serum anti-PRP titer $\geq 1.0$ mcg/ml was correlated with long-term protection.[44]
7. Occasionally it has been possible to demonstrate convincingly the protective capacity of a vaccine by "before and after" demonstration of the effect of mass vaccination with the new vaccine. When Sabin attenuated poliovirus vaccine strains were used in early Phase 2 clinical trials, there was unequivocal demonstration of person-to-person transmission of the vaccine strain. At the time (1950s), this was considered a positive attribute of that vaccine. However, the facile transmission to contacts precluded performing a classical Phase 3 efficacy trial design with random allocation of vaccine or placebo at the level of the individual and the use of cluster-randomized trials had not yet gained credence as a concept. Accordingly, Sabin and coworkers performed mass vaccination with his attenuated strains in multiple venues where there were seasonal epidemics and demonstrated that following mass vaccination there was a precipitous fall in cases and curtailment or disappearance of wild type virus from sewage surveillance. This was done on multiple occasions in Mexico and Eastern Europe and the collective data were sufficient to convince the FDA and other national regulatory authorities of the safety, immunogenicity, and effectiveness of the Sabin vaccine strains in preventing paralytic poliomyelitis.[45] The relatively inexpensive cost of goods of the Sabin vaccine and its ease of administration and acceptance by populations generated widespread support for its licensure and its implementation post-licensure globally.

8. *Volunteer challenge studies*: Single-dose live oral cholera vaccine strain CVD 103-HgR (*Vibrio cholerae* O1 classical biotype Inaba) originally manufactured by the Swiss Serum and Vaccine Institute was licensed by multiple national regulatory authorities (Switzerland, Canada, Australia, New Zealand, among others) based on efficacy results derived from experimental challenge studies in adult volunteers who ingested *V. cholerae* O1 of classical or El Tor biotype and Inaba or Ogawa serotype. A single-dose of CVD 103-HgR was well-tolerated, elicited a high seroconversion rate of vibriocidal antibodies (the best immunologic correlate of protection) and conferred significant protection against experimental cholera. Protection began as early as 8 days after the dose of vaccine. PaxVax of San Diego, CA is the new manufacturer of CVD 103-HgR which is expected to be licensed by the FDA in 2016.
9. *FDA "Animal model rule"*: For some infectious diseases for which the development and licensure of a vaccine is considered desirable, for example for some biodefense vaccines (eg, anthrax), there is not enough natural disease to be able to generate efficacy data through field trials and for ethical reasons experimental challenge studies in volunteers are not possible. For such vaccines, it is theoretically possible to document efficacy of the vaccine in a relevant animal model and to bridge the serological response between animals and humans.[46] In Nov. 2015, the FDA approved a vaccine for the first time based on the animal rule. The already licensed Anthrax vaccine Biothrax® (Anthrax Vaccine Adsorbed) was approved for administration in conjunction with antibiotics following known or likely exposure to *Bacillus anthracis* spores.[47] Efficacy must be documented through animal model challenges rigorously performed under Good Laboratory Practices with impeccable documentation. Another vaccine candidate that could be licensed in this way is Chimpanzee adenovirus 3 vector expressing Ebolavirus Zaire glycoprotein (ChAd3-EBO-Z). The combination of extensive safety and immunogenicity data in humans being accumulated, high level efficacy in a nonhuman primate model against challenge with wild type Zaire ebolavirus and an immunologic correlate of protection, make this feasible.[20,21,48,49]
10. *Accelerated FDA approval*: Some candidate vaccines that target the prevention of serious and life-threatening infectious diseases for various reasons cannot be tested for efficacy in field trials involving natural challenge. For such vaccines the US FDA offers yet another option to license the product under the "accelerated approval" provisions (21 CFR 601.40/41),[50] if data support the contention that meaningful benefit over existing treatment is likely. By the time several candidate Ebola vaccines were ready for preliminary trials to test their efficacy in West Africa, the incidence of disease had plummeted to the point where the epidemiologic assumptions for the efficacy trial designs were no longer relevant. Thus, an Ebola vaccine candidate that could not be tested in an efficacy trial with clinical endpoints

could seek licensure under the "accelerated approval" provisions, if data from well-controlled clinical trials establish an effect of the product on a surrogate endpoint such as a particular immune response that is *reasonably likely* to predict clinical benefit.[51] For example, ELISA titers achieved in vaccinated nonhuman primates that correlate with protection from highly lethal challenge could help identify an immunogenicity endpoint in humans that is deemed reasonably likely to predict protection of humans.[21] Well-designed post-licensure (Phase 4) studies would have to be undertaken at some point in the future to verify the clinical benefit of the vaccine.

## 7 ISSUES TO BE CONSIDERED IN DESIGNING A PHASE 3 VACCINE EFFICACY TRIAL

Since large-scale, randomized, double-blind, placebo-controlled field trials remain the preferred means of establishing the efficacy of a vaccine, whenever that design is possible, some of the salient issues in the design, performance, and analysis of such trials will be briefly discussed.

### 7.1 Selection and Preparation of the Study Site

Usually the selection of a site for a large-scale field trial is initiated and driven by the sponsor (eg, the manufacturer) looking for a suitable site to test their vaccine. However, there are instances where Ministries of Health have contacted developers of a vaccine to explore the possibility of having the vaccine tested in a particular population where the infection is causing a major disease burden. Live oral typhoid vaccine Ty21a came to be tested in Alexandria, Egypt, Santiago, Chile, and Plaju, Indonesia consequent to health authorities in each of these sites reaching out to the manufacturer of the vaccine candidate. This ultimately resulted in field trials of efficacy of the vaccine in each of these settings.[52–57]

### 7.2 Gathering Baseline Epidemiologic Data

It is critical to gather beforehand as much epidemiological data as possible about the incidence rate of the disease of interest, its seasonality, modes of transmission, the adequacy of health care, the microbiology infrastructure, variations in serotype from year to year (where relevant). It is also important to gather demographic data on age structure, recent census information and on migration in and out of the potential field site.

### 7.3 Protocol Design

Since the Phase 3 field trial of efficacy will be considered by the national regulatory authority to be a "pivotal study," it must be designed carefully with exquisite attention to details. The Principal Investigator takes responsibility for this

but typically with a team of key colleagues representing relevant disciplines (vaccinology, epidemiology, biostatistics, microbiology, immunology, pediatric, or adult medicine).

## 7.4 Financing

Large-scale Phase 3 field trials to evaluate vaccine efficacy are extremely expensive. While the costs vary depending on the vaccine, the number of participants in the trial, the duration of the trial and other features, the costs typically run in the tens or scores of millions of dollars. If the vaccine is targeting a disease for which there is much public clamor for the vaccine and if the disease affects industrialized country populations such that a mature market for the vaccine exists or is likely, industry will fund the vaccine trial, anticipating a return on their investment through post-licensure sales. In contrast, for vaccines directed against infections that overwhelmingly occur in impoverished developing country populations, either the public sector alone or public–private partnerships must be encouraged to fund the trials and to create markets for the vaccine. Gavi—the vaccine alliance, and its vaccine fund play a key role in guaranteeing such markets.

## 7.5 Ethical Issues

In certain situations, as in trying to set up an efficacy trial of a candidate vaccine against a highly lethal infection during an explosive outbreak, it can become very difficult to argue for a classical randomized, placebo-controlled (or other active vaccine-controlled) design. This was the situation early in the Ebola epidemic in West Africa. In such instances one may have to explore innovative designs.[39,58]

## 7.6 Nurturing Political Commitment and Ownership

Large-scale field trials are very visible and therefore it is critical to obtain political support from government leaders at the highest level possible but also to maintain close communication with community and neighborhood leaders to assure continuing support at the grass roots level.

## 7.7 Logistics and Management

The execution of a large-scale field trial of efficacy of a candidate vaccine requires detailed planning, competent management, and attention to logistics. No matter the attributes of the candidate vaccine, superior management is required to assure a well-run field trial.

## 7.8 Interaction with the Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB) must be established to oversee the field trial and assure the safety of the subjects and the integrity of the field trial.

The study sponsor must prepare a charter for the DSMB with formal rules for meetings, periodic reviews of safety data, and voting on actions and recommendations. The composition of the independent DSMB members who must have no conflict of interest should include a biostatistician along with individuals representing key technical areas of expertise. The DSMB Chair and Co-Chair should ideally be highly experienced individuals of unimpeachable character and integrity. These qualities become invaluable should an unexpected set of events occur during the field trial.

## 7.9 Management and Analysis of the Data

Enormous amounts of data will be collected in the course of the field trial. The FDA has recently published regulatory guidance that specifies the electronic format for the reporting of clinical trials data. This means that an experienced data management group must be identified to provide support for the trial. During the past three decades a number of Contract Research Organizations have emerged with expertise in data management and other areas necessary to support large-scale field trials. Failure to partner with a data management team that has depth, infrastructure, and experience in supporting a large-scale field trial can result in delays and frustrations as the trial unfolds.

## 7.10 Post-trial Commitments

It is imperative that the trial sponsor and Principal Investigator have a candid dialog with leaders in the Ministry of Health and other government leaders to discuss the expectations of the host government with respect to availability of the vaccine following completion of the trial and positive results. Governments often expect that when the vaccine becomes licensed and available, some free doses or discounted doses will become available in proportion to the size of the field trial and for some period of time as a compensation for government assistance and support provided during the field trial in their population. It is important that these expectations be discussed and negotiations on any future commitments completed before the trial begins, lest there be misunderstandings during or after the trial that will be counter-productive for all stakeholders.

## 7.11 Primary Aim(s) of the Phase 3 Field Trial Protocol

The primary aim of the trial must be clear, precise and achievable. It is preferable to have just a single primary aim or two related coprimary aims, while there can be multiple secondary and tertiary aims. Positive results that address the primary aim of the field trial will provide the evidence base for licensure of the vaccine (as efficacy results from a pivotal trial). In addition, results from addressing the properly crafted primary aim can strongly guide public health use of the vaccine post-licensure when it may be introduced at some future point in time.

## 7.12 Sample Size

The sample size needed to achieve the primary aim is a critical feature of the trial protocol, as it has implications for the cost of the trial, its duration, the complexity of the logistics, the difficulty in managing the trial dataset, and the likelihood of successfully achieving the primary aim.

Some of the factors that influence the sample size include:

1. Number of study groups and comparisons. There is often strong pressure from various stakeholders to argue for including additional groups in the field trial to allow additional comparisons. In general, this needs to be resisted to focus on the primary aim, diminish costs of the trial and simplify logistics.
2. Out migration of participants can endanger the ability to address the primary aim successfully. One must try to obtain realistic estimates of out-migration and then assume it may be even greater. An overestimate is preferable to underestimating out migration.
3. The statistical power used to detect a true difference is an important consideration in calculating the sample size. Most modern field trials incorporate 90% power in their calculation of sample size. Dropping the power to 80% drops the sample size needed. Nevertheless, whenever possible, we recommend that a power of 90% be used in calculating sample size.
4. The alpha value used in the sample size refers to the likelihood that a difference detected is real. A two-sided alpha of 0.01 or 0.05 is commonly used depending on the specific vaccine, anticipated incidence rate, etc. The lower the alpha incorporated in the calculation, the larger the sample size.
5. A 95% confidence interval (95% CI) will be calculated around the point estimate of efficacy for the primary aim of the Phase 3 trial. The primary aim can be written so as to define a lower limit of the 95% CI above which the primary aim is achieved. For example the primary aim can state that the lower limit of the 95% CI around the point estimate must not be lower than 30% or lower than 20%. While this stipulation drives up the sample size, if that sample size is deemed logistically and financially feasible, the data that emerge will give public health authorities powerful information for making a future decision about introduction of the vaccine with a high degree of confidence about its protective nature.
6. More and more in vaccinology we are coming to recognize the powerful indirect protective effects widespread use of a vaccine can have on transmission of the pathogen, particularly if transmission is person-to-person. Thus, if a Phase 3 field trial succeeds in enrolling a large proportion of the target population within a population or subpopulation, the actual disease incidence in the control group during the field trial may fall and be much lower than prior to the trial. This should be taken into account in the calculation of the sample size by lowering the predicted incidence for the control group from the incidence rate obtained during pretrial epidemiologic surveillance.

## 7.13 Selecting the Control Preparation for Vaccine Efficacy Trials

One of the most important decisions to be made in designing the Phase 3 efficacy trial is the selection of the preparation to be given to the controls in the study. Several options are given later along with recognized advantages and drawbacks of each.

## 7.14 True Placebo

There are two unequivocal advantages that derive from administering a true placebo that otherwise appears identical to the candidate vaccine being tested. The first is that there is no chance that the placebo will offer any protection against the target disease. The second is that it will allow the best assessment of the safety and reactogenicity profile of the test vaccine, since placebo recipients receive an inert material. If there are important questions about the safety or the clinical acceptability of the candidate vaccine, the clearest data will come from comparison of the frequency and type of adverse reactions compared to a true placebo group. Yet another advantage of placebo is if the sponsor can arrange a placebo preparation that is identical in appearance to the test vaccine. This enhances the ease of maintaining a double blind. By contrast, the most notable drawback to use of a true placebo is that the control group participants receive no direct biological benefit, despite providing the same time commitment, etc., as members of the test vaccine group who may have a diminished risk to the disease of interest if the vaccine proves to be effective. Another drawback is the difficulty sometimes in identifying a placebo that appears identical to the test vaccine to be able to maintain double blindness.

## 7.15 A Licensed Vaccine Against Another Infection that will have No Effect on the Study Outcome Events

In some populations offering no direct benefit to study participants, as would occur with use of a true placebo, is not well accepted. Indeed, some ethical review committees frown upon the use of a true placebo for the control group. In such situations one can look for a vaccine that is not routinely used in that population and that can offer protection against another prevalent infectious disease, while offering no possibility of cross protection against the primary outcome infection. An excellent example can be seen in the description of the design of a recent large-scale Phase 3 trial in two pediatric age groups of assessing the efficacy of RTS,S malaria vaccine in preventing confirmed episodes of *P. falciparum* malaria. Since this trial was conducted in a vulnerable population (children in resource poor communities in countries in sub-Saharan Africa), it was deemed ethically important to provide other vaccines to the control children that would provide some potential benefit without affecting susceptibility to malaria. Infants aged 6–12 weeks and children aged 5–17 months were randomly allocated (1:1:1) to one of three groups. One

group was given RTS,S/AS01 malaria vaccine at months 0, 1, and 2, followed by a booster dose at month 20; a second group got the RTS,S/AS01 primary vaccination series but received meningococcal serogroup C conjugate vaccine (Menjugate, Novartis, Basel, Switzerland) as the booster at age 20 months, instead of RTS,S/AS01; the third group received only comparator vaccines including rabies vaccine (Verorab, Sanofi Pasteur, Paris, France) for children and meningococcal serogroup C conjugate for young infants.[59] Thus, in this trial all control participants received active preparations that conferred benefit against other prevalent infectious disease risks, albeit not malaria. Drawbacks to this approach include difficulty of finding a vaccine that can be given to the target age at the same immunization schedule as the test vaccine and maintaining the double blind character of the study if that is deemed a high priority.

### 7.16 An Experimental Vaccine Against Another Infection that will have No Effect on the Study Outcome Events

There have been well-designed and executed field trials where another nonlicensed experimental vaccine was used as the product that was administered to control subjects. Thus two separate unlicensed vaccines were being tested. One example is the randomized large-scale efficacy trial of 7-valent pneumococcal conjugate vaccine performed in Northern California in which an unlicensed meningococcal C conjugate vaccine was administered to participants in the control group.[60] The advantages of this approach include the provision of a product of potential benefit to all participants in both arms of the trial and the opportunity to gain safety and efficacy information on two different vaccines. The main drawback is that since both vaccines are unlicensed, there is a considerable amount of additional regulatory oversight work to be performed. Another drawback is that it is difficult to gain insights on the relevance of differences (or lack of differences) in reactogenicity, since the comparator is an unlicensed product and limited prior safety data may be available.

## 8 PHASE 4 SURVEILLANCE AND STUDIES TO MONITOR PRODUCT SAFETY AND THE IMPACT ON DISEASE BURDEN WITH VACCINE USE POST-LICENSURE

Following licensure of a vaccine and its increasing use, careful surveillance can detect rare serious adverse events that were not detected during prelicensure Phase 1–3 clinical trials. In the United States two surveillance systems are geared to monitor the safety of newly introduced vaccines including the Vaccine Adverse Event Reporting System (VAERS)[61] and the Vaccine Safety Datalink (VSD).[6,7] VAERS is a passive reporting system jointly maintained by the Centers for Disease Control and Prevention (CDC) and the FDA, with reports coming mainly from healthcare practitioners and vaccinated individuals. By

contrast, VSD represents a highly coordinated denominator-based surveillance system operated by the CDC and nine healthcare systems that collectively perform post-licensure monitoring of vaccine safety that involves ~3% of the US population but its demographic make-up is representative of the entire population. VSD has both detected associations between specific vaccines,[62] and has refuted incriminations.[63]

There are multiple ways to document the effectiveness and impact of vaccines once they have been introduced post-licensure. The diminution of the incidence of confirmed disease and of deaths can be monitored through surveillance systems both in industrialized countries and in developing countries. Following the introduction of routine infant immunization with seven-valent pneumococcal conjugate vaccine in the United States in 2000, not only was a significant drop observed in the incidence of invasive pneumococcal disease due to vaccine serotypes in the target infant population but a powerful indirect effect was noted as invasive disease due to vaccine serotypes also fell significantly in the parent and grandparent age groups.[12] The consequence of documentation of indirect protection extending to adults was to show that the vaccination of infants with an expensive pneumococcal conjugate vaccine was much more cost-effective than had been predicted prelicensure.[64]

In Bamako (Mali), the incidence of confirmed severe invasive infections due to Hib requiring hospitalization were shown to fall by 88% within 3 years of the introduction of Hib conjugate into the routine EPI in Mali.[11] Over the same period the prevalence of titers of Hib PRP antibody in random samples of infants 6–7 months of age rose from 0.5% to > 80%, documenting how the vaccine modified the susceptibility of the young infant population previously at high risk.

Another way to assess the effectivenesss of vaccines post-licensure is to perform case-control studies either in relation to routine use of the vaccine or following a mass vaccination.[9,65] There have also been a few randomized controlled large-scale post-licensure selective vaccination and intensive surveillance studies. One such Phase 4 study allowed a high level of efficacy to be demonstrated for Hib conjugate in Santiago (Chile), where the vaccine was introduced in the EPI units of approximately one-half of the health centers of the city.[8] Demonstration of 90% effectiveness from routine use of the vaccine in these health centers accompanied by an economic analysis,[66] led Chile to become the second nonindustrialized country (at that time) to introduce Hib conjugate.

Various creative designs have allowed the demonstration of the effectiveness of vaccines in post-licensure mass vaccinations, including reactive vaccination with a live (CVD 103-HgR) oral cholera vaccine in Micronesia,[10] and with an inactivated oral cholera vaccine in Guinea.[67] These post-licensure reactive vaccination studies demonstrated that reactive vaccination with oral cholera vaccine can play an important adjunct role in cholera control, thereby dispelling prior misconceptions.

## 9 SUMMARY NOTE

During the past four decades the vaccine development paradigm based on a succession of clinical trials of increasing size and complexity leading to licensure of the vaccine and subsequent post-licensure studies intended to assess the impact and safety following large-scale use of the vaccine has evolved. Just as advances in basic research have revolutionized the generation of new vaccine candidates, so has the increased sophistication of vaccine clinical trial methodologies improved the ability to evaluate the safety, immunogenicity, and efficacy of candidate vaccines. Vaccines remain the most cost-effective weapons of mass prevention.

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Chapter 7

# Immunological Correlates of Vaccine-Mediated Protection

**Michael J. Carter, MRCPCH, Christoph J. Blomke, PhD, Andrew J. Pollard, FRCPCH, PhD**

*University of Oxford, Oxford Vaccine Group, Centre for Clinical Vaccinology and Tropical Medicine, Department of Paediatrics, Oxford, United Kingdom*

## Chapter Outline



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## 1 INTRODUCTION

Today considerable effort is undertaken during vaccine development to identify and measure potential mechanisms of immunological protection. These proposed measurements are then validated by correlation with protection from disease after natural exposure or passive protection, or alternatively related to vaccine efficacy or effectiveness endpoints. The desire to identify correlates of protection, especially early in the development of a vaccine, is more than scientific curiosity. The importance of correlates of protection is evident by the reduction of the financial risk associated with clinical development of new vaccines, support of the use of a vaccine in new populations, and the pivotal role in the assessment of the public health impact of vaccines.

If a correlate of protection is already known, for example, the level of antibody administered passively or induced following previous exposure that subsequently prevents reinfection, vaccine developers have a target antibody concentration that is likely to be associated with a successful product. Similarly, if a functional correlate is known (eg, bactericidal antibody in prevention of meningococcal disease), developers can target programs to induce the appropriate level of the functional antibody level. If such protective responses are documented, there is a low risk in moving to Phase 3 clinical efficacy trials and a high likelihood that the investment will provide data to support licensure of the product. In some cases, the cost of expensive efficacy trials may be circumvented entirely (eg, licensure of Group B meningococcal vaccines) and a license granted entirely based on the correlate. However, in the absence of a correlate, the only way in which the likelihood of vaccine efficacy can be tested is to undertake a randomized controlled Phase 3 efficacy trial at financial risk, since some products will fail. If no correlate has been identified prior to an efficacy trial, the efficacy trial itself may allow identification of a correlate. The availability of such a measure can be very important for programmatic implementation of vaccines, where some jurisdictions will expect to see local data generated that supports the use of the vaccine in the new population. The cost of repeating efficacy trials may be prohibitive, but, if a correlate has been established, bridging from a pivotal trial undertaken elsewhere becomes possible. Similarly, if a new product is developed that is in competition with an existing product, the pathway to licensure is more straightforward if there is a correlate of protection that allows head-to-head noninferiority trials to be undertaken with immunological endpoints, which may support licensure without an efficacy trial.

Following successful licensure of a new vaccine and its widespread use in a population, various vaccine factors (eg, changes in vaccine quality) and environmental factors (interference from other vaccines in the immunization schedule or reduction in circulation of the pathogen) may affect the immune response to the vaccine, and thus vaccine efficacy. Monitoring of protective levels of antibody (or T cells) in individuals and populations may be very valuable in planning changes in immunization programs that support maintenance of effectiveness.

In practice, the absence of a correlate may hinder clinical development of a vaccine because the cost of undertaking a large clinical trial in the absence of strong evidence that it will work may make investment unfavorable. Furthermore, the implementation of vaccines may be more difficult in the absence of a correlate that reassures public health authorities that the vaccine will provide protection in a new population. These points underpin the importance of correlates of protection in vaccine development and the value of robust correlates being available in the years following licensure of a new vaccine.

## 2 DEFINITIONS

### 2.1 Correlates of Protection

A variety of different terms, definitions, and interpretations of them exist in the literature surrounding correlates of protection. The lack of a consistent approach to defining correlates leads to confusion but also reflects the increasing complexity of knowledge of the host response that determines protection. Here we use a simple practical definition: a correlate of protection is a biomarker that is statistically associated with protection. Thus, in theory, any measurement that can be made of the host response pathways from the first interaction of a pathogen with the host (perhaps binding to a pattern recognition receptor on dendritic cells) through to the effector of the human immune system (eg, a bactericidal antibody response), could be related statistically to protection and be a correlate (Fig. 7.1). It is also important to consider that there may also be bystander effects of the activation of the immune response, which are not responsible for protection either directly or indirectly, but could be statistically related to protection and thus a correlate. While this latter category has not been much considered in the past, the current use of transcriptomic and proteomic approaches to analyze immune responses is likely to result in the identification of such activated pathways that are not driving the protective responses but are statistically related to them. While it may appear that any of the previously

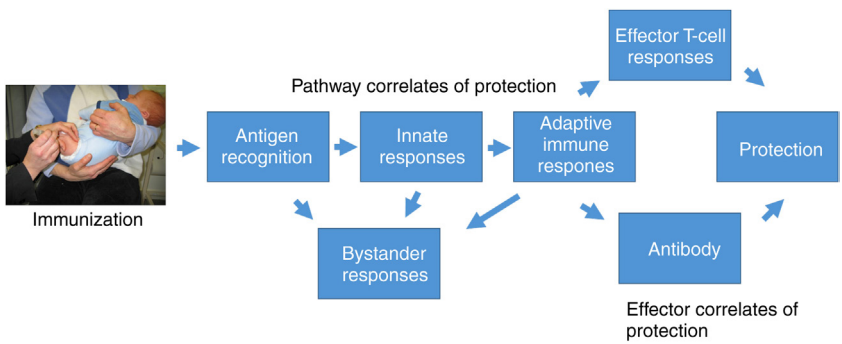


**FIGURE 7.1** **Pathway and effector correlates of protection.**

**TABLE 7.1** Examples of Types of Correlates of Protection

| Types of correlate of protection | Examples of correlates of protection |
|---|---|
| Effector correlate | Antibody: bactericidal antibody, neutralizing antibody, opsonophagocytic antibody, high avidity antibody, antibody concentration, antibody-dependent cellular cytotoxicity |
| | T cells: cytotoxic T cells, CD4 T-cell proliferation |
| Effector pathway correlate | T helper cells, TfH cells, memory B cells, plasma cells, cytokines |
| Undefined correlate | Transcription factors, cytokines, transcriptional, or proteomics profiles |

mentioned measures that correlate consistently with protection would be a useful marker, there are advantages in identifying the effector(s) of protection, as these are by definition the most robust measurements to provide assurance of effectiveness (Fig. 7.1 and Table 7.1).

## 2.2 Relative and Absolute Correlates

An additional important consideration is that correlates may be relative or absolute. An absolute correlate is one in which there is a defined and accepted threshold above which there is protection and below which there is not. Unfortunately, some "absolute" correlates of protection are not absolute, as will be discussed later. A relative correlate is where the protective biomarker has been defined but there is no threshold that relates to absolute protection, although generally higher numbers (eg, higher level of RSV antibody) are related to more protection. For relative correlates, there may be some individuals who are protected even with rather low levels of antibody, resulting in a frequency distribution of protection.

## 2.3 Co-correlates

In some circumstances there is more than one mechanism of protection that can be measured. Indeed, it seems likely, especially with live vaccines, that there are multiple pathways to protection that could be induced. For example, live viral vaccines may induce both neutralizing antibodies and cytotoxic T cells, which could both be important in early defense against infection. It is plausible that high avidity antibodies produced in response to meningococcal conjugate vaccines could provide some protection through bactericidal activity, opsonophagocytosis, and antibody-dependent cellular cytotoxicity, yet only bactericidal activity is firmly accepted as the major contributor to protection.

## 2.4 Surrogates of Protection

Surrogates of protection, from the dictionary definition of the word "surrogate," are measurements that substitute or are a proxy for protection, which would make this essentially the same as the definition of a correlate of protection described earlier. However, others have considered that the word "surrogate" relates to the "correlate" (rather than the protection) and therefore a surrogate may be defined as a marker that substitutes for the correlate of protection, but does not itself confer protection.[1] The terms correlate and surrogate have often been used interchangeably in the primary literature leading to much confusion. Qin et al.[2] defined correlates of protection as either *correlates of risk* (for correlates not in the mechanistic pathway to protection), or as different levels of surrogates of protection that relate laboratory measurements to vaccine efficacy (Table 7.2). For Qin et al. a surrogate of protection is necessarily on the mechanistic pathway to protection, with a Level 1 surrogate of protection representing a correlate on the mechanistic pathway to protection (our terminology) from a single setting (eg, a single vaccine trial), and a Level 2 surrogate of protection representing a correlate on the mechanistic pathway to protection that is predictive of vaccine efficacy across a number of settings. Although useful in highlighting the importance of validating correlates across different settings, their terminology have been recently simplified, as discussed later.[3]

The major regulatory authorities have included the term "surrogate" in their own definitions, but are not consistent in their usage (Table 7.2). The World Health Organization defines a surrogate as "a marker that is statistically associated with clinical protection and that lies on the causal pathway leading to protection" whereas a *correlate* is "a marker that is statistically associated with clinical protection, but not necessarily on the causal pathway leading to protection,"[4] which is almost exactly the opposite of the definition given earlier and of the US Food and Drug Administration (FDA) definition (Table 7.2).[4] While some have argued that the term surrogate should be abandoned as a result of this discrepancy in definition, it is established in official definitions and it is therefore important to recognize that there are substantial differences in the definitions used both in the primary literature, and reviews and official documents.

## 2.5 Mechanistic and Nonmechanistic Correlates

Plotkin and Gilbert have recently proposed that correlates are simply divided into mechanistic and nonmechanistic correlates.[3] The former being those assessments that directly measure the effector mechanism of protection and the latter being measurements of other responses that correlate with protection, but are not responsible for it. An example of a mechanistic correlate, according to Plotkin and Gilbert, is meningococcal bactericidal antibody (measured using the serum bactericidal assay), which is believed to be the effector of vaccine-induced protection after immunization with meningococcal vaccines and correlates with protection. Meningococcal antibodies can also be measured

**TABLE 7.2 Various Definitions of Correlates of Protection**

| References | Definitions recently described in literature on correlates of protection |
|---|---|
| Qin et al. *J Infect Dis* 2007;**196**:1304 | *Correlate of risk (CoR):* An immunological measurement that correlates with the rate or level of a study endpoint used to measure VE in a defined population |
| | *Level 1 surrogate of protection (SOP):* An immunological measurement that is a CoR within a defined population of vaccinees and is predictive of VE in the same setting as the trial; validation entails showing either Level 1 SOPS or Level 1 SOPP (given later) |
| | *Level 1 SOPS:* The relationship between the immunological measurement and the risk of the study endpoint is the same in vaccinees and nonvaccinees |
| | *Level 1 SOPS:* (1) groups of subjects with no or the lowest vaccine effect on the immune response have no VE (vaccine efficacy) and (2) groups of subjects with a sufficiently large vaccine effect on the immune system have positive VE |
| | *Level 2 SOP:* An immunological measurement that is a Level 1 SoP and that is predictive of VE in different settings (eg, across vaccine lots, human populations, viral populations, species) |
| Plotkin. *Clin Infect Dis* 2008;**47**(3):401 | *Correlate of protection*: A specific immune response to a vaccine that is closely related to protection against infection, disease, or other defined endpoint |
| | *Absolute correlate:* A quantity of a specific immune response to a vaccine that always provides near 100% protection |
| | *Relative correlate*: A quantity of a specific immune response to a vaccine that usually (but not always) provides protection |
| | *Cocorrelate:* A quantity of a specific immune response to a vaccine that is 1 of ≥2 correlates of protection and that may be synergistic with other correlates |
| Plotkin. *Clin Vacc Immunol* 2010;**17**(7):1055 | *Correlate:* An immune response that is responsible for and statistically interrelated with protection |
| | *Absolute correlate:* A specific level of response highly correlated with protection; a threshold |
| | *Relative correlate*: A level of response variably associated with protection |
| | *Cocorrelate:* One of two or more factors that correlate with protection in alternative, additive, or synergistic ways |
| | *Surrogate:* An immune response that substitutes for the true immunologic correlate of protection, which may be unknown or not easily measurable |

**TABLE 7.2 Various Definitions of Correlates of Protection (*cont.*)**

| References | Definitions recently described in literature on correlates of protection |
|---|---|
| Plotkin and Gilbert. *Clin Infect Dis* 2012;**54**(11):1615 | *Correlate:* An immune marker statistically correlated with vaccine efficacy (equivalently predictive of vaccine efficacy) that may or may not be a mechanistic causal agent of protection |
| | *Mechanistic correlate:* A correlate of protection that is mechanistically and causally responsible for protection |
| | *Nonmechanistic correlate:* A correlate of protection that is not a mechanistic causal agent of protection |
| US Food and Drug Administration | *Correlate:* Generally, a laboratory parameter that has been shown to be associated with protection from clinical disease |
| | *Surrogate endpoint:* Laboratory or physical sign that is used in therapeutic trials as a substitute for a clinically meaningful endpoint that is the direct measure of how a patient feels, functions or survives and that is expected to predict the effect of the therapy[a] |
| International conference on harmonisation (EU, Japan, USA) | *Validated surrogate endpoint:* An endpoint which allows prediction of a clinically important outcome but in itself does not measure a clinical benefit. When appropriate, surrogate outcomes may be used as primary endpoints[b] |
| European Agency for the Evaluation of Medicinal Products | *Immunological correlate of protection:* For example, specific antibody titer correlating with protection |
| | *Serological surrogate:* A predefined antibody concentration correlating with clinical protection |
| WHO Department of Immunization, Vaccines and Biologicals: 2013 | *Correlate:* The term *correlate* is favored to describe markers that are statistically associated with clinical protection, but are not necessarily on the causal pathway leading to protection |
| | *Surrogate:* The term surrogate refers to markers [that are statistically associated with clinical protection and] that lie on the causal pathway leading to protection |

[a]*Accelerated approval of vaccines can be given by the FDA if well-controlled trials have shown that the surrogate endpoint is considered "reasonably likely" to predict clinical benefit, subject to the requirement that the applicant studies the vaccine further to demonstrate clinical benefit.*
[b]*The strength of evidence for a surrogate includes consideration of: (1) the biological plausibility of the relationship, (2) the demonstration in epidemiological studies of the prognostic value of the surrogate for the clinical outcome, and (3) evidence from clinical trials that treatment effects on the surrogate correspond to effects on the clinical outcome.*

by enzyme-linked immunosorbent assay (ELISA) and these in turn also correlate with protection, but since antibodies, measured in this way, raised by vaccination do not necessarily actually confer protection, ELISA antibodies are considered in this framework to be a nonmechanistic correlate of protection. Conversely, in the context of the herpes zoster (shingles) vaccine, these authors have argued that T cells are responsible for protection providing a mechanistic

correlate, but the most convenient measurement to make clinically, which also correlates with protection, is the antibody response, which is not thought to be responsible for protection and is therefore a nonmechanistic correlate. Both of these examples highlight difficulties in understanding the correlate. The mechanistic correlate, meningococcal bactericidal antibody, is a subset of the antibodies contained in the total antibody measured by ELISA, leading to the conclusion that both of these measurements contain mechanistic correlates. In the case of the shingles vaccine, it is possible that there is some minor contribution of antibody-mediated viral neutralization (preventing spread between cells) to the containment of the varicella zoster virus (VZV) after immunization and thus the nonmechanistic correlate may also be a partial mechanistic correlate.

## 2.6 Pathway and Effector Correlates of Protection

In view of the difficulties mentioned earlier in defining correlates and surrogates of protection, and the different understanding about the terms among individuals and official agencies, it is important that authors routinely define the terms when using them in order to maintain clarity. We suggest that a more precise and descriptive definition of correlates, as proposed by Plotkin and Gilbert,[3] might improve understanding of the differences in meaning that have appeared in the literature. However, to be prepared for the rapid expansion in measurements that will be produced following the widespread adoption of the new technologies, we propose the following descriptive definitions (Fig. 7.1)

1. Established mechanistic correlates of protection:
   a. Effector correlate of protection: measurement that is the effector mechanism of protection and does correlate with protection (eg, meningococcal bactericidal antibody)
   b. Pathway correlate of protection: measurement of a biomarker that is on the pathway of responses that leads to the protective response and does correlate with protection (eg, T follicular helper cells)
2. Undefined correlate of protection: measurement of a biomarker that is not established as directing the protective response but does correlate with protection (eg, a gene expression profile that correlates with protection)

There are many different types of response that may be an effector correlate but an almost unfathomable number of correlates that may be pathway or as yet undefined correlates (examples are show in Table 7.1).

## 2.7 Protection Endpoints

Before discussing correlates of protection further, it is important to consider the endpoint: protection. It is readily assumed that a correlate of protection relates to the defined endpoint of sterilizing immunity at the individual level, that is, if the biomarker is present at a certain level then the individual is fully protected

from infection. While this might be an ideal situation, the reality is that not all vaccines deliver sterilizing immunity and, furthermore, obtaining this endpoint might be clinically unimportant or logistically impossible. For example, the trials of rotavirus vaccines (which enrolled more than 60,000 infants) focused on prevention of hospitalization and severe disease as achievable endpoints,[5] and had a correlate been established, this would have been the protective endpoint defined. Mild rotavirus infection is not clinically important and did still occur in the clinical trials, despite high efficacy against severe disease. By contrast, the endpoint for the original trial of the pneumococcal conjugate vaccine (PCV7),[6] involving over 37,000 infants, was invasive pneumococcal disease, measured by blood culture. This study led to the definition of a level of antibody (0.35 μg/mL) as the correlate of protection that has been widely adopted. From a global perspective, invasive pneumococcal disease is not actually the most important clinical endpoint, since most of the hospitalizations and deaths from pneumococcal disease are caused by pneumonia. Pneumococcal pneumonia in children, where approximately only 10% of cases are blood culture positive, is difficult to define with high specificity but the development of a consensus on a WHO radiological definition of endpoint pneumonia has made the use of pneumonia accessible as a useful clinical endpoint.[7]

It is now established that herd immunity (herd protection) is an important component of the protection of populations against almost all communicable diseases that are acquired through contact with other humans. In the case of pneumococcal disease, herd immunity is most readily measured by studying the reduction in nasopharyngeal carriage of vaccine-type pneumococci among vaccinated populations,[8] and is now the basis of effectiveness studies being undertaken in many settings to assess the impact of the roll out of new PCV10/13 vaccine programs.[9,10] Once colonization of toddlers with vaccine serotypes is blocked by vaccine-induced antibody, disease rates fall among children and adults who are vaccinated and those who are not as transmission is interrupted.[11,12]

Suitable clinical endpoints, with which a biomarker might be usefully correlated, therefore include: (1) absolute prevention of infection, (2) prevention of death, (3) prevention of severe disease/hospitalization, (4) prevention of sequelae, (5) prevention of certain syndromes associated with the infection (eg, pneumonia), and (6) a clinical measure of herd immunity (eg, blocking of colonization).

While the gold standard for establishing a correlate is to consider individual protection in randomized controlled clinical trials, some biomarkers are best established after licensure, particularly where there has been no prelicensure efficacy trial. In this circumstance, the correlate of protection will relate only to population protection and cannot quantitate the level of the correlate that is required to confer individual protection. A good example of this is the capsular Group C meningococcal vaccine that was evaluated after licensure in the United Kingdom in an effectiveness study.[13] The proportions of the population in different age groups

whose serum bactericidal antibody titer was over the putative protective threshold (≥1:8 with rabbit complement) was related to the population estimate of protection to derive a threshold for protection at the population level.

## 3 INDENTIFYING CORRELATES OF PROTECTION

### 3.1 Correlates of Protection From Natural Infection and Vaccination

A correlate of protection may be identified from careful observational studies of naturally acquired infection, passive immunization studies, or derived from measurements of the immune response following vaccination (either in challenge studies or a field trial).

Immunization through naturally acquired infection and immunization through vaccination differ. Naturally acquired infection requires replication-competent pathogens and is likely to involve exposure to diverse and highly numerous antigens (3–4000 proteins in many bacterial pathogens), over a period of days (before the infection is cleared), and often with a bacteremia or viremia, stimulating a wide variety of immunological tissues in addition to the site of pathogen entry to the host. Immunization through vaccination with attenuated replicating live organisms maintains some of these features, particularly the diversity of antigens and the exposure over a potential period of days before clearance of infection. However, these organisms contain attenuating gene deletions and may not display the full array of antigens that occur with natural infection. Inactivated (killed) vaccines maintain a diversity of antigens, but exposure to these antigens is only over a short period and at a single site since the organism does not replicate. Many more recently developed vaccines contain a small number of purified antigens, such as in acellular pertussis vaccines or the protein–polysaccharide conjugate vaccines for *Haemophilus influenzae* type b (Hib), *Streptococcus pneumoniae*, and *Neisseria meningitidis.* The differences between vaccination and natural infection, and between different types of vaccine, hamper the search for correlates of protection.

### 3.2 Correlates Identified From Natural Experiments

Perhaps one of the most difficult issues in hunting for correlates of protection is knowing where to start. There are a large number of potential effector mechanisms for protection (eg, different types of T- and B-cell responses) and many pathways that are activated during the immune response that may correlate with protection. Experiments of nature, such as in various forms of inherited and acquired immunodeficiency, provide considerable insight into the likely effector mechanisms that are important in susceptibility or protection from infection. For example, asplenic individuals are highly susceptible to pneumococcal disease, but much less to infection with other invasive bacterial pathogens such as meningococcus and Hib. This observation led to the identification of opsonophagocytic antibodies as the likely effector of protective immunity, requiring

the presence of splenic macrophages bearing Fc and complement receptors to facilitate the removal of antibody and complement opsonized pneumococci from the circulation. In contrast, complement deficiency renders an individual highly susceptible to infection with *N. meningitidis*, supporting the laboratory finding that complement replete immune sera are able to kill meningococci in vitro and that this correlates with protection after vaccination. Predictably, patients receiving eculizumab, a monoclonal antibody which blocks terminal complement component function, are susceptible to meningococcal disease.[14]

Individuals with T-cell immunodeficiency are especially susceptible to severe viral diseases, which might indicate that T-cell immunity is important for front line protection. However, it appears that T-cell immunodeficient individuals may have susceptibility to viral infection that is similar to that experienced by other naïve individuals, but are less able to contain viral replication after infection occurs, leading to severe disease. An increasing number of primary immunodeficiencies are being discovered using genomic approaches to diagnosis, providing new clues about mechanisms of protective immunity that can be applied in the search for correlates of protection for current and new vaccines.

## 3.3 Correlates of Protection From Observational Studies of Naturally-Acquired Infections in Humans

Observational studies, either prospective cohort or case-control studies could provide information on correlates of protection to naturally acquired infection. Unfortunately, to investigate correlates of protection, blood samples should ideally be taken prior to the incident infection (otherwise distinguishing pre- and postinfection immune status is not be possible). This has tended to limit observational studies to rare opportunistic studies, the indirect cohort method, or those embedded within randomized trials of vaccine efficacy; and to infectious diseases where infants and children are subsequently naturally exposed to infection at a relatively high incidence.

Opportunistic observational studies have generally occurred in well-defined outbreaks of infectious diseases. The derivation of a measles antibody titer of $\geq$120 mIU/mL (as measured by the plaque reduction neutralization test) as an absolute correlate of protection from classical measles occurred through the analysis of sera from blood donated from university students immediately prior to an outbreak of measles at the university.[15] Students with a preoutbreak measles antibody titer of $\geq$120 nIU/mL were protected from clinical measles disease, but only those with a titer $\geq$1052 mIU/mL appeared to be fully protected from infection (and therefore, possibly only a titer $\geq$1052 mIU/mL is sufficient to fully prevent measles transmission). Students with a measles antibody titer of $<$120 mIU/mL developed measles disease at high incidence.

An alternative method to derive correlates of protection from observational studies is to use immunogenicity data from a representative sample of the population of interest, and apply this to changes in the incidence of disease of interest in

the larger population. This is termed the indirect cohort method, and is of particular use in the context of epidemiologically rare disease (such as proven invasive bacterial disease). Andrews et al., in a recent example of such a study of pneumococcal disease in infants in England, Wales, and Northern Ireland, used 706 cases of PCV7-type invasive pneumococcal disease, 292 cases due to the additional 6 serotypes in PCV13, and 414 cases due to nonvaccine-type pneumococci.[16] Immunogenicity data, using ELISA in microgram per liter and opsonophagocytic antibody titers, were used from serum samples from infants previously vaccinated with PCV7 and PCV13 as representative UK samples. Vaccine effectiveness was calculated by using cases of PCV7 and PCV13 and comparing with controls with nonvaccine-type disease. Second, serotype-specific correlates of protection vaccine-types were derived by applying the calculated vaccine-effectiveness to the distribution of serotype-specific IgG 1 month after the first dose of PCV priming (either PCV7 or PCV13). Thus framed, the serotype-specific absolute correlate of protection against the endpoint of invasive pneumococcal disease could be calculated for all incident vaccine serotypes. This exemplifies the use of studies of the change in disease incidence following vaccine introduction (vaccine impact studies) to inform our understanding of correlates of protection in addition to providing important health policy data.

## 3.4 Correlates Derived From Randomized Controlled Trials

Observational studies embedded within randomized controlled trials of vaccine efficacy provide an excellent opportunity for the delineation of immune correlates of vaccine-mediated protection. Endpoints for these studies can be either clinical, such as pertussis disease, or invasive bacterial disease, or epidemiological, such as prevention of nasopharyngeal colonization by Hib.

An important example is provided by pertussis (due to infection with *Bordetella pertussis*). Serum antipertussis IgG was identified as an important correlate of protection against pertussis in the 1950s on the basis of measurement of agglutination of *B. pertussis* when exposed to sera from children successfully vaccinated (and protected) with various whole cell pertussis vaccines.[17] Further case-control studies were embedded within a randomized trial of acellular pertussis vaccines in Sweden. Following vaccination with acellular pertussis vaccine and later natural household exposure to pertussis, children who developed severe pertussis had a median antipertussis toxin (PT) IgG concentration of 79 U/mL, children with mild pertussis had a median anti-PT IgG concentration of 156 U/mL, and children who did not develop pertussis a median anti-PT IgG concentration of 246 U/mL.[18] Thus serum anti-PT (in addition to antibodies to other pertussis antigens derived from similar studies) acts as a relative correlate of vaccine-mediated protection against pertussis, with an IgG concentration between 156 and 246 U/mL probably equating to an absolute correlate of vaccine-mediated protection.[19] The importance of antipertussis IgG as an effector of protection has been suggested by the success of maternal vaccination with

acellular pertussis at preventing disease in early infancy.[20] Although decreased infant exposure to pertussis by augmenting maternal immunity to pertussis (cocooning) has some effect, the transplacental transfer of maternally derived antipertussis IgG is also likely to be of considerable importance.

Peltola et al. provided early data on the use of unconjugated polysaccharide vaccines against Hib invasive disease by embedding an immunogenicity study within a randomized trial in infants and children in Finland in the 1970s.[21] They found postvaccination mean anti-Hib anticapsular antibody concentrations to be excellent correlates of protection against invasive Hib disease in 98,000 vaccinated Finnish children and controls. However, vaccine responsiveness was highly age-dependent, with children less than 18 months failing to achieve anti-Hib antibody concentrations of 0.15 μg/mL, and consequently continuing to be at risk of invasive Hib disease. Defining the failure of unconjugated polysaccharide Hib vaccines in infants, as a failure to elicit the correlate of protection that contains the effector (anti-Hib antibodies), informed the development of highly efficacious protein–polysaccharide conjugate Hib vaccines that recruit T cells.[22] T-cell recruitment with Hib protein conjugate vaccines is necessary for the production of anti-Hib antibody in infants, but not in older children,[23] probably as a result of the immature splenic marginal zone in early life where polysaccharide-recognizing T-independent marginal zone B cells develop. The correlates, derived from studies of unconjugated polysaccharide vaccine have remained in use for almost 40 years. However, the isotype and quality of the antibody induced by the conjugate vaccines is not the same, and these historical correlates may not be a true reflection of the absolute level of protection, as discussed later.

Correlates of protection have also been delineated on the basis of passive immunization with pathogen-specific immunoglobulin. Such correlates of protection from passive immunization may, or may not, equate to those achieved by active immunization with vaccines or secondary to natural infection. Passive immunization is largely used to protect vulnerable individuals who have been very recently exposed to a pathogenic virus or other organism and who are at exceptionally high risk of developing severe disease, such as through immunocompromised or immaturity. Therefore correlates of protection derived from passive immunization (antibody levels) may overestimate the antibody level as a correlate of protection achieved by active immunization. An example is that of passive immunization against varicella disease.

The protective effects of antivaricella antibodies are complicated. Trials of varicella zoster immunoglobulin in the 1960s established that the administration of very high doses of antivaricella antibodies in the serum of adults convalescing from herpes zoster prevented infection in exposed children.[24] Since then, this has been an important part of the management of at-risk groups exposed to varicella. However, considerably lower concentrations of antivaricella antibodies correlate with protection following active immunization with the live attenuated varicella vaccine,[25] suggesting that either autologous antibodies may be more efficacious, or that cellular immunity has a role in protecting from varicella.

Natural observation studies of children with agammaglobulinemia and other isolated antibody production defects (who are at no increased risk for complicated varicella infection) and children with combined immunodeficiencies (who are at high risk of complicated varicella infection) suggest that cellular immunity is at least required for limitation of, and recovery from, varicella disease. The increased risk of zoster reactivation with increasing age (especially in recipients of a single dose only of live attenuated varicella vaccine) supports this. Thus, correlates of protection against infection with varicella virus include antibody data [either a titer of fluorescent antibody-to-membrane antigen (FAMA) of ≥1:64, or anti-gpELISA seroconversion] and CD4+ T cell related data.

Human challenge studies (ie, vaccination of a cohort of volunteers before artificially exposing them to infection) represents the most direct and efficient method of assessing the efficacy of a vaccine. The certain exposure of the cohort to infection and the ability to take a number of samples over varying time points allows high quality data to be collected with a small number of volunteers. Nevertheless, challenge studies are limited to assessing the vaccine under ideal conditions, mainly against clinical endpoints (as epidemiological endpoints are difficult to infer in such a setting) and with a dose of the pathogen during challenge that may not reflect normal environmental exposure. Unimpeachably high standards of ethical and regulatory guidance are required to ensure that the safety of participants and investigators remains paramount.[26]

The development of an oral cholera vaccine in the 1980s and 1990s exemplifies the use of a challenge model. Prior to the development of a vaccine, serological surveys in East Pakistan (now Bangladesh) had established the importance of serum vibriocidal antibodies as correlates of protection against cholera disease in the Bangladeshi community. In this community, vibriocidal antibodies were also protective against cholera disease, even in the absence of antitoxin antibodies.[27] A recombinant live oral cholera vaccine elicited strong vibriocidal and antitoxin antibodies in volunteers in the USA, equivalent to those that protected from clinical infection in Bangladesh.[28] Subsequent oral challenge with *Vibrio cholerae* at infective doses showed a vaccine efficacy of 91% against diarrhoea.[29] Translating the success of this vaccine for the short-term protection of travelers into a vaccine for endemic regions, with longer-lasting immune memory has taken further decades of research. Whether immunological memory can be considered a correlate of protection is discussed later.

## 4 STATISTICAL METHODS FOR EVALUATING CORRELATES OF PROTECTION

### 4.1 Criteria for Endpoints in Clinical Trials

Prentice has outlined four criteria on which to judge surrogate endpoints in clinical trials.[30] Although they were not specifically developed for vaccines, they have been translated into the field of vaccinology.[4]

1. Protection against the clinical endpoint is significantly related to having received the vaccine.
2. The correlate of protection is significantly related to the vaccination status.
3. The correlate of protection is significantly related to protection against the clinical endpoint.
4. The full effect of the vaccine on the frequency of the clinical endpoint is explained by the correlate of protection, as it lies on the sole causal pathway.

Although these criteria have considerable value, they are limited by being focused on the individual (ie, clinical) endpoints. For example, interruption of transmission of an infectious disease achieved with only moderate levels of a correlate of protection (ie, levels insufficient to be an absolute correlate) would not meet criterion 3. Additionally, the redundancy of the immune system makes criterion 4 very difficult to fulfill. We prefer to consider absolute and relative correlates of protection that are endpoint specific.

## 4.2 Absolute and Relative Correlates of Protection

Heterogeneity among individuals and populations, infecting dose of organism, programmatic issues (particularly in field trials of vaccines), and unmeasured stochastic effects ensure that, in a sense, protection from infectious diseases is always relative. Nonetheless, and as described earlier, there is utility in describing a correlate of protection as absolute against a specified endpoint. Such a threshold provides a basis against which to assess novel vaccines to a pathogen (or existing vaccines in vaccine-naïve populations).[31] An early example of an absolute correlate of protection is provided by the development of antitetanus toxoid vaccine. Here, passive immunization with antitetanus toxoid and field trials of antitetanus toxoid vaccines show that an antitoxin concentration of 0.1 μg/mL provides almost complete protection against tetanus infection (the exception to the rule: occasional mild cases in patients with deep necrotizing wounds).

As discussed earlier, an "absolute" correlate of protection has been derived for Hib of 0.15 μg/mL of antibody as measured by antipolyribosylribitol phosphate (PRP) ELISA. However, the total ELISA antibody concentration does not necessarily reflect the level of the antibody effector, thought to be high avidity bactericidal antibody, as the ELISA also detects low avidity nonfunctional anti-PRP IgG. It is therefore possible that an individual can have a high ELISA concentration but a low level of anti-PRP and vice versa, and so the absolute correlate may not confirm or refute the level of protection in an individual, just that they are more likely to be protected than not.

Furthermore, not all vaccinees will achieve immune responses equating to an absolute correlate of protection against a clinical endpoint. The relevance of this is disease specific: for example, the Gaussian distribution of antibody responses to live measles vaccine leaves a small proportion of the vaccinated population

susceptible to measles infection—possibly due to later loss of measles-specific immunoglobulin. For these children and adults, protection is maintained by their membership of a population with herd immunity measured by an immunological correlate of protection (measles antibody titers) to an epidemiological endpoint (measles virus transmission).

Alternatively, immune responses and their correlates of protection may be distributed in a bimodal manner. Such a situation typically arises with nonresponders to active immunization (either natural infection or vaccination), for example, nonresponders to hepatitis B vaccine[32] (those who do not achieve hepatitis B surface antibody concentrations of ≥10 mIU/mL, which themselves correlate with helper T-cell pathway responses). Attempting to augment the immune response of nonresponders to hepatitis B vaccine through further doses may aid up to 90% of these individuals to achieve the defined absolute correlate of protection. However, some vaccinees remain apparently nonimmune despite multiple additional doses.

One of the fundamental problems in vaccinology is the induction of long-lasting immune responses to provide defense against rapidly invasive organisms (*N. meningitidis*) and strong recall responses to provide defense against organisms (like hepatitis B) with a long incubation period. Immune memory should therefore be incorporated into the concept of a correlate of protection. An ideal correlate of protection would provide information on both the vaccine-mediated immunity in the short term (following vaccination) and also vaccine-mediated immunity over the years and decades following vaccination. For example, antihepatitis B serum antibodies are conventionally used as a correlate of vaccine-mediated protection against hepatitis B virus. However, breakthrough hepatitis B infections are extremely rare, for at least several decades after infant immunization despite waning of these vaccine-induced antibodies over time, due to the persistence of antihepatitis-specific memory B cells.[33] This suggests that pathogen-specific memory B cells may represent a more temporally accurate correlate of protection than serum antibodies—although these responses are considerably harder to quantify.

## 5 CORRELATES OF PROTECTION AS EFFECTOR OR PATHWAY CORRELATES

Many immune markers correlated with naturally acquired or vaccine-mediated protection from disease are likely to be collateral to the effector correlate of protection. This is particularly the case for naturally acquired infection and live vaccines due to the plethora of resulting cellular processes. This is not a problem for the identification of pathway correlates of protection; such correlates are identified by statistical association only (and are presumed to be mechanistically related to the effector unless clearly demonstrated otherwise). Pathway correlates of protection may be perfectly adequate for the assessment of vaccine efficacy (such as prior to licensure of a novel vaccine).

The identification of effector correlates of protection is considerably more difficult and is likely to involve experimental immune modulation in animal and cell models, and identification of genetic traits that predispose to infection (or

provide protection from infection) in case-control studies of disease incidence (so-called Mendelian randomization). Additionally, the redundancy of the immune system means that identification of a correlate of protection on one pathway does not preclude the identification of correlates of protection lying on other pathways. Nonetheless, effector correlates of protection are vital for our understanding of the immunology of vaccination.

The advent of systems biological methods has the ability to transform our understanding of correlates of vaccine-mediated protection, and other fundamental problems in immunology. Such methods include quantification and analysis of the global postvaccination (or infection) transcriptomic (ie, RNA expression) response, and sequencing of the B-cell receptor repertoire. The global transcriptomic response to active immunization could clarify known mechanistic immunological pathways to protection, and illuminate numerous others. However, the large volume of data will challenge our conception of correlates of protection by the sheer number of cocorrelated gene transcripts, and the challenge of distinguishing the signal of pathway or undefined correlates of protection from the noise. Li et al.[34] have attempted to delineate the peripheral blood mononuclear cell (PBMC) transcriptional responses to meningococcal serogroup C vaccine, and quadrivalent meningococcal vaccine (serogroups A, W, Y, and W-135). Their approach involved the modeling of correlated gene transcripts into biologically relevant modules in a control dataset of 30,000 PBMC transcriptomes, before applying these modules to their postvaccination transcription data. Their identification of various transcriptional modules that cocorrelated with standard correlates of protection (such as induction of antibody against polysaccharide antigens in the meningococcal vaccines) illuminates the genes (and cellular processes) necessary to elicit these correlates. Furthermore, the identification of unexpected transcriptional modules (such as the involvement of myeloid dendritic cells in the antipolysaccharide antibody response) highlights the power of hypothesis-free systems' biological approaches to identify new areas of research for "traditional" immunological and epidemiological techniques. Such approaches also stress the need for our concepts of correlates protection to be fit for this new era of immunology.

## 6 KNOWN CORRELATES OF PROTECTION FOR VACCINES

The accepted correlates of protection for almost all licensed vaccines in 2015 are measurements of functional or total antibody level using various different laboratory methods. This is an important observation and indicates that antibody is an essential first line of defense for prevention of a wide array of both viral and bacterial infections, and in sufficient quantity, can provide sterilizing immunity against many pathogens. This also indicates that T cells have an essential role in effecting the immune response to contain infection, rather than prevent infection *per se*. However, the situation may be more complicated in vivo—for example, CD4+ T cells can confer protection against pneumococcal infection in animal models, in the absence of antibody.[35] Table 7.3 describes an overview of correlates of protection for currently used vaccines.

**TABLE 7.3 Vaccine-Mediated Correlates of Protection**

| Vaccine | Assay | Endpoint | Correlate | References |
|---|---|---|---|---|
| Anthrax | Toxin neutralization; ELISA | Anthrax disease | Not validated | [36] |
| Diphtheria | Toxin neutralization | Diphtheria disease | 0.01–0.1 IU/mL | [37] |
| Hepatitis A | ELISA | Hepatitis | 10 mIU/mL | [38] |
| Hepatitis B | ELISA | Hepatitis, chronic carriage | ≥10 mIU/mL | [39] |
| Herpes zoster (shingles) | ELISA/T-cell proliferations | Shingles | Not validated | [40] |
| Hib (conjugate) | ELISA | Invasive disease | ≥0.15 (short term)/≥1 μg/mL (long term) | [21,41] |
| Human papillomavirus | ELISA, T-cell proliferation | Infection and pathology | Not validated | [42] |
| Influenza | Hemagglutinin inhibition antibody titer | Influenza disease | 1:40 dilution (adult)/1:100 dilution (child) | [43] |
| Japanese encephalitis | Plaque reduction neutralization titer | Encephalitis | ≥10 titer | [44] |
| Lyme disease | ELISA | Lyme disease | Not validated | [45] |
| Measles | Plaque reduction neutralization titer | Classical measles disease | ≥120 mIU/mL | [15] |
| Meningococcus (A/B/C) | hSBA/rSBA | Invasive disease | ≥1:4/≥1:8 | [13,46] |
| Mumps | Neutralization/ HAI titer | Mumps disease | Not validated | [47] |
| Pertussis | ELISA (PT) | Pertussis disease | 150–250 U/mL | [19] |
| Pneumococcus (conjugate) | ELISA/OPA | Invasive disease | ≥0.35 μg/mL (or serotype specific)/≥1:8 | [16,48] |
| Polio | Neutralization titer | Poliomyelitis | ≥1:8 | [49] |
| Rabies | Neutralization | Rabies disease (death) | 0.5 IU/mL | [50] |

**TABLE 7.3** Vaccine-Mediated Correlates of Protection (*cont.*)

| Vaccine | Assay | Endpoint | Correlate | References |
|---|---|---|---|---|
| Rotavirus | Serum IgA | Severe gastro-enteritis | Not validated | [51] |
| Rubella | Immunoprecipi-tation | Clinical rubella | 10–15 mIU/mL | [47] |
| Tetanus | Toxin neutralization titers | Tetanus disease | ≥0.1 UI/mL | [52] |
| Tick-borne encephalitis | ELISA | Encephalitis | Not validated | [53] |
| Tuberculosis | Interferon release/ trancriptomics | Tuberculosis disease | Not validated | [54] |
| Varicella zoster | ELISA/FAMA | Varicella disease | ≥5 U/mL (ELISA); ≥1:4 (FAMA) | [55,56] |
| Yellow fever | Neutralization titers | Yellow fever disease | 1:5 | [57] |

ELISA, enzyme-linked immunosorbent assay; hSBA, human complement serum bactericidal assay; rSBA, rabbit complement SBA; OPA, opsonophagocytic assay; and FAMA, fluorescent antibody-to-membrane antigen.
Correlate represents, where possible, an "absolute" correlate of individual protection against the endpoint. Not validated indicates a lack of broad acceptance of a measurement as a proven correlate of protection across human populations (which may subject to change in future).
**Source:** Adapted with modifications from Plotkin.[1]

## 6.1 Bacteria With Polysaccharide Capsules

Encapsulated bacteria (Hib, pneumococci, and meningococci among others) express a polysaccharide capsule on the bacterial surface that is antiphagocytic and reduces risk of dehydration during transmission. In general, infants mount a poor immune response to plain polysaccharide antigens, making them high risk for invasive disease from encapsulated bacteria, especially once maternal antibody has waned.

Hib, the predominant cause of invasive *H. influenzae* disease, is a commensal that colonizes the nasopharyngeal tract early in life. The inverse correlation between serum anti-Hib serum antibodies (primarily against the PRP capsule) and disease; and passive immunization studies in individuals with agammaglobulinemia, presented convincing clues that antibody responses can protect from Hib disease.[23,58] An anti-PRP antibody concentration of ≥0.15 μg/mL was established as correlate of protection against Hib disease in older children using the plain-polysaccharide vaccine[21] (as noted, infants only responded to Hib vaccines constructed of a polysaccharide antigen conjugated to protein). In

both infants and older children, higher antibody concentration (≥1 μg/mL) correlated better with long-term protection from invasive disease. Two important observations indicate that anti-PRP antibodies are the mechanism of protection. First it is well established that anti-PRP antibody concentrations correlate with serum bactericidal activity (SBA, the ability of serum from vaccinees, in addition to complement, to kill Hib bacteria in vitro).[59] Second, vaccine failure and lack of SBA activity is associated with low avidity of antibodies raised following vaccination.[41] Thus, vaccine-induced anti-PRP antibodies appear to represent an effector correlate of protection against invasive Hib disease.

Over 90 distinct pneumococcal serotypes have been identified, although invasive pneumococcal disease in children is mainly confined to a small proportion of these serotypes.[60] The first PCV used against pneumococcus contained polysaccharide antigens from seven serotypes (PCV7) and was highly immunogenic and efficacious against invasive disease for these serotypes. A total antipneumococcal IgG concentration of ≥0.35 μg/mL (measured by ELISA) was established as correlate of protection against invasive pneumococcal disease, based on pooled data from vaccine efficacy trials.[48] Serotype-specific antibody correlates of protection have been recently derived for PCV13 and show that vaccine-induced correlates of protection vary considerably among serotypes.[16]

Antibodies seem likely to be the primary mechanism of protection against pneumococci, however the established serological threshold measured by ELISA only moderately correlates with opsonophagocytic assay (OPA, a functional measure of the ability of antibodies to opsonize bacteria prior to phagocytosis by neutrophils in vitro).[61] OPA has been proposed as a second correlate of protection, for assessment of PCVs, and a titer of ≥1:8 was used to license PCV13.[62] Given the varying antibody-derived absolute correlates of protection against specific serotype of pneumococci,[16] the OPA titer may require further postlicensure analysis.

Meningococcal disease is a major global health problem, against which conjugate vaccines were developed in the 1990s. Work in the 1960s highlighted the inverse correlation between SBA activity and invasive meningococcal disease, illustrating that complement-mediated antibody-dependent lysis of the bacterium is the primary mechanism of protection.[46] Natural protection was observed in army recruits who had an SBA titer of ≥1:4 against capsular Groups A, B, and C; a titer that was subsequently validated in various efficacy trials including a Norwegian trial using an outer membrane vesicle vaccine.[63] However, SBA measures are technically sensitive and depend on the exogenous complement source and the bacteria used as a target in the assay. The titer of ≥1:4 was established using human serum as complement source (hSBA) which has been suggested to be equivalent to a threshold of ≥1:8 when using baby rabbit complement. Furthermore, protective antibody increases may depend on seropositivity prior to vaccination[64] with a fourfold increase in SBA, also a correlate of protection. Titers of ≥1:4 and ≥1:8 are both accepted by the regulatory authorities as validated absolute correlates of protection and have been used in recent licensures of vaccines against meningococcal disease.[64]

Current evidence suggests that functional antibody is an effector correlate of protection and SBA reflects the mechanism of protection; however, some emerging data may argue against this. Although only small-scale studies have been conducted, it seems that vaccination of complement-deficient individuals also confers protection, strongly indicating that other potential mechanisms such as opsonization play a role in protection.[65] Clearly, the redundancy of the immune system may mean that both bactericidal and opsonizing activity of the antibodies (with and without complement) are effectors of protection.

## 6.2 Toxin-Producing Bacteria

Tetanus is caused by blockade of spinal inhibitory neurons following neuronal uptake of toxins released by the bacterium *Clostridium tetani*. Tetanus toxin can be inactivated to produce tetanus toxoid, which has been used as monovalent vaccine when combined with an alum adjuvant. The "gold standard" measure of antitoxin antibodies uses an in vivo neutralization assay in mice, and is thus expensive and impractical. More commonly an ELISA is used to measure IgG against tetanus toxin, which correlates well with the in vivo neutralization assay.[66] Correlates of protection have not been systematically determined, but a level of ≥0.01 UI/mL is a relative correlate of protection and ≥0.1 UI/mL a near absolute correlate of protection.[4]

Diphtheria toxin is a potent toxin produced by the bacterium *Corynebacterium diphtheriae*. The disease differs from tetanus in the context that antitoxin can be measured following clinical disease or carriage of the bacterium. As with tetanus, protection is mediated through production of antidiphtheria toxin antibodies, which neutralize the toxin. Antibody functionality is determined by neutralization tests using either skin injection (Schick test) or tissue culture and monitoring of the ability of serum to inhibit cell death induced by the toxin (both complex tests). A tissue culture level of ≥0.01 IU/mL is considered a relative correlate of protection and reflects a negative Schick test result.[37] However, a level of ≥0.1 IU/mL may be a better correlate of long-lasting vaccine-mediated protection.[67]

## 6.3 Intracellular Bacteria

Intracellular pathogens hide inside specific host cells in order to evade the immune system. A short transition time through the extracellular space, and the hijacking of host cellular mechanisms makes immunological clearance of infection difficult. In such situations, antibodies may represent effector correlates of protection by preventing initial invasion of host cells, but cellular immunity will be responsible for clearance of infection. As such, there is a strong argument for the measurement of cellular immunity for the derivation of correlates of protection for intracellular bacteria.

It has proven to be difficult to produce new vaccines with efficacy against tuberculosis. Bacille–Calmette–Guérin (BCG), a live vaccine derived from serial passage of *Mycobacterium bovis*, has widely varying estimates of vaccine efficacy against *Mycobacterium tuberculosis* (Mtb) in infants and young children.[68] Protection against Mtb is largely mediated through cellular immunity. For example, genetic defects in interferon-gamma signaling (largely a role of CD4+ T cells) and acquired defects of CD4+ T-cell function (such as with HIV infection) dramatically increase susceptibility to tuberculosis disease.[69] Many other pathways and immune cells have also been implicated in pathways to protection (or susceptibility) in tuberculosis, highlighting the complexity of this disease. While BCG induces host responses similar to natural infection, no correlates of protection have been described to date.

## 6.4 Viruses Transmitted by Arthropods

Tick-borne encephalitis vaccines are available in Northern Europe and are widely used with evidence of effectiveness from observational studies. The vaccines that are highly immunogenic and neutralizing antibody, determined in randomized controlled immunogenicity studies, is used as the endpoint and has also become accepted as the effector of protection, although no absolute correlate of protection has been defined and validated.[53]

Recent Phase 3 clinical trials of dengue vaccines have demonstrated substantial efficacy (95% against severe disease), although protection was not equal for all four serotypes.[70] The developers have a challenge in defining correlates of protection since antibodies are implicated both in protection, as well as the process of enhanced disease; and a recent report suggests that hospitalization may be higher in year 3 after vaccination among children under 9 years of age.[71] Planned safety reviews during long-term follow-up of trial participants are currently being undertaken.

## 6.5 Viruses Invading Human Blood via the Mucosae

Although viruses are intracellular pathogens, most viral life cycles require high numbers of cell-to-cell transmission events during acute infection to provide a sufficient viral load to facilitate spread to a new host. This provides a window of opportunity for the host immune response to clear the infection. Immunity against viruses can be conferred through antibody-mediated mechanisms, but since viruses are intracellular pathogens, T cell-mediated immunity is often a requirement for clearance of an infection. Antibodies against viruses (if functional and at sufficient concentrations) tend to prevent de novo infection; whereas T cell-mediated immunity is needed for elimination of virus-infected cells.

Protection from VZV disease requires immunity against primary infection (chickenpox) and herpes zoster. The effect of varicella zoster immunoglobulin

administration in susceptible newborns, infants, and older age groups, indicate that humoral responses may be sufficient to provide effective protection against VZV infection.[24] However, during advanced stages of varicella, T cell-mediated immunity is also required to eliminate infected cells.[72] Live attenuated varicella vaccine, used routinely in the USA since 1995, has significantly reduced varicella infection and mortality in the population.[73] The vaccine induces persistent antibodies, with a 6-week postvaccination glycoprotein (gp)-ELISA concentration of ≥5 U/mL ELISA suggested as a reasonable correlate of protection against breakthrough infection.[74] However, the gp-ELISA can produce false positive results and the fluorescent antibody-to-membrane antigen (FAMA) methodology with a titer of ≥1:4, although labor intensive, may be more reliable.[55]

Following primary infection, VZV is sequestered in the ganglions of peripheral nerves before, in some cases, reemerging as a painful vesicular rash in a dermatomal distribution—herpes zoster (shingles). The Shingles Prevention Study Group demonstrated that VZV-specific cell-mediated immunity is induced in older individuals following zoster vaccination, declines with age, is associated with reduced severity of zoster episodes, and does not correlate with antibody levels following zoster vaccination.[75,76] These findings, when combined with our knowledge of the role of cellular immunity in clearing intracellular infections, suggest that T-cell proliferation assays may be a more accurate and effector correlate of vaccine-mediated protection against herpes zoster.

Poliomyelitis (polio) is on the verge of eradication due to vaccination against poliovirus. Two vaccines exist against polio—the inactivated polio vaccine (IPV) and the live attenuated oral polio vaccine (OPV). IPV induces higher levels of serum neutralizing antibodies, but OPV is more effective in limiting intestinal infection.[77] OPV has also been responsible for extremely rare cases of vaccine-related poliomyelitis (through type 2 poliovirus in previous trivalent OPV formulations), a problem not associated with IPV. The choice of IPV versus OPV (or both) illuminates the importance of endpoint: OPV is more efficacious at interrupting poliovirus transmission through enhanced mucosal immunity, but IPV is required to maintain humoral immunity of children to poliovirus once transmission is interrupted. Correlates of protection are difficult to establish for these two vaccines, partly because of the importance in distinguishing whether a clinical or epidemiological endpoint is paramount. However, because both vaccines induce neutralizing antibodies, a titer of 1:8 is considered a reasonable correlate of protection against clinical disease.[1] Because of the additional stimulation of mucosal immunity there may exist more suitable, undiscovered, correlates of protection for OPV.

## 6.6 Viruses Limited to Replicating on the Mucosae

The annual development and administration of vaccines to circulating strains of influenza to prevent a proportion of the disease burden is an important public health intervention, despite offering only partial individual protection.[78]

Two different types of vaccines are available—a tri- and a quadrivalent inactivated vaccine (TIV and QIV), as well as a live-attenuated influenza vaccine. While influenza vaccines induce several aspects of the host immune response, antibodies against viral hemagglutinin glycoprotein are routinely measured by hemagglutination inhibition (HAI) assay to assess vaccines. HAI titers have been established as a relative correlate of protection in natural infection studies; however, the degree of correlation with protection has been questioned for inactivated vaccine.[79] A titer of 1:40 after immunization with TIV/QIV in adults correlates with a vaccine efficacy of 50–70% but titers considerably higher ($\geq$1:100) appear to be more appropriate in children,[43] and fold-rise in titer may be more applicable as this takes preexisting antibody levels into account. Age-related differences in protective mechanisms may also be important. Correlation between HAI titers and protection is lacking in older individuals, likely because of the decline of cellular immunity with age, and measurements of cytotoxic T-cell responses may be a better correlate of protection following influenza vaccination in the elderly.[80,81] For regulatory purposes HAI titers are still used, with consideration of preexisting titers and age-specific differences, and for licensure of pandemic influenza vaccines (in Europe), demonstration of virus neutralization is used.[43]

## 6.7 Malaria

It has proven difficult to develop vaccines for protection against malaria and other protozoa. RTS,S has recently been licensed by the European Medicines Agency, as a malaria vaccine following efficacy trials. RTS,S is a monovalent recombinant protein vaccine containing circumsporozoite protein (CSP) and hepatitis B surface antigen. A large trial including 11 African study sites showed moderate protective efficacy against clinical disease and severe malaria that waned over time, and induced anti-CSP antibodies in >99% of vaccines a month after the third dose.[82,83] The mechanism of protection is not entirely clear and besides anti-CSP responses, CD4+ T-cell responses are induced and has been indicated in contributing to protection.[83,84] While these immunological aspects are certainly induced by the vaccine, none of them have been identified as an absolute correlate of protection. The RTS,S vaccine furthermore highlights an interesting issue related to the genetic diversity of the parasite and that efficacy may be CSP allele specific, adding another level of complexity to the delineation of correlates of protection.[85]

# 7 CONCLUSIONS

Correlates of vaccine-mediated protection are an invaluable commodity in vaccinology and vaccine-assessment; however, they are often difficult to identify and measure, and unfortunately rarely absolute. Furthermore, the literature is littered with confusion over the definition of the words that are used to define correlates of protection, so as it is not always clear what is meant by the author

or required by the regulator. Nevertheless, there are established correlates of protection for many of the routinely administered vaccines that are used today, and those correlates are almost exclusively measures of antibody level or function. The field of correlates is at an exciting place with the oncoming storm of big data from transcriptomic, proteomic, and mass cytometry studies about to release a large number of biomarkers that are likely to correlate with effector immune responses and potentially protection. To identify a correlate of protection three questions should be addressed. First, what is the clinical endpoint with which the biomarker should correlate? Second, can an absolute (or relative) level of protection be identified? Finally, can the biomarker be defined as an effector of vaccine-mediated protection or does it lie on the mechanistic pathway of protection, or is it undefined? Given the importance of persistence of protection for some vaccines, and immunological memory for others, and of herd immunity for most, it is also important to determine these additional critical correlates. Having established correlates of protection, perhaps using the new technologies to reinvestigate our current vaccines as well as study of new vaccines, it is likely that we have obtained new insight into vaccine development possibilities and acquired a new understanding of the incredible immune response.

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Chapter 8

# How Vaccine Safety is Monitored

**Daniel A. Salmon, MD, Neal A. Halsey, MD**
*Institute for Vaccine Safety, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

## Chapter Outline



## 1 INTRODUCTION

Safety expectations for vaccines are high because they are administered to healthy and sometimes vulnerable populations such as pregnant women, infants, and the elderly. Also, vaccines are endorsed or required by most governments, further raising safety expectations. Although no biologic or medical intervention is perfectly safe, vaccines are generally very safe and the risks of side effects are almost always greatly outweighed by the benefits derived from vaccination to prevent disease. Vaccine safety is evaluated at all stages in the development of vaccines, including after the vaccines have been approved by regulatory authorities and introduced into widespread use (Fig. 8.1).[1,2]

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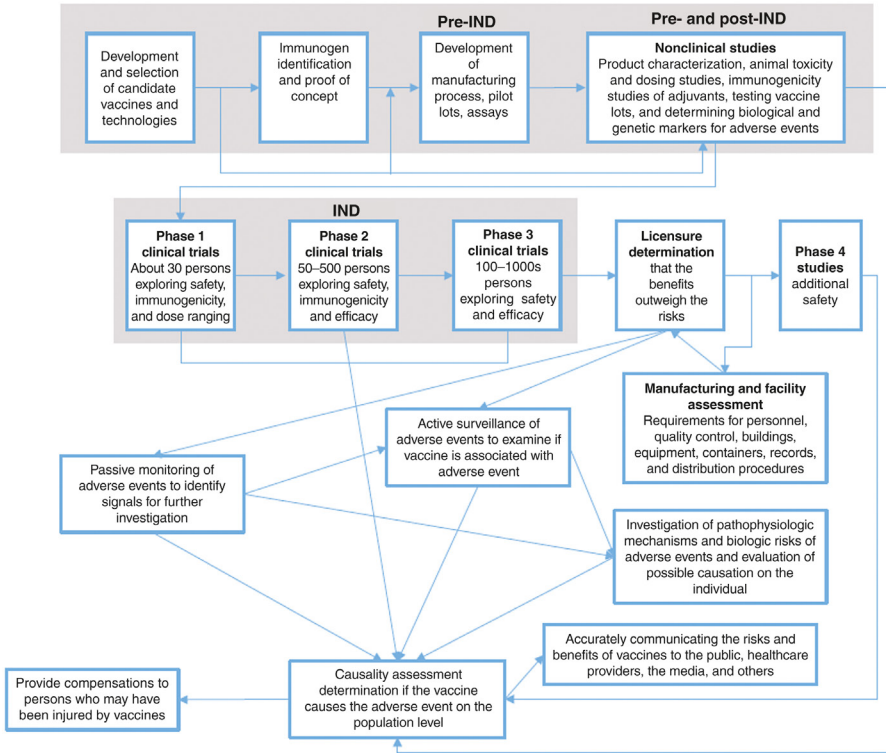


**FIGURE 8.1 Vaccine safety activities throughout the product life cycle.**[2] *(Adapted from a Comprehensive Review of Federal Vaccine Safety Programs and Public Health Activities. http://archive.hhs.gov/nvpo/nvac/documents/vaccine-safety-review.pdf).*

## 2 VACCINE DEVELOPMENT

Safety is important in determining the type of vaccine to be developed, the selection of antigens, and all other ingredients in the final product. For example, recombinant hepatitis B vaccines replaced plasma-derived vaccines to avoid theoretical concerns about adventitious agents in blood. For some diseases, such as Ebola and HIV, live attenuated strains of the naturally occurring viruses are not used due to theoretical concerns the vaccine could cause disease. Vaccine ingredients are carefully scrutinized for real or potential risks from adventitious agents and the potential to cause adverse events.

Regulatory authorities require manufacturers to assure safety, purity, and potency of all vaccine products.[3] Prior to testing experimental vaccines in humans, products are characterized by physical, chemical, and biological methods. Animal studies are conducted to identify potential toxicities. Novel adjuvants usually require more extensive safety testing in animal studies prior to obtaining approval for human studies. If the vaccine is intended for use in women of

reproductive age and/or pregnant women, animal studies are conducted to assess potential adverse effects on pregnancy outcomes.

## 3 INVESTIGATIONAL NEW DRUG (IND) APPLICATION

Prior to conducting human studies, vaccine developers must submit all of the data from the preclinical studies and the plans for conducting human studies to regulatory authorities in the form of an IND application. The regulatory authorities [Food and Drug Administration (FDA) in the United States, European Medicines Agency (EMA) in Europe] require the organization, manufacturer, or individual developing the vaccine to demonstrate that the preclinical data support clinical trials. An IND application contains information about the vaccine, the methods for manufacturing, quality control testing, potential study subjects, toxicology data and clinical protocols, and investigators expertise. The sponsor of the trial must annually report adverse events and rapidly report serious or unexpected adverse events to the regulatory agency. The regulatory agency typically specifies "stopping rules," which will halt the trials if serious adverse events occur at predetermined unacceptable levels.

## 4 CLINICAL TRIALS

Phase I trials typically include 20–100 healthy subjects and, from a safety standpoint, are designed to ensure there is no gross toxicity and to gather safety data on dose-related immune response. Phase II clinical trials typically include 10–100 and up to 1000 health subjects. These studies are designed to assess common, short-term side effects and explore interactions between the investigational vaccine and already licensed vaccines, and may involve different age groups. Phase III clinical trials typically include 1,000–20,000 or more persons to evaluate efficacy and safety. The incremental phases of clinical trials minimize exposure of study participants to theoretical risks from previously untested vaccines, which could have unanticipated adverse effects.

Clinical trials are the gold standard for assessing safety as they are randomized (some people get the new vaccine being investigated and some do not, based solely on chance) and double blind (neither the patients nor the investigators know who received the study vaccine). Double blind, randomized trials greatly reduce potential confounding and bias because host factors predisposing to adverse effects should be equally distributed to the different study groups. These studies are usually restricted to healthy individuals of a restricted age group and the results may not be generalizable to the entire population.

The strict inclusion and exclusion criteria in most trials result in uncertainty regarding safety in groups that were excluded from the trials such as persons with concurrent medical conditions. Limited follow-up of clinical trial participants limits the ability to identify adverse health outcomes with delayed onset. The size of clinical trials limits the ability to evaluate uncommon adverse

**TABLE 8.1** Sample Sizes Needed to Detect an Increase in Rate of Adverse Events and Numbers Potentially Affected for a Vaccine Administered Universally

| Rates—Baseline versus Increase | Sample size[a] | Number potentially affected[b] | | |
|---|---|---|---|---|
| | | United States | Europe | India |
| 0.1 versus 0.2 | 50,000 | 4,000 | 5,100 | 23,000 |
| 0.1 versus 0.3 | 17,500 | 8,000 | 10,200 | 46,000 |
| 0.05 versus 0.1 | 100,000 | 2,000 | 2,550 | 11,500 |
| 0.01 versus 0.02 | 500,000 | 400 | 510 | 2,300 |
| 0.01 versus 0.03 | 175,000 | 800 | 1,020 | 4,600 |

Births: USA, 4 million (2014); EU, 5.1 million (2014); India, 23 million (2015).
[a]*Two-arm trial, power 80%, alpha (2 sided) = 5%*
[b]*Entire birth cohort vaccinated.*
Adapted from Ref. [4].

events. For a medical condition that occurs at a background rate of 1 in 1,000 a trial with 50,000 persons would be required to identify a doubling of risk (Table 8.1). Missing a doubling of this risk for such an event would result in 4,000 persons potentially affected every year in the United States, about 5,100 persons potentially affected every year in Europe, and about 23,000 persons affected every year in India if the vaccine was administered to all infants.

## 5 REGULATORY APPROVAL

If a vaccine is shown to be safe and effective in clinical trials, the vaccine sponsor or manufacturer submits an application to national regulatory authorities (NRA) for licensure or registration of the vaccine. In the United States this application is called a biological license application and is submitted to the FDA. In Europe, the application is called a marketing authorization and is submitted to the EMA, which provides approval for European Union Member States as well as European Economic Area countries. The World Health Organization (WHO) can prequalify vaccines for licensure for countries that lack rigorous NRAs. However, the vaccine still requires licensure in each country where it will be used. NRAs must meet with WHO to review the licensure application and provide authorization. National Immunization Technical Advisory Groups (NITAGs), where they exist (about half of countries in Africa), can provide recommendations to NRAs in making vaccine licensure decisions. Ultimately, the Minister of Health must sign off on licensure before the vaccine can be used in the country.

NRAs carefully review the results of clinical trials as well as the chemistry, manufacturing and controls, description of the manufacturing facility,

and results of tests to demonstrate manufacturing consistency and product specifications. NRAs review and approve package labeling and advertising to help assure that the vaccine will be used in accordance with the approval granted to the manufacturers. Careful consideration is also given to the manufacturing facility to ensure that the facility can consistently produce a vaccine that is safe, pure, and potent. Regulations typically cover the facility's personnel, quality control, buildings, equipment, containers, records, and distribution procedures. Manufacturers must meet current Good Manufacturing Practices (cGMP) standards to ensure that vaccine manufacturing practices utilize advances in processes, techniques, and vaccine production technology that improve over time. NRAs often regularly inspect vaccine manufacturing facilities and require manufacturers to conduct tests on every lot of vaccine prior to release to assure the absence of contamination. Also, manufacturers must store samples of every lot for future testing in the event of safety or potency concerns raised after the vaccine has been used.

## 6 VACCINE RECOMMENDATIONS

Government authorities usually rely on expert advisory committees to make recommendations for the use of vaccines, such as the Advisory Committee on Immunization Practices (ACIP) in the United States. European countries make recommendations at the national level rather than across the European Union. WHO provides guidance for use of vaccines in developing countries. These advisory committee recommendations provide guidance on vaccine use in different ages and risk groups as well as information on what is known about the safety of the vaccine. Recommendations include guidance on groups or individuals who should not receive the vaccine due to safety concerns. For example, guidance is given for individuals with underlying conditions that might predispose to serious adverse events in order to prevent possible vaccine-associated injuries. Generally live vaccines are not given to persons with serious immune deficiency disorders, but some live vaccines are safe in those with less serious immunological conditions. Vaccine recommendations may include guidance for use in populations not studied in clinical trials, such as pregnant women. There is wide variability in vaccine recommendations globally as there are many differences in the burden of disease, and considerations of risks and benefits from vaccines.

## 7 POSTMARKETING SURVEILLANCE AND SPECIAL STUDIES

Vaccine safety monitoring after regulatory approval is implemented to identify rare serious complications that occur at rates too small to be detected in prelicensure studies and to examine the safety of the vaccine in populations excluded from clinical trials. Monitoring is important to detect safety problems that could occur due to changes in manufacturing practice. NRAs often require

manufacturers to conduct postlicensure active surveillance studies in defined populations to obtain additional information on vaccine safety and effectiveness as described later. NRAs may also require pregnancy registries for vaccines that are intended for women of reproductive age.

## 7.1 Passive Surveillance

Health-care providers should report adverse events following immunizations to national health authorities. In the United States, this system is called the Vaccine Adverse Event Reporting System (VAERS), which is jointly maintained by the FDA and the Centers for Disease Control and Prevention (CDC). Canada has the Immunization Monitoring Program, ACTive (IMPACT) run by the Canadian Paediatric Society with funding from the Public Health Agency of Canada. European countries have surveillance systems, which are often integrated into drug safety surveillance, and all European member states report to the EMA.[5] For example, the United Kingdom has a system referred to as the "yellow card" administered by the Medicines and Healthcare Products Regulatory Agency. Argentina, Brazil, Mexico, Panama, and Venezuela use a passive system called SANEVA. A 2012 report from WHO indicates that about 80% of low- and middle-income countries (LMIC) report spontaneous or passive safety surveillance systems; however, only about half of these countries report detection of vaccine adverse event reports.[6] WHO has developed a multiphase plan for improved surveillance and investigation of vaccine safety in LMIC.[7] The WHO Collaborating Centre for International Drug Monitoring or the Uppsala Monitoring Center (UMC) is an independent foundation and center for service and research whose activities include improving passive safety surveillance internationally.

Generally, anyone can make reports to passive systems, including physicians, other health-care providers, and the public. Personnel in the responsible agencies or organizations monitor reports of adverse events following immunization (AEFI) to identify individual cases or clusters of cases that are possible signals of unanticipated events that may warrant further follow-up. Passive systems also meet the expectations of the public and health-care providers to have a place to report adverse health outcomes that they believe may be caused by vaccines.

There are many limitations to passive surveillance systems. These systems suffer from underreporting (in which only a subset of cases are reported) and overreporting (many adverse health outcomes that are not related to the vaccine are reported). Passive systems lack denominator data on how many people are vaccinated and consequently rates of adverse events after vaccination cannot be well established. Doses distributed are sometimes used to estimate rates for reports, but there are often large differences between the number of doses distributed and the number that were actually administered. Efforts are sometimes made to compare the rates of adverse health outcomes reported to expected

background rate in the population. However, care should be taken in making such comparisons as the underreporting rate is seldom understood and consequently such comparisons cannot be interpreted. Additionally, passive vaccine safety systems do not capture information on people who are not vaccinated making it impossible to compare rates between vaccinated and unvaccinated persons. Passive surveillance systems are prone to misinterpretation by the media and the public because of the common misperception that reported adverse events based on a temporal relationship are causally related.

## 7.2 Active Surveillance

Active surveillance systems are conducted in health-care systems where all medical encounters are captured so that investigators can determine the rate of adverse events in persons who receive specific vaccines as well as rates in comparison populations who have not received the vaccine. Studies can also be done to determine if there are increased rates of the adverse health outcomes of interest in time windows following the vaccine as compared to control time windows among vaccinated persons. Signals that arise from passive surveillance or public concerns are often investigated in active systems. Examples of active surveillance systems are shown in Table 8.2.[8–10] Information on vaccine exposure, hospitalization, outpatient visits, and laboratory data allow for well-designed studies able to consider the many potential biases and confounders that are necessary for rigorous studies. These systems are not established for research purposes and consequently are usually based on procedural or diagnostic

**TABLE 8.2 Examples of Active Surveillance Systems, 2014**

| Location | System |
|---|---|
| US | Vaccine Safety Datalink (VSD) |
| US | Postlicensure Rapid Immunization Safety Monitoring (PRISM) Network |
| Canada | Canadian Network for Observational Drug Effect Studies (CNODES) |
| Canada | Vaccine and Immunization Surveillance in Ontario (VISION) |
| EU | Exploring and Understanding Adverse Drug Reactions by Integrative mining of Clinical Records and Biomedical Knowledge (EU-ADR) Alliance |
| EU | Vaccine Adverse Event Surveillance and Communication (VAESCO) |
| UK | Vigilance and Risk Management of Medicines (VRMM) Division |
| UK | Drug Safety Research Unit (DSRU) |
| Asia | Asian Pharmacoepidemology Network (AsPEN) |
| Asia | Shanghai Drug Monitoring and Evaluation System (SDMES) |

Adapted from Ref. [8].

codes. Chart review using well-designed case definitions are usually required. Given the considerable infrastructure required for these active surveillance systems, they are only available in developed countries.

The methods utilized for active surveillance studies vary, including case-control studies and cohort studies comparing the rates of outcomes among vaccinated to unvaccinated persons. These studies can suffer from the healthy vaccinee effect and health-care utilization biases.[11,12] Self-controlled methods have been developed to compare the risk of an outcome in one time period to another time period, often including only individuals who received the vaccine and developed the illness.[13] These methods eliminate confounding by all time-independent variables (including the healthy vaccinee effect and health-care utilization bias) and typically offer the power and simplicity of the cohort method and economy of the case-control method.[14] The VSD has also developed rapid cycle analysis in which prespecified outcomes are examined on a weekly basis and compared to historic rates of the same outcomes.[15] This method has been particularly useful to examine the safety of newly licensed vaccines and influenza vaccines that require focused safety surveillance efforts every year due to annual changes in the vaccine.

## 8 STANDARDIZED CASE DEFINITIONS

The Brighton collaboration develops standardized case definitions for health outcomes of interest in vaccine safety studies.[16] Using standardized definitions allows for comparability within and across clinical trials, surveillance systems, and postlicensure clinical studies. Case definitions typically are categorized by level of diagnostic certainty. As of December 2015, the Brighton collaboration has developed 28 standardized case definitions, which are widely used in vaccine safety studies.

## 9 CLINICAL ASSESSMENT AND INDIVIDUAL-LEVEL CAUSALITY ASSESSMENT

Several countries have established expert committees or panels to review serious adverse event following vaccines. In Canada, the Advisory Committee on Causality Assessment, a committee composed of independent experts in infectious diseases, public health, vaccine safety, epidemiology, pathology, neurology, and paediatrics review reports of hospitalization, deaths, and other selected adverse events for causal associations with vaccines.[1] In the United States, the Clinical Immunization Safety Assessment (CISA) Network is a collaboration between the CDC and academic medical centers.[17] Vaccine safety experts review unusual adverse events for evidence of causal associations using a standardized algorithm approach.[18] WHO has also adopted an algorithm approach to investigation of individual adverse events in resource-poor environments.[19]

These investigations help to identify the cause of individual serious adverse events and have resulted in identification of causes other than the vaccine for serious adverse events. Also, these reviews can lead to special studies to investigate pathogenic mechanisms for true causally related events. Having expert panels carefully investigate serious adverse events can often provide reassurance to the public that public-health authorities are carefully investigating individual adverse events that are commonly assumed to be caused by vaccines.

## 10 COMPENSATION FOR VACCINE INJURIES

Many developed countries (19 countries in 2011) provide no-fault compensation for people who may have been injured by vaccines.[20] These compensation programs are typically built on the premise that, while society shares the benefit of vaccination, a very small number of people suffer the burden of very rare but serious adverse events. Compensation programs thus distribute this burden. There is wide variability in how these programs are administered and funded, eligibility is determined, the process for decision making, standard of proof, elements of compensation, and litigation rights. In countries like the United States without a single health-care payer, medical costs throughout a lifetime can be a burden and are often a substantial component of the compensation provided by the program. In countries like the United Kingdom with a single payer health-care system, compensation may be for areas such as income support and child care. Vaccine injury compensation programs typically have a much lower standard for causality assessment (such as more likely than not) than is used scientifically. Consequently a substantial proportion of those compensated may not be due to vaccination. While the level of proof for compensation is an important policy decision and there is value in over rather than under compensating, doing so can be confusing to the public who often interprets compensation as recognition by the government that the vaccine caused the adverse health outcome.

## 11 COORDINATION

A broad range of parties contributes to vaccine safety assessment and the vaccine safety system. The primary responsibility typically lies in federal health authorities who are responsible for vaccine licensure, recommendations, and postlicensure assessment. Vaccine manufacturers are essential to making the safest possible vaccines and also contribute to postlicensure safety assessment and vaccine risk communication.[21] Health-care providers are responsible for communicating the vaccine risks and benefits of vaccines to patients and reporting possible adverse reactions to passive surveillance systems. Professional medical associations frequently contribute to vaccine risk communication and make vaccine recommendations that may be harmonized with federal vaccine recommendations. Academic researchers conduct a broad range of safety studies and often assist in making vaccine recommendations. Federal safety

infrastructure and activities are often coordinated through a policy or programmatic office, such as the National Vaccine Program Office in the United States. The WHO Global Advisory Committee on Vaccine Safety coordinates vaccine safety activities internationally.[22]

## 12 INVESTIGATIONS OF REPORTS OF MMR AND AUTISM: AN EXAMPLE

In 1998, a gastroenterologist in England published a report of 12 children with pervasive developmental disorders associated with gastrointestinal symptoms, 8 of whose parents or physicians reported that the onset of behavioral issues were temporally associated with MMR vaccination.[23] The lead author issued a press release and actively promoted in the media the concept that MMR caused autism.[24,25] He also published other studies suggesting that measles vaccine virus persisted in the intestine of children with autism.[26] Public concern grew quickly and many parents withheld MMR from their children resulting in outbreaks of measles in Europe[27] and to a lesser extent in the US.[28] Investigations revealed that the reports of persistent measles virus in intestinal tissue were based on flawed methods.[29,30] The epidemiological evidence has convincingly demonstrated that vaccines do not cause autism. There have been six methodologically sound, controlled epidemiological studies showing no association between MMR vaccine and autism.[31–36] Investigations into the original report revealed that some of the data were fraudulent and the primary author had multiple undisclosed conflicts of interest.[37–42] In 2010, the gastroenterologist's license to practice medicine in the UK was revoked by the British General Medical Council and his original publication was retracted by the Lancet. Many medical and public health associations, national health authorities, and the WHO have concluded that vaccines do not cause ASD.[43,44]

## 13 SUMMARY

In summary, safety is evaluated at all stages of vaccine development, including the use of vaccines in immunization programs. Questions about vaccine safety will undoubtedly continue to occur and continuous monitoring will be needed to address these questions with rigorous scientific methods to help assure the public that vaccines are made as safe as possible.

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Chapter 9

# Vaccination and Autoimmune Diseases

**Paul-Henri Lambert, MD*, Michel Goldman, MD****

**University of Geneva, Centre of Vaccinology, Department of Pathology and Immunology, Geneva, Switzerland; **Institute for Interdisciplinary Innovation in Healthcare, Université Libre de Bruxelles, Belgium*

## Chapter Outline



## 1 INTRODUCTION

At a time when vaccines save annually millions of child's lives, it is paradoxical that in many industrialized countries more public attention is given to the possible risks of adverse effects of vaccination than to its beneficial effects. This attitude often leads to reducing vaccination coverage in some countries or particular communities and may result in disease outbreaks.

Autoimmune diseases, that is, diseases caused by immune responses against host self-antigens, are often at the center of such controversies. This is reflected in the large number of publications that describe cases of autoimmune disease arising following vaccination. Most of the time, these are cases characterized by a temporal relationship between two events but without demonstration of causality. The risk of fortuitous coincidence is particularly brought up by the increase of adolescent and young adult vaccination since several autoimmune diseases are often initially diagnosed in these age groups. In these circumstances, it is critical to properly estimate the real risk of a causal relationship between a particular vaccination and autoimmune events.

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Autoimmune diseases might be either tissue-specific (eg, thyroiditis, type 1 diabetes, multiple sclerosis), or systemic (systemic lupus erythematosus, vasculitis). Collectively, disease manifestations caused by an autoimmune process may affect 5–9% of the population in Western countries.[1] These disorders represent a growing burden as their incidence significantly increased over the last years. For example, the annual incidence of type 1 diabetes is increasing globally by 2.3% per year.[2] A similar increase is seen for multiple sclerosis.[3]

It is generally assumed that autoimmune disorders result from complex interactions between genetic traits and environmental factors. Although there is a frequent concordance of autoimmune diseases among monozygotic twins,[4] the concordance rate is lower-than-expected. Similarly, changes in the incidence of type I diabetes and multiple sclerosis when children from a given population migrate from one region to another[5,6] strongly suggest a critical role for environmental causes in addition to genetic predisposition. In most autoimmune diseases the trigger has not been formally identified, leaving room for hypotheses and allegations not always substantiated by facts.

Mechanisms leading to autoimmune responses and to their occasional translation into autoimmune diseases are now better understood. Autoimmune responses result from the combined effects of antigen-specific stimulations of the immune system and of an antigen-nonspecific activation of antigen-presenting cells in the context of a genetically determined predisposition and of a somewhat deficient immune regulation. Most often such responses are not followed by any clinical manifestations unless additional events favor disease expression, for example, a localized inflammatory process at tissue level. Therefore the demonstration of autoantibodies or autoreactive T cells does not imply their involvement in a disease process. The role of infections has been occasionally demonstrated either as etiologic factor or as triggering event in autoimmune diseases. Prototypic examples are the poststreptococcal rheumatic heart disease or the Guillain-Barré syndrome that follows *Campylobacter jejuni* infections. Such observations have emphasized the multifactorial immunological pathogenesis of secondary autoimmune pathology. First, there is a potential role of antigenic similarities between some microbial molecules and host antigens. Second, infection-related signals that trigger innate immunity appear to play an essential role in enhancing the immunogenicity of host antigens or of host-mimicking epitopes, and in possibly overcoming regulatory mechanisms that limit autoimmune responses. It should be stressed that postinfectious autoimmune responses are not infrequent whereas associated autoimmune diseases remain rare events and often require additional infection-related inflammatory processes.

It is on the basis of such observations that questions were raised regarding the potential risk of autoimmune responses and autoimmune diseases following vaccination that include exposure to microbial products or antigens. Is there a significant risk that some vaccines may induce autoimmune responses through the introduction of microbial epitopes that cross-react with host antigens? Can adjuvant-containing vaccines trigger the clinical expression of an underlying

autoimmune process through a "nonspecific" activation of antigen-presenting cells and the release of inflammatory cytokines? Until now, answers to these questions have been largely based on data collected during pharmacovigilance studies in the context of postmarketing surveillance Many autoimmune diseases have a relatively low natural incidence. Although rheumatoid arthritis may reach 1% prevalence, others such as multiple sclerosis or systemic lupus erythematosus are much less frequent (around 0.1%) and many others are rare diseases. Therefore, only large epidemiological studies or huge clinical trials may allow for a consistent assessment of the relative risk of vaccine-related effects.

Understanding the mechanisms by which autoimmune responses are generated and how they may or not lead to autoimmune diseases is of paramount importance for defining the real risk of vaccine-associated autoimmune reaction. During the course of vaccine development, comprehensive and multidisciplinary approaches may help to reduce to a minimum the risk that a new vaccine would induce autoimmune manifestations. Later, once the new vaccine is largely used in public health programmes, systems are now in place in many countries to readily assess observations or allegations of unexpected autoimmune adverse effects.[7] Although in the last few years, there was a dramatic increase in the number of allegations regarding links between vaccination and autoimmune disease, it was somehow reassuring that autoimmune adverse effects were confirmed in only very few instances.

## 2 UNDERSTANDING INFECTION-ASSOCIATED AUTOIMMUNITY

Several autoimmune diseases are known to result from an infection. This is the case for rheumatic fever, including rheumatic heart disease, which appears in up to 0.3% of children following infection by group A *Streptococcus*.[8] A neurologic disease, the axonal form of Guillain-Barré syndrome, can occur in the course of *Campylobacter jejuni* enteritis. Similarly, autoimmunity was demonstrated in HTLV-1-associated myelopathy/tropical spastic paraparesis.[9] Although there is suggestive evidence that viruses might contribute to the pathogenesis of type I diabetes and multiple sclerosis, a clear-cut relation between the onset of tissue-specific autoimmunity and viral infection has not been firmly established. On the other hand, the role of infections in the exacerbation of a preexisting autoimmune disorder is rather well established. For example, in multiple sclerosis, epidemiological data strongly suggest that relapses of the disease can be triggered by both bacterial and viral infections.[10,11]

There is now a better understanding of immunological mechanisms in infection-associated autoimmunity. This is helpful to assess and reduce the potential risk of inducing autoimmune diseases with vaccines that aim at preventing these infections. The main characteristic of the immune system is its capacity to recognize a considerable number of antigenic moieties due to highly efficient gene rearrangement mechanisms during the maturation of

B- and T cells. Antibody responses to multiple infectious agents demonstrate the remarkable recognition capacity of the B cell repertoire. A parallel recognition of autologous antigenic moieties is largely avoided by tolerogenic signals during early steps of B cell development.[12] However structural homology with autoimmune consequences has been identified. Rheumatic heart disease which appears following infection by group A *Streptococcus* is associated with the an antistreptococcal immune response that cross-reacts with host cardiac myosin[13] The axonal form of Guillain-Barré syndrome that occurs in the course of *Campylobacter jejuni* enteritis is mediated by antibacterial lipopolysaccharide antibodies that cross-react with human gangliosides.[14] Similarly, antibodies directed against the Tax protein of the human T-lymphotropic virus type 1 (HTLV-1) that cross-react with the heterogeneous nuclear ribonucleoprotein-A1 (hnRNP-A1) self-antigen were demonstrated in HTLV-1-associated myelopathy/tropical spastic paraparesis.[9] More recently, antibodies to influenza nucleoprotein were shown to cross-react with the human hypocretin receptor 2 and proposed to contribute to the pathogenesis of narcolepsy occurring after administration of an influenza pandemic vaccine.[15] Therefore, it is obvious that B cell epitope cross-reactivity between host and microbial proteins can occur and occasionally lead to pathological consequences. Cross-reacting B-cell epitopes are more likely to generate autoimmune responses when they are linked with T-cell microbial epitopes that can recruit efficient T-cell help.

It is generally assumed that activation and clonal expansion of autoreactive T lymphocytes represent critical steps in the pathogenesis of cell-mediated autoimmune diseases. Infections might be responsible for these key events through several nonmutually exclusive mechanisms including molecular mimicry, enhanced presentation of self-antigens, bystander activation, and impaired T-cell regulation.[16]

The molecular mimicry hypothesis is based on sequence homologies between microbial peptides and self-antigen epitopes. At the T-cell level, this concept was initially established in an experimental model in which rabbit immunization with a hepatitis B virus polymerase peptide containing a 6 amino-acid sequence of rabbit myelin basic protein (MBP) elicited an anti-MBP T-cell response leading to autoimmune encephalomyelitis.[17] It was also suggested that a viral infection in itself could lead to autoimmune pathology caused by cross-reactive T cells in herpes simplex keratitis in which pathogenic autoreactive T-cell clones were shown to cross-react with a peptide from the UL6 protein of the herpes simplex virus.[18]

However, the significance of T-cell epitope mimicry is limited. We know that the recognition of self peptide-MHC (pMHC) complexes play an essential role in positive and negative selection of maturing T cells in the thymus. Only T cells with a sufficient affinity for self-MHC peptides presented by cortical thymic epithelial cells will survive.[19] Shaping the T-cell repertoire is then pursued in the thymic medulla. T cells undergo a negative selection that leads to the

elimination of T cells with a high affinity for self pMHC complexes presented on medullar epithelial and dendritic cells. At the end of this process, weakly autoreactive T cells will leave the thymus and constitute the T-cell repertoire. These T cells will react to diverse microbial attacks essentially through cross-reactions.[20] Therefore, one could consider that without any host cross-reactivity, the immune system would not be able to cope with infections.

Infection can also promote processing and presentation of self-antigens by several mechanisms. First, cellular damages locally induced by viral or bacterial infection can result in the release of sequestered self-antigens that stimulate autoreactive T cells. This was clearly demonstrated in autoimmune diabetes induced by coxsackievirus B4 infection in mice.[21] Second, the local inflammatory reaction elicited in tissues by microbial products can trigger dendritic cell maturation, which represents a key step in the induction phase of immune responses. Microbial products that engage toll-like receptors on dendritic cells can induce the upregulation of membrane expression of MHC and costimulatory molecules and the secretion of cytokines, which promote T-cell activation and differentiation.[22] Third, a T-cell response directed toward a single self-peptide can "spread" to other self epitopes during an inflammatory reaction. This process of "epitope spreading" has been well documented in murine models of encephalomyelitis.[23]

Special attention has been paid to cytokines of the IL-12 family as those mediators can promote bystander activation of memory T cells and occasionally trigger autoimmune reactions when such autoreactive cells do preexist. Using murine models of encephalomyelitis, Shevach et al. demonstrated that quiescent autoreactive T cells could differentiate into pathogenic Th1 effectors in presence of microbial products that induce IL-12 synthesis.[24,25] Likewise, it was shown that viral infections inducing IL-12 production could elicit relapses of autoimmune encephalomyelitis (EAE), in a nonantigen specific manner, in myelin-primed animals. A salient feature of bystander activation is its limited duration. In order to observe an exacerbation of EAE one should provide the triggering signal within a relatively restricted window of time after the aetiological stimuli that "primed" the animal for disease. In addition disease exacerbation occurs within weeks after bystander activation and it is not usually seen after longer delays.[26] In recent years, much attention has been paid to IL-23, another member of the IL-12 family that is induced by microbial products[27] and emerged as a key mediator of several human autoimmune diseases.[28]

The development of pathogenic autoimmune T-cell responses is largely reflecting individual defects in regulatory mechanisms that control the activation of autoreactive T-cell clones.[29] Regulatory T cells are instrumental in controlling autoreactive T cells both in neonates and adults.[30,31] It is clear that infectious agents can have profound influences, either positive or negative, on the balance between effector and regulatory T cells, as recently reviewed in the context of skin disorders.[32]

## 3 THE RISK OF VACCINE-ASSOCIATED AUTOIMMUNITY

Diagnosing vaccine-related autoimmune disease can only be done on a case-by-case basis. In general, appropriate epidemiological studies are essential before seriously considering that a particular autoimmune clinical condition might be associated with a given vaccination. This can then be supplemented by the determination of known biological markers of the identified autoimmune disease in other vaccinees. However, when feasible, it is particularly important to collect data on the natural incidence of autoimmune diseases in populations and age groups in the absence of vaccination. It is also relevant to compare the level of vaccine-related risk to that associated with the corresponding natural infection.

Criteria underpinning vaccine adverse event causality assessment have been established by WHO.[33] Some of these criteria particularly apply to autoimmune diseases and may be summarized as follows:

1. *Consistency*. The association of a purported autoimmune event with the administration of a vaccine should be consistent, that is, the findings should be replicable in different localities, by different investigators not unduly influencing one another, and by different methods of investigation, all leading to the same conclusion(s).
2. *Strength of the association*. The association should be strong in the magnitude of the association (in an epidemiological sense).
3. *Specificity*. The association should be distinctive and the adverse event should be linked uniquely or specifically with the vaccine concerned, rather than its occurring frequently, spontaneously, or commonly in association with other external stimuli or conditions. An adverse event may be caused by a vaccine adjuvant or additive, rather than by the active component of the vaccine. In this case, it might spuriously influence the specificity of the association between vaccine and adverse event.
4. *Temporal relation*. There should be a clear temporal relationship between the vaccine and the adverse event, in that receipt of the vaccine should precede the earliest manifestation of the event or a clear exacerbation of an ongoing condition. The timing is important; long delays (over 2 months) are not the rule. Indeed, the induction or the acceleration of autoimmune tissue lesions that have been observed following some acute infections (eg, *Campylobacter jejuni* or influenza) have always occurred within weeks after the infectious event.

An association between vaccine administration and an autoimmune adverse event is most likely to be considered strong when the evidence is based on:

1. Well-conducted human studies demonstrate a clear association in a study design that is determined a priori for testing the hypothesis of such association. Such studies will normally be one of the following, in descending order of probability of achieving the objective of the study: randomized controlled clinical trials, cohort studies, case control studies, and controlled

case-series analyses. Case reports, however numerous and complete, do not fulfil the requirements for testing hypotheses. When autoimmune events appear attributable to a vaccine, it is important to determine whether there is a predisposed set of subjects (by age, population, genetic, immunological, environmental, ethnic, sociological, or underlying disease conditions). Such predisposition is most likely to be identified in case-controlled studies.

2. An association that is demonstrated in more than one human study and consistent among the studies. The studies would need to have been well conducted, by different investigators, in different populations, with results that are consistent, despite different study designs.
3. In the case of future vaccines against infections known to be associated with autoimmune complications (eg, postgroup A streptococcal rheumatic heart disease), vaccine-associated autoimmune adverse events which closely resemble these infection-associated complications.
4. A nonrandom temporal relationship between administration and the adverse incident.

There should be a strict definition of the autoimmune adverse event in clinical, pathological, and biochemical terms, as far as that is achievable. The frequency in the nonimmunized population of the adverse event should be substantially different from that in the immunized population.

## 4 VACCINE-ATTRIBUTABLE AUTOIMMUNE DISEASES

The old rabies vaccine that was produced using rabbit brain tissue was associated with the occasional (0.33/1000) development of immune-mediated encephalitis and antimyelin T-cell responses.[34] This is not observed anymore with presently used rabies vaccines produced on cell lines. In fact, it is only in a few rare cases that autoimmune pathology has been firmly considered as attributable to the use of modern vaccines. For example, a form of Guillain-Barré Syndrome (GBS, polyradiculoneuritis) was found associated with the 1976–77 vaccination campaign against swine influenza using the A/New Jersey/8/76 swine-flu vaccine.[35] The estimated attributable risk of vaccine-related GBS in the adult population was just under one case per 100,000 vaccinations and the period of increased risk was concentrated primarily within the 5-week period after vaccination ( relative risk: 7.60). Although this original Centers for Disease Control study demonstrated a statistical association and suggested a causal relation between the two events, controversy has persisted for several years. The causal relation was reassessed and confirmed in a later study focusing on cases observed in Michigan and Minnesota[36] The relative risk of developing GBS in the vaccinated population of these two states during the 6 weeks following vaccination was 7.10, whereas the excess cases of GBS during the first 6 weeks attributed to the vaccine was 8.6 per million vaccinees in Michigan and 9.7 per million vaccinees in Minnesota. The pathogenic mechanisms involved

are still unknown. With subsequent influenza vaccines, no significant increase in the development of GBS was noted[37] and it is currently assumed that the risk of developing the GBS following vaccination (one additional case per million persons vaccinated) is substantially less than the risk for severe influenza and influenza-related complications. However, a slight increased risk of GBS after influenza A (H1N1) 2009 monovalent inactivated vaccine was detected in a US surveillance system.[38]

Another example of adverse effect of vaccination is idiopathic thrombocytopenia (ITP) that may occur after the first dose of measles-mumps-rubella (MMR) vaccination. In a recent UK study, the reported frequency of clinically apparent ITP after this vaccine is around 1 in 22,500 doses in the 6-week postimmunisation period,[39] There was no increase nor recurrence after a second dose of MMR.[40] The clinical course of MMR-related ITP is usually transient but it is not infrequently associated with bleeding and, as shown in a study conducted in Finland, it can occasionally be severe.[41] In this latter study, there was an increase in platelet-associated immunoglobulin in 10 of 15 patients whereas circulating antiplatelet autoantibodies, specific for platelet glycoprotein IIb/IIIa, were detected in 5 of 15 patients. These findings are compatible with an autoimmune mechanism triggered by immune response to MMR vaccination. However, it should be noted that the risk for thrombocytopenia following natural rubella (1/3000) or measles (1/6000) infections is much greater than after vaccination.[42] Patients with a history of previous ITP are prone to develop this complication and in these individuals the risk of vaccination should be weighed against that of being exposed to the corresponding viral diseases.[43]

Narcolepsy is the most recent example of a possible autoimmune-mediated pathology related to vaccination. Indeed, a slightly increased incidence of narcolepsy (a dysregulation of sleeping) in 5–18 years old children was observed in association with the use of one of the pH1N1-2009 AS-03 adjuvanted pandemic vaccine, primarily in Nordic countries. Narcolepsy is a rare disease characterized by the disappearance of a group of neurons of the hypothalamus that produce a protein, hypocretin, which participates in sleep regulation. The affection is closely associated with the presence of a specific HLA haplotype (HLA-DQB1*06:02), and an autoimmune aetiology is highly suspected. A cross-reactivity of antibodies to influenza nucleoprotein with the hypocretin receptor[15] has been recently reported. However such antibodies were shown in healthy children as well as in those affected by narcolepsy. Their link with narcolepsy is questionable. On the other hand, the implication of the adjuvant contained in the vaccine was not confirmed.[44] One should note that an increased incidence of narcolepsy was also observed, at a lesser level, in China, in temporal association with the H1N1/2009 pandemic crisis, but in the absence of vaccination.[45] One cannot exclude that postvaccination narcolepsy would be the result of combined effects of a silent viral exposure followed by an active immunization against viral antigens in susceptible individuals.

## 5 ALLEGATIONS OF AUTOIMMUNE ADVERSE EFFECTS

The introduction of new vaccines and the increasing number of highly publicized reports that claim a link between certain immunizations and autoimmune diseases have led to public concern over the risk of inducing autoimmune disease by immunization. For example, 20 years ago, special concerns were expressed in France regarding the potential association of multiple sclerosis with hepatitis B vaccination. Similarly, the influence of childhood vaccination on type 1 diabetes was questioned in United States. Such allegations were not confirmed but had detrimental effects on vaccination programmes at a global level.[46,47]

More recently, the so-called ASIA syndrome has also elicited concerns about safety of vaccines, including the recently introduced vaccines against human papillomavirus (HPV). ASIA stands for autoimmune/inflammatory syndrome induced by adjuvants and was first reported in 2011.[48] While this syndrome attempts to associate a spectrum of immune-mediated diseases with an adjuvant stimulus, the authors recently emphasized that the clinical proof of causality remains a challenge.[49]

Because of their recent introduction in large population of adolescents (over 178 million doses were distributed worldwide), it is not surprising that HPV vaccines were subject of a series of allegations over last years, especially regarding their putative role in the induction of multiple sclerosis and other autoimmune neurological disorders. Extensive studies to assess the safety of the vaccine in routine practice were conducted since licensure, including more than one million preadolescents, adolescents, and adults from various countries. A recent metaanalysis of available postlicensure safety data found no increase in the incidence of autoimmune disorders postvaccination compared with background rates.[50]

## 6 NEW GENERATION VACCINES AND AUTOIMMUNITY: APPROACHES TOWARD EARLY RISK ASSESSMENT

During the course of vaccine development, a comprehensive and multidisciplinary strategy may help to reduce the theoretical risk that a new vaccine would induce autoimmune manifestations. It is essential to present appropriate documentation to regulatory authorities,[51] with specific considerations for adjuvanted vaccines.[52] First, one should question whether clinical manifestations of an autoimmune nature are known to be associated with the infectious disease that will be the target of the new vaccine. If such events have been reported, for example, for group A streptococcal diseases, attention should be given to avoid reproducing the natural disease pathogenic process. This may include the identification and the exclusion of naturally pathogenic epitopes. Second, potential molecular and immunological mimicry between vaccine antigens and host components should be critically analyzed through an intelligent combination of bioinformatics and immunological studies. One should keep in

mind that, by itself, an identified mimicry is of little pathogenic significance. Information should be gathered on the relative ability of such epitopes to bind to human MHC molecules, to be processed by human antigen-presenting cells and to be recognized by autoreactive T cells. One should keep in mind that molecular mimicry will not be sufficient to trigger autoimmune pathology as demonstrated by a recently developed Lyme disease vaccine that was shown to contain an immunodominant epitope of the outer surface protein A of *Borrelia burgdorfi* (Osp A) displaying significant homology with human LFA-1, an adhesion molecule. This vaccine was not associated with autoimmune adverse effects.[53] Indeed, other factors intrinsic to infections such as tissue damage and long-lasting inflammatory reaction must be present for autoimmune pathology to develop. Different vaccine formulations and adjuvants can be compared regarding their potential capacity to induce or enhance the expression of pathology in relevant models. For example, there are models of experimental allergic encephalitis, which are sensitive to the administration of microbial products and can help to compare the nonspecific effects of different adjuvants or vaccine formulations.[26] However, it appears now that the value of such observations in animal models is very limited in relation to human vaccinology.[54] Fourth, appropriate immunological investigations (eg, autoimmune serology) may be systematically included in Phase I-II-III clinical trials. On an ad hoc basis, clinical surveillance of potential autoimmune adverse effects may have to be included in the monitoring protocol. Such surveillance will have to be extended through the postmarketing stage if specific rare events have to be ruled out. Fifth, at postmarketing stage, a particular attention should be given to vaccinated patients with a known autoimmune disease. Absence of disease exacerbation following vaccination is a good indication of vaccine safety in the context of preexisting autoimmunity.[55]

## 7 VACCINATION IN PATIENTS WITH CHRONIC AUTOIMMUNE DISEASES

Natural infection, unlike vaccination, is a proven risk factor for exacerbating preexisting autoimmune diseases. Influenza and other acute respiratory infections are also commonly associated with an increased frequency of relapses in patients with relapsing multiple sclerosis.[56] This risk is markedly reduced in patients that received the seasonal influenza vaccine.[57] Likewise, HPV vaccines were reported to be efficacious and safe in most of the patients affected by autoimmune diseases.[58]

Indeed, a recent metaanalysis concluded that several vaccine-preventable infections occurred more often in patients with autoimmune disease, that vaccines were efficacious in these patients, and that there did not appear to be an increase in vaccination-related harm compared to nonvaccinated patients.[59] Therefore, there is a general agreement that important vaccination should be performed in such patients.

## 8 CONCLUSIONS

Although the benefit of vaccination against infectious diseases largely outweighs the potential risk of autoimmune adverse effects, this risk deserves a particular attention. The recent introduction of vaccines for adolescents and young adults has increased the risk of purely coincidental association of vaccination and incipient autoimmune diseases, for example, multiple sclerosis, and this led in some countries to overwhelming and detrimental concerns about vaccination.

This concern does not appear justified for vaccines that are now in regular use. Occasional autoimmune manifestations are and will be seen after vaccination but an association cannot be claimed in the absence of an extensive causality assessment. The mere occurrence of autoimmune markers (autoreactive antibodies or T cells) is a frequent phenomenon in a normal population and their pathological expression, that is, the development of an autoimmune disease, is by far much less frequent. Several approaches are now available to reduce to a minimum the risk of autoimmune effects with newly developed vaccines. It is important to note that vaccination (eg, influenza) is commonly recommended for patients with chronic autoimmune diseases and other inflammatory processes.

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Chapter 10

# Maternal Immunization: Protecting Vulnerable Populations

**Janet A. Englund, MD**
*University of Washington, Department of Pediatrics and Seattle Children's Hospital, Division of Pediatric Infectious Diseases, Seattle, WA, United States*

## Chapter Outline



## 1 INTRODUCTION

Immunization of pregnant women to protect both the mother and infant from infection is a unique strategy to prevent disease in high risk, vulnerable populations. This approach, utilized for decades to prevent puerperal and neonatal tetanus, is increasingly considered as an immunization strategy worldwide.[1] Morbidity and mortality due to infections during pregnancy and the first few months of life contribute substantially to overall mortality worldwide. In particular, infectious causes of respiratory disease and sepsis

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are responsible for approximately one-quarter of mortality in neonates[2] with infections occurring during a period when active infant immunization is not expected to be protective. Neonates during the first weeks of life are uniquely at risk for disease due to infections from multiple pathogens during a time in which their immune system is still relatively ineffective. Multiple doses of routine childhood vaccines are required prior to the establishment of protective efficacy but disease within the first 2 months of life represents a substantial burden of disease overall. Alternative approaches to provide protection to newborns at the time of birth include active immunization, an approach that has rarely been successful in providing protective efficacy by a young age, and passive immunization with immunoglobulin or antibody products, an expensive and more difficult immunization strategy. This chapter discusses the rationale and data currently available regarding maternal immunization, and ongoing and potential strategies for its use.

## 2 BACKGROUND

### 2.1 History of Maternal Immunization

Immunization of women during pregnancy is not a new idea. Infants have long been felt to be protected against diseases that mothers had previously had. Smallpox disease has been reported to be severe in pregnant women, with case reports of severe disease in pregnant women dating back to the 19th century.[3] Smallpox vaccination in pregnant women was first reported in an uncontrolled series in Germany in 1879, when Burkhardt reported that infants born to pregnant women who had been immunized with smallpox vaccine using "Jennerian vaccination" were not infected by smallpox vaccine during the first days following vaccination.[4] Clinical trials of whole cell pertussis vaccine in pregnant women were conducted during the 1930s and 1940s, although antibody assays to pertussis were problematic even then. These investigators demonstrated safety in the infant, with transmission of potentially protective antibody to the infant.[5,6] A description of reactions or safety in the women, some of whom received multiple doses of whole cell pertussis vaccine, was not reported. Routine immunization with multiple vaccines including influenza vaccine and polio vaccine during pregnancy was commonplace during the 1950s and 1960s in the United States, a time when polio was active and the Hong Kong influenza pandemic had demonstrated increased mortality in pregnant women. The benefits of protecting the mother against polio and influenza were perceived to be high. The safety and benefit of inactivated and live polio vaccine was demonstrated in a long-term prospective study of more than 3,000 women in the United States during that time period[7] as well as during polio outbreaks in Finland and Israel, in which approximately 25,000 pregnant women received polio vaccine.[8–11] The safety of live and inactivated polio vaccine has been described in a recent WHO review.[12] Mass vaccination against meningococcal A disease was carried out in the 1970s in 90 million persons including pregnant women during a devastating meningococcal

A outbreak in Brazil, with vaccine provided by Sanofi Pasteur.[13] Although prospective surveillance for safety or adverse events in pregnant women and their offspring was not carried out, investigators studied maternal and cord blood levels and subsequent infant levels of antibody at 3 and 6 months of age, concluding that vaccination of the mother increased specific meningococcal antibody levels in the infants threefold compared to babies born to unvaccinated mothers, and that the antibody levels in these infants declined to ~80% of cord levels by 3 months of age.[14] Historically, the type and intensity of surveillance and reporting of potential adverse events associated with vaccine was less rigorous than today. As the perceived risks for pregnant women and their infants diminished, recommendations and uptake for maternal vaccination also decreased with the exception of tetanus toxoid vaccine in developing countries.

## 2.2 Considerations for Pregnant Women

There is currently widespread international agreement that pregnant women deserve appropriate routine medical care for their pregnancy as well as for other, nonpregnancy related conditions. Pregnant women are, in general, younger, healthier, and more likely to seek medical care than the overall female population and the availability of antenatal care facilities has been increasing over the past decades such that care is potentially available for the vast majority of women today. Societies such as the American College of Obstetrics and Gynecology advocate that women should receive treatment for medical conditions as indicated for nonpregnant women, such as antibiotics for acute infections, treatment against HIV if indicated, as well as care and prevention against important pathogens causing more frequent or severe disease in pregnant women including influenza[15] and pertussis[16] (Table 10.1). Pregnant women should not be excluded from potentially beneficial therapies based solely on their pregnancy status, an issue that has been controversial over the past decades in areas such as clinical trials for antiretroviral therapy for HIV-infected women.[17,18]

Pregnant women have mature immune systems, which are more competent than the immune systems of the fetus or neonate, and have good immune responses to protein polysaccharide, and protein-conjugate vaccines. Immune responses during pregnancy have been studied, although generally in small studies or for responses to specific pathogens such as malaria. It is known that physiologic changes during pregnancy include increased heart rate, increased stroke volume and decreased pulmonary functions overall but with an increase in oxygen carriage. These women have an increase in blood cortisol levels due to decreased clearance, and decreased cell-mediated immunity that is relatively minor but appears to predispose pregnant women to infection with listeria, tuberculosis, and toxoplasmosis.[19] Pregnant women have a decrease in total IgG antibody due to hemodilution, which appears to return to normal shortly after delivery. Most studies indicate that the immune responses to vaccines during pregnancy are similar to those during the nonpregnant state.[20] A recent

**TABLE 10.1 Potential Vaccines That Could be Used During Pregnancy**[a]

| Vaccines | Risk to mother | Risk to fetus | Comments |
|---|---|---|---|
| **Vaccines routinely administered** | | | |
| Tetanus (T)/ diphtheria (D) toxoid | None reported | None reported | Effective; administered routinely worldwide |
| Inactivated influenza | None reported except anaphylaxis (rare) | None reported | Effective; administered widely; given in all trimesters |
| Acellular pertussis combined with T, D, and potentially inactivated poliovirus (UK) | None reported | None reported | Given widely in USA/ UK; effective in preventing neonatal pertussis; infants with good immune response after 12–15 M booster |
| **Administered if indicated under special circumstances**[b] | | | |
| *Bacterial vaccines* | | | |
| Meningococcal conjugate vaccine | None reported | None reported | Administered during outbreaks or mass campaigns |
| Pneumococcal conjugate vaccine | Local reactions | None reported | Studied in controlled clinical trials |
| Inactivated typhoid vaccine | Unknown | Unknown | To be considered only if exposure or outbreak |
| *Viral vaccines* | | | |
| Hepatitis A vaccine | None reported | Unknown | Pregnancy does not alter recommendations for use; consider risk of severe hepatitis disease during pregnancy |
| Hepatitis B vaccine | None reported | None reported | Pregnancy does not affect recommendation for use |
| Oral or inactivated poliovirus vaccine | None reported | None reported | Has been studied; recommended during outbreak situation |
| Rabies vaccine | Unknown | None reported | Administered as for nonpregnant persons after exposure |
| Yellow fever vaccine | Well tolerated | Potential transmission of vaccine virus to infant | Given in outbreak settings but use risk assessment before administration; consider postponing elective travel |

[a]*Munoz FM, Englund JA. Vaccines in pregnancy.* Infect Dis Clin North Am *2001;***15***:253–271.*
[b]*Potential for administration if residence in or travel to endemic areas, known exposure, or outbreak situations. Data in pregnancy are limited for most vaccines. Should weigh theoretical risk of vaccination against benefit of preventing disease and risk of disease in the pregnant woman.*

**TABLE 10.1 Potential Vaccines That Could be Used During Pregnancy[a] (*cont.*)**

| Vaccines | Risk to mother | Risk to fetus | Comments |
|---|---|---|---|
| **Vaccines under investigation** | | | |
| Group B streptococcal conjugate | Studies ongoing | Ongoing | |
| Pneumococcal conjugate vaccine | Studies completed | None known | No evidence of adverse events |
| RSV F protein vaccine | Studies ongoing | Ongoing | |
| Other potential vaccines: HIV, HSV, CMV | | | |
| **Contraindicated** | | | |
| Rubella | None reported | None confirmed | |
| Measles–mumps–rubella | None reported | None confirmed | |
| Varicella or zoster vaccine | None reported | None confirmed | |

[a]*Munoz FM, Englund JA. Vaccines in pregnancy.* Infect Dis Clin North Am *2001;***15***:253–271.*
[b]*Potential for administration if residence in or travel to endemic areas, known exposure, or outbreak situations. Data in pregnancy are limited for most vaccines. Should weigh theoretical risk of vaccination against benefit of preventing disease and risk of disease in the pregnant woman.*

study of inactivated influenza vaccine demonstrated that pregnant women do not have impaired humoral responses to influenza antigens and that plasmablast circulation may be even increased following immunization.[21] Importantly, pregnant women are capable of determining benefits for themselves and their unborn child and in concert with their family, have the ability to understand the advantages of medical care including immunization and should be permitted to receive this benefit.

Rates of morbidity and mortality in pregnant women have decreased globally over the past decades due to improvements in access to prenatal care, delivery at medical centers, and improvements in medical care overall, but pregnancy still remains a time of risk for pregnant[22] women. An estimated 289,000 women died during late pregnancy or childbirth in 2013 (WHO, UNICEF 2014). Infectious causes such as group B streptococcal infections or influenza have been documented to contribute to morbidity and infections in this population, but it is clear that better understanding of causes of maternal complications is needed. It is also known that increased access to antenatal care and delivery assistance may assist in improved outcomes in pregnant women and their infants. An important

goal internationally has been improved antenatal care for pregnant women, with rates from 2010 showing that 78% of women globally receive at least one antenatal care visit (with rates ranging from 69% in the lower socioeconomic sites to 94%) in higher income locations.[23] The accessibility of pregnant women to receive medical care can result in improvement in prenatal care, and be cost-effective for preventing neonatal deaths.[24] Proven interventions for pregnant women include packages including education, vitamins, clean birth kits, community or clinic based delivery, and tetanus immunization prior to delivery. The contribution of maternal infections to premature onset of labor globally remains uncertain.[25] Rates of preterm delivery, defined as childbirth occurring at less than 37 weeks gestation, has not been well documented in developing countries and identification of causes of preterm delivery in these regions is considered to be a high priority to prevent neonatal mortality.

## 2.3 Infants

Infants during the neonatal period, or the first 28 days of life, are most vulnerable to serious consequences related to preterm delivery, complications of delivery, and infection. It is estimated that 41% of all deaths in children under 5 years of age occur during the neonatal period.[26] Recent global estimates show that preterm delivery is an important factor contributing to neonatal mortality, with rates of preterm delivery ranging widely from 6.4 to 17.5% around the world, with rates highest in sub-Saharan Africa. Progress in reducing childhood mortality is ongoing, with increasing vaccination of young children. The rate of decline of 5% per year in some of the highest risk countries has been documented.[27] Nonetheless, mortality due to infection during the first months of life remain an important and potentially preventable cause of childhood deaths and innovative approaches with vaccines seem to be a promising and cost-effective measure. Strategies including multivalent vaccines, increased availability of childhood vaccination centers, and enhanced surveillance for vaccine uptake and vaccine-preventable diseases are potential implementation strategies undertaken in multiple countries.

Infant vaccine schedules vary globally, but the Expanded Programme of Immunization (EPI) schedule starts with vaccination at 6 weeks of age with subsequent doses given at 10 and 14 weeks of age. Protection from infections covered by the primary injectable vaccination series in the youngest infants is due either to preexisting maternal antibody in the infant (which generally decreases quickly over the first months of life) or to the active immune response to vaccine antigens. Typically, these vaccines consist of the pediatric diphtheria-whole cell or acellular pertussis–tetanus toxoid vaccine (DPT or DaPT), hepatitis B vaccine, *Haemophilus influenzae* type b conjugate (Hib) vaccine, and the multicomponent pneumococcal conjugate vaccine and protective antibody levels do not appear until at least after the second dose and more generally after the third dose. Despite tremendous progress, global coverage remains below the target of 90% coverage of infants with three doses of diphtheria–tetanus–pertussis vaccine.[28]

Pathogens responsible for high rates of disease in the first several months of life may differ in various geographical regions. This variation may be due to standards of medical care, such as widespread utilization of clean birth techniques and adult and/or maternal immunization policies (eg, tetanus), routine individual surveillance of pregnant women [eg, HIV testing or prophylaxis for maternal Group B streptococcus (GBS) carriage], and herd immunity achieved by population-wide immunization (eg, Hib or pneumococcus). Nonetheless, common pathogens appreciated worldwide during the first months of life consist of Gram-negative bacterial infections such as *Escherichia coli* or *Klebsiella*, Gram-positive bacterial infections such as *Staphylococcus* pneumococcus, or GBS, parasitic infections such as malaria, and viral infections such as respiratory syncytial virus (RSV).

Although vaccine uptake in many countries is increasing overall, the timeliness of vaccine administration continues to be an issue in both developed and developing countries and thus, young infants may be unprotected against diseases such as pertussis for months between birth and the development of specific antibody following the third infant dose of vaccine. Delay in receipt of a vaccine series is common worldwide; in the United States almost half of children had some delay in receiving a DTaP vaccine dose and 16% were delayed in vaccine receipt for more than 6 months in the first 2 years of life.[29] A longitudinal study in Ghana reported that while coverage for three doses of DTP was 95% at 12 months, only 10% of infants were vaccinated within 1 week of the scheduled time of 14 weeks; and the median delay for the third dose of DTP was 4 weeks.[30]

## 2.4 Transplacental Antibody Transfer

Maternal IgG antibody is actively transported across the placental using specific receptors.[31] Active transport of maternal IgG occurs primarily after 32 weeks gestation; infants born before this time have low levels of maternal antibody.[32,33] By the time of delivery of a full-term infant in a healthy mother, the level of IgG is generally higher in the infant than the mother due to this active transport.[33] Multiple factors influence the transfer of maternal IgG during pregnancy, including placental integrity, total maternal IgG concentration, IgG subtype, and if vaccine is administered, the timing of vaccination relative to delivery. The presence of maternal infection with HIV or malaria can reduce antibody transfer by reducing the ability of the placenta to transport IgG through impairment of Fc receptor function.[34] The impact of HIV infection in reducing neonatal antibody titers to *Bordetella pertussis*, tetanus, and pneumococcal antibodies in South Africa has been shown to lead to a reduction in antibody levels of 15–40%.[35] Higher levels of total maternal IgG may also reduce transfer of antigen-specific IgG by competitive binding to placental Fc receptors.[36]

The maternal transfer of IgG subtypes varies. $IgG_1$, which is induced primary by protein antigens such as tetanus toxoid, is most efficiently transferred

while $IgG_2$, induced by polysaccharide antigens such as pneumococcus, is least efficiently transferred.[37] Infant antibody titers rise approximately 2 weeks after maternal vaccination. In a study of Hib conjugate vaccine, transmission of antibodies was greatest in mothers vaccinated more than 4 weeks before delivery.[38] Vaccination with a conjugate vaccine at between 28 and 32 weeks gestation may optimize the amount of disease-specific IgG present at time of delivery and ensure the greatest period of protection for neonates. For diseases where seasonality plays an important role such as influenza, and where there is substantial risk to the pregnant woman and fetus as well as the infant, the US Advisory Committee on Immunization Practices (ACIP) recommends vaccination at the beginning of the seasonal epidemic.[39]

Maternal immunization with Hib vaccine was studied in the 1990s (Table 10.2). Epidemiological evaluation of reduction of Hib disease due to decreased carriage and herd immunity provided by pediatric immunization with the extremely effective conjugate Hib vaccine makes maternal immunization less likely to benefit infants. Nonetheless, maternal Hib vaccine studies have demonstrated the importance of timing of maternal vaccine, differential rates of IgG subtype antibody transfer, the lack of neonatal or infant priming by maternal immunization with protein or protein-conjugate vaccines, and the higher rates of antibody transferred to the fetus when immunization takes place during rather than prior to pregnancy[40–42] (Table 10.2).

**TABLE 10.2 Immunization During Pregnancy Results in Higher Cord Antibody Titers Compared to Immunization Prior to Pregnancy: The Experience With *Haemophilus influenzae* Type b (Hib) Vaccines**

| When/where was Hib vaccine given? | Antibody level: IgG Anti-PRP (μg/mL) | | |
|---|---|---|---|
| | Mother | Infant | Transmission from mother to infant (%) |
| Prior to pregnancy | | | |
| Sacaton, AZ[a] | 20 | 11 | 73 |
| Third trimester | | | |
| Houston, TX[b] | 78 | 47 | 60 |
| The Gambia[c] | 4 | 2 | 61 |

[a]*Santosham M, Englund JA, McInnes P, et al. Safety and antibody persistence following* Haemophilus influenzae *type b conjugate or pneumococcal polysaccharide vaccines given before pregnancy in women of childbearing age and their infants.* Pediatr Infect Dis J *2001;***20***:931–940.*
[b]*Englund JA, Glezen WP, Turner C, Harvey J, Thompson C, Siber GR. Transplacental antibody transfer following maternal immunization with polysaccharide and conjugate* Haemophilus influenzae *type b vaccines.* J Infect Dis *1995;***171***:99–105.*
[c]*Mulholland K, Suara RO, Siber G, et al. Maternal immunization with* Haemophilus influenzae *type b polysaccharide-tetanus protein conjugate vaccine in The Gambia.* JAMA *1996;***275***:1182–1188.*

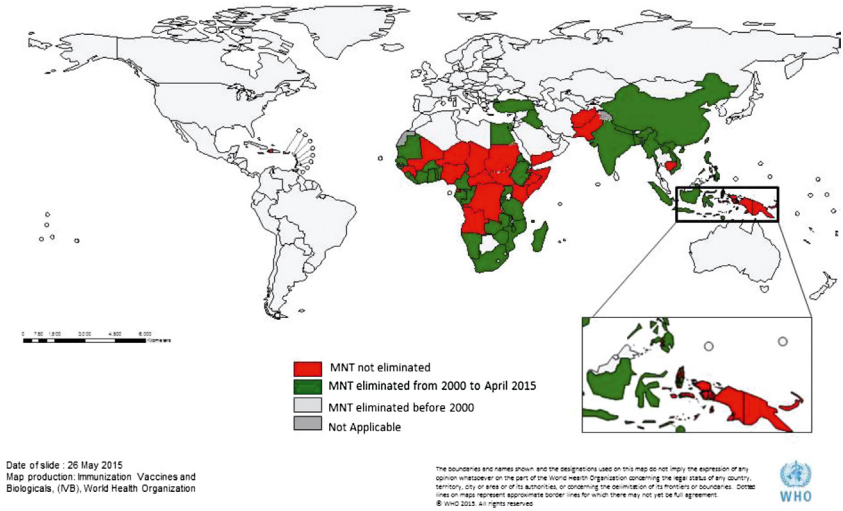


**FIGURE 10.1** **Countries (37 in number) that eliminated MNT between 2000 and May 2015.** Note: Ethiopia except Somali region, 30 provinces out of 34 in Indonesia, and 16 regions out of 17 in Philippines; leaving 22 countries yet to eliminate MNT. *(WHO/UNICEF Database.)*

## 2.5 Why Immunize a Pregnant Woman?

Several vaccines are currently recommended for use in pregnant women (Table 10.1). Immunization during pregnancy has been utilized for more than 50 years to prevent tetanus disease in mothers and infants, a disease that was responsible for up to 30% of deaths in developing countries up to the middle of the 20th century. Ongoing campaigns against tetanus lead by UNICEF, WHO, and other organizations have resulted in the eradication of neonatal tetanus from many countries, and the Western Hemisphere (Fig. 10.1). Experience from the tetanus vaccine campaigns have shown that immunization during pregnancy is feasible, as it may be integrated into with routine prenatal care. This approach has also shown that immunization during pregnancy has the potential to protect two individuals—the mother and the infant—during a vulnerable period of life at a minimal cost. Furthermore, pregnant women are increasingly accessible to medical care. Access to antenatal care, a Millenium Development Goal of the United Nations, is increasing and becoming more widely available even in the lowest income countries and this access, which typically occurs during the last months of pregnancy, provides an opportunity to provide vaccine as well as other health care to these women. Finally, maternal immunization strategies are far safer and less expensive than the administration of exogenous immunoglobulin products to the infant. The use of monoclonal antibodies against RSV, for example, in preterm infants has been effective in reducing RSV-related lower respiratory tract disease[43] but at a cost exceeding resources in most settings.

## 3 VACCINES CURRENTLY ADMINISTERED TO PREGNANT WOMEN

### 3.1 Tetanus

Tetanus disease and mortality has been well documented worldwide because of the readily identifiable clinical characteristics of disease; and a marked decrease in the incidence of neonatal tetanus associated with improved vaccination of adults and pregnant women has been clearly documented (Fig. 10.1). The landmark trial in maternal immunization was conducted using a using a village randomization design in New Guinea by Schofeld et al.,[44] with impressive reduction in neonatal disease from an incidence of neonatal tetanus of 16/160 (10%) to only 1/175 (0.6%) infants using three doses of a tetanus toxoid vaccine administered to the mother during pregnancy. The mortality attributed to neonatal tetanus mortality has been reduced by 92% with the advent of universal TT administration during pregnancy in combination with improved hygienic birthing practices.[45] TT is a protein-based vaccine that elicits an IgG1 immune response, with antibody actively transported across the placenta with >100% efficiency. The WHO recommends the administration of two doses of TT in the first pregnancy and one in each subsequent pregnancy for a maximum of five doses. Implementation of TT is widely used in resource-limited settings with 80% coverage of pregnancies worldwide (Table 10.1). Administration of TT alone during pregnancy in the United States is not indicated for protection against tetanus in women who have completed the recommended immunization series prior to conception. TT is not routinely administered but may be given as part of the tetanus–diphtheria–acellular pertussis vaccine (Tdap) during pregnancy given primarily for protection against neonatal pertussis. The infrastructure for delivery of TT in resource-limited settings has the potential to introduce other maternal immunization programs for other vaccines, such as influenza.[46]

### 3.2 Influenza

Pregnant women are at high risk for more severe complications from influenza than the general population, and women in the third trimester of pregnancy are at highest risk.[47] Influenza infection during pregnancy in the 2009 H1N1 pandemic was associated with an increased risk of maternal and fetal death, while administration of the H1N1 and multivalent inactivated influenza vaccines has been shown to be safe and efficacious in pregnancy.[48–50] Only inactivated influenza vaccine, with or without adjuvant, has been studied in pregnant women. Influenza vaccination during pregnancy generates a potentially protective antibody response in the mother[51] and decreases clinical illness in both mothers and infants, including mothers who are HIV-positive and their infants.[50] Vaccination with one dose of inactivated monovalent 2009

H1N1 vaccine during pregnancy produces a protective antibody response in 93% of pregnant women, with efficient transplacental antibody transfer and generation of protective antibody responses in 87% of infants.[52] Influenza vaccination during pregnancy also reduces the risk of an influenza diagnosis in the mother.[48,50]

The effect of influenza vaccination on birth outcomes, including a potential effect on decreased incidence of small for gestational age (SGA), preterm birth, and low birthweight infants in pregnant women, has been analyzed in retrospective cohort studies[52,54,55] and several prospective randomized controlled trials.[49,50,52,53] In the prospective study in Bangladesh, receipt of TIV during pregnancy was associated with an increased birth weight of 200 g and decreased incidence of SGA by 34%. Retrospective studies have also shown a decreased risk of PTB with both H1N1 vaccine and TIV administration during pregnancy.[54,55] Results from the South African study demonstrate efficacy in both pregnant women and their infants but did not demonstrate an impact on birth weight or incidence of small for gestational age.[50] Prospective clinical trials are underway in Mali and Nepal and will add further data regarding the effect of vaccination on birth outcomes.[53]

## 3.3 Pertussis

The highest rates of morbidity and mortality for pertussis disease are seen in young infants. In the United States the majority of hospitalizations and deaths from pertussis have occurred in infants under 2 months of age who were unvaccinated.[56] Administration of Tdap to postpartum mothers and family members to prevent infant pertussis disease has been recommended by the ACIP since 2005, but was logistically difficult to implement and did not provide protection during the first few weeks of the infant's life prior to generation of an antibody response.[57,58] Studies evaluating infant cord blood pertussis antibody levels showed significantly higher antibody titers in those born to mothers who were vaccinated with Tdap during pregnancy.[59] Additional studies of pertussis booster administration to healthy adolescents and adults showed that levels of antibody remained sustained for several months after vaccination.[60] Based on these studies, the ACIP modified their recommendations in 2011 to include administration of Tdap to all unvaccinated pregnant women, and then subsequently updated these recommendations in 2012 to include vaccination of all pregnant women, regardless of previous immunization status, during the third trimester of pregnancy.[61] This modification was in response to concerns of potentially inadequate vaccination histories, as well as additional data showing that mothers who were vaccinated before the third trimester of the current pregnancy did not transfer sufficient protective antibody titers to their infants.[62]

In the United Kingdom, increased neonatal pertussis deaths were reported beginning in 2011 and increasing into 2012. Routine immunization of pregnant

women against pertussis using a multivalent vaccine containing pertussis antigens between 28 and 38 weeks gestation was rapidly implemented in Oct. 2012. Surveillance for vaccine uptake and safety showed vaccine coverage the first year to be 64%. To date, there has been good uptake of vaccine with a good safety profile. Overall, with more than half a million pregnant women in the United Kingdom immunized with a vaccine containing Tdap and inactivated trivalent polio vaccine (REPEVAX, Sanofi Pasteur, Lyon, France).[57] The number of fatal infant pertussis cases in the United Kingdom decreased from 12 deaths in 2012 to 2 deaths in 2013; both fatalities in 2013 were infants born to mothers who were not immunized.

Immunogenicity studies conducted in pregnant and nonpregnant women have shown similar antibody responses to Tdap vaccine.[63] In another study, immunization of pregnant women with Tdap between 27 and $30^{+6}$ weeks was associated with highest umbilical cord geometric mean concentration of IgG antibody to pertussis toxoid and filamentous hemagglutinin, compared with immunization beyond 31 weeks.[64] Studies conducted on infants following maternal immunization have shown increased antibody levels through 2 months of age compared to infants whose mothers were not vaccinated.[63,65] Slightly decreased infant antibody levels were seen following the primary immunization series to some but not all antigens, and in several prospective studies, antibody levels to all vaccine antigens were similar following the booster dose of Tdap.[63,65]

Current unresolved issues include the effect of maternal Tdap administration on long-term (4 years of age and older boosters) to diphtheria, tetanus, and acellular pertusiss (DTaP) immunization, as well as lack of data regarding safety and potential adverse events associated with repeat administration of Tdap during closely spaced pregnancies.

## 4 POTENTIAL MATERNAL VACCINES

### 4.1 Group B Streptococcus

GBS causes invasive disease in young infants and is also responsible for bacteremia, urinary tract infections, chorioamnionitis, and endometritis in pregnant women. GBS is the most common cause of invasive disease in infants less than 3 months of age in the United States. Rectal or vaginal GBS carriage is a prerequisite to invasive infection, with US carriage rates around 20%.[66] GBS carriage is common in other countries including developing countries when it is actively tested for; with wide rates of maternal carriage from 12% in a refugee camp in the Thai–Myanmar border[67–69] to 21% in HIV-infected pregnant women in Malawi, and 30% in Quebec, Canada. Early onset neonatal disease presenting within the first 7 days of life can be prevented with intrapartum antibiotics in women with rectal carriage of GBS, although this does not prevent late disease in infants or disease in pregnant women themselves. Early studies of polysaccharide vaccines

showed variable immunogenicity.[70] A monovalent polysaccharide-conjugate vaccine has been studied in pregnant women, with results showing safety and immunogenicity, efficient transplacental antibody transfer to the fetus, and persistence of antibody until 2 months of age.[71] Currently, a trivalent polysaccharide conjugate vaccine composed of capsular serotypes Ia, Ib, III is in Phase II clinical trials sponsored by Novartis in Europe and Africa.[72] The combination of these three serotypes causes the majority of early onset GBS disease, and cost-effectiveness studies show that if such a vaccine were immunogenic and efficacious, maternal vaccination with such a vaccine would substantially reduce the burden of infant GBS disease and be very cost-effective based on WHO guidelines.[73] In this analysis, intrapartum antibiotic use was estimated to only prevent 10% of GBS disease.

## 4.2 Respiratory Syncytial Virus

RSV is responsible for respiratory distress, bronchiolitis, and pneumonia in children, causing high rates of hospitalization worldwide for infants under 6 months of age.[74] No vaccine is yet available to prevent RSV, and treatment is mainly supportive. A monoclonal IgG antibody directed against the F protein of RSV, palivizumab, protects against RSV disease in high-risk infants such as premature infants or those with underlying congenital heart disease,[43] but this antibody is expensive and requires administration by monthly injection. Higher levels of maternal IgG are associated with disease of less severity in infants, and prophylaxis with palivizumab is effective in reduction of hospitalizations due to RSV.[75] A formalin-inactivated vaccine was tested in infants in the 1960s, and caused augmentation of disease after subsequent wild-type infection in vaccinees, putting a halt to vaccine studies for many years.[76] Vaccine development and clinical studies in infants under the age of 6 months have been difficult due to poor immunogenicity of vaccine candidates in this age group, with live attenuated RSV vaccines the most likely candidate vaccine for young infants.[77] However, live RSV vaccines are unlikely to be considered as a maternal vaccine candidate.

Maternal vaccination appears to be an ideal strategy to prevent disease in neonates and young infants. The extensive experience with the use of palivizumab indicates the potential safety and benefit of an RSV fusion protein vaccine during pregnancy. A purified fusion protein (PFP-2) vaccine for RSV has been shown to be safe in pregnancy, though did not significantly increase neutralizing antibody titers to RSV. Currently, multiple new RSV vaccines are under development. A small placebo-controlled study in pregnant women of the F-protein vaccine manufactured by Novavax (Gaithersburg, Maryland, USA) has been completed, with a larger clinical trial in pregnant women currently underway. New studies have clarified the conformational structure of the RSV fusion protein and identified novel antigenic sites that have the potential for vaccine development.[76]

### 4.3 Herpes Simplex Virus

The highest risk of neonatal herpes occurs in women who have a primary infection during pregnancy.[78] Most women with genital herpes are asymptomatic or have subclinical infection. Most infants are infected with herpes simplex virus (HSV) through exposure in the genital tract during vaginal delivery, and neonatal infection is associated with a 60% mortality rate if untreated, and frequently with significant sequelae even if treated. An appropriate vaccine candidate would prevent acquisition of HSV-1 and HSV-2 in pregnant women. HSV vaccine candidates in the past have not been successful in preventing primary infection from both HSV-1 and HSV-2, although several candidate vaccines are under development.[79,80]

### 4.4 Cytomegalovirus

Congenital cytomegalovirus (CMV) infection is the most common infectious cause of developmental delay in children and is responsible for substantial rates of hearing loss and neurodevelopmental sequelae in children.[61] Seronegative pregnant women are at highest risk to become infected with CMV during pregnancy. Women acquire CMV via close contact with secretions, and may be infected by young children who acquire CMV in social settings, and those with primary infection during pregnancy are at highest risk to transmit CMV to the fetus. A vaccine administered to young children or adolescent females prior to pregnancy would prevent primary infection in pregnant women. Several candidate CMV vaccines are under development,[81] with some vaccines in clinical trials in nonpregnant persons. A subunit vaccine, gB/MF59, targets a glycoprotein complex involved in fusion of the virus with the host cell membrane. In a Phase II randomized clinical trial conducted in seronegative postpartum women, this vaccine had an efficacy of 50% in preventing acquisition of primary CMV infection: congenital CMV infection was detected in 1% of infants born to mothers in the vaccine group, and 4% in the placebo group.[82] Currently, additional Phase II trials are ongoing in healthy, adolescent female volunteers.

## 5 SAFETY OF MATERNAL IMMUNIZATION

The safety of maternal immunization remains an important issue in the assessment of vaccination strategies. Safety concerns include immediate local and systematic reactions in the pregnant woman as well as immediate, intermediate, and long-term effects in the fetus and infant. There is emerging scientific information and theoretical considerations indicating that many vaccines are safe for pregnant women and fetuses, but discussions of safety is challenging because of perceived potential risks and temporal association of adverse outcomes that may or may not be related to vaccination itself. In many clinical studies, maternal immunization is administered after at least 28 weeks gestation

so as to not be even temporally associated with fetal development or spontaneous abortions. In research studies, the inclusion of ultrasound prior to immunization to provide accurate dates and to document an intact, viable fetus has been utilized to provide protection to both the subject and the study. The safety of a wide range of commercially available vaccines has been reviewed by a committee of the WHO.[12] This review evaluated inactivated vaccines including tetanus toxoid vaccine, conjugate meningococcal vaccines, inactivated nonadjuvanted and adjuvanted influenza vaccines, and inactivated polio vaccines, as well as live vaccines including rubella, oral polio, and yellow fever vaccines. This review assessed the gaps preventing accurate assessment, and concluded that no evidence of adverse pregnancy outcomes has been identified in pregnant women or their infants as a result of maternal immunization. Nonetheless, ongoing clinical trials and routine administration in clinical practice require due diligence by investigators, clinicians, and public health officials to ensure that maternal vaccines are safe and effective, and to assure the continued safety of this practice.

## 6 POTENTIAL OBSTACLES FOR MATERNAL IMMUNIZATION

Potential obstacles for successful maternal immunization platforms remain. The most important obstacle is a lack of effective vaccines against common yet important pathogens. The immune response to many vaccines, such as influenza and pertussis, appears short-lived necessitating intrapartum vaccination and repeated immunization. Regulatory and legal issues remain a hurdle, with the perception that licensure of a vaccine for pregnant women has even more hurdles than routine vaccines.[83] Finally, issues affecting interactions with pharmaceutical companies and liability issues in individual countries pose another problem.

## 7 CLINICAL TRIAL DESIGNS

Pregnant women and their fetuses deserve appropriate medical care and preventative care, but obtaining clinical data to justify maternal immunization is not simple. Pregnant women are considered a vulnerable population in the United States and other countries where clinical research is performed and therefore, there is intense scrutiny and attention to the design, implementation, conduct, and follow-up of clinical trials involving pregnant women. Prior to the initiation of clinical trials, vaccines that are safe, immunogenic, and relatively nonreactogenic must be developed. Importantly, fever is an important marker of disease in pregnant women and fever associated with a maternal vaccine would not be considered acceptable in most situations. Vaccines need to be developed and evaluated in nonpregnant women of child-bearing age in populations similar to that where maternal immunization will be carried out; immunogenicity needs to be assessed carefully to assure that antibody levels will rise promptly and be available for transplacental transfer to the infant.

Clinical trials need to be carried out by experienced investigators who are familiar with vaccination studies as well as familiar with the area where the studies are conducted. In general, consideration should be given for a placebo arm of the trial but this will depend on the location of the study. Certainly, standard of care must be implemented for all study participants including use of tetanus or influenza vaccine if that is standard practice in that area. Input from local public health and governmental as well as neighborhood, religious, political, or tribal officials should be sought prior to conducting clinical trials in this visible and vulnerable population because of the potential for noncausal but potentially serious adverse events during pregnancy—even in placebo subjects.

The issue of consent must be considered early in maternal immunization trials. In many settings, consent from both the mother and father should be obtained but this may depend on the exact regulations and practices of the study locale. In the USA, strategies or methods for obtaining exemptions from obtaining paternal consent are generally considered before the trial is instituted because partners may no longer be available for some women. In some settings the family patriarch or tribal head should be involved. Consent must be obtained in a careful conversation using words familiar to the participant. Similarly, strategies to assess potential adverse outcomes need to be considered prior to study implementation. The potential for early miscarriages or spontaneous abortions exists in all women and is highest during the first trimester. Utilizing dating systems to postpone study enrollment until the second or early third trimester or later may assist in decreasing potential adverse events that are unrelated to the study vaccine. Methods such as ultrasound may be used to document fetal size, limbs, and head size as another strategy as ultrasound becomes more widely available, but this must be considered within the rules of the study site, as ultrasound in some areas is not permitted because of concerns regarding sex selection.

Considerations for clinical trials in pregnant women should assess the risk and consequences of the disease to both the mother and the infant.[84] The background rates of spontaneous abortions, major congenital anomalies, and stillbirths should be known or at least have been studied in the area where trials are conducted.[85] It must be acknowledged that temporal relationships, rather than causation, will be difficult to prove or disprove. For vaccines already licensed and approved for use in adults, specific approval by governmental regulatory agencies for use during pregnancy to prevent disease in the mother and/or infant may have a significant impact on the uptake and usage of vaccines in pregnant women.[83] In addition, FDA or other agency approval for use during pregnancy would result in labeling that would serve as a resource for practitioners and would facilitate the safe and effective use of the vaccine during pregnancy. However, to date, no vaccines have been licensed for use in pregnant women. In addition, there is some consideration given that a vaccine should be studied in pregnant women only if the disease is extremely serious in the mother; the

concept of immunizing a mother to protect against mild maternal disease is becoming more acceptable, at least by US regulators.[83]

## 8 SOCIAL AND ETHICAL ISSUES

Immunization remains a validated and safe approach for the prevention of infectious in the current age. However, widespread social concerns often amplified by social media and personalities have put the scientific basis of immunization into a different light. Many concerns of immunization even in nonpregnant persons are not scientifically justified but frequently are in the media. In addition, utilizing vaccination during pregnancy—even during the last few months of pregnancy—has the potential to be associated with adverse events that may not be related to vaccines at all. There is an expectation today in most countries and from every parent that their child will be healthy and a concern that someone or something is responsible if this is not the case. The background of a litigious society makes supporting clinical studies and/or national policies difficult for manufacturers, but those involved in the care of pregnant women and young infants see the potentially preventable diseases in their work place daily. Consideration for indemnification by governmental authorities needs to be considered before companies will be able to participate in production and testing of vaccines specifically aimed at pregnant women.

## 9 SUMMARY

The concept of maternal immunization to prevent infectious diseases in the mother and infant during a period of increased vulnerability is supported by historical experience and small but carefully conducted studies of various viral and bacterial vaccines. Candidate diseases that are targeted should be documented in the location where clinical trials are carried out to document the increased risk to the mother and/or infant, and clinical studies should document clinical effectiveness and transplacental antibody transmission in the populations under study. Candidate vaccines for use in pregnant women should be minimally reactogenic, as well as immunogenic, and safe, and must undergo careful, prospective, longitudinal clinical studies to assure the safety and long-term effectiveness of this approach. Access to antenatal care and health education must be achieved if maternal immunization is to succeed as a disease prevention strategy. Maternal immunization studies and recommendations must be carefully considered and conducted using adequate public information and transparency because events related to pregnancy and childbirth can become well known. Events that are related only temporally but not causally to vaccination can potentially become an issue, and liability issues with the manufacturer must be discussed with public health and governmental agencies before large scale use of these vaccines. The potential to prevent serious disease in mothers and infants with this approach remains promising.

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Chapter 11

# New Challenges for Pertussis Vaccines

**Scott A. Halperin, MD**
*Dalhousie University, Department of Pediatrics; Department of Microbiology and Immunology; Canadian Center for Vaccinology, IWK Health Centre, and Nova Scotia Health Authority, Halifax, Nova Scotia, Canada*

## Chapter Outline



## 1 HISTORICAL PERSPECTIVE

The effort to control pertussis through vaccination has been ongoing for more than 100 years, since the first description of the causative bacterium, *Bordetella pertussis*.[1] The whole-cell pertussis vaccine, produced by heat killing and chemical detoxification came into use in the 1930s and became a routinely used vaccination in the 1940s.[2] With its widespread use, reported cases of pertussis declined dramatically to less than 10% of prevaccination levels.[3] Efficacy of the whole-cell pertussis vaccine was demonstrated in a series of clinical trials sponsored by the Medical Research Council in the United Kingdom;[4] efficacy of the vaccine correlated with protection in the mouse intracerebral test,[5] which is now used to measure the potency of whole-cell vaccines. In the intracerebral protection assay, immunized mice are injected intracerebrally and monitored for death.[6] Current regulatory standards for whole-cell vaccines require that each lot of vaccine demonstrate no less than[7] mouse protective units in the intracerebral challenge test in order to be released.[8] While increased potency of whole-cell

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vaccines in the intracerebral assay correlate with increased efficacy, there is a similar correlation with increased rates of adverse events.

Beginning in the 1970s, there was increasing public concern about the safety of whole-cell vaccines. High profile allegations of damage related to whole-cell vaccine led to decreased rates of pertussis vaccination in some countries and removal of the vaccine from the routine immunization schedule others. In Japan, following two infant deaths following immunization in 1975, pertussis vaccination was halted and the vaccine was reintroduced at 2 years of age rather than 3 months. Vaccine coverage dropped from 85% in 1972 to 13.6% in 1976. This resulted in a resurgence of disease, with more than 13,105 cases and 41 deaths by 1979.[9] In the United Kingdom, rates of pertussis vaccination dropped dramatically from 77% in 1974 to 30% by 1978 after allegations that the whole-cell vaccine was the cause of encephalopathy and permanent brain damage. Despite a large study that was unable to demonstrate a link between permanent brain damage and pertussis vaccine (National Collaborative Encephalopathy Study),[10,11] it was years until pertussis vaccine coverage rates increased to their previous levels and, in the interim, high rates of pertussis and infant deaths occurred.[9] In the United States, pertussis immunization rates also declined following television "documentaries" like "A Shot in the Dark" and advocacy by antivaccination groups such as "Dissatisfied Parents Together (DPT)."[12]

Spurred by the allegations and concerns with whole-cell pertussis vaccine, there was a concerted effort in the 1980s and 1990s to develop new and improved pertussis vaccines. Led by Japanese investigators, new pertussis vaccines were developed comprising purified components of *B. pertussis*. These so-called acellular pertussis vaccines included between one and five pertussis components. All of the acellular pertussis vaccines contained pertussis toxoid, the inactivated toxin that is a major virulence factor of the organism and causes many of its biological effects.[7] Clinical trials with the first acellular vaccines were undertaken in Sweden in the 1980s but resulted in a lower than desired efficacy.[13] Second-generation acellular vaccines were studied in a series of Phase 2 studies and leading candidates were selected for Phase 3, randomized, controlled trials which were undertaken in Sweden, Germany, Italy, and Senegal. All of these studies demonstrated that the acellular vaccines were less reactogenic than the whole-cell vaccine comparators and elicited antibody responses to their component antigens equivalent to or greater than that of the whole-cell vaccines.[14] Efficacy of the acellular vaccines was in the range of 85%; efficacy of the whole-cell vaccines was far lower, equivalent, or higher.

## 2 CHANGING EPIDEMIOLOGY

With the licensure of acellular pertussis vaccines, a rapid shift from whole-cell to acellular vaccine use occurred in high-income countries including the United States, Canada, Europe, the United Kingdom, Australia, and New Zealand. The incidence of pertussis continued to fall, likely related to improved uptake

(eg, in the United States) or replacement of a poorly performing whole-cell vaccine (eg, in Canada).[15] With improved control of pertussis in preschool- and school-aged children, increased outbreaks of pertussis were reported among adolescents. In order to control pertussis in adolescents, adult formulations of acellular pertussis vaccine were developed, which had decreased content of diphtheria toxoid and decreased quantities of pertussis antigens (Tdap vaccines). Implementation of universal adolescent vaccination programs with Tdap led to control of pertussis in these age groups.[16] Recommendations for Tdap immunization of adults were made in several countries to control pertussis throughout the life span and to decrease transmission of pertussis from adults to children.[17,18] Most recently, Tdap has been recommended during pregnancy to induce high levels of maternal antibodies which are transferred transplacentally to the fetus and provide protection during the first months of life, prior to initiation of the infant immunization series.[19] These recommendations were implemented in the United States and United Kingdom in response to large outbreaks that caused increased infant deaths;[20,21] the effectiveness of this intervention was demonstrated recently in observational[22] and case control studies.[23]

In the last 5 years, there have been large outbreaks of pertussis in some but not all areas that have exclusively used acellular pertussis vaccine. Beginning in 2010, California reported more than 9000 cases, including 10 deaths.[21] Within several years, most states in the United States reported an increased incidence of pertussis; in 2012, outbreaks in Oregon, Washington, Wisconsin, and Minnesota reported rates not seen since the early days of pertussis vaccination in the 1940s.[24–27] Of concern, a cohort effect was observed whereby those children who had received all of their doses with acellular vaccines had a higher incidence than slightly older children who had received one or more doses of whole-cell vaccine prior to the switch from whole-cell to acellular vaccines.[28] Also of concern was that protection after the preschool acellular pertussis vaccine dose began to decrease within 4–5 years, much shorter than had previously been predicted.[29,30] Duration of protection after the Tdap vaccine in adolescents was even shorter, decreasing after only 2 years.[31] Despite these reports, increased incidence of pertussis was not seen in all countries using acellular pertussis vaccines.[32]

## 3 CURRENT USE OF PERTUSSIS VACCINES

Currently, whole-cell pertussis vaccines are primarily used in low- and middle-income countries (LMIC) that follow the Expanded Program on Immunization (EPI) recommendations. The World Health Organization (WHO) recommends that all infants receive pertussis vaccine at 6, 10, and 14 weeks of age and countries consider a booster dose in the second year of life when coverage for the three primary doses is high and a booster dose is feasible.[33] In many middle-income countries, acellular vaccines are used in the private market, even when not available through the national vaccination programs. In countries with

emerging economies, there has been a tendency to move national programs from whole-cell to acellular pertussis vaccine; with the recent outbreaks of pertussis in countries using acellular pertussis vaccine, this trend has been discouraged by WHO.[33]

While reemergence of pertussis has been observed in countries exclusively using acellular pertussis vaccines, it is not clear that this is entirely related to the vaccine. Some countries with long-standing acellular pertussis vaccination programs have not yet observed dramatic increases but rather are reporting typical 3- to 5-year pertussis cycles. As well, increased rates of pertussis are also being reported in countries such as Brazil and Columbia that use whole-cell pertussis vaccines.[33] It is not clear, however, whether the increases in those countries are related to the vaccine or due to decreased vaccine coverage in certain regions of the country.

## 4 RELATIVE MERITS OF ACELLULAR AND WHOLE-CELL PERTUSSIS VACCINES

Whole-cell and acellular pertussis vaccines each have advantages and disadvantages. There are differences in their reactogenicity, immunogenicity, efficacy, duration of protection, nature of the immune response, acceptability, manufacturing reproducibility, and cost. Use of pertussis vaccines may also affect the nature of the bacterium itself. Differences in pertussis vaccine performance may be the result of a number of factors.

While the impetus for development of acellular pertussis vaccines was the unsubstantiated and ultimately refuted causative link to encephalopathy and permanent brain damage, acellular vaccines were consistently less reactogenic and better tolerated than whole-cell pertussis vaccines in randomized, double-blind, controlled clinical trials. Common adverse events such as injection-site redness, pain, and swelling were substantially less frequent as were postvaccination fever, crying and irritability.[34] Less common adverse events such as prolonged crying, hypotonic-hyporesponsive episodes, and febrile seizures were also less common after acellular pertussis vaccines.[35] Antibody levels after acellular pertussis vaccines tended to be higher and more consistent than those after whole-cell pertussis vaccines, leading to minimal lot-to-lot variability.[36] In contrast, the antibody response to whole-cell pertussis vaccines varied widely between different vaccines and antibody determination was not part of the regulatory evaluation (instead, the mouse intracerebral test was used).[37] Some whole-cell vaccines were found to induce high levels of anti-pertussis toxin (PT) antibodies while others evoked virtually no anti-PT antibodies and elicited primarily antifimbriae antibodies.[37]

While efficacy of the multicomponent acellular vaccines was demonstrated to be relatively consistent (around 85% in randomized controlled trials), efficacy of whole-cell pertussis vaccines has not been as rigorously determined. In clinical trials of the acellular vaccines, the whole-cell pertussis vaccine efficacy

ranged from 36% to more than 90%, depending on the study and whole-cell pertussis vaccine used.[38] In contrast to acellular pertussis vaccines, the efficacy of most whole-cell pertussis vaccines in current use worldwide has never been tested in clinical trials.

Recent reports of decreased duration of protection after acellular pertussis vaccines are of particular concern in regard to the increasing incidence of pertussis in countries with longstanding acellular pertussis vaccination programs. Data from the 1960s to 1980s suggested that protection after whole-cell pertussis vaccine began to drop off dramatically after several years and was no longer detectable 10 years after immunization.[39,40] This is not dissimilar to the current observations in children who have received all of their doses as acellular vaccine.[28,30,31,41–43] Longer duration of protection was associated with having received at least one dose of whole-cell pertussis vaccine prior to receiving any doses of acellular pertussis vaccine.[30]

The observation that a single dose of whole-cell pertussis vaccine improves the duration of protection may be related to differences in the type of immune response elicited by acellular and whole-cell pertussis vaccines.[43] All currently licensed acellular pertussis vaccines contain an alum adjuvant, a strong inducer of Th2 immune responses. Th2 responses are characterized by high antibody levels, relatively poor induction of memory B cells, and little cell-mediated responses. In contrast, whole-cell pertussis vaccines, despite also being alum adjuvant, induce a Th1 predominant response characterized by increased memory induction and a mixed antibody and cell-mediated response. The Th1-biased response of whole-cell pertussis vaccines may be related to the presence of residual lipooligosaccharide of *B. pertussis*, one of the many pertussis antigens present in the whole-cell vaccine.[44] Lipooligosaccharide, similar to lipopolysaccharides (endotoxin) is a potent inducer of a Th1 response and likely is able to shift the immune response away from the alum-induced Th2 bias.[45]

The recent development of a nonhuman primate model of pertussis has significantly advanced our understanding of pertussis transmission and the nature of the immune response to natural infection and postimmunization.[46] Baboons infected with *B. pertussis* develop an illness with clinical and physiological similarities to human disease including severe paroxysmal cough, lymphocytosis, and a correlation between young age and disease severity.[47] The model has been used to demonstrate airborne transmission between animals and the nature of the protective immunity after infection, particularly the importance of the Th1 and Th17 response postinfection.[47–49] A finding in the baboon model with potentially important implications for understanding human transmission and epidemiology is that animals immunized with acellular pertussis vaccines were protected from severe disease but not colonization or the ability to transmit infection. In contrast, whole-cell-pertussis-vaccinated animals and naturally infected animals were protected against both subsequent colonization and diseases and were unable to transmit infection.[50] If these findings reflect the effect of vaccination in humans, it may in part explain the resurgence of pertussis in

highly vaccinated populations in which acellular pertussis vaccines are used routinely. Development of a human pertussis challenge model may enable the validation of the findings in the baboon model in humans and assist in further understanding the nature of pertussis infection and immunity.[51]

Currently, there is a substantial difference in cost between whole-cell and acellular pertussis vaccines. While whole-cell pertussis vaccines can be supplied to LMIC at pennies a dose, acellular pertussis vaccines cost in the range of $16–36/dose,[52] making them unaffordable in LMIC, even if they are superior products. There continues to be efforts to produce acellular vaccines that can be used in LMIC, if not for universal infant immunization then perhaps for maternal immunization since whole-cell pertussis vaccines may not be acceptable because of their increased rates of fever and adverse pregnancy outcomes associated with maternal fever during pregnancy.[53]

There has been much speculation about the effect of pertussis vaccination on *B. pertussis* and whether immune pressure has led to substantial changes in the expression of different *B. pertussis* antigens. Both whole-cell and acellular pertussis vaccines have been implicated in causing these changes in circulating strains. Studies early in the 20th century identified agglutinogens on the surface of *B. pertussis*, and antibodies against these surface proteins correlated with protection.[54,55] Agglutinogen types 1, 2, and 3 were identified as the major agglutinogen types, and agglutinin antibodies were thought to correlate with efficacy of whole-cell pertussis vaccines.[56] Increased incidence of pertussis in some areas was thought to be the result of circulation of *B. pertussis* expressing agglutinogens not present in the vaccine,[57] and outbreaks have been attributed to a mismatch of vaccine and the agglutinogen type of the circulating bacteria.[58] Most whole-cell vaccines now contain strains expressing the three major agglutinogen types to avoid this immune pressure away from the vaccine strain.

With advancement of technology and identification of specific *B. pertussis* antigens, it has been demonstrated that agglutinogens 2 and 3 primarily comprise fimbriae with some contribution to agglutinating antibodies provided by pertactin and lipooligosaccharide.[12] Other antigens, such as pertussis toxin, filamentous hemagglutinin, adenylate cyclase toxin, and tracheal cytotoxin have been found to be *B. pertussis* virulence factors and some have been demonstrated to be protective antigens and included in acellular pertussis vaccines.[12] Mutations in the pertactin and pertussis toxin genes have been observed, and it has been hypothesized that these changes in the organism have contributed to the resurgence of pertussis in some countries such as the Netherlands.[59] However, those strains have been shown to be circulating with similar frequencies in other countries that have not observed an increase in pertussis incidence.[60] More recently, pertactin-negative strains have emerged and are now the predominant circulating strains in some countries.[61–64] In the pivotal Phase 3 clinical trials that led to licensure of the acellular pertussis vaccines, pertactin was the antigen that correlated best with protection.[65,66] Concerns have been raised that this mutation may have been caused by immune pressure from pertactin-containing

vaccines which now dominate the market in countries that use acellular pertussis vaccine and may be the cause of the large outbreaks being observed. While the predominance of pertactin-negative strains may be contributing to the increased incidence of pertussis, it cannot be the only (or even primary) reason. In the United States the large 2010 outbreak that occurred in California was caused almost entirely by pertactin-expressing strains. In 2012 the organisms associated with the outbreaks in Washington and elsewhere in the United States were primarily pertactin-negative.[63,64] Although pertactin-deficient *B. pertussis* may have a selective advantage, there is no evidence yet that the epidemiology or the clinical disease manifestations are different with these strains.[67] While changes in the antigenic composition of *B. pertussis* need to be monitored, the extent of their contribution to the changing epidemiology of pertussis and the relative effects of whole-cell and acellular vaccine remain uncertain.[68]

Given some perceived benefits of the whole-cell pertussis vaccine, some have advocated a return to whole-cell pertussis vaccine in high-income countries or at least reintroducing whole-cell pertussis vaccine for the first dose.[69] The rationale for the latter strategy is that the initial dose primes the immune system for the subsequent nature of the immune response. After a first dose of whole-cell pertussis vaccine, subsequent doses with acellular vaccine evoke a Th1 response rather than the Th2 response that occurs if the first dose is with acellular vaccine. Interestingly, if the first dose of vaccine is with acellular vaccine, the Th2 response induced cannot be changed by subsequent doses with whole-cell pertussis vaccine.[70] Despite this immunological rationale for reintroducing whole-cell pertussis vaccine, it is unlikely that the public in many countries would tolerate a return to the whole-cell vaccine and its increased reactogenicity.

## 5 REMAINING GAPS IN CONTROL OF PERTUSSIS

Multiple gaps remain in the control of pertussis. Outbreaks affecting school-aged children and adolescents continue to occur. *B. pertussis* continues to circulate among adults, causing either atypical or typical disease; infected adults continue to be the reservoir of infection for young infants. Deaths still occur in these young infants who have not yet completed their primary series. The extent of the problem and the true burden of disease are obscured by inadequate surveillance and identification of pertussis illnesses, particularly in LMIC. The cause of this failure to control pertussis is multifactorial and includes inadequacies of the vaccine (type of immune response, duration of protection, and composition) and the nature of the organism (mutations resulting in altered antigen expression). Given our current understanding of the epidemiology of pertussis and the nature of the currently available vaccines, it is unlikely that these gaps in pertussis control can be adequately addressed by the currently available vaccines; in fact, some of the gaps may be caused by the current vaccines. New vaccines that address the deficiencies in the current vaccines

(reproducibility, reactogenicity, and duration of protection of the whole-cell vaccines and nature of the immune response, duration of protection, and level of efficacy of the acellular vaccines) are required.

## 6 PROSPECTS FOR NEW PERTUSSIS VACCINES

There is much renewed interest in developing new pertussis vaccines that will address the identified gaps. However, most of this work is based in academia and it remains unclear whether the major vaccine manufacturers will become engaged in the effort. A number of strategies are being pursued that address the performance gaps of the current vaccines.

A live, attenuated *B. pertussis* vaccine has been developed and demonstrated to be immunogenic and protective in the mouse model of pertussis.[71] BPZE1 contains genetic alterations that eliminate or inactivate three *B. pertussis* virulence factors: the dermonecrotic toxin gene was deleted, the PT gene was genetically modified abolishing the enzymatic ADP-ribosyltransferase activity of PT, and the *B. pertussis* ampG gene was replaced by *Escherichia coli* ampG eliminating production of the tracheal cytotoxin.[72] BPZE1 is nonpathogenic in mouse models but is able to colonize the mouse respiratory tract like the virulent parent strain. A single nasal dose of BPZE1 protects mice against challenge with virulent *B. pertussis*.[73] The potential advantage of such a vaccine is the close simulation of natural infection with a broad, balanced Th1/Th2/Th17 response against multiple antigens expressed by the bacteria.[74,75] An additional advantage may be the use of the vaccine in newborns prior to beginning the primary series with acellular vaccines as part of a prime-boost strategy, tilting the immune response toward a Th1 bias.[76] This vaccine has now moved into clinical trials; in a Phase 1 clinical trial, the higher doses of the vaccine strain consistently colonized the nasopharynx. The vaccine was well tolerated; however, the immune response was lower than desired. Modifications of the dose schedule are planned for subsequent studies.[77]

Other strategies for novel pertussis vaccines include the use of adjuvants other than alum in order to direct the immune response toward a Th1 bias. As part of one of the Bill and Melinda Gates Foundation Grand Challenges to develop a single-dose, neonatal vaccine against pertussis, a triple adjuvant combination with PT comprising CpG, a host defense peptide, and polyphosphazene induced an immune response of greater magnitude than multiple doses of a licensed acellular pertussis vaccine and provided superior protection in mouse and pig models of pertussis. The triple adjuvant PT vaccine was able to induce protection in neonatal animals and overcome the suppression of the active immune response from high levels of maternal antibodies.[78,79] In addition to novel adjuvants, there is interest in the use of additional antigens to improve the protection provided by acellular pertussis vaccines. Adenylate cyclase toxin, outer membrane vesicles, detoxified lipooligosaccharide, and tracheal cytotoxin have all been proposed as additional vaccine components.[80–83]

Proponents of the whole-cell vaccine have also proposed the development of new and improved whole-cell pertussis vaccines. There is interest in vaccines being produced from *B. pertussis* strains modified to express a less toxic lipopolysaccharide.[84]

Bringing a new pertussis vaccine to market is a daunting task, which may explain the reticence of major vaccine manufacturers. While agglutinogens roughly correlated with protection from whole-cell vaccines,[54–57] and "high" compared to "low" antibodies against PT, pertactin, and fimbriae correlated with protection after acellular pertussis vaccines,[65,66] there is no single defined antibody level or other correlate of protection for evaluation of pertussis vaccines.[85] All currently available pertussis vaccines were licensed for use based on randomized, controlled, efficacy studies, and subsequent modification of those vaccines (eg, expanded combination vaccines made by the addition of other vaccine antigens) were approved by bridging studies comparing antibody response in the new formulations with antibodies in sera from those original clinical trials. New vaccines that include different antigens will not be able to be assessed by this type of bridging; even those that contain the same antigens but have novel adjuvants will no longer be able to be bridged in this way because of depleted stocks of those stored sera from the original efficacy studies. Vaccines that protect by mechanisms other than antibody (eg, cell-mediated immunity) will be impossible to bridge to the original efficacy studies. Performance of new efficacy studies will be difficult because there are no longer countries that do not recommend pertussis vaccination so the use of a placebo is no longer ethical, vastly increasing the sample size required to demonstrate efficacy. Despite the increased burden of pertussis, outbreaks are still sporadic and unpredictable, so identifying where and when an efficacy study could be undertaken is virtually impossible.

Given these challenges, the regulatory pathway to licensure of a new pertussis vaccine is not clear. In the absence of efficacy studies, alternate pathways will be required. If an immunological correlate of protection is known, vaccine efficacy can be demonstrated using a serological or other immunological assay. This has been done with vaccines against *Haemophilus influenzae* type b, hepatitis B, and others. With *B. pertussis*, however, a correlate of protection is not known. While protection after acellular pertussis vaccination has been correlated with higher antibody titers against pertactin, pertussis toxin, and fimbriae,[65,66] an antibody level to a given antigen above which a person is protected has not been established. Animal models of infection are useful for assessing the efficacy of vaccines but are most useful when the model simulates the clinical disease in humans. Mouse models of pertussis have been very useful in understanding the immune response to pertussis antigens; however, the model does not correlate well with the clinical manifestations of whooping cough. The recently established baboon model of pertussis does have features that are similar to those in humans and may provide a potential method for demonstrating vaccine efficacy.[46–50] However, it is not clear whether the so-called "animal

rule" will be accepted for licensure of new pertussis vaccines.[86] More likely, the baboon model will be an excellent tool to dissect further the nature of the immune response to pertussis and establish a predictive correlate of protection.

An alternative to the baboon model or, more likely, an adjunct to the baboon model is the development of a human challenge model of pertussis. Human challenge studies have been established for multiple infectious diseases including salmonella, norovirus, malaria, and respiratory infections such as rhinovirus and influenza.[87] Pertussis meets the criteria that have been proposed for selecting pathogens that are medically and ethically acceptable for human challenge such as well characterized clinical course, availability of a treatment regimen that is highly effective in eliminating the infection, and manifestations that are well tolerated prior to rescue treatment.[88] A pertussis human challenge model could be very useful in establishing correlates of protection, particularly in validating data generated in the baboon model where more severe and well-established infections can be studied.[51] Establishment of such a model is underway, and its potential contribution to understanding immunity to pertussis and assessing vaccine efficacy is highly anticipated.

## 7 INTERIM MEASURES FOR THE CONTROL OF PERTUSSIS

In view of the lack of correlates of protection and the resultant regulatory hurdles and the absence of any pertussis vaccines in late-stage clinical trials, new pertussis vaccines with higher efficacy and longer duration of protection will not be available anytime in the near future. Increased size of the cyclical 3- to 5-year peaks in pertussis incidence and continued regional outbreaks are likely to occur. Therefore, interim measures are needed to better control pertussis around the world. Optimal use of the currently available vaccines will likely be the most achievable goal while awaiting novel products.

In low- and middle-income countries, WHO's Strategic Advisory Group of Experts (SAGE) on immunization has recommended continued use of whole-cell pertussis vaccines, halting the trend toward changing to acellular vaccines as economies emerge.[33] In view of the data demonstrating increased duration of protection after whole-cell pertussis vaccines, this seems prudent. However, while increased pertussis activity is being reported more from countries using acellular pertussis vaccine than those that use whole-cell pertussis vaccine, surveillance, and reporting also tend to be better in countries using acellular pertussis vaccine. Improved surveillance in LMIC is critically important to better understand and compare the epidemiology of pertussis in the presence of acellular and whole-cell pertussis vaccines. Regardless of which vaccine is being employed, improving vaccine coverage rates to maximize the benefit from these suboptimal vaccines is a useful and worthwhile interim measure.

Focusing on the most vulnerable populations should also be a priority until better vaccines are available. Protecting infants in the first 6 months of life is the highest priority because this is where all of the mortality and most of the

morbidity occurs. Data are emerging that even a single dose of pertussis vaccine in infancy has a substantial protective effect against death, even if it does not completely protect against disease.[89] While neonatal immunization with pertussis vaccine may provide benefit, clinical trials have suggested that an acellular vaccine combined with diphtheria and tetanus toxoids may result in lower antibody titers after the routine infant series[90] and that increased levels are only achieved when a pertussis-only vaccine is used.[91,92] Because there are no licensed pertussis-only vaccines, neonatal immunization is not an immediate solution to prevent neonatal pertussis. Ensuring timely administration of the first dose in the routine infant series thus is an important goal in all jurisdictions. Protecting infants prior to their first pertussis vaccine dose is possible through immunization of women during pregnancy. Tdap during pregnancy has been demonstrated to be safe,[93–96] provide high levels of transplacental antibodies,[97–100] and be effective in preventing neonatal pertussis.[22,23] Maternal immunization was implemented as an outbreak control response in the United Kingdom and is recommended during all pregnancies in the United States.[19,22,23] WHO's SAGE considered recommending maternal immunization in LMIC; however, no recommendation was made because of the lack of an inexpensive acellular pertussis vaccine for use in LMIC and the lack of data on the burden of neonatal pertussis because of inadequate surveillance.[33]

Presently, in countries using acellular vaccine, pertussis is reasonably well controlled in children 6 months to 7–8 years of age who receive 3 doses of pertussis vaccine in the first year of life, a reinforcing dose in the second year of life, and a preschool booster at 4–6 years of age. Outbreaks continue to occur in children between 8 and 16 years of age, particularly in children who received all of their doses with acellular pertussis vaccines. Duration of protection after the preschool DTaP booster has been calculated to be 3–6 years;[29,101] duration of protection after the early adolescent Tdap vaccine was estimated to be only 2–3 years.[31,102] While decennial Tdap boosters with a Tdap duration of protection of 10 years have been estimated to be cost-effective,[103,104] more frequent booster doses are unlikely to be feasible and cost-effective, particularly if needed every 2–3 years. Innovative approaches may be required; given the predictable 3- to 5-year pertussis cycles, regional immunization campaigns targeting school-aged children in advance of predicted peak years may be more appropriate than boosters targeted to a single age cohort. These mass immunization campaigns have been very effective for control of other epidemic infectious diseases.

## 8 SUMMARY AND CONCLUSIONS

Pertussis control with currently available vaccines remains better than in the preimmunization era, with decreases in both morbidity and mortality. Despite widespread use of pertussis vaccines, 3- to 5-year cyclical outbreaks continue to occur, accounting for a substantial burden of disease. Outbreaks continue to occur in schools, daycare centers, institutions, and in the wider community;

these outbreaks consume substantial public health resources. New vaccines are being developed but they will not be available in the short term or midterm. In the interim, pertussis control will be improved by optimal use of the currently available vaccines (on-time immunization, high coverage) and effective new uses of the currently available products (maternal immunization). Innovative changes in the routine schedule may improve control of pertussis, particularly in school-aged children and adolescents, and adults.

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Chapter 12

# Pneumococcus, Pneumococcal Disease, and Prevention

**Katherine L. O'Brien, MD, MPH[*], Meena Ramakrishnan, MD, MPH[**], Adam Finn, MD PhD[†], Richard Malley, MD[‡,§]**

*[*]International Vaccine Access Center, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States; [**]Consultant, Philadelphia, PA, United States; [†]University of Bristol, Bristol, United Kingdom; [‡]Kenneth Macintosh Chair in Pediatric Infectious Diseases, Division of Infectious Diseases, Boston Children's Hospital, Boston, MA, United States; [§]Harvard Medical School, Boston, MA, United States*

## Chapter Outline



## 1 INTRODUCTION

First isolated in 1880 by Pasteur in the saliva of a patient with rabies, *Streptococcus pneumoniae* (also known as the pneumococcus) has been branded as the "captain of the men of death" by William Osler, for the nefarious role this organism plays in causing the demise of so many people particularly among the elderly. While certainly evocative, this description does not fully capture the intricate interaction between the pneumococcus bacteria and its human host, one characterized by repeated and persistent nasopharyngeal colonization events that

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start at the earliest age and can be documented throughout life. In the context of this relatively friendly coexistence, the pathology caused by this bacterium, which ranges from relatively benign (though thoroughly unpleasant) mucosal infections like otitis media and sinusitis to serious and potentially fatal conditions such as pneumonia, bacteremia, and meningitis, are relatively rare events in the host–pathogen relationship. Along with the great apes, humans are the main natural host for the pneumococcus; when other mammals develop pneumococcal disease, they are usually animals in captivity and acquire the organism through their handlers. The bacterium's relatively limited host range creates the potential for effective control by vaccination and it is precisely through the ability of newer vaccines to prevent or reduce the likelihood of nasopharyngeal colonization that the greatest impact on prevention of pneumococcal disease has been achieved.

In this chapter, we review the organism, pathogenesis, and epidemiology of pneumococcal disease, as well as recent and potential future advances in immunization strategies.

## 2 THE ORGANISM AND ASSOCIATED PATHOLOGY

*S. pneumoniae* is a Gram-positive, lancet-shaped coccus, which is often but not always seen microscopically in pairs. The organism is characterized by its polysaccharide capsule, which defines the serotype although some strains are unencapsulated (Fig. 12.1). Identification of the capsule can be done serologically, using specific antisera (Quellung reaction) although more recently, molecular genetic approaches have been developed and are becoming widely used so that the standard term "serotype" should perhaps now be replaced with "capsular type." Based on a combination of strategies, more than 94 serotypes have been identified (and more may be identified in the future). There are two systems used to classify the different capsular serotypes: the Danish system classifies similar,

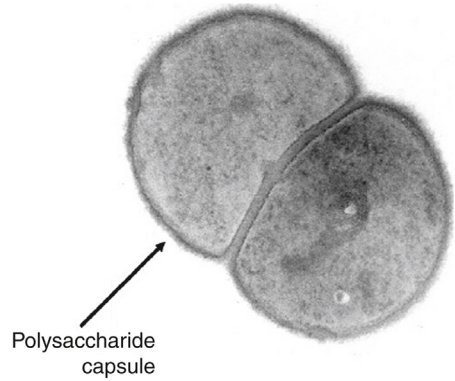


**FIGURE 12.1 Electron micrograph of *Streptococcus pneumoniae* with its surrounding polysaccharide capsule.**

potentially (but not necessarily) cross-reacting serotypes into serogroups (eg, serotypes 6A, B, and C within serogroup 6), whereas the US system numbers serotypes in the order in which they were discovered (the Swedish 6A is 6 in the US system, whereas 6B is assigned the number 26). Because the Danish system is the one most commonly used worldwide, this chapter will follow that nomenclature.

Pneumococcus causes a variety of diseases in humans, with a predilection for infants, young children, the elderly, and the immunocompromised. The most common pneumococcal disease is acute otitis media (AOM, middle ear infections), with pneumococcus isolated from 28 to 55% of middle ear aspirates. The economic importance of otitis media, and pneumococcal otitis media in particular, cannot be overstated, as this disease is the most common reason for pediatric office visits in the United States, with more than 20 million visits annually. Before their first birthday, 60% of children in the United States have had at least one episode of otitis media albeit only a fraction of these are caused by pneumococcus. Sinusitis is also a frequent medical presentation of pneumococcal disease, although precise estimates of incidence are difficult to obtain, due to the general inability to obtain microbiologic confirmation. For very much the same reasons, while it is generally accepted that pneumococcal pneumonia is the next most common pneumococcal disease, precise estimates of incidence in different age groups are lacking. The classic presentation of an adult patient with pneumococcal pneumonia includes high fever, a single episode of rigor, and cough (which may be productive of purulent, rusty sputum). Other symptoms include chest pain, rapid breathing, and weakness. The picture of lobar or segmental opacification on the chest radiograph is classically associated with pneumococcal pneumonia although it is widely accepted that other less well-defined abnormalities often also occur. Pneumococcal pneumonia may be associated with bacteremia (presence of bacteria in the blood) in 5–15% of cases. The overall case-fatality rate in the United States and Europe is between 5 and 7%, and even higher in the elderly. Morbid complications of pneumococcal pneumonia are not uncommon and include infection of the pleural space (empyema), the heart (endocarditis), the sac surrounding the heart (pericarditis), and very rarely lung abscess formation. There is an increased risk of cardiac events up to one year after hospitalization for pneumococcal pneumonia, which is not explained by preexisting comorbidities, suggesting that infection with this organism may elicit cardiac complications as well.

The most serious forms of the pneumococcal disease include septicemia without pneumonia and infection of the lining of the brain (meningitis). Both of these diseases are relatively rare compared to other forms of pneumococcal infections, but have a much higher case-fatality rate. For example, bacteremia in the absence of pneumonia in adults has an overall fatality rate of 20% (60% in the elderly); similarly, meningitis in adults also has about a 20% fatality rate. While the mortality rate of pneumococcal meningitis in children is lower (around 8%), this disease is often associated with significant morbidity, such as developmental delay, deafness, and seizure disorders.

It is important to note that, while pneumococcal disease afflicts individuals in every geographic location, the majority of the burden of disease occurs in the developing world, where respiratory infections are the leading cause of death in children under 5 years of age. It has been estimated that in 2000 pneumococcus caused between 1 and 4 million cases of pneumonia in the developing countries of Africa alone, and more than 800,000 deaths in developing countries worldwide.[1,2]

## 3 EPIDEMIOLOGY OF PNEUMOCOCCAL DISEASE

Pneumococcus is an organism whose clinical manifestations, age distribution of disease, serotype distribution, and mode of person-to-person community transmission have been studied and documented for more than 60 years across heterogeneous epidemiologic settings. This evidence base forms the foundation on which pneumococcal control and prevention strategies, vaccines in particular, have been developed.

### 3.1 Colonization

The central epidemiologic condition that drives pneumococcal disease, and which increasingly is the focus of disease control through vaccination, is the upper respiratory colonization state. However, the relationship between colonization and disease is complex; it varies according to host, environmental, and community attributes. All humans are episodically colonized with pneumococcus in the upper respiratory tract, beginning in infancy or early childhood, serially acquiring and eliminating different strains of pneumococcus with colonization events lasting days, weeks, and in some cases months before they are cleared. As the infant host is increasingly exposed and colonized with different strains, and as her immune system matures, there is a growing immunity to the organism in the form of both type-specific antibodies and a cellular immune response. The effect of this serial exposure and immune system experience is that the age distribution of pneumococcal colonization forms a curve, with highest rates in early childhood, low rates in adolescence and adult years (albeit with higher rates among parents of young children), and an increase among the elderly.[3]

The age at first colonization varies substantially by community setting; in some developing country settings most children are colonized within the first days or weeks of life, whereas in high-income countries the mean age at first acquisition is generally 6 months or older.[4] The prevalence of colonization is maintained at this level through approximately 2–3 years of age in high-income settings. By contrast in other settings, where crowding is common, respiratory exposures are frequent, malnutrition is prevalent, and other risk factors for colonization are widespread, the high rates of colonization found in infancy are maintained for longer periods of time, sometimes through late childhood before they start to fall to a nadir in adolescence and early adulthood.[5]

Since presence in the upper respiratory tract is the necessary precondition for development of disease in an individual, the serotype distribution of colonization is of substantial interest for understanding the serotype distribution of disease-causing strains, notwithstanding the fact that the two are not directly correlated. Studies of pneumococcal nasopharyngeal colonization in the prevaccine setting reveal that not all pneumococcal carriage strains are equally abundant, that some strains are rarely found in the colonization state in spite of their prevalence as disease-causing strains in some settings (eg, serotype 1 and 5), that more than one strain can cause colonization at any given time, but that the frequency of multiple serotype colonization varies by community. A dominant strain is usually present and the density of colonization is an important attribute of the colonization state, which varies widely between individuals and over time.[6] Furthermore, the distribution of serotypes causing colonization is much broader than that of disease causing strain.[5,7] The invasiveness of a pneumococcal strain is described by the frequency of a given strain to cause disease relative to the frequency at which it is detected in colonization.[8] Substantial effort has been made to identify the determinants of invasiveness (ie, capsular or noncapsular characteristics) and therefore the likelihood that these characteristics could change over time with the introduction and use of capsule-based vaccines. The evidence to date favors that invasiveness is primarily an inherent characteristic of the capsule and does not vary substantially over time or epidemiologic setting.[9] More heavily encapsulated serotypes tend to be more prevalent colonizers in young children but are less likely to cause invasive disease.[3,8]

## 3.2 Disease Descriptive Epidemiology

Pneumococcal disease is an uncommon but important result of upper respiratory tract acquisition of the pneumococcus and presents clinically as any one of various syndromes; pneumococcal disease events are categorized as either noninvasive or invasive, distinguished by whether the body site of infection is a normally sterile site (Fig. 12.2). Invasive pneumococcal disease (IPD) is defined as the isolation of pneumococcus from a normally sterile body fluid such as blood, cerebrospinal fluid, pleural or joint fluid, among others. Pneumococcal pneumonia is of particular importance because it constitutes the greatest disease burden of all serious pneumococcal infections and because it is detected and characterized as an invasive infection only when the pneumococcus is isolated from the blood, a lung tap, or pleural fluid. Pneumococcal pneumonia events that are not associated with detection of the organism from a normally sterile body fluid, but instead detected through analysis of sputum, are not characterized as IPD events, even though the lower portions of the lung are generally characterized as a sterile body site.

The age distribution of pneumococcal disease follows a U-shaped curve with the highest burden of disease found in young infants, then in young children, and an increase found again in the elderly.[10] Mortality due to the pneumococcus

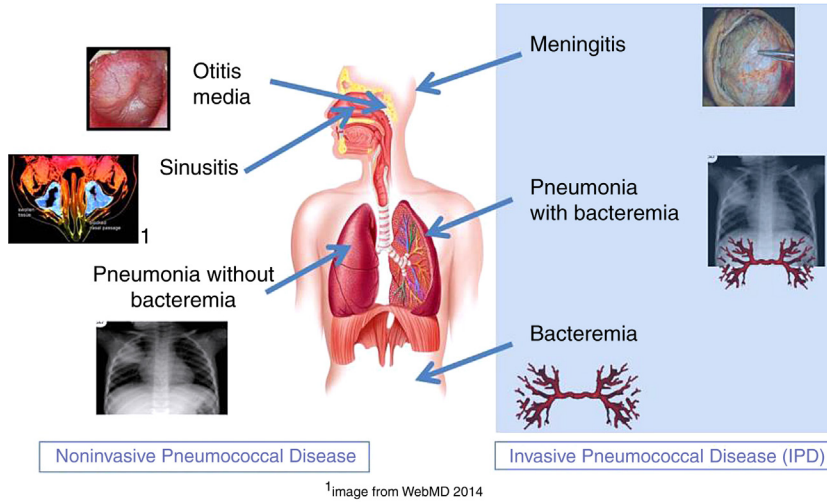


**FIGURE 12.2** **Invasive and noninvasive pneumococcal disease syndromes.**

is highest among the elderly, followed by young infants[10] and it is a significant contributor to overall deaths in children under 5 years old (U5). In 2008, it was estimated that there were 541,000 deaths due to the pneumococcus (uncertainty range 376,000–594,000 deaths) in children U5 worldwide, representing about 9% of all deaths in children U5 after the neonatal period.[11]

## 3.3 Risk Factors for Pneumococcal Disease

Apart from age, there are numerous individual conditions or community factors that increase the risk of pneumococcal disease either directly or indirectly through another mediator; these include:

- HIV infection
- Sickle cell disease
- Malnutrition
- Exposure to smoke
- Micronutrient deficiencies
- Lack of breast-feeding
- Day-care attendance (ie, crowding or increased exposure to other children)
- Coinfection with certain viral pathogens

In some settings, ethnic or racial group is also a risk factor, but this is likely a marker of socioeconomic status and therefore of other biologically relevant risk factors.

The very high risk of IPD in children with HIV infection has been reported in many studies from South Africa and the United States. In a 2008 review, in the absence of antiretroviral treatment (ART), HIV infection was associated

with a 9- to 43-fold increase in IPD rate compared to HIV-uninfected children.[12] Use of ART in South Africa has coincided with a 50% reduction in the incidence of IPD in children with HIV.[13] There is also evidence that infants who are HIV-exposed (eg, born to HIV-positive mothers) but uninfected (HEU) have a somewhat increased risk of IPD and mortality compared to infants who are HIV-unexposed and uninfected (HUU).[14]

## 3.4 Serotype Distribution of Pneumococcal Disease

As described earlier, there are more than 94 pneumococcal serotypes that are immunologically distinct and vary in prevalence of nasopharyngeal (NP) colonization, clinical disease syndromes, and geographical distribution. Antibody-mediated anticapsular immunity to pneumococci is generally serotype-specific, although there is some cross-protection within certain serogroups.[15] In a 2010 systematic review of data on serotypes causing IPD in children U5 by WHO geographic region, prior to the introduction of pneumococcal conjugate vaccines (PCV), 6–11 pneumococcal serotypes accounted for more than 70% of IPD.[7] Fig. 12.3 shows the proportion of IPD due to serotypes in rank order in the 73 countries that have been eligible for vaccine support from GAVI, the Vaccine Alliance (GAVI). The seven most common serotypes in GAVI-eligible countries—serotypes 1, 5, 6A, 6B, 14, 19F, and 23F—accounted for 65% of IPD in Africa and 60% in Asia, prior to the introduction of pneumococcal vaccine (PCV).[7] Furthermore, in every region, serotypes included in the currently licensed PCV products accounted for 70–82% of IPD disease events.

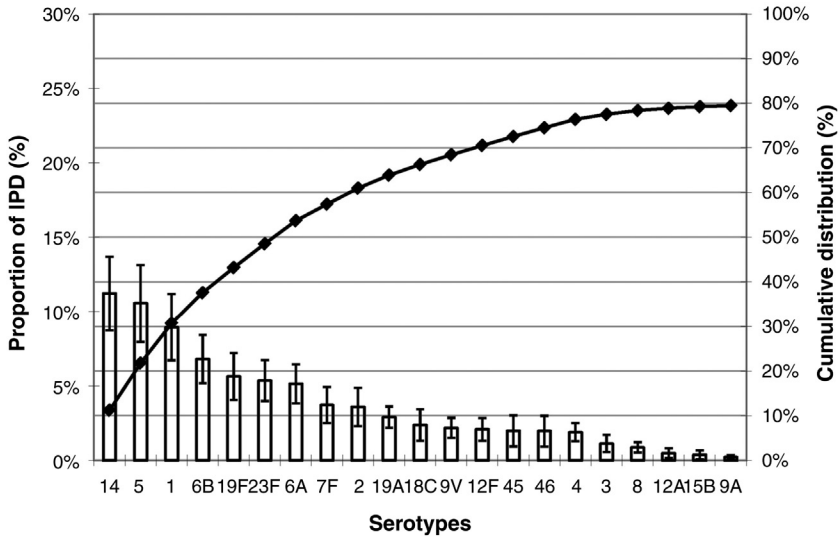


**FIGURE 12.3** **Serotypes causing IPD in children U5 years in GAVI-eligible countries, 1980–2007.**[7]

## 4 IMMUNOLOGY

While the natural human immune response to pneumococcal colonization and disease is directed against a number of antigens, much research has focused on responses to the polysaccharide capsules as these have been the antigens used in licensed vaccines to date. Antibodies to the polysaccharide capsule bind to the organism, resulting in activation of complement, and increased ingestion and killing of the bacteria by professional phagocytes such as polymorphonuclear neutrophils. Clearance of bacteria in the liver and the spleen is critical, and patients with liver disease or with anatomic or functional asplenia (eg, as a result of sickle cell disease) are at much higher risk for pneumococcal invasive disease and associated mortality. Currently available vaccines against the pneumococcus aim to induce antibodies against the most common capsular types of pneumococci.

Anticapsular antibody responses are, by their nature, very specific, limited in their scope to the pneumococci that bear that particular capsule (or those closely related). Since the 1980s, investigators have been interested in identifying genetically conserved, common pneumococcal antigens that may confer broad or even universal protection against pneumococcal disease that is not dependent on serotype. There have been numerous preclinical studies and a few clinical trials evaluating whether antibodies to conserved antigens could confer such broad protection. Current evidence in support of this approach comes from animal studies and functional assays performed with sera obtained from immunized subjects. Potential organism characteristics that might limit efficacy include the possibilities of shielding of the antigen by the polysaccharide capsule in vivo, differential expression of antigens in animal models compared to humans and progressive selection of antigen-negative strains over time.

More recently, the existence of another pathway of acquired immunity to pneumococcus in humans has been identified. It has long been recognized that patients with deficient or defective T-cells, such as individuals with di George syndrome (22q11.2 deletion)[16] or HIV/AIDS, are also at increased risk of pneumococcal infection. This was assumed to be due to concomitant antibody production deficiency. However, laboratory studies have demonstrated that memory Th17 cells, which can be induced by immunization with live or killed bacteria, or purified antigens, mediate resistance to pneumococcal colonization in mice that is independent of antibody production (see review in Ref. [17]). The subsequent demonstration that patients with Hyper IgE syndrome (a condition that predisposes to pneumococcal and other infections) have a marked defect in the generation of memory Th17 cells provided additional suggestion that the cellular arm of the immune response may be playing an important role in conferring protection against pneumococcal infection.[18]

# 5 VACCINES AGAINST PNEUMOCOCCUS

## 5.1 Conceptual Basis

Recognizing that protection against pneumococcal disease can be achieved by the presence of high concentrations of circulating type-specific antibodies, vaccines against pneumococcus have been pursued for more than 70 years. The first pneumococcal vaccines were designed to address the adult disease burden and began with the development of a killed whole cell pneumococcal vaccine that did not reach licensure. This was followed by the development of a 14-valent polysaccharide vaccine that was licensed on the basis of immunogenicity and was later broadened to include 23 serotypes that were the most common causes of adult disease. Polysaccharide antigens alone stimulate a T-cell independent immune response, which is weak in young children and does not substantially protect against colonization. Consequently this licensed pneumococcal polysaccharide vaccine, although widely used in elderly adults, was not considered an effective tool for reducing the pediatric pneumococcal burden of disease. Following the success of *Haemophilus influenzae* type b polysaccharide-protein conjugate vaccine, where the polysaccharide antigen of interest is covalently conjugated to a protein carrier effective in stimulating T-cells, the same approach to formulation was pursued for pneumococcal vaccine. Such conjugation generates vaccine antigens capable of inducing T-cell dependent responses characterized by strong type-specific antibodies with higher affinity, even in early infancy, memory B-cell induction and enhanced antibody class (isotype) switching. In this way the impediments of previous unconjugated pure polysaccharide vaccines for effective use in very young infants were overcome with consequent protection against pneumococcal disease at the very ages when disease risk is highest.

Different licensed pneumococcal vaccine products vary in the number of serotypes included, the protein carriers to which the polysaccharide antigens are bound, and the chemistry used for conjugation. Three products have reached licensure to date; Prev(e)nar-7 (Pfizer Inc.), Synflorix-10 (Glaxosmithkline) and Prev(e)nar-13 (Pfizer Inc.) (Fig. 12.4). Only the latter two remain commercially available. Many other manufacturers have pursued PCV development, with varying success and although none have yet licensed a PCV product, the product pipeline is quite robust.

## 5.2 Immunogenicity

Many immunogenicity studies of PCV products have explored the impact of age, interval, and number of doses on the type-specific antibody concentration, functional nature, and affinity for antigen. The main findings of such immunogenicity studies are that PCVs are highly immunogenic, stimulating the production of type specific antibody after the administration of a primary series,

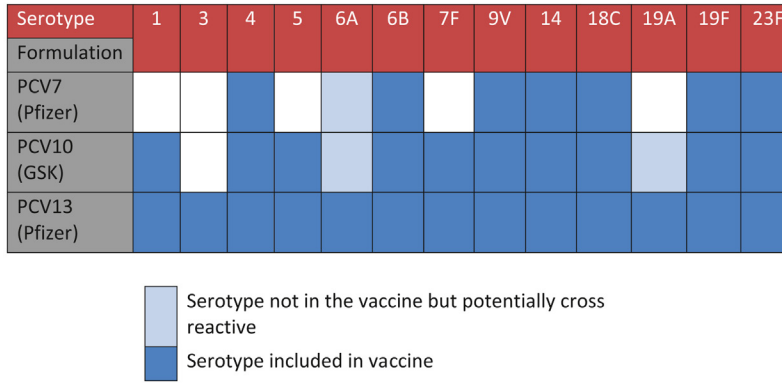


**FIGURE 12.4** **Serotypes included in PCV products that have reached licensure.**

consisting of two or three doses given in an interval of 4–8 weeks, and that this primary immune response is a T-cell dependent one that can be boosted by subsequent administration of type specific polysaccharide either in the form of another PCV dose or a pneumococcal polysaccharide vaccine dose. Furthermore immunogenicity varies by serotype, as does the functional activity of the antibodies. Prior colonization with a homologous serotype, maternal antibody transfer to young infants, and geographic setting (perhaps due to differing prevalence of circulating serotypes therein) affect immune responses to PCVs. While the correlate of protection linking immunogenicity to risk of disease or carriage is not straightforward and varies between serotypes, immunogenicity data is the basis for licensure of new PCV products.

## 5.3 Efficacy

Vaccine efficacy is the proportionate reduction in disease incidence in a vaccinated group compared to an unvaccinated group. Ideally, estimates of vaccine efficacy are based on data from double-blind, randomized controlled trials (RCT) that represent the "best case scenario" of vaccine protection under controlled conditions. In an RCT, the administration of the intervention (vaccine) can be monitored, the disease incidence closely studied, and confounding factors reduced by the randomization process.[19] However, RCTs may not accurately represent the effectiveness of the intervention in the general population or under "real world" scenarios in part because of exclusion criteria for participation in the studies and in part because individually randomized studies cannot measure population-wide effects nor efficacy beyond the duration of study follow up.

PCVs have been tested in RCTs in a variety of settings to determine the vaccine efficacy against numerous disease outcomes: IPD, pneumococcal (bacteremic) pneumonia, radiologically confirmed pneumonia, clinical pneumonia, and AOM (Fig. 12.5). PCVs have also been studied to determine their efficacy in reducing

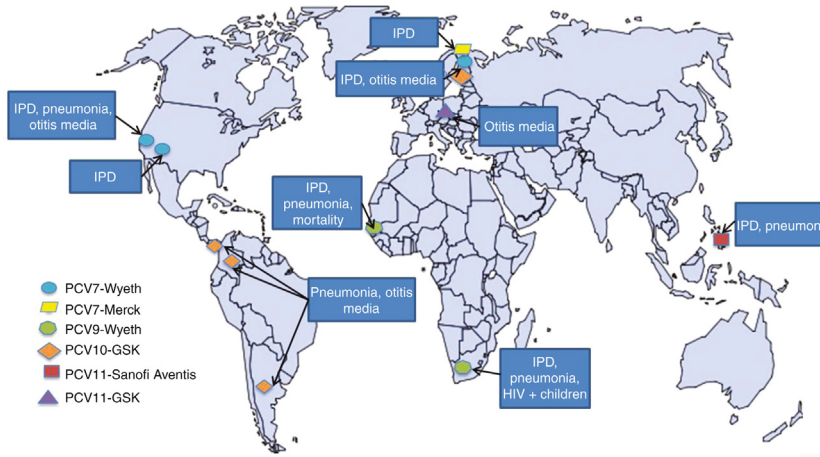


**FIGURE 12.5** **Map of randomized clinical trials of PCV products and disease outcomes assessed.**

pneumococcal nasopharyngeal carriage, which although not a disease reflects effects on transmission and exposure within the population. Many of the RCTs with clinical outcomes were done using earlier 7- and 9-valent formulations of PCV whereas licensure of current PCV products PCV10 and PCV13 was largely supported by immunogenicity studies, demonstrating their noninferiority to PCV7. PCV10 has been tested for efficacy against IPD, pneumonia, and AOM in placebo controlled RCTs, but these trials did not form the basis for product licensure.

PCV efficacy varies by disease outcome; for outcomes more specifically attributable to vaccine serotype pneumococcus, efficacy estimates are higher. This relies on the fact that immune protection for pneumococcus is serotype-specific, and PCVs largely only protect against those serotypes contained within the vaccine, though some cross-protection between related serotypes (such as 6A, 6B, and 6C) can occur.[15] In a 2009 metaanalysis of RCTs in children less than 2 years of age, the pooled vaccine efficacy of PCV7, PCV9, and PCV11 against IPD caused by serotypes contained in the vaccine was 80% in HIV-1 negative children.[20] The vaccine efficacy against overall IPD (caused by any serotype) was 58% driven by the proportion of IPD caused by vaccine serotypes in each of the trial settings which was always less than 100%.

For outcomes based on clinical rather than microbiological diagnoses, the magnitude of the vaccine efficacy estimates are lower than those specific for vaccine type pneumococci. Pneumococcus is one among several etiological agents for clinical syndromes such as pneumonia or otitis media, so measurable vaccine impact depends both upon the proportion of cases caused by pneumococcus and the proportion of those caused by the appropriate vaccine serotypes. In a 2009 metaanalysis, PCV pooled efficacy for radiologically confirmed pneumonia was 27% among children less than 2 years old.[20] In early infancy, randomized studies

of PCV7 showed modest efficacy against all-cause AOM of up to 7%, with a more pronounced effect against pneumococcal AOM.[21] Vaccine formulations, including polysaccharides conjugated to a carrier protein derived from nontypeable *Haemophilus influenzae* (another bacterium causing pediatric AOM), have shown better efficacy against all cause AOM (34%[22] and 19%[23]).

Notably, in one RCT conducted in the Gambia using PCV9 in young children, all-cause mortality was reduced by 16% in the vaccinated group compared to the control group over a two-year follow-up period. This equates to seven deaths prevented for every 1000 children vaccinated in the study.[24] Other RCTs have not shown similar reductions in all-cause pediatric mortality, but none of the studies had enough subjects or background mortality and so sufficient power to investigate this outcome.[20]

## 5.4 PCV Effectiveness/Impact

As of October 2015, PCVs are administered routinely to infants through national immunization programs in 132 countries using several different dosing schedules, comprised of either two or three primary doses with or without a booster dose in the second year of life (Fig. 12.6, VIMS/IVAC). PCV introduction into routine infant immunization schedules has been effective in reducing IPD caused by vaccine serotypes, pneumonia and antibiotic resistant pneumococcal disease in young children (Fig. 12.7).[25] The overall effectiveness of PCV on IPD has been predictably reduced by an increase in IPD caused by serotypes not included in the vaccines; a phenomenon termed serotype replacement.[26] Higher valency PCV formulations (containing more serotypes) were developed in part to respond to this phenomenon by including disease-causing serotypes not in PCV7. Data from many countries are amassing on the impact of higher valency PCVs following licensure and implementation and suggest

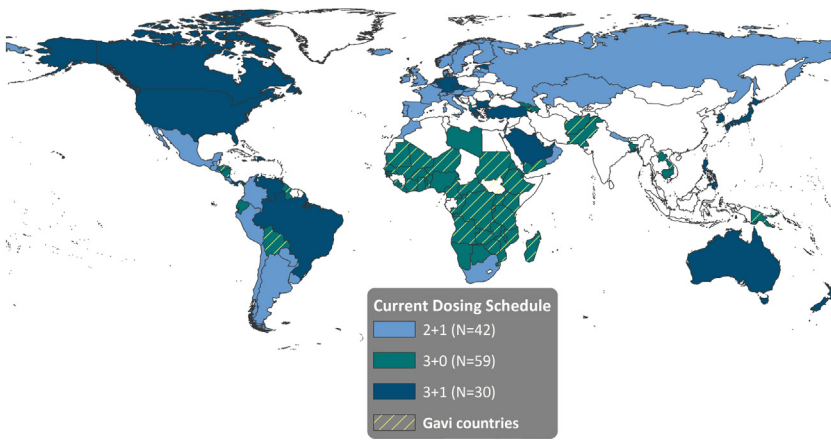


**FIGURE 12.6** **PCV dosing schedules, by country (Oct. 2015).**

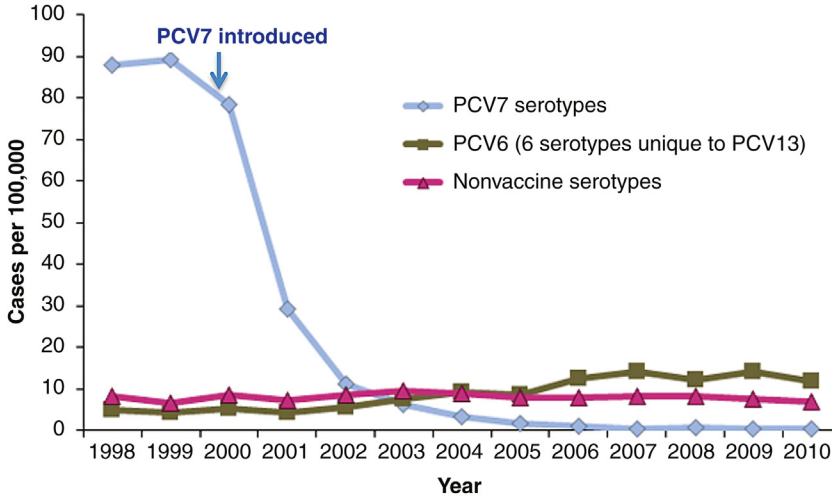


**FIGURE 12.7** **IPD cases in the United States in children under 5 years old before and after the routine use of PCV7, 1998–2010.**[29]

the vaccines are causing further decreases in IPD burden similar to those seen with PCV7 (Fig. 12.8).[27,28]

The public health impact of PCV introduction in national immunization schedules has exceeded the expected reduction in vaccine serotype disease based on vaccine efficacy studies. This amplification of the health impact of PCV results from the reduction in colonization and transmission with vaccine serotype (VT pneumococci) among vaccinated children and the reduced

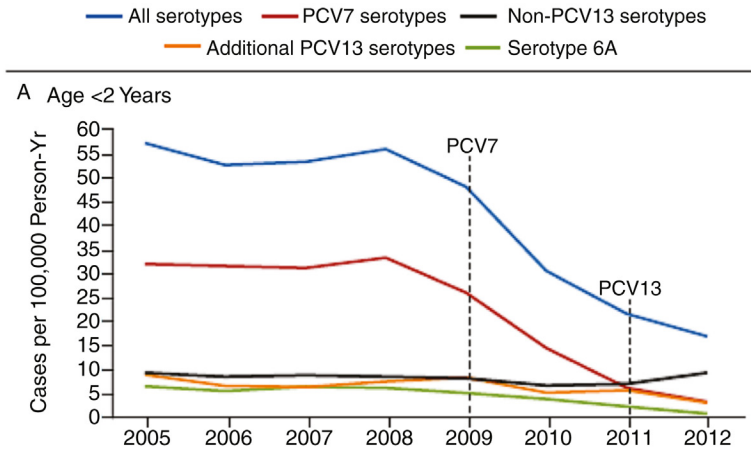


**FIGURE 12.8** **Changes in the incidence of IPD in children under 2 years old in South Africa, 2005–2012. Vaccine coverage estimates for 3 doses of PCV in infancy were 10% for 2009, 64% for 2010, 72% for 2011, and 81% for 2012.**[28]

transmission of these strains to others in the community. This is called the indirect effect and has been shown to result in substantial reductions in vaccine-type disease among older children and adults in the community.[30,31] There are several mechanisms by which PCVs act to reduce disease at the population level, a combination of direct effects in vaccine recipients and indirect effects, extending to persons who are not vaccinated. First, PCV decreases nasopharyngeal acquisition of VT-pneumococcus in vaccinated individuals, thereby decreasing the individual risk of disease (direct effect). Second, PCV reduces the density of VT-colonization in vaccinated individuals, even when they do acquire these strains; pneumococcal colonization density has been associated with development of pneumococcal disease and may also be associated with higher rates of onward transmission to others (direct and indirect effects). Third, PCV provides systemic protection in those who are vaccinated, reducing the likelihood of disease even in colonized individuals (direct effect). Fourth, with reduced VT carriage in vaccinated individuals, there is less transmission to, and carriage among, unvaccinated persons, thereby reducing their risk of pneumococcal disease (indirect effect). Finally, vaccinated individuals also benefit from the reduced circulation of VT-pneumococci in the community and thus have an even lower chance of pneumococcal acquisition than expected from the direct benefits of the vaccine (indirect effect).[30] In a systematic review of data on the indirect effect of PCV use in 14 countries, VT-IPD consistently decreased after PCV introduction across all age groups (Fig. 12.9).[30]

PCV implementation has also indirectly reduced the burden of VT-IPD among HIV-infected adults as demonstrated in the United States, Spain, and South Africa.[30,32,33] Neonates and infants too young to be immunized with PCV have also benefited from the routine use of PCV, although replacement disease has been observed to some extent as shown in the United Kingdom, United States, and Denmark.[34–36]

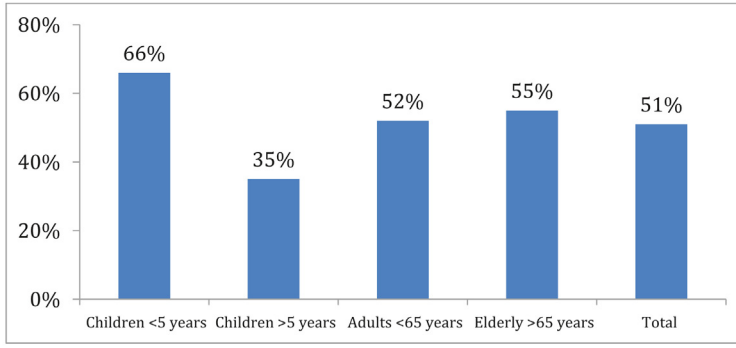


**FIGURE 12.9 Impact of indirect effects as demonstrated by the percent reduction in IPD by age group, data from 14 countries.**[30] *(Adapted from Davis SM, et al. Impact of pneumococcal conjugate vaccines on nasopharyngeal carriage and invasive disease among unvaccinated people: review of evidence on indirect effects.* Vaccine *2013;***32***:133–145.)*

### 5.5 Health Economic Impact of PCV

As well as health benefits, PCVs also have substantial economic benefits. Economic evaluation is the process by which the costs and benefits associated with health interventions and programs are assessed, measured and modeled in order to compare their net impact and inform rational policy and purchasing decisions. Cost-effectiveness studies usually express the cost of an intervention per disability adjusted or quality adjusted life year gained. Commonly an intervention whose cost effectiveness (CE) is less than the per capita gross domestic product (GDP) is considered highly cost effective and those with CE less than threefold the per-capita GDP are considered cost effective.[37] Findings from cost effectiveness analyses (CEA) of PCVs are highly dependent on the parameters of vaccine price, vaccine efficacy, disease incidence, and indirect effects used in the model. Findings from CEA have shown that PCV10 and PCV13 are expected to be cost effective in all 72 Gavi-eligible countries and highly cost effective in all but one country.[38] Another study of PCV CE found PCV10 and PCV13 to be cost-effective in 77 middle-income countries with PCV10 highly cost effective in 68 countries and PCV13 in 71.[39] While PCV13 may prevent more cases of IPD—to an extent dependent on local serotype prevalence and the extent of cross-serotype protection—PCV10 may prevent more cases of AOM if it proves that it does reduce AOM due to nontypeable *Haemophilus influenzae* (NTHi).[40] Evidence on both these uncertainties is still emerging.

### 5.6 Pneumococcal Vaccines for Adults

The use of PCV among adults has recently been evaluated. In addition to immunogenicity studies, an RCT of PCV13 among adults has been completed demonstrating efficacy against IPD and pneumonia.[41] Some high-income countries are assessing the role of giving PCV to adults in their disease prevention portfolio, while the United States has issued recommendations for the product use in those 65 years and older.[42]

Recommendations and evidence for use of the 23-valent polysaccharide vaccine in adults are published.[42–45] Although definitive evidence is lacking, there is a consensus that administration of this vaccine can prevent vaccine type IPD for at least a year following administration, but a definitive role for the prevention of pneumonia has not been shown. Although country specific recommendations vary, it is still widely recommended.

## 6 FUTURE VACCINE APPROACHES

Following the success of pneumococcal conjugate vaccines in reducing pneumococcal invasive disease, and the rise in some nonvaccine type strains, vaccine manufacturers are considering the development of conjugate vaccines with greater valency; one company (MSD) in particular is currently in clinical trials

with a 15 valent pneumococcal vaccine. It is likely that other groups will try to expand on the coverage of the currently available vaccines with the addition of polysaccharides from strains that are now responsible for disease in countries that have implemented universal PCV vaccination. However, the limit in the number of serotypes that can be included may ultimately be reached either on the basis of manufacturing limitations, cost, concentration of carrier protein, interference between serotypes, or other biologic or nonbiologic attributes.

At the same time, various groups, including pharmaceutical companies, have been exploring the potential benefit of conserved "universal" pneumococcal antigens, either as stand-alone vaccines or combined with conjugate vaccines (see review in Ref. [46]). Examples of such efforts include a killed whole cell vaccine (consisting of killed bacteria), purified protein antigens, the combination of conjugate vaccines with purified proteins, or the use of conserved proteins as "carriers" for the polysaccharide. In particular, an unencapsulated killed whole cell vaccine developed by the nonprofit group PATH in collaboration with Boston Children's Hospital is currently undergoing a Phase II clinical trial in Kenyan toddlers. This potential approach, if successful, could have important advantages, as it may offer the possibility of universal coverage (irrespective of the capsular type) at very low cost.[47] Results of this and other clinical trials including many of these approaches are expected in the near future, and may provide further opportunities for control of pneumococcal diseases worldwide.

## 7 CONCLUSIONS

*S. pneumoniae* remains an important cause of morbidity and mortality globally. The advent of effective pneumococcal conjugate vaccines has provided an extremely important weapon against this disease, one that has now been introduced in more than 130 countries worldwide. The development of these vaccines has required improved understanding of the immunology of pneumococcus, together with remarkable advances in conjugation technology, manufacturing, and implementation of universal immunization programs. At the same time, efforts to improve on existing vaccines continue, as do attempts to develop broader (and possibly less complex and more inexpensive) vaccines. Further knowledge about the organism, the pathogenesis of disease, and the mechanisms required to generate long lasting immunity may provide an even broader defense against the organism in the future.

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Chapter 13

# Human Papillomavirus Vaccines

**Margaret Stanley, PhD**
*University of Cambridge, Department of Pathology, Cambridge, United Kingdom*

## Chapter Outline



## 1 INTRODUCTION

Human papillomaviruses (HPVs) are a large group of viruses that infect both cutaneous and mucosal squamous epithelia and have an exclusively intraepithelial infectious cycle.[1] More than 170 HPVs have been isolated from clinical biopsies;[2] they are classified by DNA sequence and numbered in the sequence in which they were isolated for example, HPV 1, HPV 2 etc. About 30–40 HPV types regularly or sporadically infect the mucosal surfaces of the anogenital tract. A subset of these mucosal HPVs 16, 18, 31, 33, 35, 52, 58, 39, 45, 59, 56, 66, and 51, are described as high risk or oncogenic HPV types since a rare, but important, consequence of infection with one of this subset is invasive cervical cancer in women, the third most common cancer in women worldwide.[3] Two types, HPV 16 and 18 cause more than 70% of carcinoma cervix with HPV 16 detected in more than 50% and HPV 18 in ≥12% of cases irrespective of the geographical location.[4] Although cervix cancer is the major consequence of oncogenic HPV infection, a proportion of cases of carcinoma of the penis, vulva, vagina, anus, and oropharynx are attributed to HPV with HPV 16 the

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major player.[5] The contribution to the cancer burden is very significant but the disease burden of those mucosal HPVs rarely associated with cancers; the low risk HPVs–mainly types 6 and 11 the cause of genital warts (GWs)—should not be underestimated. GWs are the commonest viral sexually transmitted infection with a life-time risk of acquisition of 10% and they constitute a huge disease burden for which there is inadequate treatment.[6,7]

## 2 BURDEN OF HPV-ASSOCIATED DISEASE

Disease caused by both low- and high-risk mucosal HPV infections constitutes a global public health problem (Fig. 13.1).

### 2.1 Genital and Laryngeal Warts

GWs are the commonest viral sexually transmitted infection with a peak incidence between 15–25 years and an overall population incidence per annum of 0.16–0.2% in economically developed countries.[5,9] Methodologically robust HPV detection and typing assays reveal that 96% or more of GWs are caused by HPV 6 or 11.[10]

Recurrent respiratory papillomatosis is a rare disease with an incidence rate of 0.5/100,000 live births.[11] HPVs 6 and 11 are the causal agents with HPV 11 predominating.[12] Although the lesions are histologically benign, morbidity is

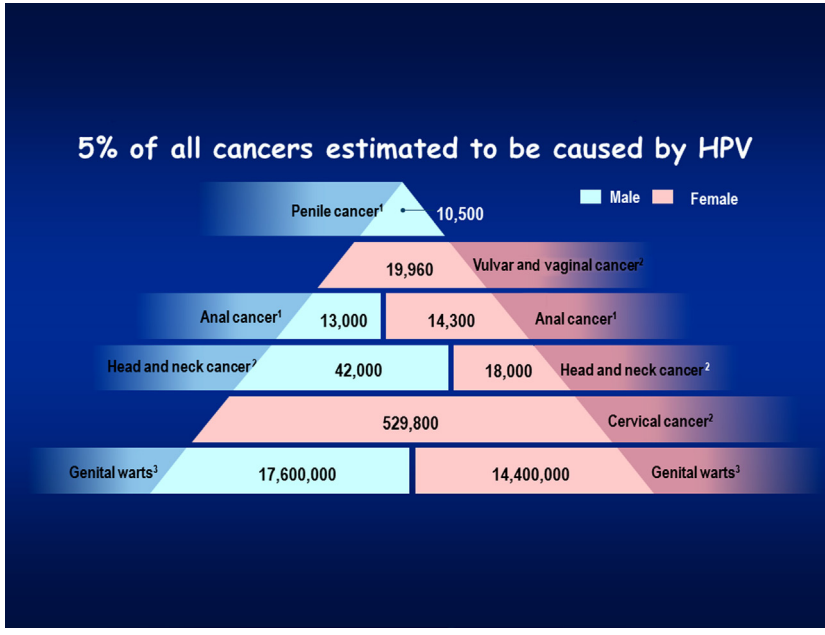


**FIGURE 13.1 Estimated annual prevalence rates for HPV-associated cancers and GWs among males and females.** *(Data from Forman et al.,[5] Guan et al.,[8] and Hartwig et al.[9])*

significant since the frequent recurrence and often confluent spread of these lesions make treatment difficult and patients usually require multiple surgical interventions for excision of lesions.[13]

## 2.2 Invasive Cancer

Globally, it is estimated, that more than 610,000 cancer cases per annum are attributable to HPV infection. The vast majority of these, 530,000 cases, are cervical cancer followed by anus, oropharynx, vulva, penis, and vagina (Fig. 13.1).[5,8] More than 86% of cervical cancers occur in economically undeveloped countries.[5] This discrepancy in cervical cancer incidence between economically developed and undeveloped can be attributed very largely to cervical cancer screening programs in developed countries. Invasive cervical cancer is preceded by epithelial atypia: cervical intraepithelial neoplasia (CIN) in squamous cell carcinoma and adenocarcinoma in situ (AIS) in adenocarcinoma. CIN represents a spectrum of atypia in squamous epithelia ranging from mild (CIN1), moderate (CIN2) to severe or high grade (CIN3); CIN3 and AIS are usually accepted as the obligate precursor lesions of invasive cervical carcinoma. The objective of cervical cancer screening is to detect these high grade lesions and remove them by ablative or excisional procedures, thus interrupting progression to malignant disease in the screened population.

Intraepithelial atypia comparable to the cervical spectrum precede anal, vulval, vaginal, and penile cancers[14] but the natural history of these precursor lesions and progression to malignant disease is not well-documented and understood as for the cervix. Screening is either not available or not feasible for noncervical HPV-associated cancers and the precursor lesions and the available data from economically developed countries show that the incidence of these cancers is rising in these locales. Thus the incidence of anal cancer has risen by 2–3% per annum over the past 3–4 decades irrespective of age in the United Kingdom, the Nordic countries, the USA, Australia, and Canada to name but a few. The incidence of oropharyngeal squamous cell carcinoma (OPSCC) has more than doubled over the past decade in some European countries, the USA, Canada, and Australia.[15] These increases correlate strongly with the rise in the proportion of HPV positive OPSCC over the period from 1980 onward. The rise is greater, two- to threefold, in men than women and in contrast to HPV negative cancers, HPV positive OPSCC occur in younger age groups (<60 years), is unrelated to tobacco use but is associated with oral sex consistent with the evidence that sexual behaviors have changed among the recent birth cohorts in developed countries.[16]

## 2.3 HPV-Type Distribution in HPV-Associated Cancers

Large international studies that employed robust, centralized methodologies for HPV testing and histopathology consistently show that HPV 16 and 18 are the major oncogenic types contributing to approximately 70% of invasive cervical cancers irrespective of geographical locale.[4,8] HPV 16 is the most prevalent

detected in 50% or more of ICC followed by HPV 18 (≥12%). HPVs 31, 45, and 33 occupy positions 3–5 in all continents with the exception of Asia where HPVs 58, 33, and 52 were the commonest types after HPVs 16 and 18.[17] In noncervical-associated cancers, HPV 16 is the major player.

## 3 HPV VACCINES: RATIONALE

Prophylactic vaccines that generate virus-specific neutralizing antibody are the most effective means to control viral diseases. HPV should, in theory, be no exception but the exquisite host and tissue tropism and complex biology of the papillomaviruses differentiates them from most other viruses against which vaccination has proved successful. The HPV life cycle is exclusively intraepithelial and only a fully differentiated squamous epithelium supports the complete infectious cycle and the production of infectious particles.[1] There is no detectable viremia; virus particles are shed from mucosal surfaces far from lymphatics and vascular channels and, not surprisingly, systemic cellular and humoral immune responses to HPV antigens are poor.[18] Serum neutralizing antibody to the major capsid protein L1 is generated in genital HPV infections but neutralizing antibody titers are very low and only about 50–70% of infected women seroconvert.[19] The degree of protection and the duration afforded by antibody in natural infections is not known, reinfection with the same HPV genotype and reactivation of latent virus is thought to occur, even in seropositive individuals.

In natural papillomavirus infections in animals, neutralizing antibodies directed against L1 the major capsid protein are protective. Since these viruses cannot be grown in bulk in tissue culture and viral particles particularly of the oncogenic types are sparse in lesions, the generation of native, or properly folded L1 protein, was challenging. The challenge was met by the demonstration that if the L1 gene was expressed via a viral or yeast vector, the L1 protein was produced in large amounts and self-assembled into a macromolecular structure, a virus-like particle (VLP) an empty capsid that is geometrically and antigenically almost identical to the native virion.[20,21] These VLPs were shown to generate neutralizing antibody in the animal models and immunized animals were protected against high-level virus challenge.[22,23]

## 4 LICENSED PROPHYLACTIC HPV VACCINES

The currently licensed prophylactic HPV vaccines are comprised of VLPs formed of the L1 protein and are made using recombinant technologies in which the L1 gene of specific HPV types is recombined into the host genome of the yeast *Saccharomyces cerevisiae* or the insect virus baculovirus and the L1 protein expressed via these recombinant vectors. The chemistry of the expressed protein is such that it spontaneously assembles into VLPs that are morphologically and antigenically similar to the wild-type virus particle illustrated in Fig. 13.2. However, VLPs lack DNA and are noninfectious and nononcogenic.[24]

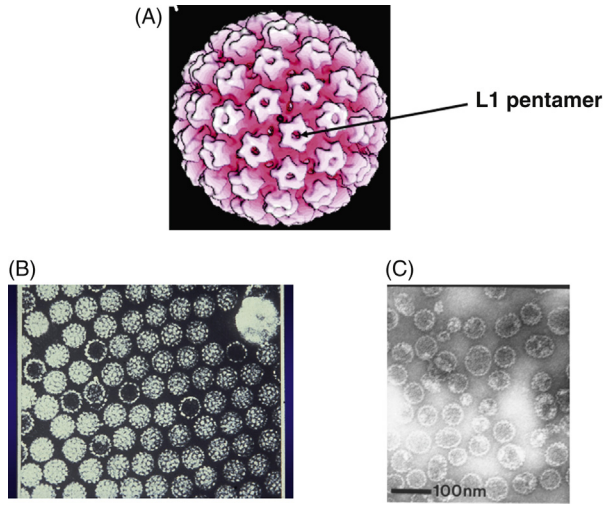


**FIGURE 13.2** (A) A model of the papillomavirus coat or capsid. There are two coat proteins L1 and L2. The rosette like surface structures (*arrowed*) are pentamers each consisting of five molecules of L1; one molecule of L2 fits into the central dimple of each pentamer. (B) Papillomavirus particles, both full (contain DNA) and empty particles can be seen. (C) HPV 16 L1 VLPs made by expressing the HPV 16 L1 gene in baculovirus. The L1 protein so expressed spontaneously assembles into empty capsids or VLPs that are morphologically similar to the empty virus particles seen in part (B). *(From Stanley et al.[24] with permission.)*

Two prophylactic HPV L1 VLP vaccines have been licensed since 2006/2007. These are Cervarix®, a bivalent HPV (bHPV) 16/18 product from GlaxoSmith-Kline Biologicals licensed by the Federal Drugs Administration (FDA) in 2009, and Gardasil® (also known as Silgard), a quadrivalent HPV (qHPV) 6, 11, 16, 18 product from MSD licensed by the FDA in 2006 (Table 13.1). These products are licensed and marketed in more than 160 countries. A third product Gardasil9, a ninevalent HPV (nHPV) 6, 11, 16, 18, 31, 33, 45, 52, 58 VLP vaccine from MSD (Table 13.1) was licensed by the FDA in Dec. 2014 for use in 9–26-year-old females and 9–15-year-old male.

## 5 VACCINE EFFICACY

All vaccines have undergone large, randomized, placebo controlled, double blind Phase III trials (RCTs) in young women (15–26 years old).[25–28] For a detailed review of the bHPV and qHPV trials see Ref. 29. The bHPV and qHPV vaccines in these trials (Tables 13.2A and B) have demonstrated remarkable efficacy in individuals naïve for the HPV types in the relevant vaccines at trial entry and at the completion of the three-dose immunization regimen. The qHPV vaccine has also undergone trials in 16–23-year-old men to determine efficacy against external GWs in heterosexual men[30] and anal intraepithelial neoplasia in men who have sex with men.[31]

**TABLE 13.1** Prophylactic HPV VLP Vaccines: Profiles

| | Cervarix (bivalent vaccine) | | Gardasil (quadrivalent vaccine) | | Gardasil9 (nonovalent vaccine) | |
|---|---|---|---|---|---|---|
| Manufacturer | Glaxo Smith Kline | | Merck | | Merck | |
| Volume | Per dose | 0.5 mL | Per dose | 0.5 mL | Per dose | 0.5 mL |
| Adjuvant | $AsO_4$ | | Amorphous | | Amorphous | |
| | $Al(OH)_3$<br>MPL® | 500 mg<br>50 mg | Aluminum hydroxyphosphate sulfate® | 225 mg | Aluminum hydroxyphosphate sulfate | 500 mg |
| Antigens | L1 HPV 16<br>L1 HPV 18 | 20 µg<br>20 µg | L1 HPV 6<br>L1 HPV 11<br>L1 HPV 16<br>L1 HPV 18 | 20 µg<br>40 µg<br>40 µg<br>20 µg | L1 HPV 6<br>L1 HPV 11<br>L1 HPV 16<br>L1 HPV 18<br>L1 HPV 31<br>L1 HPV 33<br>L1 HPV 45<br>L1 HPV 52<br>L1 HPV 58 | 30 µg<br>40 µg<br>60 µg<br>40 µg<br>20 µg<br>20 µg<br>20 µg<br>20 µg<br>20 µg |
| Expression system | Hi-5 Baculovirus | | Yeast: *S. cerevisiae* | | Yeast *S. cerevisiae* | |
| Schedule | Intramuscular 0, 1, 6 months | | Intramuscular 0, 2, 6 months | | Intramuscular 0, 2, 6 months | |

**TABLE 13.2A Efficacy of Gardasil Against HPV 6, 11, 16, 18 Genital Infection and Disease in Women and Men (Per Protocol Efficacy Population)**

| Women | | | Men | | |
|---|---|---|---|---|---|
| Age 16–23 years | | | MSW (age 16–23 years) | | |
| Mean follow up 42 months | Efficacy (%) | 95% CI | Mean follow up 36 months | Efficacy (%) | 95% CI |
| HPV 16/18 CIN 2 | 100 | 94.7, 100 | HPV 6, 11, 16, 18 | | |
| | | | External genital lesions | 90.4 | 69.2, 98.1 |
| HPV 16/18 CIN 3 | 96.8 | 88.1, 99.6 | HPV 6, 11, 16, 18 | | |
| | | | Persistent genital infection | 85.6 | 73.4, 92.9 |
| HPV 16/18 AIS | 100 | 30.9, 100 | MSM (age 16–23 years) | | |
| HPV 16/18 VIN3/VaIN3 | 100 | 82.6, 100 | HPV 6, 11, 16, 18 | | |
| | | | AIN any grade AIN2/3 | 77.5 | 39.6, 93.3 |
| HPV 6, 11, 16, 18 | | | HPV 6, 11, 16, 18 | | |
| VIN1,VaIN1 | 100 | 86, 100 | Persistent anal infection | 94.9 | 80.4, 99.4 |
| External genital lesions | 99 | 97, 100 | | | |
| Age 25–45 years | | | | | |
| Mean follow up 4 years | | | | | |
| HPV 6, 11, 16, 18 | | | | | |
| CIN 2/3 | 83.3 | −37.6, 99.6 | | | |

(*Continued*)

**TABLE 13.2A Efficacy of Gardasil Against HPV 6, 11, 16, 18 Genital Infection and Disease in Women and Men (Per Protocol Efficacy Population) (*cont.*)**

| Women | | | Men | | |
|---|---|---|---|---|---|
| External genital lesions | 100 | 30.8, 100 | | | |
| HPV 6, 11, 16, 18 | | | | | |
| Persistent genital infection | 89.6 | 79.3, 95.4[a] | | | |

Per protocol: received all three vaccinations, seronegative to appropriate HPV type at day 1, PCR negative to appropriate HPV type on all swabs/biopsies from day 1 through month 7, no protocol violations.
CIN, cervical intraepithelial neoplasia; VIN, vulval intraepithelial neoplasia; VaIN, vaginal intraepithelial neoplasia; AIN, anal intraepithelial neoplasia; MSW, men who have sex with women; and MSM, men who have sex with men.
Persistent infection: detection of same HPV type in genital swab or tissue specimen collected on two or more consecutive occasions at least 6 months apart.
Data for CIN2/3 and AIS are a combined analysis of four randomized clinical trials comprising 20,583 women randomized to receive vaccine or placebo.
[a]*97.5% CI.*
**Source:** Data from Kjaer et al.,[27] Munoz et al.,[43] Castellsague et al.,[28] Giuliano et al.,[30] and Palefsky et al.[31]

**TABLE 13.2B Efficacy of Cervarix Against Cervical HPV 16/18 Infection and Disease in Young Women in the PATRICIA Trial**

| Age 15–23 years (mean follow up 40 months) | Cervarix | Control | Efficacy (%) | 95% CI |
|---|---|---|---|---|
| HPV 16/18 endpoint | *n/N* | *n/N* | | |
| CIN2+ | 5/7338 | 97/7305 | 94.9 | 87.7, 98.4 |
| CIN3+ | 2/7338 | 24/7305 | 91.7 | 66.6, 99.1 |
| Persistent infection | | | | |
| 6 months | 35/7182 | 588/7137 | 94.3 | 92.0, 96.1 |
| 12 months | 26/7082 | 354/7038 | 92.9 | 89.4, 95.4 |

*N* = number of subjects included in each group and *n* = number of cases.
CIN, cervical intraepithelial neoplasia.
Persistent infection: the detection of HPV DNA in swab or biopsy on two occasions at least 6 or 12 months apart.
According to Protocol Cohort: End of Study—ATP: includes women who received three doses of vaccine, were DNA negative and seronegative at month 0 and DNA negative at month 6 to the relevant HPV type (HPV 16 or HPV 18).
**Source:** Data from Lehtinen et al.[25] and Wheeler et al.[26]

**TABLE 13.2C 9vHPV VLP Vaccine: Efficacy on Cervical, Vulvar, and Vaginal Disease and Persistent Infection with Vaccine HPV Types**

| Per protocol efficacy population | 9vHPV vaccine | qHPV vaccine | Vaccine efficacy |
|---|---|---|---|
| Endpoint | No. of cases (*n*) | No. of cases (*n*) | CI (%) |
| All CIN, VIN, VaIN | | | |
| Related to 31, 33, 45, 52, 58 | 3/6016 | 103/6017 | 97.1 (91.8, 99.2) |
| CIN2+,VIN2+ VaIN2+ | | | |
| Related to 31, 33, 45, 52, 58 | 1/6016 | 30/6017 | 96.7 (80.9, 99.8) |
| Related to 6, 11, 16, 18 | 1/5883 | 3/5898 | 66.6 (−203.0, 98.7) |
| 6 month persistent infection | | | |
| Related to 31, 33, 45, 52, 58 | 35/5939 | 810/5953 | 96.0 (94.4, 97.2) |
| Related to 6, 11, 16, 18 | 59/5812 | 80/5830 | 26.4 (−4.3, 47.5) |

Per protocol: received all three vaccinations, seronegative to appropriate HPV type at day 1, PCR negative to appropriate HPV type on all swabs/biopsies from day 1 through month 7, no protocol violations.
CIN, cervical intraepithelial neoplasia; VIN, vulval intraepithelial neoplasia; and VaIN, vaginal intraepithelial neoplasia.
Persistent infection: detection of same HPV type in genital swab or tissue specimen collected on two or more consecutive occasions at least 6 months apart.
**Source:** Data from Joura et al.[32]

The pivotal Phase 3 efficacy study of the 9vHPV vaccine was conducted in 14,000 women, 16–26 years of age equally randomized to receive 9vHPV or the 4vHPV vaccine.[32] A placebo was not appropriate in this study since the standard of care for the prevention of HPV 6, 11, 16, 18 infection and disease is the 4vHPV vaccine. Vaccine efficacy against disease and infection was assessed separately for HPV 6, 11, 16, and 18 compared to the new types. A direct comparison between 4vHPV and 9vHPV for efficacy against 6, 11, 16, and 18 infection and disease was not feasible, since 4vHPV is >90% efficacious against the 4 HPV types. Thus the efficacy findings for 4vHPV were bridged to 9vHPV based on the noninferiority of the antibody response at 7 months. Noninferiority was met for all four HPV types. Efficacy against 16, 18 related CIN grades 1, 2, and 3 and vulval intraepithlial neoplasia (VIN) or vaginal intraepithelial neoplasia (VAIN) grades 2, 3 for 9vHPV was non-inferior to 4vHPV (normalized to the historic placebo). Efficacy of 9vHPV vaccine was assessed against a composite endpoint of HPV 31/33/45/52/58 disease (Table 13.2C). The epidemiological data indicate that the bHPV and qHPV should reduce cervical cancer incidence by 70% or more and the nHPV vaccine by 88%.[17]

## 6 IMPLEMENTATION AND EFFECTIVENESS

HPV vaccines are prophylactic, not therapeutic, preventing not treating infection and they are not effective in individuals with already established infections. Genital HPV infection is usually sexually transmitted and the most important risk period for acquisition of a genital HPV is soon after the onset of sexual activity.[33] The average age of sexual debut varies widely between societies but to be assured that the vaccine recipients receive protection, young adolescents in the 9–14-year age group should be targeted. Immunization before puberty with HPV vaccines is immunologically optimal and 2 times greater antibody responses in the three-dose regimen are achieved in 9–13-year-old adolescents compared to 15–23-year-old women.[34–36] Recommendations for HPV vaccination in most countries—both in the developing and developed world—recognize this and are remarkably uniform in targeting 12–14-year-old females as the primary group for immunization.[37,38] Catch up programs are recommended in some countries but there is variability in the age of the catch up populations.[37]

## 7 VACCINE IMPACT

At the time of writing HPV vaccination has been incorporated into the National Immunization Programme in more than 62 countries covering all continents and evidence of the impact on disease and infection is becoming available. The population level and herd effects of female only vaccination have been assessed in a recent systematic review and metaanalysis of 20 studies from high income countries representing >140 million person years of follow up.[39]

In countries achieving >50% vaccine coverage, HPV 16 and 18 infections decreased by 68% and anogenital warts by 61% in girls (13–19 years of age). Significant reductions in infections with the nonvaccine HPV types 31, 45, 33 were also recorded suggesting some cross protection, a phenomenon demonstrated in the RCTs for both vaccines.[40] In addition reductions in anogenital warts in men <20 years of age and women 20–39 years in populations immunized with the qHPV vaccine were reported implying herd effects. In countries with <50% vaccine coverage, significant reductions in 16/18 infection and anogenital warts occurred in women <20 years but no crossprotection or herd effects were demonstrated. Vaccine coverage is crucial and the highest coverage in the studies reported was achieved consistently with vaccine delivery via school programs.

Reductions in cervical cancer will only be seen in the long term—decades after vaccination—but reductions in precancerous lesions caused by vaccine HPV types should be detectable in the medium term. Reductions in cervical abnormalities have been observed following the Australian National Vaccination Programme, a school-based program targeted to girls aged 12–13 years with a catch up over 2 years for 13–26-year-old young women achieving ≥70% coverage in the school cohort. In a retrospective cohort analysis between Apr. 2007 and Dec. 2011, the effectiveness of the HPV vaccine against CIN1, CIN2, CIN3, and AIS (histologically diagnosed) was assessed in vaccinated and unvaccinated women in the state of Victoria.[41] Vaccine effectiveness was highest in the cohort vaccinated at the youngest age (less than 14 years) with 75% reduction in any high grade histology (CIN2/3 or AIS) compared to 32% in those vaccinated at 17 years. Overall the data from this study and other Australian states[42] indicate that in the vaccinated cohorts (age 12–26 years) high grade cervical abnormalities (CIN2/3, AIS) have decreased by about 48%, a situation predicted by data from the earlier randomized controlled trials.[43] Similarly with the bHPV vaccine in Scotland reductions of 50% for CIN2 and 55% for CIN3 in 20/21-year-old females were observed in the catch up cohort (15–18 year olds) with a mean vaccine coverage of 66%.[44]

The rationale for immunizing only one gender (females) against a sexually transmitted infection is that where immunization coverage is high enough this generates herd protection by blocking transmission to effectively protect the sexual partners.[45] Female only vaccination by definition cannot achieve herd immunity since heterosexual men are not immune but protected if their sex partners are immune or uninfected but remain susceptible to infection if this scenario changes. Men who have sex with men (MSM) are left entirely unprotected by the female only approach and HPV genoprevalence remains high in this group. HPV vaccines are highly effective and, in common with most vaccines, depend for their impact at the population level upon the indirect effects of reducing transmission and carriage. If transmission efficiency of vaccine HPV genotypes is to be reduced to an R0 that is less than 1 at the population level then gender neutral vaccination is the required strategy.

## 8 VACCINE-INDUCED IMMUNE RESPONSES

The current assumption is that antibody is the mechanism of protection afforded by HPV VLP vaccines.[46] Rigorous evidence for this is based at present on preclinical studies in animals that showed that passive immunization of naive recipients with serum immunoglobulin purified from VLP immunized animals protected against high dose viral challenge.[22,47] Only intact VLPs could generate protective antibody and this and other data provided evidence that conformational epitopes in L1 are required to generate neutralizing antibodies and that neutralizing antibody was required for protection reviewed in Ref. 46.

In contrast to natural infections in which the humoral immune response is slow and weak and not all individuals seroconvert, systemic immunization with L1 VLP vaccines generates high serum antibody concentrations at least 50–1000 times greater than those measured in natural infections[48–50] and virtually all vaccinees seroconvert[51–54] (Fig. 13.3). Following the three-dose immunization schedule geometric mean titers (GMTs) for antibodies to the vaccine HPV types peak at month 7. GMTs then wane until 18–24 months at which there is a plateau level at about 10 times natural infection,[51,53] which remains stable for at least 8–9 years after the primary immunization.[55,56] This pattern of antibody response is consistent with the notion of the generation after the three-dose immunization schedule of a large population of antibody secreting plasma cells with varying life spans, some of which have the phenotype of long-lived plasma cells that migrate to the bone marrow and survive for life, maintaining a low but constant antibody production. Antigen challenge at 60 months postdose 1, with

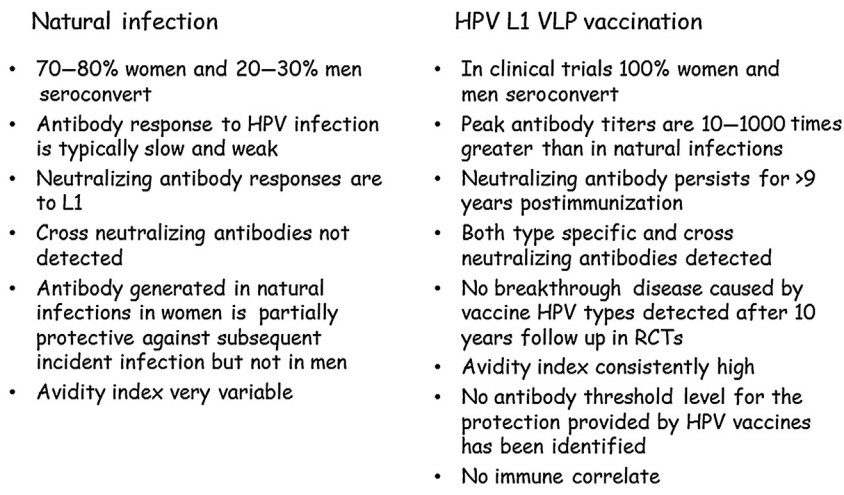


**FIGURE 13.3 Antibody responses in natural genital HPV infection and after HPV VLP vaccination.** *(Data from Stanley et al.[46] and Scherpenisse et al.[50])*

either vaccine, results in a rapid and robust anamnestic response with antibody concentrations rising within a week to levels greater than that achieved at peak (1 month postdose 3) in the initial immunization schedule, demonstrating the presence of reactive memory B cells.[57,58] Serum neutralizing antibody persists with GMTs about 10 times greater than natural infection for the 7–9-year duration of the published studies.[56,59] Mathematical modeling predicts slow decay of antibody over a 30–50-year period and potentially, therefore, protection over that time. Both type specific and cross neutralizing antibodies are generated by VLP vaccines although concentrations of cross neutralizing species are on average 100 times lower than type specific.[60] However, although the HPV VLP vaccines are highly efficacious and immunogenic, no breakthrough cases of disease have been reported from the follow up studies of the RCTs and there is no immune correlate of protection against infection or disease; the minimum level of antibody needed for such protection and the role of B-cell memory if antibody wanes have yet to be established.

Experimental animal data using rodent cervicovaginal infection and challenge models may be informative. In these models microwounds are induced at the cervical squamocolumnar junction in mice and macaques.[61,62] The animals are then challenged vaginally with HPV pseudovirions, L1/L2 VLPs that have packaged a plasmid encoding a reporter molecule such as red fluorescent protein. Using sensitive longitudinal in vivo imaging technologies, the course of HPV pseudovirion infection and the effect of passive transfer of antibody to prevent infection can then be followed in living animals. Recent data using this model and passive immunization with sera from animals immunized with the commercially available vaccines show that very low concentrations of antibody are protective.[63] Such concentrations in vivo are up to 100 times less than those measured in vitro by the gold standard pseudovirion neutralizing seroassay.[64] This suggests that very low levels of vaccine generated antibody below our capacity to measure at the present time will be protective.

## 9 SAFETY

The safety profile of both vaccines was assessed extensively in the RCTs and by robust pharmacovigilance in the postlicensure setting using both passive[65] and active vaccine surveillance.[66–68] The most commonly reported vaccine related adverse events (AE's) are injection site reactions including pain, swelling, erythema, these are usually of short duration and resolve spontaneously; systemic AE's, such as myalgia, fatigue, have been mild and self-limited.[69] Postvaccination syncope has occurred and is considered to be a psychogenic reaction[70] and it is recommended that after vaccination there is a 15-min observation period. No associations with new onset chronic conditions such as autoimmune or neurological disease have been identified in large well-conducted population-based studies.[71]

HPV vaccines are now given to boys in the Australian National Immunization Programme and the safety profile parallels that observed in girls. The Global Advisory Committee on Vaccine Safety (GACVS) of WHO recently published a safety update on HPV vaccines and commented "In summary, 4 years after the last review of HPV vaccine safety and with more than 170 million doses distributed worldwide and more countries offering the vaccine through national immunization programs, the Committee continues to be reassured by the safety profile of the available products."

## 10 ALTERNATIVE DOSAGE SCHEDULES

In view of the overwhelming data on efficacy from the RCTs and the emerging data on population effectiveness, the focus of discussions about the current vaccines is no longer about efficacy but rather about implementation, access, and affordability. In this context changing schedules and/or reducing the doses is of contemporary interest. HPV vaccines are delivered in three doses at 0, 1–2, and 6 months, a "prime, prime boost" schedule with the extended period between dose 2 and 3 required for the generation after dose 3 of high concentrations of high affinity antibody and robust immune memory. Several studies have shown that the interval between doses 2 and 3 can be extended (but not reduced) to 12 and even 24 months.[72–74] In many settings this flexibility is important for implementation and high uptake of the vaccines.

Antibody responses in young adolescents before or at the time of puberty are optimal with antibody titers twice those achieved in the 16–26-year-old women in whom efficacy has been demonstrated in the RCTs.[34] Studies have investigated the feasibility in the young adolescent cohort of changing from the three-dose "prime, prime, boost" to a two-dose "prime, boost" at 0 and 6 months.[36,75] The evidence for both vaccines from these studies is that in 9–14-year-old girls two doses at 0 and 6 months, antibody responses (titers and avidity) are noninferior over a 3- or 4-year period to those achieved after three doses in 16–26-year-old women.[75,76]

At their meeting in Apr. 2014 the Strategic Advisory Group of Experts on Immunization (SAGE) of WHO considered HPV vaccine schedules and made the following recommendations:

> *SAGE reiterated the importance of providing human papillomavirus immunization to girls as early as necessary, i.e. in girls aged 9 to 13 years prior to sexual debut, based on local data and patterns of sexual activity. Upon review of the evidence, SAGE recommended a 2-dose schedule for girls, if vaccination is initiated prior to 15 years of age. A 3-dose schedule remains necessary if immunization is initiated after the girls' 15th birthday. The recommended minimal interval between the 2 doses is 6 months. This interval may be extended to 12 months if this facilitates administration. A 3-dose schedule (i.e. at 0, 1-2, and 6 months) remains recommended for immunocompromised individuals, including those known to be HIV-infected.*[77]

## 11 CONCLUDING REMARKS

The HPV vaccine story is a remarkable story of scientific achievement, entrepreneurial drive, and commercial and scientific interaction. HPV 16 and HPV 18 DNAs were cloned from cervical carcinoma biopsies in Harald zur Hausen's laboratory in 1983 and 1984 starting the explosion in HPV molecular biology and epidemiology that showed unequivocally that the oncogenic HPVs were the cause of cervical cancer. HPV VLPs were first made in 1991 and 1992 and prophylactic HPV VLP vaccines were first licensed in 2006—15 years later. More than 170 million doses of these vaccines have been distributed to date and millions of men and women can expect to be protected against HPV-induced disease.

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Chapter 14

# Rotavirus Vaccines

**Umesh D. Parashar, MBBS, MPH**
*National Center for Immunizations and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States*

## Chapter Outline



Rotavirus is the leading cause of severe childhood gastroenteritis worldwide, accounting for about one-third of diarrhea episodes requiring hospitalization. Although rotavirus is equally prevalent worldwide, the vast majority of rotavirus deaths occur in developing countries, because of suboptimal access to health care. Orally administered live attenuated vaccines have been developed to provide protection against rotavirus. Two licensed rotavirus vaccines have been available since 2006 and have been implemented in 77 countries as of Aug. 2015. In this chapter, we review the epidemiology of rotavirus, progress with vaccine development, and outline remaining issues and challenges to achieving optimal control of rotavirus disease through vaccination.

## 1 BIOLOGY OF ROTAVIRUS

Rotaviruses are 100 nm, nonenveloped RNA viruses belonging to the family *Reoviridae*.[1,2] They were identified in humans in 1973 by Bishop and coworkers who used immune electron microscopy to demonstrate wheel-shaped particles

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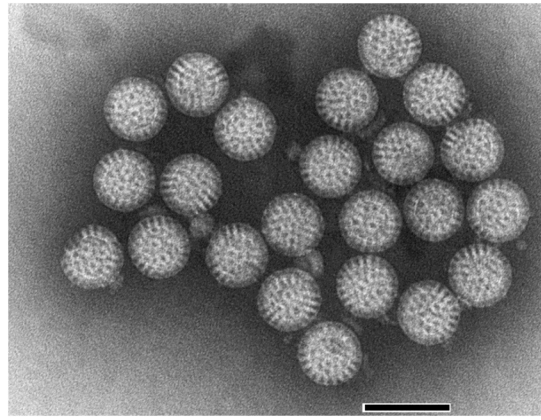

**FIGURE 14.1** **Electron micrograph of rotavirus particles in stool specimens.**

(rota = wheel in Latin) in biopsies of duodenal mucosa from infants with gastroenteritis in Australia (Fig. 14.1).[3,4] Rotavirus particles contain a triple-layered capsid surrounding a viral genome consisting of 11 segments of double-stranded RNA. These RNA segments code for six structural proteins (VP1–VP4, VP6, and VP7) and six nonstructural proteins (NSP1–NSP6). The VP6 protein comprises the middle layer of the capsid and is the protein to which common immune diagnostics are directed. Eight groups of rotavirus have been described (A–H) based on genetic and antigenic differences in the VP6 protein.[2] Only rotaviruses in groups A, B, and C are known to cause disease in humans, with group A rotaviruses being the principal cause of human disease. The VP7 protein (a glycoprotein, or G-type protein) and VP4 protein (a protease-activated protein, or P-type protein) comprise the outer layer of the capsid. These proteins form the basis of binary classification (G and P types) of rotavirus. To date, >20 G serotypes and >30 P genotypes have been described,[5] and theoretically they could form more than 600 different G/P combinations by segregation. However, globally five G types (G1–4 and G9) and three P types (P[4], P[6], and P[8]) predominate[5–7], and five combinations of these common types generally account for more than 90% of circulating viruses: P[8]G1, P[4]G2, P[8]G3, P[8]G4, and P[8]G9.

## 2 ROTAVIRUS DISEASE AND TREATMENT

The clinical spectrum of rotavirus infection ranges from subclinical illness or mild, watery diarrhea of limited duration to frequent, profuse diarrhea with vomiting and fever that can result in dehydration with shock, electrolyte imbalance, and death. Rotavirus illness usually begins with acute onset of fever and vomiting, followed 1–2 days later by frequent, watery stools. Up to one-third of children may have a moderate fever (temperature > 102°F or 39°C).

Vomiting usually lasts less than 1–2 days and other gastrointestinal symptoms generally self-resolve in 3–7 days. While gastroenteritis is the chief manifestation of rotavirus infection, neurologic features—including benign convulsions, encephalitis/encephalopathy, and cerebellitis—have been described in children with rotavirus gastroenteritis.[8]

The management of acute rotavirus gastroenteritis primarily focuses on the treatment and prevention of dehydration. In most situations the clinician will not be aware at the start of treatment whether the etiologic agent is rotavirus or another pathogen. Initial assessment therefore focuses on determining the degree of dehydration because this will both guide and monitor treatment. It is important that appropriate feeding continue throughout rehydration and maintenance phases of treatment.

## 3 BURDEN AND EPIDEMIOLOGY OF ROTAVIRUS

Rotavirus is the leading etiologic agent of severe childhood gastroenteritis globally, causing an estimated 25 million clinic visits, 2 million hospitalizations, and 180,000–450,000 deaths in children <5 years of age each year (Fig. 14.2).[9–11] Rotavirus infects nearly all children—in both developed and developing countries—by 3–5 years of age. Neonatal infections occur, but are often asymptomatic or mild, possibly because of protection from antibodies acquired from the mother or from breastfeeding. The incidence of clinical illness peaks among children ages 4–23 months, who are also at greatest risk for severe disease requiring hospitalization. Repeat infections are common (eg, 3 or more rotavirus infections occurred in about 42% of children by 2 years of age in one follow-up study in a cohort of Mexican children[12]); however, symptoms are milder with each subsequent infection.[12,13] Therefore, rotavirus infections of adults are usually subclinical or mild, but can be severe, particularly in immunocompromised persons and the elderly.

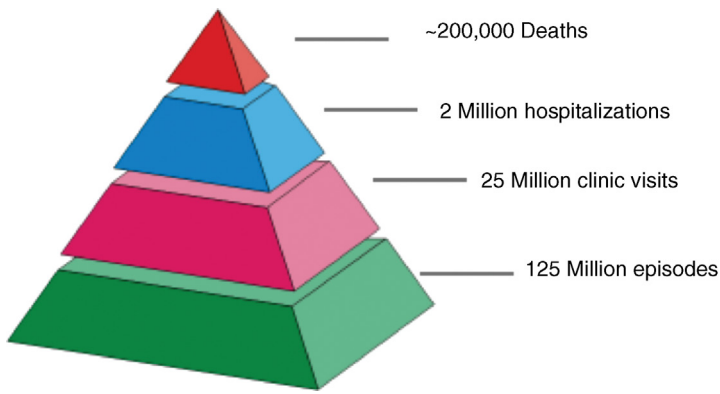


**FIGURE 14.2** **Global burden of rotavirus disease.**

Rotavirus is the leading cause of hospitalization for gastroenteritis, accounting for 33–49% of hospitalizations for gastroenteritis in countries in different geographic regions and with varying levels of child mortality.[10] However, >90% of global deaths from rotavirus occur in low income countries in sub-Saharan Africa and South Asia, likely because of suboptimal access to health care including basic hydration therapy. In addition, compared with industrialized countries, severe rotavirus gastroenteritis occurs at a younger age in developing countries and coinfections with other enteric pathogens are more common. In temperate climates, rotavirus gastroenteritis shows prominent seasonality, occurring mainly during the fall and winter, with little disease activity during summer months.[14] In tropical countries, rotavirus occurs year-around, although seasonal increases in incidence during the cool, dry months are often seen even in these settings.

## 4 RATIONALE FOR ROTAVIRUS VACCINE DEVELOPMENT

Vaccines to prevent rotavirus disease have been developed for several reasons. First, because rotavirus infects nearly all children in both industrialized and developing countries early in life, improvements in hygiene and sanitation alone are considered inadequate for prevention. Second, follow-up studies of birth cohorts of infants indicated that, although children can be infected with rotavirus up to 4–5 times in the first 2 years of life, the incidence of severe rotavirus gastroenteritis is reduced with each repeat infection.[12,13] Therefore, orally administered, live attenuated, rotavirus vaccines have been developed to mimic the effect of natural infection and prevent severe rotavirus disease.

## 5 THE FIRST LICENSED ROTAVIRUS VACCINE—ROTASHIELD

A rhesus-human reassortant rotavirus vaccine (Rotashield, Wyeth) was licensed in the United States in 1998 after demonstrating high efficacy against severe rotavirus gastroenteritis in randomized clinical trials, and was recommended for routine immunization of US infants the same year.[15] However, this vaccine was abruptly withdrawn a year later in 1999 after it was given to about 1 million US infants because it was associated with a severe adverse event, intussusception.[16,17] Intussusception is a form of bowel obstruction that frequently requires surgical treatment and is associated with high fatality if not treated. The risk of intussusception was elevated almost 30-fold during the 3–7 day period after administration of the first dose of Rotashield,[16] translating to an estimated one excess intussusception case from vaccination of 10,000 infants.

## 6 CURRENT INTERNATIONALLY LICENSED ROTAVIRUS VACCINES—ROTARIX™ AND RotaTeq

Two other live oral rotavirus vaccines—a pentavalent bovine-human reassortant vaccine (RotaTeq, Merck and Co.) and a monovalent human vaccine (Rotarix, GSK Biologicals)—were in advanced stages of clinical testing when Rotashield

**TABLE 14.1 Features of Rotarix (GSK Biologicals) and RotaTeq (Merck) Rotavirus Vaccines**

| Features | Rotarix | RotaTeq |
|---|---|---|
| Composition | Single human rotavirus strain (P1A[8], G1) | Five human G/P reassortants with bovine rotavirus strain WC3 (P7[5], G6):<br>G1 × WC3<br>G2 × WC3<br>G3 × WC3<br>G3 × WC3<br>P1A[8] × WC3 |
| Number of doses | 2 oral doses | 3 oral doses |
| Schedule* | Dose 1: Minimum 6 weeks of age<br>Dose 2: ≥4 weeks later<br>Complete by 24 weeks of age | Dose 1*: 6–12 weeks of age<br>Doses 2 and 3: ~4–10 week intervals<br>Complete by 32 weeks of age |
| Dose | Each dose (1–1.5 mL) contains at least $10^6$ median cell culture infectious doses | Each dose (2 mL) contains at least 2.0–2.8 × $10^6$ infectious units per reassortant |
| Shelf life | 36 months | 24 months |
| Storage | 2–8°C, protected from light | 2–8°C, protected from light |
| Contraindications | • A demonstrated history of hypersensitivity to the vaccine or any component of the vaccine<br>• History of uncorrected congenital malformation of the gastrointestinal tract that would predispose the infant to intussusception<br>• History of Severe Combined Immunodeficiency Disease (SCID).<br>• History of intussusception. | • A demonstrated history of hypersensitivity to the vaccine or any component of the vaccine<br>• History of Severe Combined Immunodeficiency Disease (SCID)<br>• History of intussusception. |

**Ages for vaccine doses vary to some extent according to individual country recommendations and vaccination schedules.*

was withdrawn (Table 14.1). RotaTeq and Rotarix each underwent large randomized clinical trials of ~60,000–70,000 infants to assess risk of intussusception prior to licensure.[18,19] No elevation in intussusception risk was found during 42 and 30 days after vaccination after any of the 3 doses of RotaTeq or any of the 2 doses of Rotarix, respectively. The vaccines demonstrated 85–98% efficacy against severe rotavirus gastroenteritis in these trials conducted in the Americas and Europe, with good protection against disease caused by rotavirus strains not included in the vaccines (heterotypic immunity). These findings

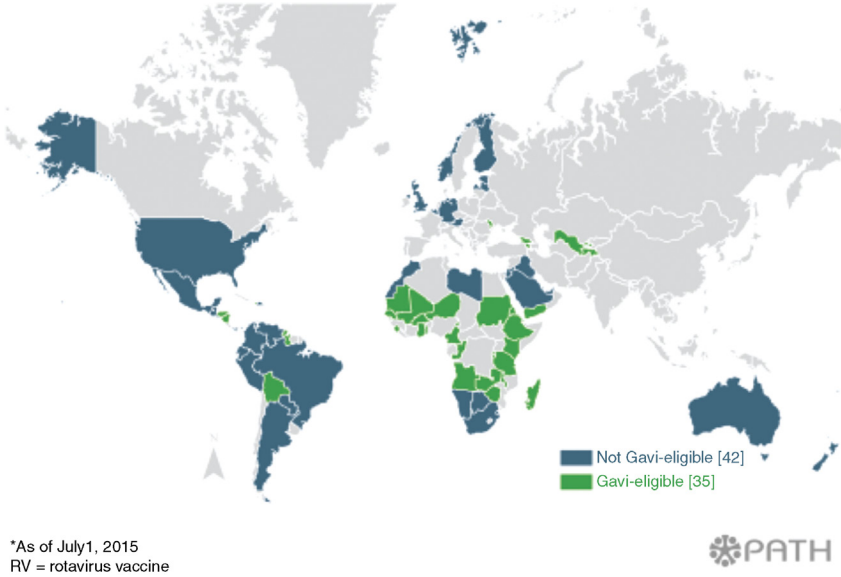


**FIGURE 14.3 Countries that have implemented rotavirus vaccination as of Aug. 2015.**

supported vaccine licensure and recommendations for use by policy groups in the United States and Europe, and by the World Health Organization (WHO).[20]

As of August 2015, 77 countries around the world have implemented rotavirus vaccines in their national immunization programs[21] (Fig. 14.3), and several have documented rapid and sharp declines in the burden of severe gastroenteritis after vaccine implementation. For example, data from national laboratory surveillance in the United States—the first country to implement rotavirus vaccination—have demonstrated delayed, shorter rotavirus seasons and a sustained reduction in the number of rotavirus tests through eight rotavirus seasons following vaccine implementation compared with prevaccine years (Fig. 14.4).[22] A systematic review of data from eight countries that have implemented routine rotavirus vaccination reported a 49–89% decline in laboratory-confirmed rotavirus hospitalizations and 17–55% decline in all-cause gastroenteritis hospitalizations among children <5 years within 2 years of vaccine introduction.[23] As an unanticipated positive surprise, rotavirus vaccination of young infants has also resulted in the added benefit of declines in rotavirus disease among children who missed vaccination and older children and even adults who were not vaccine-eligible.[24] This phenomenon, known as herd protection, is likely related to reduction in community transmission of rotavirus because of vaccination. In addition, studies from Mexico and Brazil have shown a 35 and 22% decline in childhood deaths from diarrhea, respectively, since implementation of rotavirus vaccine [25,26]; in Mexico, these declines have been sustained for 4 years after vaccine introduction. These findings are particularly noteworthy as vaccine efficacy against diarrhea mortality was not evaluated in prelicensure trials.

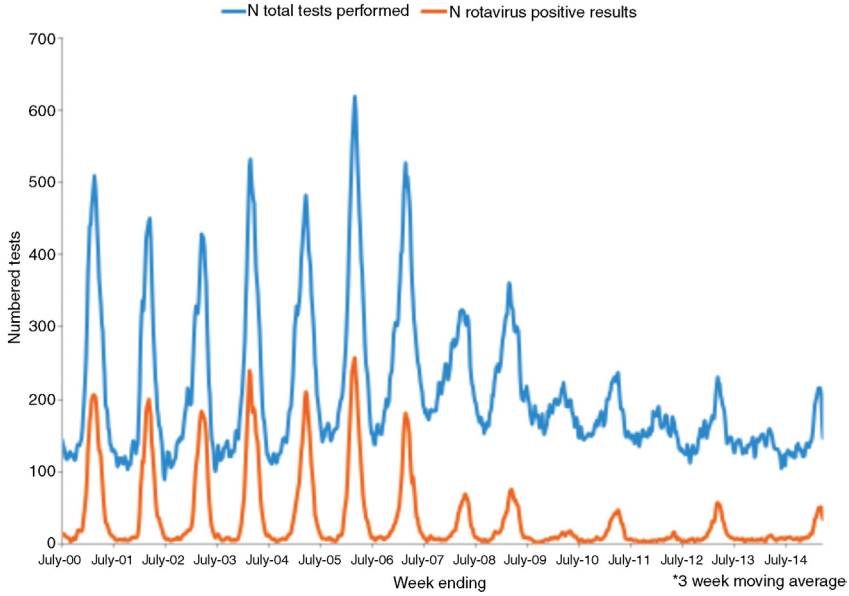


**FIGURE 14.4** **Total number of rotavirus tests and positive results*, United States, Jul. 2000–Mar. 2015.**

## 7 REMAINING ISSUES AND CHALLENGES FOR ROTAVIRUS VACCINES

Despite the remarkable early success of rotavirus vaccines in reducing the burden of severe gastroenteritis in countries that have implemented vaccination, several issues and challenges remain to be fully addressed to realize the public health value of vaccination globally.

### 7.1 Reduced Efficacy of Rotavirus Vaccines in Developing Countries

Live, oral vaccines against many diseases, such as polio, typhoid, and cholera, have performed less well in developing country settings compared with industrialized countries. The reasons for this variability are not completely understood, but the diminished vaccine performance in developing countries may be related to interference in vaccine take by greater levels of maternal antibody or concurrent enteric infections or to diminished immune response in infants because of comorbidities or malnutrition, including micronutrient deficiencies.[27,28]

Because of these concerns, randomized efficacy trials of both RotaTeq and Rotarix were conducted in developing countries of Africa and Asia.[29–31] These trials showed modest vaccine efficacy (50–64%) against severe rotavirus

gastroenteritis. Notably, despite the diminished efficacy, the public health benefits of vaccination in terms of the number of severe rotavirus gastroenteritis episodes prevented for every 100 vaccinated infants were greater in developing compared to industrialized countries because of the substantially greater rate of severe rotavirus gastroenteritis in developing countries. These considerations led WHO to issue a global recommendation for vaccine use in 2009 and have prompted several low-income countries to include rotavirus vaccination in their immunization programmes.[20]

Emerging data from the first low-income countries in Africa that have implemented routine rotavirus vaccination show promising findings. Rotarix introduction in South Africa in 2009 resulted in at estimated 45–50% reduction in rotavirus hospitalizations among infants in first two years following introduction, and in Blantyre, Malawi, an estimated 43% reduction in the incidence of rotavirus hospitalizations among infants occurred in the second season following RV1 introduction in 2012.[32,33] Using the case-control methodology, two doses of Rotarix were found to be 57% effective against rotavirus hospitalization among South African children aged <2 years, with similar results in the first and second year of life and in HIV exposed-uninfected and HIV unexposed-uninfected children.[34] In Malawi, two Rotarix doses were 64% effective against rotavirus hospitalization in young children.[33] As rotavirus vaccines are introduced in immunization programs of low income countries globally, assessing the real-world impact of vaccination is important to better understand vaccine effectiveness in a range of settings.

## 7.2 Impact of Rotavirus Strain Diversity on Rotavirus Vaccine Performance

Rotarix and RotaTeq differ in composition, with Rotarix containing a single human G1P[8] strain and RotaTeq containing five reassortant rotaviruses developed from human and bovine parent rotavirus strains. Four of the bovine-human reassortant rotaviruses express human virus VP7 from serotypes G1, G2, G3, or G4, whereas the fifth reassortant virus contains VP4 (P[8]) from a human rotavirus strain. Given its monovalent composition, a priori concerns existed about how well Rotarix would protect against rotavirus disease caused by strains partially (different G or P type) or fully (different G and P type) heterotypic to the vaccine strain. In particular, concerns focused on protection against fully heterotypic G2P[4] strains that also have a different overall genomic RNA constellation defined by RNA–RNA hybridization assays and hence belong to a different genogroup than the G1P[8] Rotarix strain.

In the pivotal prelicensure trial in Latin America, Rotarix prevented 87–91% of severe rotavirus diarrhea caused by partially-heterotypic G3P[8], G4P[8], and G9P[8] strains as well as fully-homotypic G1P[8] strains.[18] While fully heterotypic G2P[4] strains were uncommon, protection appeared to be lower (45%) against these strains. However, in a later trial conducted in Europe[35] as well as a meta-analysis study integrating all previous trials,[36] RV1 provided

statistically significant protection against severe rotavirus diarrhea caused by G2P[4]. In the African clinical trial of Rotarix conducted in Malawi and South Africa,[30] great diversity of circulating rotavirus strains was observed, with the G1P[8] vaccine-type strains accounting for 57% of strains detected in South Africa and only 13% of strains in Malawi. Nevertheless, the vaccine demonstrated good efficacy against a range of G types—efficacy against G1, G12, and G8 types of 64, 52, and 64%, respectively—as well as a range of circulating P types—efficacy against P[8], P[4], and P[6] of 59, 71, and 55%, respectively.[37]

During the first 2 years after the introduction of Rotarix in Brazil, a nationwide predominance of G2P[4] strains was reported.[38–40] A similar observation was made following vaccine introduction in Australia, where a higher prevalence of G2P[4] strains was seen in states that exclusively used Rotarix compared to states using RotaTeq, where G3P[8] was the predominant strain.[41,42] These reports prompted an international discussion over the potential linkage of the appearance of these strains with the introduction of rotavirus vaccines.[43,44] However, with additional years of monitoring, further strain changes were observed and G2P[4] strains no longer remained the predominant strain in either Brazil or in Australian states using Rotarix.[45–47] The observed strain changes may thus represent natural secular variation in rotavirus strain that has been well documented in the years prior to introduction of rotavirus vaccines,[48] rather than vaccine selection pressure. Additional evidence of good cross-protection from Rotarix comes from observational studies in several countries of Latin America that have demonstrated high vaccine effectiveness against rotavirus disease caused by nonvaccine type strains.

In summary, no clear pattern of sustained vaccine-associated rotavirus strain shift have been documented and both vaccines appear to provide a high level of protection against severe rotavirus disease from a variety of heterotypic strains. However, data from middle- and low-income countries on these issues are sparse, and ongoing postintroduction surveillance is crucial for interpreting data on emergence novel or unusual strains, assessing vaccine impact, and strain specific vaccine effectiveness.

## 7.3 Intussusception Risk

Neither Rotarix nor RotaTeq was found to be associated with intussusception in prelicensure trials of 60,000–70,000 infants each. However, despite their large size, a low level risk of intussusception could not be excluded and further monitoring was recommended in countries implementing vaccination. Postlicensure observational studies in several countries, including the United States, Australia, Mexico, and Brazil, have identified a low risk of intussusception with both rotavirus vaccines.[49–56] The evidence of risk for the two vaccines is difficult to directly compare because of different populations where the studies were conducted and differences in study design. In general, the overall risk is about 1–5 excess intussusception cases per 100,000 vaccinated infants and risk

has been observed with both rotavirus vaccines. Several countries with documented intussusception risk have assessed the risk against data on real-world health benefits of vaccination from the same setting (Table 14.2). Considering the substantial and well documented health benefits of vaccination against a low intussusception risk, policy makers in countries with documented risk, as well as global health authorities, such as WHO, continue to strongly support rotavirus vaccination of infants.

### 7.4 Vaccine Supply and Affordability

Assuring adequate supply of affordable rotavirus vaccines is vital to sustain global vaccine implementation. In this regard, it is encouraging, in addition to the two licensed multinational vaccines, several manufacturers in emerging markets, including India, China, Vietnam, Indonesia, and Brazil, are developing candidate rotavirus vaccines.

In 2014, India licensed an indigenously manufactured rotavirus vaccine based on natural bovine-human reassortant rotavirus strain, 116E (ROTAVAC). In a multicenter phase III trial that enrolled infants from three cities in India—New Delhi, Pune, and Vellore—ROTAVAC showed 56% efficacy (95% CI 37–70%) against severe rotavirus gastroenteritis during the first year of life, with sustained efficacy in the second year of life (49%, 95% CI 17–68%).[58,59] ROTAVAC provided protection against a wide variety of vaccine mismatch strains, including G1P[8], G2P[4], and G12P[6], which were the most common circulating strains during the trial. ROTAVAC was not associated with any serious adverse events, including intussusception, in the phase III trial. However, since a relatively small number of infants (~4500) were vaccinated in the trial, a low risk of intussusception cannot be excluded. ROTAVAC has been licensed for use in India and has been recommended for inclusion in the Universal Immunization Program of India. The manufacturer has committed to pricing the vaccine at <US$1 per dose for a 3-dose series, a price that is substantially lower than the price of the multinational vaccines.

## 8 CONCLUSIONS

During the past decade, significant progress has been made in the prevention and control of rotavirus diarrhea through vaccination. The introduction of rotavirus vaccines into the national immunization programs of more than 75 countries has resulted in substantial declines in diarrhea-related morbidity and mortality. Despite this, the full public health impact of these vaccines has not been realized, as many countries, including some with the highest disease burden, have not yet introduced rotavirus vaccines into their national immunization programs. Several key research activities may help to address remaining questions about rotavirus vaccine use under field conditions and inform vaccine introduction decisions, especially in low-income countries. These include: (1) establishing

**TABLE 14.2** Risk-Benefit Estimates of Rotavirus Disease and Intussusception Outcomes by Country*

| Countries | Outcome | Rotavirus outcomes averted | Intussusception outcomes caused | Rotavirus outcome averted: intussusception outcome caused | References |
|---|---|---|---|---|---|
| Mexico | Hospitalizations | 11,551 | 41 | 282 : 1 | [51] |
| | Deaths | 663 | 2 | 331 : 1 | |
| Brazil | Hospitalizations | 69,572 | 55 | 1265 : 1 | [51] |
| | Deaths | 640 | 3 | 213 : 1 | |
| Australia | Hospitalizations | 6,528 | 14 | 466 : 1 | [49] |
| | Deaths | NR | NR | NR | |
| United States | Hospitalizations | 53,444 | 35–166 | 322–1530 : 1 | [57] |
| | Deaths | 14 | 0.1–0.5 | 28–134 : 1 | |

NR: Not reported.
**Estimates based on one vaccinated birth cohort to age 5 years.*

effectiveness/impact and safety of rotavirus vaccines in low-income settings; (2) identifying potential strategies to improve performance of oral rotavirus vaccines in developing countries; and (3) pursuing alternate approaches to oral vaccines, such as parenteral vaccines to improve vaccine efficacy in developing countries. Addressing these questions and additional policy- and program-level barriers will ensure that countries are able to make informed decisions regarding rotavirus vaccine introduction and to help realize the full potential impact of these vaccines.

## DISCLAIMER

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention (CDC).

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Chapter 15

# Antiviral Vaccines: Challenges and Advances

Ali H. Ellebedy, Rafi Ahmed

*Emory University School of Medicine, Emory Vaccine Center and Department of Microbiology and Immunology, Atlanta, GA, United States*

## Chapter Outline



## 1 INTRODUCTION

Vaccination is the most effective means of preventing and controlling viral infections.[1] The eradication of smallpox and the significant progress made toward polio eradication are clear examples of the great impact of antiviral vaccines.[2,3] However, viral infections remain a major public health threat and a significant cause of death. Most of the antiviral vaccines introduced over the past century were empirically developed.[4] Poliomyelitis, measles, mumps, and rubella are examples of diseases that are now largely controlled thanks to these empirically developed vaccines.

The common factor among our most effective antiviral vaccines is that they were developed to mimic our natural immune response to the pathogen. For example, a single episode of measles confers lifelong immunity in the survivors. Hence, what we needed to do is induce a similar immune response.

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It is when we have to do better than "mother nature" that we have been facing substantial challenges in developing successful vaccines. For example, the immune response against viruses such as HIV, influenza, and respiratory syncytial virus (RSV) is either inadequate or outpaced by the pathogen's evolution. And while developing a broadly protective vaccine against such pathogens has been a colossal task, it is not impossible and similar missions have been successfully accomplished as in the case of anti-HBV and anti-HPV vaccines.

There is a growing list of emerging and reemerging viral infections against which an effective vaccine is yet to be developed. Recent technological advances in the areas of immunogen design, single cell transcriptomics, systems biology, gene delivery, epigenetics, nanoparticles, and adjuvants expanded our understanding of how vaccines work and provide potentially new platforms that could be harnessed to develop vaccines against challenging and emerging viral pathogens.[1]

## 2 TYPES OF CURRENTLY LICENSED ANTIVIRAL VACCINES

1. *Live viral vaccines*. Live virus vaccines are prepared from viral strains that have been attenuated, but retain their ability to replicate in the human host and thus their ability to induce protective immune responses.[5] Out of the 15 viruses against which antiviral vaccines are currently licensed in the United States, nine are live attenuated (Table 15.1). There are several immunological advantages for utilizing the live attenuated antiviral vaccine platform; (1) the replication of the attenuated vaccine strains in host cells allows for the potential activation of antigen-specific $CD8^+$ T-cell responses; (2) the potential of eliciting a mucosal immune response (eg, IgA), where the portal of entry for many viruses resides. Several methods have been used to attenuate virus strains in order to be safely used as human vaccines. One method depended on the use of viral strains that are specific to a different host as vaccine strain. The oldest example of such strategy is the use of cowpox virus to vaccinate humans against smallpox.[6] Another strategy relied on attenuation of the virus by passaging it in unnatural host or cells. Examples of this approach are the development of 17D, the yellow fever vaccine strain and polioviruses.[7] Introducing the virus via unnatural route is a strategy used to develop adenovirus Types 4 and 7 vaccine, which is given orally.[8] Finally, generation of temperature sensitive mutants such as the live attenuated influenza vaccines.[9]
2. *Inactivated whole viral vaccines*. Whole inactivated virus preparations are prepared by simply inactivating viral particles by heat, UV irradiation or by special chemical treatments. Formalin and beta-propiolactone are the most commonly used chemicals for this purpose. Vaccines against polioviruses and influenza were among the first to be prepared using this strategy.[10,11] Immunogenicity of these viral preparations is usually robust as they contain

**TABLE 15.1** List of the Various Characteristics of Currently Licensed Antiviral Vaccines in the United States[a]

| Virus | Number of serotypes included per disease | Platform | Adjuvant | Route of administration | Test used to measure the correlate of protection | Trade name |
|---|---|---|---|---|---|---|
| Adenovirus | 2 (Types 4 and 7) | Live attenuated | No | Oral | Neutralization | No trade name, Barr Labs |
| Hepatitis A | 1 | Inactivated | Aluminum salts | Intramuscular | ELISA | Havrix, GSK |
| | 1 | | | | | VAQTA, Merck |
| Hepatitis A | 1 | Inactivated | | | | Twinrix, GSK |
| Hepatitis B | 1 | VLP | | | | |
| Hepatitis B | 1 | VLP | Aluminum salts | Intramuscular | | Recombivax HB, Merck |
| | 1 | | | | | Engerix-B, GSK |
| Papillomavirus | 4 (Types 6, 11, 16, 18) | VLP | Aluminum salts | Intramuscular | | Gardasil, Merck |
| | 9 | | | | | Gardasil 9, Merck |
| | 2 (Types 16 and 18) | | AS04 | | | Cervarix, GSK |
| Influenza | 1 (2009 pH1N1) | Split | No | Intramuscular | HAI[b] | No trade name, CSL |
| | | Live attenuated | No | Intranasal | | No trade name, MedImmune |
| | | Split | No | Intramuscular | | No trade name, ID Biomedical |
| | | Subunit | No | | | No trade name, Novartis |
| | | Split | No | | | No trade name, Sanofi Pasteur |

(*Continued*)

**TABLE 15.1** List of the Various Characteristics of Currently Licensed Antiviral Vaccines in the United States[a] *(cont.)*

| Virus | Number of serotypes included per disease | Platform | Adjuvant | Route of administration | Test used to measure the correlate of protection | Trade name |
|---|---|---|---|---|---|---|
| | 1 (H5N1) | Split | No | | | No trade name, Sanofi Pasteur |
| | | Split | AS03 | | | No trade name, ID Biomedical |
| | 3 (H1N1, H3N2, and type B) | Subunit | MF59 | | | FLUAD, Novartis |
| | | Split | No | | | Afluria, CSL |
| | | Split | No | | | FluLaval, ID Biomedical |
| | | Live attenuated | No | Intranasal | | FluMist, MedImmune |
| | | Split | No | Intramuscular | | Fluarix, GSK |
| | | Subunit | No | Intramuscular | | Fluvirin, Novartis |
| | | Subunit | No | Intramuscular | | Agriflu, Novartis |
| | | Split | No | Intramuscular or Intradermal | | Fluzone, Sanofi Pasteur |
| | | Subunit | No | Intramuscular | | Flucelvax, Novartis |
| | | Recombinant | No | Intramuscular | | Flublok, Protein Sciences |
| | 4 (H1N1, H3N2, and two type B strains) | Live attenuated | No | Intranasal | | FluMist Quadrivalent, MedImmune |
| | | Split | No | Intramuscular | | Fluarix Quadrivalent, GSK |
| | | Split | No | Intramuscular | | Fluzone Quadrivalent, Sanofi Pasteur |
| | | Split | No | Intramuscular | | FluLaval Quadrivalent, ID Biomedical |

| | | | | | | |
|---|---|---|---|---|---|---|
| Japanese Encephalitis | 1 | Inactivated | Aluminum salts | Intramuscular | Neutralization | Ixiaro, Intercell Biomed |
| | | | No | Subcutaneous | | JE-Vax, BIKEN-Osaka |
| Measles and mumps[c] | 1 | Live attenuated | No | Subcutaneous | Neutralization | M-M-Vax, Merck |
| Measles, mumps, and rubella | 1 | Live attenuated | No | Subcutaneous | Neutralization (measles and mumps) Immunoprecipitation (rubella) | M-M-R II, Merck |
| Measles, mumps, rubella, and varicella | 1 | Live attenuated | No | Subcutaneous | Neutralization (measles and mumps) Immunoprecipitation (rubella) FAMA gp ELISA (varicella) | ProQuad, Merck |
| Poliovirus | 3 (Types 1, 2, 3) | Inactivated | No | Intramuscular or Subcutaneous | Neutralization | IPOL, Sanofi Pasteur |
| Rabies | 1 | Inactivated | No | Intramuscular | | Imovax, Sanofi Pasteur |
| | 1 | | | | | RabAvert, Novartis |
| Rotavirus | 1 | Live attenuated | No | Oral | Serum IgA | ROTARIX, GSK |
| | 5 [G1, G2, G3, G4, and P1A(8)] | Live attenuated | No | Oral | | Rotateq, Merck |
| Smallpox | 1 | Live attenuated | No | Percutaneous | Neutralization | ACAM2000, Sanofi Pasteur |
| Varicella | 1 | Live attenuated | No | Subcutaneous | FAMA gp ELISA | Varivax, Merck |
| Yellow fever | 1 | Live attenuated | No | Subcutaneous | Neutralization | YF-Vax, Sanofi Pasteur |
| Zoster | 1 | Live attenuated | No | Subcutaneous | CD4 T cell Lymphoproliferation | Zostavax, Merck |

[a]*Vaccines that have been licensed, but their production has been discontinued are not included.*
[b]*HAI stands for hemagglutination inhibition assay.*
[c]*Measles, mumps, and rubella are also licensed to be used in combination with other antibacterial and antipoliovirus vaccines under different trade names that are not included in this table.*

multiple pathogen-associated molecular patterns (PAMPs) that could engage several of the host innate immune receptors such as the toll-like receptors (TLRs).[12] For polio, an incident of incomplete inactivation of the vaccine preparation resulted in an outbreak of paralytic poliomyelitis in the United States, the so-called "Cutter Incident."[13,14] Hence, safety of such preparations has always been a concern.

3. *Subunit vaccines*. Due to the increased risk of reactogenicity associated with whole inactivated virus vaccine preparations, purified preparations that contain the main targets of protective immune responses were developed.[15] Subunit vaccines that contain the surface glycoproteins of influenza and hepatitis B viruses are currently licensed (Table 15.1). Subunit vaccines show an improved reactogenicity profile compared to whole inactivated virus preparations, but this is usually at the expense of the immunogenicity of the vaccine. When administered with adjuvants, immune responses to these vaccines can be significantly enhanced.[16]
4. *Recombinant viral proteins*. The advance in methods of protein manufacturing made it possible to express desired viral proteins on a large scale to be used as vaccine antigens. Bacterial, yeast, insect, and mammalian cell lines have been used for this purpose.[17] A recombinant vaccine that contains the main surface glycoprotein of influenza viruses, the hemagglutinin or HA, Flublok,[18] has recently been licensed in the United States (Table 15.1). As discussed later in the chapter, some recombinant viral proteins such as the surface antigen of hepatitis B viruses tend to form virus-like particles upon expression.
5. *Virus-like particles (VLPs)*. VLPs are multimeric structures assembled from viral structural proteins. They often display viral surface proteins in a high-density repetitive manner on their surface, which may play a role in the enhanced immunogenicity observed with this kind of vaccines compared to recombinant viral proteins.[19–22] In 1986, the first antiviral VLP vaccine (against hepatitis B) had been licensed.[23] The vaccine is based on the hepatitis B surface antigen or HBsAg, which upon expression in yeast forms spherical VLPs that are then adsorbed onto alum as adjuvant. Recently, another antiviral VLP vaccine against human papillomavirus has been licensed.[22]

## 3 HOW ANTIVIRAL VACCINES MEDIATE PROTECTION?

Viral infections can be broadly classified into three main categories depending on the nature of the infection:

1. Acute infections caused by antigenically stable viruses. Infection with- or vaccination against such viruses provides a lifelong immunity to clinical reinfection. Examples of such viruses include smallpox, yellow fever, measles, mumps, rubella, and polio. Developing effective vaccines against these viruses has been relatively a straightforward process.

2. Acute infections caused by rapidly mutating viruses. The immunity acquired against such viruses through infection or vaccination is usually short-lived because of the antigenic changes, and recurrent immunization is often required. The clearest example for such viruses is influenza.[24]
3. Chronic infections caused by rapidly mutating viruses. HIV and HCV are prime examples for such viruses. Developing vaccines against such viruses have proved to be a very daunting task.[25–27]

Two main effector arms of the adaptive immune response that are induced by antiviral vaccines mediate protection against viral infections: antibodies and T cells.[1,28] While we will briefly discuss these two arms later in the chapter, it is important to understand that other immune effectors such as cytokines secreted by innate immune cells activated by the vaccine itself or by coadministered adjuvants could also directly contribute to controlling the viral burden. Also, the initial innate immune recognition of the vaccines/adjuvants is essential not only for triggering the adaptive immune responses, but also for determining the quality and duration of such responses.[28]

## 3.1 Antibodies

Given the speed with which most viruses replicate, possessing protective levels of preformed antibodies is the best strategy to protect against most viral infections. Therefore, a major immunological goal for antiviral vaccines is to elicit high and durable levels of antigen-specific antibodies.[29] Preferably these antibodies are induced at the portal of virus entry. To date, all human vaccines that have shown considerable success in combating viral infections depend on antibodies as the primary mediators of protection.[1] The process of generating these antibodies starts when a vaccine antigen encounters and binds to its specific B cell. In the presence of cognate CD4 T-cell help, these vaccine specific B cells start to expand.[30] Some of the activated B cells differentiate into plasmablasts whose function is to secrete an early protective wave of antigen-specific antibodies.[30] In a primary vaccination, those early antibodies are mostly IgM and bind to the vaccine antigen with a relatively low affinity. A subset of the activated B cells will continue expanding forming specially organized structures in the secondary lymph nodes known as germinal centers (GCs).[30] GCs are where vaccine-specific B cells with the highest antigen binding affinity are preferentially selected and also where the majority of antibody isotype-switching from IgM to IgG and IgA occurs.[31]

Antibodies can protect against viral infections via several ways:

1. When induced to sufficient levels, antibodies prevent infection by blocking the binding of viruses to their receptors on host cells. These are called “neutralizing” antibodies, and their target epitopes lie primarily within the surface glycoproteins of enveloped viruses or the capsid proteins of non-enveloped ones. The target epitopes of neutralizing antibodies are usually conformational.

2. Opsonization and phagocytosis of viral particles by neutralizing and non-neutralizing antibodies that bind to the surface of viral particles.
3. Lysing infected cells that express viral antigens on their surface via the complement pathway or through antibody-dependent cellular cytotoxicity (ADCC). For ADCC, cells mediating the lysis of infected cells such as natural killer (NK) cells recognize the antibody labeling infected cells via Fc receptors.[32]

The cells responsible for the maintenance of antigen-specific serum antibody levels following vaccination and infection are long-lived plasma cells. These cells are generated during the germinal center reaction and reside mainly in the bone marrow.[33–36]

### 3.2 T Cells

The main two subsets of T cells are $CD4^+$ and $CD8^+$ T cells. Through at least one of these two subsets T cells participate in the protection mediated by all antiviral vaccines. The main function of T cells is to provide help to B cells ($CD4^+$) or clear the infection ($CD8^+$), and not to prevent the infection. In contrast to antibodies that recognize epitopes in 3-dimensional conformation, T cells recognize linear peptides from the infecting agent that are expressed on MHC molecules on the surface of virus-infected cells. Some of these peptides come from viral proteins that do not exhibit extensive antigenic variation making T cells an important mechanism of protection against rapidly evolving viruses.[37] $CD4^+$ T cells contribute to antiviral vaccine effectiveness in several ways; secreting cytokines such as IFN-γ and TNF and supporting the activation of B cells and $CD8^+$ T cells (Th1); secretion of IL-4, IL-5, IL-13, and other cytokines to support B-cell activation and differentiation (Th2); triggering the formation and maintenance of the GC reaction via the expression of CD40L and secretion of IL-21 (Tfh). $CD8^+$ T cells, on the other hand, clear virus infected cells by directly killing those cells (through the release of perforins and granzymes) or indirectly by secreting inflammatory cytokines. $CD8^+$ T-cells can control viral burden and thus limit the severity of the disease. In the United States, there is currently no licensed antiviral vaccine that works solely via the induction of T cells, but they significantly contribute to the protective effect of several antiviral vaccines such as those against the measles and zoster viruses.

## 4 MODERN APPROACHES TO STUDYING IMMUNE RESPONSES INDUCED BY ANTIVIRAL VACCINES

1. *Systems vaccinology*. Systems biology is the integrative analysis of all the components involved in a complex biological process.[38] It includes the analysis of the genes (eg, transcriptomics), the molecules (eg, proteomics) and cells (eg, multiparameter flow cytometry) that were "perturbed" in the course

of a certain biological process such as an active immune response.[38] Systems vaccinology refers to the use of systems biological approaches in analyzing human immune responses to vaccination.[38] Advantages of using systems vaccinology approaches include; (1) gaining new insights about the mechanisms of antiviral vaccine-mediated immunity; (2) defining new molecular signatures triggered by the immune response to various vaccines and adjuvants; (3) the potential use of those molecular signatures as alternative correlates of protection.[38] Moreover, applying systems biology approaches highlighted the important role played by the early innate immune responses in triggering adaptive immune responses to various antiviral vaccines. This role is usually overlooked when assessing the effectiveness of such vaccines using traditional correlates of protection (Table 15.1). Analysis of the immune response to the yellow fever YF-17D vaccine was one of the earliest examples of utilizing systems vaccinology and it provided a proof of concept for such approach.[39] The approach was later applied to other antiviral vaccines such as those against influenza.[40] In summary, these exciting advances highlight the potential of systems biology to transform our understanding of not only how antiviral vaccines work, but also the mechanisms of immune regulation in general.

2. *Multiparameter flow cytometry*. The introduction of flow cytometry has revolutionized how we analyze immune responses.[41] It allowed us to examine not only the physical parameters of various immune cells, such as cell size and granularity at different states, but also the expression levels of many proteins either on the cell surface or inside the cells simultaneously. These analyses provide us with enormous insights about a variety of biological processes that an immune cell experiences such as activation, proliferation, differentiation and death when responding to a foreign antigen. At the beginning the number of fluorescent dyes (which are conjugated to antibodies so that each dye could be assigned to one molecule) that could be used simultaneously was limited to one or two. This number has dramatically expanded (up to 18) over the past two decades.[41] These advances came from the introduction of novel fluorescent dyes that provided additional excitation and emission spectra to be used. Perhaps one of the most important insights that came from flow cytometric analyses is the defining of the multiple lineages of B and T cells that are elicited by various antiviral vaccines.[42] Also, how the differentiation status and fates of these lineages change over time.

   The number of parameters that can be measured per cell has recently been expanded to more than 40 by the integration of mass spectrometry with single-cell fluidics (eg, CyTOF).[43] In CyTOF, antibodies are labeled with heavy metal ion tags instead of fluorochromes. Another major advantage of CyTOF is the elimination of signal interference resulted from spectral overlap of the various fluorescence dyes.[43] Reports using these new technologies are already revealing new insights about the complexity and interconnectedness of the different subsets of virus-specific T cells generated after infection.[44]

3. *Single-cell transcriptomics*. While the aforementioned systems approaches have provided exceptional insights on how our immune system works, some of them measure only the "average" of the response from sometimes a highly heterogeneous cell population. Gene expression analyses for example are performed using total mRNA purified from highly heterogeneous cell populations. In the latter scenario, the end result of the analysis would probably be biased toward the most abundant fraction of the heterogeneous cell population because of their larger contribution to the overall RNA content. Therefore, identifying rare subsets of cells using such technologies is arguably impossible.[42] Single-cell RNA sequencing (RNA-seq) is an important extension of the gene expression arrays technologies. It enabled us to interrogate the genome-wide expression profile of individual cell mRNA in an unbiased way. In addition, single-cell RNA-seq revealed some other transcriptional features in single cells, such as splice variants, allele-specific expression, and the potential discovery of previously uncharacterized genes.[42]
4. *Epigenetic regulation of immune responses*. Epigenetics refer to histones and DNA modifications, which regulate the access of different transcription factors and polymerases to transcriptional regulatory elements in chromatin.[45] Such modifications regulate gene expression and provide cells with a mechanism to retain acquired transcriptional programs throughout cell division. Given the essential role of epigenetics in deciding and maintaining cell fate, a huge amount of interest has recently been given to studying the role of epigenetics in immune responses to viral infections and vaccines. There are several aspects through which immune responses to antiviral vaccines could be modified via epigenetics; (1) defining the epigenetic programs associated with memory B and T cells with optimal quality; (2) directing the differentiation of immune cells into the most desired fate (eg, Th1 vs. Th2 CD4 T cells); (3) reversing an undesirable fate of antigen-specific cells (eg, rejuvenation of exhausted CD8 T cells in chronic viral infections).[45] Characterization of the gene expression and epigenetic programs associated with antiviral vaccine-induced memory B and T cells will provide further insight into the protective quality of the poised effector recall response.[45]
5. *Next generation sequencing (NGS) of the B and T cell receptor repertoires*. Next generation sequencing (also referred to as deep sequencing) has significantly impacted how we analyze many biological phenomena;[46] immune response is no exception. In regard to immune responses to antiviral vaccines, deep sequencing has affected both sides of the equation: the virus/vaccine side and the adaptive immune side. Most RNA viruses such as HIV and influenza exist as quasispecies and the introduction of deep sequencing technology has afforded us a higher resolution look at such diversity instead of analyzing individual viruses.[1] Each clonal pool of antigen-specific B or T cell share a distinct junction region that is formed at the site of the B-cell receptor (BCR) heavy or T-cell receptor (TCR) beta gene segments ligation.[47]

Interrogating the B-cell repertoire by deep sequencing allowed us to study the diversity of B-cell responses to viral infections and vaccinations.[46] By diversity here we refer to how many distinct clonal pools are participating in the B- or T-cell response to a particular vaccine. This is particularly important when analyzing responses to vaccines against highly variable viruses such as influenza and HIV. Against such viruses it is better to have a polyclonal response that is directed against several epitopes than a focused response. Moreover, tracking B-cell clonal pools that secrete antibodies of desired specificity or quality has helped in studying the ontogeny and evolution of such responses.[48] Similar analyses have been performed on the alpha and beta chain of the TCRs.[49]

6. *Generation of human monoclonal antibodies (mAbs)*. More than a 100 years ago, Emil von Behring developed passive immunotherapy using serum to treat infections, such as diphtheria and tetanus and was awarded the Nobel prize in Physiology or Medicine in 1901.[50] The advent of hybridoma technology in the mid-1970s introduced the concept of generating a monoclonal antibody with a single defined specificity.[51] Over the past two decades, tremendous efforts have gone into developing technologies to generate human mAbs. The currently most widely used methodologies to generate human mAbs are:
   a. Phage display libraries: As the name indicates, phages are designed to express single-chain variable antibody fragments (scFvs) or antigen-binding fragments (Fabs) on their surface and screened for binding to the desired antigen. The libraries are constructed from the variable genes of B cells isolated from vaccinated individuals or from convalescent patients. This method has been successfully used to generate neutralizing mAbs against many viruses including West Nile, rabies, severe acute respiratory syndrome (SARS) virus, hepatitis A, HIV, Ebola, yellow fever, hepatitis C, measles and influenza.[52] A major drawback of this method is that it cannot be used to examine the repertoire or the immunodominance hierarchy of the antigen-specific B-cell response as the antibody fragments displayed were generated by random pairing of the BCR heavy and light chains and not from a naturally existing pairing.[53]
   b. B-cell immortalization: B cells can be immortalized by Epstein–Barr virus (EBV) mediated transformation.[54] Immortalized B cells can then be stimulated to secrete antibodies and those antibodies are screened for the desired specificity. B-cell pools secreting the desired antibody are then cloned by limiting dilution into single cells and the BCR genes are sequenced. This method has been used to generate mAbs against many viruses including influenza, HIV, SARS, dengue, and RSV.[55–58] While this method is effective in isolating mAbs from rare memory B cells,[59] it is labor-intensive, as it requires the screening of thousands of immortalized memory B cells in order to isolate few mAbs with the desired specificity.

c. Single cell cloning and expression of mAbs: This is the most recent technology and also the most efficient.[60,61] In this approach, the heavy and light chain genes of single-cell sorted B cells are amplified and cloned into antibody expression vectors. Single antigen-specific B cells can be sorted by flow cytometry based on their surface phenotype (eg, sorting of plasmablasts from blood following vaccination)[62] or based on their binding to a desired antigen.

Human mAbs have expanded our understanding of human B-cell responses to viral infections. Through the generation of mAbs following various viral infection and vaccination we were able to map the viral targets of our most protective immune responses. Most importantly, they revealed some of the subdominant epitopes within viral proteins that are now being extensively examined, as discussed later in the chapter, as targets for broadly neutralizing mAbs and potential cores for new immunogens.

## 5 NEXT GENERATION VACCINE PLATFORMS

1. *Structure-based immunogen design*. As mentioned earlier, the design an immunogen to be used as an antiviral vaccine has always been an empirical process. The immunogen was picked based on its ability to elicit a detectable protective immune response. While this process was sufficient for many viruses, for some challenging viruses such as HIV and influenza, a deeper analysis of the epitopes targeted by neutralizing antibodies was needed.[63] The traditional way of determining the amino acid residues within a viral protein that are recognized—and thus mediate virus neutralization—by a certain mAb is the generation of viral escape mutants. This method was instrumental in mapping the major neutralizing epitopes within the influenza HA molecule.[64] However, this method has several drawbacks; (1) some neutralizing mAbs fail to generate escape mutants such as the influenza broadly neutralizing mAbs recognizing the HA stem region (discussed later in the chapter) and therefore could not be mapped using this approach; (2) the increased risk and logistic difficulties associated with the generation of escape mutants against certain viruses, such as the highly pathogenic avian influenza viruses and Ebola; (3) given that the majority of neutralizing mAbs recognize conformational epitopes so identifying a single or few amino acid residues that contribute to binding does not provide a complete picture; (4) a change of an amino acid residue in an escape mutant does not necessarily mean that this residue is the point of contact between the viral protein and the mAb. A change of the epitope conformation induced by the change of an adjacent amino acid residue could also be responsible for the generation of an escape mutant.

   In 1990, the first crystal structure of a viral glycoprotein-antibody complex was published.[65] Solving such structures for many viral glycoproteins has allowed us to examine the binding of antibodies to their respective

epitopes at the atomic level. More importantly, it provided the basis for rationally designing viral immunogens that could—at least theoretically—induce an immune response enriched with antibodies with a desired specificity. This idea has recently been tested with partial success in the efforts to generate targeted antibody responses to HIV, RSV, and influenza.[1] These early experiments have also revealed that more work is needed to fully understand how these complex epitopes are recognized by B cells in vivo and how to minimally design an immunogen without interrupting its stability as a protein or the antigenicity of the epitope.[1]

2. *DNA- and RNA-based vaccines*. The concept of using naked DNA as a vaccine was introduced in the early 1990s.[66] It rapidly gained traction mainly due to its simplicity and versatility. While the early clinical trials demonstrated the safety of DNA vaccines, it also revealed that they were poorly immunogenic. The immunogenicity of DNA vaccines has been improved through different methods; (1) improving the efficiency of DNA delivery to enhance the cellular uptake of the plasmid DNA; (2) the use of adjuvants either in physical form or encoded on separate plasmids; (3) optimizing the sequence of the DNA vaccine to enhance the expression and immunogenicity of the encoded protein.[66] DNA vaccines against a variety of viruses are now being tested at different stages of clinical trials.

   Advances in the methods of mRNA synthesis and stabilization have paved the way for the possibility of using mRNA as vaccine platforms.[67] The ability of mRNA to stimulate several of the innate immune receptors (eg, TLR3 and TLR7/8) gives them an intrinsic adjuvant activity. The approach has been boosted by the recent introduction of self-amplifying RNA strategy, which works by delivering the alphavirus genes encoding the RNA replication machinery along with the recombinant viral target antigen resulting in enhanced antigen expression.[67]
3. *Vector-based vaccines*. Vectored-based vaccines could be considered a type of DNA vaccines where an attenuated virus or bacterium is used to introduce microbial DNA to host cells. The most commonly used virus vectors are adenoviruses, alphaviruses, and poxviruses.[68] As for bacteria, strains belonging to *Bacillus Calmette-Guerin, Listeria monocytogenes*, and *Salmonella typhi* are being tested as vectors for human vaccines.[69]

## 6 HARNESSING THE TECHNOLOGICAL ADVANCES TO DEVELOP VACCINES AGAINST CHALLENGING AND EMERGING VIRUSES

Viral pathogens against which an effective vaccine is yet to be licensed can be broadly grouped into two categories; challenging viruses and emerging viruses. Examples for challenging viruses are HIV, HCV,[70–72] RSV, CMV,[73] HSV-2,[74,75] EBV, and dengue. For a variety of reasons, developing an effective vaccine against these viral pathogens has been a formidable task despite the tremendous

**TABLE 15.2** Some of the Antiviral Vaccine Candidates That are in Advanced Stage of Development (Phase 2 or Beyond)[a]

| Virus | Name of vaccine candidate | Manufacturer or sponsor | Development phase | References |
|---|---|---|---|---|
| HCV | Ad6NSmut | GSK | Phase 1/2 | [70–72] |
| | TG4040 | Transgene | Phase 2 | |
| | GI-5005 | GlobeImmune | Phase 2 | |
| CMV | ASP-0113 | Astellas Pharma | Phase 3 | [73] |
| SV-2 | GEN-003 | Genocea Biosciences | Phase 2 | [74] |
| | HerpV | Agenus | Phase 2 | [75] |
| HIV | AGS-004 | Argos Therapeutics | Phase 2 | [76–83] |
| | HIV recombinant | GSK | Phase 2 | |
| | AIDSVAX | GeoVax | Phase 2 | |
| | Vacc-4x | Bionor Pharma | Phase 2 | |
| | VRC-hIVADV014-00-VP | GenVec/VRC | Phase 2 | |
| RSV | RSV F Protein | GSK | Phase 2 | [84,85] |
| | RSV F Nanoparticle | Novavax | Phase 3 | |
| Dengue | Dengvaxia | Sanofi Pasteur | Phase 3 (approved in Brazil) | [86,87] |
| | DENVax | Inviragen | Phase 2 | |
| Ebola | ChAd3-ZEBOV | GSK/PHAC | Phase 2/3 | [89] |
| | VSV-EBOV | New Link Genetics/Merck | Phase 2/3 | |
| Norovirus | G1-I/GII-4 VLP | Takeda Vaccines | Phase 2 | [90] |

[a]*This list is not exhaustive. For example, it does not include vaccine candidates for viruses against which successful vaccines have already been licensed such as influenza, HPV, Zoster, and rabies.*

efforts. Great amounts of resources have gone into developing a vaccine against HIV,[76–83] but this mission has proved to be the most arduous so far (challenges and prospects are discussed later in the chapter). As for influenza, effective vaccines against seasonal and potentially pandemic influenza virus strains have been licensed. However, these vaccines (as discussed later in the chapter) do not offer broad protection against these rapidly evolving viruses. Vaccine candidates against RSV[84,85] and dengue[86,87] have now entered advanced stages of clinical testing (Table 15.2).

Emerging viral pathogens include Ebola, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), hendra, nipah, Marburg, chikungunya, lassa, Crimean-Congo hemorrhagic fever, and zika viruses. Infections with most of these viruses are limited to certain endemic areas, which in turn make the decision of developing a vaccine against such viruses not an economically favorable one. However, in the wake of the 2014 massive Ebola outbreak that ravaged West Africa this perception could change. For Ebola, recent studies suggest that robust immune responses could be detected in convalescent patients,[88] indicating that developing a protective vaccine against this pathogen is doable. Indeed, many vaccine candidates have shown promising results in clinical trials.[89] Two of these candidates are ready for Phase 3 testing.[89] Other antiviral vaccines that are in advanced stages of clinical testing include vaccines developed against CMV and norovirus.[73,90] Later in the chapter we will discuss the challenges facing developing a vaccine against HIV, influenza (universal), and RSV, and how recent technological advances could help in overcoming such challenges.

## 6.1 The HIV Challenge

Efforts to develop a vaccine against HIV started in the mid-1980s[91] and the fact that there is still no licensed vaccine yet despite the plethora of resources invested shows the enormity of the task. The challenges that impede developing a vaccine against HIV stem from the following points:[92–94]

1. Like most RNA viruses, HIV viruses continually mutate and evolve leading to the emergence of new variants even within an infected individual. This necessitates that for any vaccine to be successful, it has to elicit an immune response with enough breadth to protect against such extensive diversity.
2. The correlates of protection against HIV infection are not well established. A common factor for viruses against which a vaccine has successfully been developed is that we know which immune effector mediates protection. Correlates of protection are usually defined by analyzing immune responses in individuals who have recovered from infection or showed less susceptibility to such infection. Complete recovery from HIV infection is not common occurrence, if at all. This is at least partially because the virus infects $CD4^+$ T cells, which orchestrate the two arms of adaptive immune responses: B cells and $CD8^+$ T cells.
3. There is a knowledge gap in regard to which protein/portion of the viral proteins is the most antigenic and immunogenic and thus best suited as a vaccine antigen. Also, whether a specific structural conformation is required for such protein to elicit a protective immune response is not clear.

The disappointing results of the early vaccines that were designed to solely induce $CD8^+$ T-cell responses [1] has refocused anti-HIV vaccine efforts on generating protective broadly neutralizing antibody responses. This notion was augmented by the modest success of the RV144 HIV vaccination trial conducted in

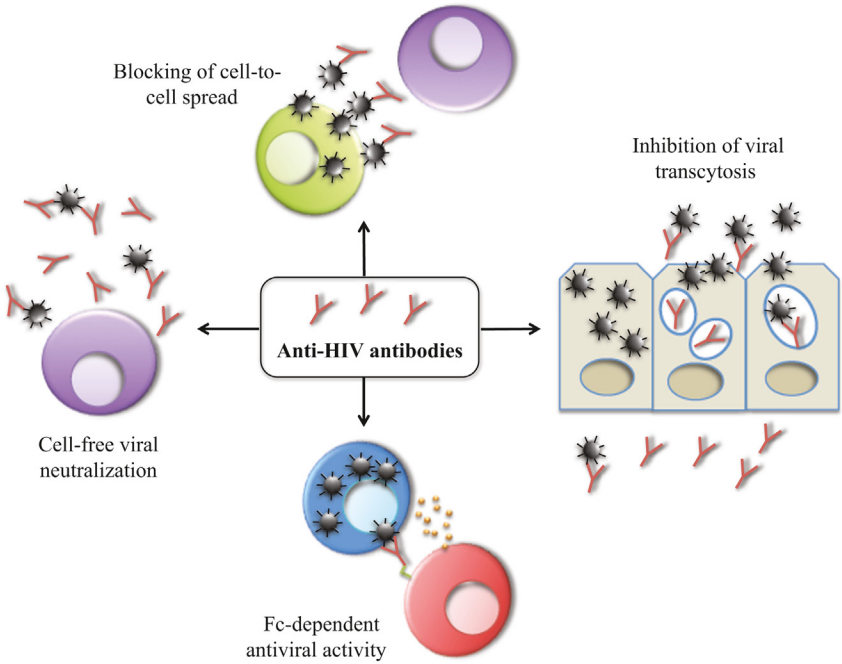



**FIGURE 15.1 Potential mechanisms of neutralizing antibody-mediated protection against HIV.** There are at least four different mechanisms by which anti-HIV neutralizing antibodies could block the virus. First, they can block the initial attachment of the virus to its receptor/coreceptor on the target cells. Second, they can block viral spread through cell-to-cell transmission. Third, they can aid in the clearance of infected cells through Fc-dependent mechanisms such as antibody-dependent cell-mediated cytotoxicity. Fourth, anti-HIV antibodies could inhibit the passage of HIV-1 from the lumen to the basolateral pole (HIV-1 transcytosis). *(Source: Adapted from Ref. [97].)*

Thailand in which subjects were primed with a replication-defective canarypox viral vector expressing HIV gp120, gag, and pol proteins, and then later boosted with the gp120 protein.[95] The rate of HIV infection among subjects who received the experimental vaccine was 30% lower than that in volunteers who received the placebo.[95] A correlation of protection with the antibody response to the V2 region of the HIV envelope protein was later established.[96]

In vivo, anti-HIV antibodies could protect in several ways; (1) neutralize cell-free virions; (2) block cell-to-cell transmission; (3) clear infected cells by antibody-dependent cell-mediated cytotoxicity or ADCC; (4) block transcytosis of the virions from the lumen to the basolateral side of mucosal cells (Fig. 15.1).[97] The RV144 trial observations resulted in a sharp increase in the number of broadly neutralizing anti-HIV human mAbs that are being isolated and characterized.[98–101] Sequence analysis of these antibodies and structural examination of their interactions with HIV antigens at the atomic level have strengthened our understanding of how these broadly neutralizing antibodies

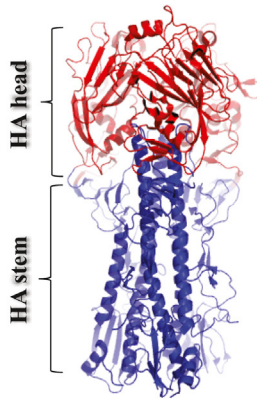
work. For example, we now know that broadly neutralizing antibodies are more likely to have either an unusually long CDR3 loop or an extremely high rate of somatic hypermutations.[102] Extensive research efforts are currently focused on learning how to elicit such antibodies by vaccination.[103] This target is being pursued from different angles:

1. The use of structurally inspired immunogens that mimic the epitopes of the broadly neutralizing mAbs. The hope is to get a B-cell immune response that is dominated by such antibodies. Early trials indicate that additional structural requirements may be needed for the desired epitopes to be recognized in vivo other than just presenting the epitopes in the right conformation.[104]
2. Possible need for a special immunization strategy that depends on sequentially exposing the immune system to different, but closely related HIV envelope proteins. The idea is to mimic how the immune system of chronically HIV-infected individuals experiences a rapidly evolving virus and ends up developing broadly neutralizing antibodies in a subset of them. Extensive deep sequencing analysis of the ontogeny of some of the broadly neutralizing antibodies has allowed us to dissect the affinity maturation steps required to attain such levels of reactivity.
3. Examining the evolution of the viral genome in infected individuals by deep sequencing. This approach would help us define the characteristics of the immunogens that should be used in the sequential immunization strategy discussed earlier.[1]

In summary, new technological advances have taught us that; (1); HIV exists as a swarm of viruses and evolves rapidly within infected individuals (deep sequencing analysis); (2) our immune system is capable of generating very potent and broadly neutralizing mAbs that could combat such viral diversity (generation human mAbs); and (3) we can design new immunogens to enrich B cell responses to HIV vaccines with the broadly neutralizing antibodies (structure-based immunogen design).

## 6.2 The Influenza Puzzle

The earliest trials to develop an influenza vaccine date back to the 1940s, shortly after the isolation of the first human influenza virus in 1933.[105] It was an inactivated whole virus preparation developed by the US military to be used in World War II.[10] Although these early vaccines were protective, controlling infections mediated by this respiratory pathogen continues to represent a formidable challenge. The dilemma stems from the ever-evolving nature of influenza viruses, which enables the viruses to escape preexisting immune surveillance.[106] Moreover, effective vaccines against influenza viruses work by eliciting antibody responses that primarily target the globular head of the HA, which is the most variable among virus proteins.[106] Therefore, it has been necessary to perform an annual revision of the antigens included in human seasonal influenza vaccines




| Epitope variability | Escape variants | HAI activity | Neutralization potency | Breadth of protection |
|---|---|---|---|---|
| High | High frequency | Yes | Strong (with few exceptions) | Narrow, with few exceptions |
| Low | Very low frequency | No | Moderate | Wide |

**FIGURE 15.2 Features of the epitopes within influenza HA globular head versus stem regions.** A structural view of influenza HA trimer with a listing of the features characterizing the epitopes within the HA globular head region versus those in the stem region.

to ensure that they match the circulating influenza strains. Currently, seasonal influenza vaccines include antigens from three (H1N1, H3N2, and an influenza B) or four (H1N1, H3N2, and two influenza B) human influenza virus strains. The need for a broadly protective influenza vaccine is clearly demonstrated by the occasional failure of these seasonal influenza vaccines to control the annual epidemics of influenza viruses, which result in about 3–5 million cases of severe illness, and up to 500,000 deaths worldwide.[106] In a more serious scenario, influenza viruses cause occasional pandemics when an antigenically novel virus spills into the human population or when, as in the case of the 2009 pandemic H1N1 virus, an influenza virus that has ceased to circulate among humans for decades reemerges.

The major neutralizing epitopes are located around the receptor-binding domain within the influenza HA globular head region (Fig. 15.2).[107] While antibodies targeting such epitopes are protective, they are mostly strain-specific and lack the broad neutralizing activity required to protect against different influenza subtypes. Structural analysis of a number of the recently isolated broadly neutralizing human HA-specific mAbs revealed that they bound to a conserved domain within the HA stem region (Fig. 15.2).[108–111] These mAbs were isolated by phage display libraries or by direct cloning of single cell sorted plasmablasts following influenza infection or vaccination. Unlike the case for HIV, influenza broadly neutralizing antibodies could be detected following infection and vaccination.[112] Therefore, it remains puzzling that despite being repeatedly exposed to such conserved domains of influenza HA either in the form of vaccination or natural infection that influenza remains a serious public health problem. One possible—among many—explanation for this puzzle is that the concentration of HA stem-specific antibodies is too low to prevent infection.[106]

This notion is supported by the observation that mAbs targeting the stem region are weaker in general than those targeting the head epitopes in terms of in vitro neutralization potency.

The question then is how to boost stem-specific responses to levels that are protective in vivo. Recent data suggest that conventional seasonal vaccines induced B-cell responses are dominated by those targeting the HA head region.[112] Therefore, new immunogens would be needed to refocus the responses on those targeting the HA stem region. The use of immunogens comprised of globular head region HA molecules derived from viruses which have not been widely circulating in the human population, such as H5N1 avian influenza viruses, combined with a stem region that is conserved among the strains can change the dominant immune response from head focused to stem focused.[112] The globular head region of H5 is significantly different from the circulating human viruses while the stem region is largely conserved. The idea is to engage stem-specific memory B cells with minimal interference of the head specific ones. While this strategy did indeed succeed in boosting the stem-specific responses, questions regarding whether this boost is enough to afford protection in vivo are yet to be addressed.[112] In addition, further boosting with H5 in the aforementioned trial restored the globular head immunodominance of B-cell responses suggesting that the globular head region might be intrinsically more immunogenic than its stem counterpart. Similar to HIV, efforts are now focused on designing structure-based immunogens that present the broadly neutralizing epitopes (those within the HA stem and the within the receptor-binding domain) to the immune system in the most relevant conformation.[113–116]

## 6.3 The Quest for a Vaccine Against RSV

As discussed earlier, the gold standard for designing a successful antiviral vaccine is to mimic natural infection. This scenario could not be applied in the case for RSV because natural RSV infection provides limited protection from reinfection.[117,118] Therefore, developing a protective vaccine against RSV has been a daunting task.[119] It has been a global public health priority for over 5 decades.[120,121] While antigenic variability is a major obstacle in developing a broadly protective vaccine against HIV and influenza, there are only two serotypes of RSV and cross-reactive antibodies could be readily detected in human sera.[1] The major hurdles that hindered the development of a licensed vaccine against RSV are:

1. Vaccine-enhanced disease: This phenomenon was first noted when a formalin-inactivated vaccine candidate (FI-RSV) was developed and tested in infants and children in the late 1960s and the immunogenicity results were promising.[118,122] However, upon natural RSV infection, 80% of the FI-RSV-vaccinated subjects were hospitalized, whereas only 5% of the control group required admission, and two children died.[118,122]

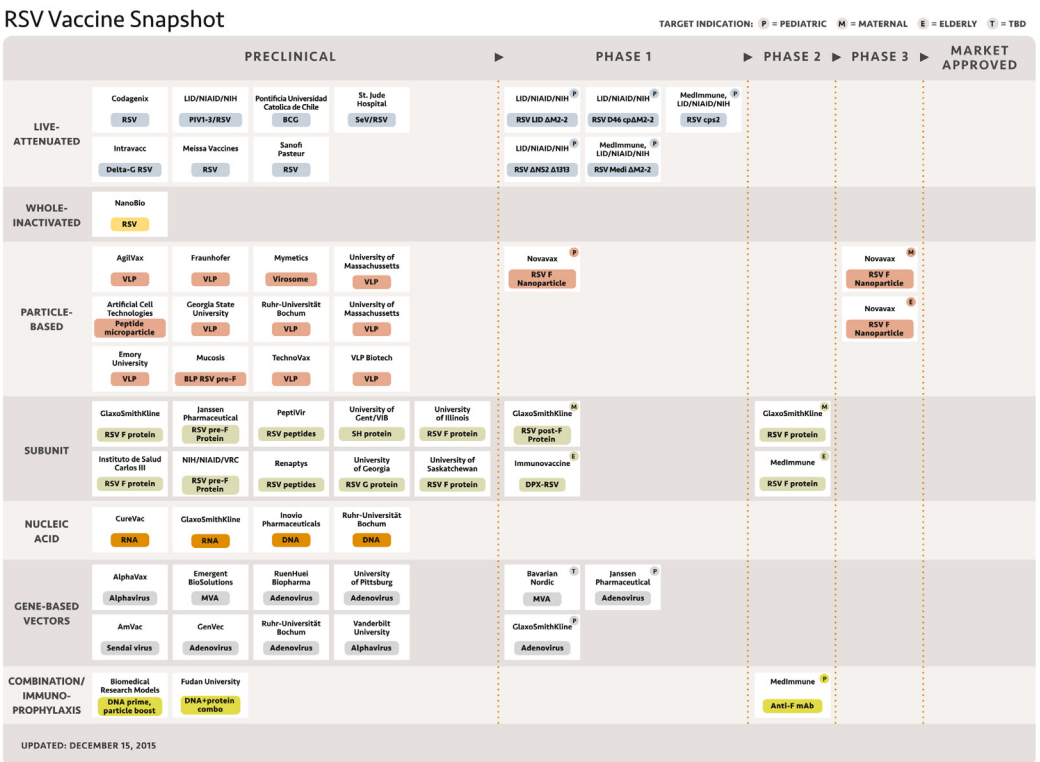



**FIGURE 15.3** **RSV vaccines currently in pipeline.** *(Source: This RSV vaccine snapshot is reproduced with permission from PATH and can be found at http://sites.path.org/vaccinedevelopment/respiratory-syncytial-virus-rsv/.)*

2. The formidable task of getting the right balance between vaccine safety and immunogenicity/efficacy especially in the most vulnerable and highest priority target population, infants.[1]
3. The lack of ideal animal models making clinical trails—which are expensive and time consuming—an imperative measure to assess candidate vaccines. Additionally, the clinical endpoints in evaluating an RSV vaccine efficacy are not very specific as the disease symptoms are shared with many other respiratory viral infections.[1]

Based on the epidemiology and burden of RSV disease there are four target populations for RSV vaccination; (1) infants (<6 months); (2) children (6–24 months); (3) pregnant women; (4) the elderly.[117,118] Each of these populations presents its own challenges in terms of how their immune systems respond to various vaccine candidates. There are several types of RSV vaccines that have been developed and many others are in the pipeline (Fig. 15.3). Many of these vaccines are still in the preclinical stage. Four types of vaccine platforms are being tested in Phase 1, 2, or 3 trials (Fig. 15.3); live attenuated virus vaccines; particle-based vaccines (VLPs); subunit vaccines; and vector-based vaccines.[123] Each of these vaccine platforms has advantages and disadvantages,[117,118] but they mostly share that they focus on eliciting antibody response to RSV F glycoprotein.[123] Palivizumab, which is a humanized mAb specific to the F glycoprotein, is a proof of principle that neutralizing antibodies could provide protection against hospitalization.[124,125] Palivizumab is licensed to be used in infants at high risk of severe disease.[124,125]

Recently, the structural insights gained by solving the atomic structure of the pre- and postfusion forms of the F proteins have invigorated RSV vaccine development efforts.[126–130] We now know that many of the neutralizing F-specific antibodies that did not bind the postfusion form of the F protein are actually specific to the prefusion form. Moreover, a novel antigenic site, termed Ø, was revealed. Antibodies targeting this epitope show a more potent neutralization capacity compared to palivizumab.[1] The efforts are now focused on preparing physically stable vaccine candidates comprised of the native F protein trimer in its prefusion form.[131]

## 7 SUMMARY

There are multiple factors that contribute to the lack of a licensed protective vaccine against any particular virus. Most of these factors originate from the nature of the virus itself. Examples include: (1) viruses that exhibit extensive genetic variations (eg, influenza); (2) viruses that evolved multiple mechanisms for evading host immune detection and response (eg, HCV and HSV); (3) viruses that integrate their genomes in that of the host (eg, HIV); (4) viruses that are capable of developing latency (HSV). Some factors stem from the inability of the host to protect against infection (and reinfection) due to the inadequacy of the immune response (eg, RSV). Other factors that are unrelated to both virus and

host include the lack of animal models that are suitable for evaluating the efficacy of vaccine candidates and economical considerations. Early antiviral vaccine success stories (eg, smallpox, rabies, and yellow fever) were based on empirical efforts in which vaccines were derived from either a live attenuated form of the virus or inactivated whole virus. It is clear that such approaches are not enough to address the current challenges. Developing an effective vaccine against many of the currently challenging viruses would require an integrative effort from scientists representing various disciplines. For example, public health studies would be needed to assess the disease burden in different populations in order to define the most vulnerable ones. Virological studies would be important for generating viral vaccine strains that lack immune evasion capacity, for example. Immunologists would define which arms of the immune response are needed to achieve protection. Bioinformaticians would need to work very closely with immunologists in order to transform high-throughput data and analyses into concrete, useful knowledge. Structural analyses would help in identifying important immune targets and how to rationally design immunogens that elicit maximal immune responses to such targets. Vaccinologists would then assess vaccine candidates in preclinical and clinical trials. Finally, engaging the pharmaceutical industry would be essential for scaling up the production of the final product.

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Chapter 16

# New Approaches for Needed Vaccines: Bacteria

Calman A. MacLennan, DPhil, FRCP, FRCPath*, Ankur Mutreja, PhD**, Gordon Dougan, DPhil, MA, FRS†

**University of Oxford, The Jenner Institute, Nuffield Department of Medicine, Oxford, United Kingdom; **The Hilleman Laboratories, New Delhi, Delhi, India; †The Wellcome Trust Sanger Institute, The Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom*

## Chapter Outline



## 1 BACKGROUND

Bacteria are still one of the most common causes of infection, associated with a range of different diseases in humans and important veterinary animals. The emergence of antibiotic resistant microbes (ARM) means that the threat bacteria pose to human health is unlikely to diminish in the near future. Thus, the design of new or improved bacterial vaccines remains high on the global health agenda, particularly as we still lack effective vaccines for many bacterial disease classes. Bacteria are relatively complex life forms in comparison to viruses as they harbor genes for thousands of proteins that provide structure, support life, and drive the synthesis of complex life-associated molecules [eg, lipopolysaccharide (LPS), capsules, etc.]. Nevertheless, individual disease-associated bacteria have been successfully targeted by vaccination. Indeed, Pasteur targeted bacterial diseases such as anthrax in his very early work on vaccine development.

Since those early pioneering days, a variety of bacterial vaccines have been developed based around different formulations. Many early vaccines, for example, typhoid and cholera, were composed of "killed" whole bacterial cells that

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were delivered parenterally by injection. Such vaccines tended to be of moderate efficacy and were quite reactogenic, in part because of the many innate toxins and immune stimulators that whole bacterial cells harbor. Perhaps the only vaccine of this type still in common use is the whole-cell pertussis vaccine based on inactivated *Bordetella pertussis* bacteria.[1–3] Killed whole bacterial cell vaccines have also been developed for oral use against enteric infections such as cholera and enterotoxigenic *Escherichia coli* (ETEC).[4] Again these vaccines tend to have moderate efficacy, although reactogenicity is less of an issue and they have found some utility both within disease endemic regions and for travelers to such regions.[5] Additionally, live whole cell vaccines have been developed that are based upon attenuated vaccine strains, such as Bacille de Calmette et Guérin (BCG), that can be delivered orally or by injection. The only live attenuated bacterial vaccine still finding broader utility is BCG, although more niche typhoid (Ty21a) and cholera vaccines are available in some regions.[6,7]

The recognition that some bacterial diseases are toxin-driven facilitated the development of early toxoid-based vaccines for diphtheria and tetanus. These vaccines are now cornerstones of the global children's vaccine program, even though the technology used to produce them is relatively crude by modern standards. The success of the toxoid approach stimulated a drive to replace some whole cell vaccines (eg, cholera and pertussis) with toxoid formulations. A good modern example was the development of acellular pertussis vaccines built around toxoided pertussis toxin[8] and several other defined *B. pertussis* surface proteins (eg, pertactin, filamentous hemagglutinin).[9,10] The development of these vaccines proved the principle of moving from whole cell to acellular bacterial vaccines based on defined antigen mixes. Other examples have followed including the recently licensed meningitis vaccine Bexsero (4CMenB), based on a combination of *Neisseria* proteins.[11] Hence, in line with other vaccines, there is a trend toward bacterial vaccines of defined antigenic composition or known mechanism of attenuation, facilitating a drive toward better quality and a stronger safety profile.[12]

Over the past decades investigations into how bacteria cause disease have intensified and many key "pathogenic mechanisms" have been defined and the proteins and other molecules that contribute to infection identified. Such studies on the molecular basis of infection/pathogenesis can contribute to vaccine design and the selection of antigens for vaccine development.[13] Thus, the molecular toolbox for vaccine development has expanded dramatically giving rise to opportunities for new vaccination approaches based on functional genomics and structural biology.[14,15] These approaches are also incentivizing interest in bacterial targets that were previously regarded as intractable or challenging.

Surface polysaccharides play a key role in the ability of pathogenic bacteria to defend themselves against attack from the host immune system. Consequently, there was an early recognition that such polysaccharides could make attractive vaccine candidates. Many polysaccharides are relatively poor immunogens and most are so-called T-cell independent antigens that are poor stimulators of T-cell

immunity. Consequently such antigens are poorly immunogenic in infants, do not effectively induce antibody affinity-maturation, efficient antibody class switching or effective immune memory. Fortunately such T-cell-independent antigens can be converted to T-cell-dependency by conjugating them to carrier molecules, normally globular proteins.[16] Such conjugates have found broad utility in the vaccine industry and now many classes of bacterial vaccines are being built using the conjugation approach.[17,18]

The first conjugate vaccine of this type to be licensed was against *Haemophilus influenzae* type B and now conjugate vaccines against *Streptococcus pneumoniae* and *Neisseria meningitidis* have been successfully launched (see other chapters in this book). A complication of the conjugate approach is that a variety of different antigenically distinct polysaccharides can be found on different clades of the same bacteria causing the same disease (eg, *S. pneumoniae*) and consequently multiple conjugates have to be formulated into the same (now multivalent) vaccine.[19] Also, only a limited number of proteins have been used as carriers in licensed vaccines. These include tetanus toxoid and a mutant diphtheria toxin known as $CRM_{197}$ (cross reactive material 197).[20] These carriers are regarded as "heterologous" in the sense that they are derived from different bacteria than the target polysaccharide antigen. It may be advantageous to use "homologous" antigens from the same bacteria in certain circumstances so searching for new carrier proteins is an ongoing endeavor. Such homologous antigens have the potential to induce pathogen-specific T cells.

## 2 GAPS AND TARGETS

Despite the success of these approaches there are still significant challenges remaining in the field of bacterial vaccines. We do not have any licensed vaccines against a range of important pathogens including *Treponema pallidum* (syphilis), *Chlamydia*, *Shigella*, *Klebsiella* and these are urgently needed as some are developing antibiotic resistance. Also, there is evidence that some pathogens, including *B. pertussis* and *S. pneumoniae* may be escaping vaccine-induced immunity.[21,22] Further, the evolution of bacterial disease is dynamic and rapid, so we can anticipate that the epidemiology of such infections will change over time. This is in part driven by the use of antibiotics and the aging human population, where increasing numbers of immunosenescent individuals present fresh challenges to vaccine developers.

A major gap exists in the area of sexually transmitted infections, where we currently have no vaccines against the more common bacterial diseases. Antigenic variation has presented a challenge to vaccine development against *Neisseria gonorrhoea* with early attempts to develop vaccines based on fimbriae/pili having little success.[23] Vaccines against *T. pallidum* and *Chlamydia* should be feasible but to date progress has been hampered by a variety of challenges, including the fastidious nature of these pathogens and the lack of good animal models.[24] However, the dramatic breakthrough made in the area of papilloma

virus vaccines indicates that the problem is tractable.[25] Another area of challenge is the healthcare associated pathogens. Here a variety of infections are emerging ranging from multiply antibiotic resistant *Staphylococci* through to *Clostridium difficile*.[26,27] Many of these infections are associated with immunocompromised or aging individuals and any vaccine development against such infections must take this factor into account. We also lack effective vaccines against many of the bacteria that cause enteric infections and here a number of approaches, including the development of live oral vaccines, have met with mixed success.[28] New vaccines would also be desirable against *Mycobacterium tuberculosis* and other *Mycobacteria* associated with infections. Here, intensive investigations are underway.[29]

## 3 CLASSICAL APPROACHES FOR MAKING BACTERIAL VACCINES

One of the challenges associated with generating vaccines against bacterial pathogens is to identify potentially protective antigens among the thousands of proteins and other antigenic molecules the bacteria can produce. In the premolecular era vaccine developers either exploited whole bacterial cells or focused their attention on a limited number of tractable antigens that could be identified by simple serological or biochemical assays. The main targets that emerged from such studies were either immunogenic surface oligosaccharides or polysaccharides that could be readily purified or toxins identified as significant drivers of disease pathology.[30]

Despite the limitations of these approaches, considerable progress was made in terms of developing vaccines against many bacteria. We have mentioned the early successes with diphtheria and tetanus toxins that were inactivated by chemical treatment to produce highly successful toxoid-based antigens. Toxoid extracts have remained in general use since the early development for these two diseases. Although attempts have been made to make improved vaccines based on either purified toxins or genetically engineered variants (eg, $CRM_{197}$ for diphtheria toxin or tetanus toxin fragment C), these have not been adopted for the two diseases.[20,31] However, as we entered the molecular genetic era in the 1970s efforts began to emerge to make improved bacterial vaccines based on more defined antigens.

The first real success in this area was the development of acellular pertussis vaccines, driven by claims that the whole-cell pertussis vaccine caused serious side effects in some individuals.[32] Searches were undertaken to identify potential antigens for inclusion in acellular vaccines. The primary candidate was pertussis toxin that had been shown to be one of the main drivers of disease and was a highly immunogenic antigen.[33] Pertussis toxin has a classical AB bacterial toxin structure and toxoided versions of the toxin were created that were both immunogenic and induced the production of toxin-neutralizing antibodies.[8] In addition, genetically modified versions of the toxin were engineered

using site-directed mutagenesis and these were also considered as vaccine candidates.[34] Several other protein antigens, including the adhesins pertactin and filamentous hemagglutinin, were also developed as vaccines leading to the generation of a series of acellular vaccines that were evaluated in clinical trials in different countries.[35,36] Eventually blends of these antigens were found to have reasonable efficacy in children and acellular vaccines were gradually licensed across the world over the following decade. Thus, acellular pertussis vaccines became the first of a new type of bacterial vaccine based on combinations of defined bacterial proteins.

Again, as biochemistry improved, vaccine developers began to search for other antigens that might be exploitable as acellular vaccines and attention turned to the polysaccharides that were expressed at the bacterial surface as capsular materials. A series of polysaccharide based vaccines were generated against diseases including *H. influenzae* (type B), *N. meningitidis* (A, C) and *S. pneumoniae* (multiple capsular types).[37] Although these vaccines had efficacy against disease they generally induced relatively short-lived protection due to the T-cell independent nature of the antigens and they were gradually replaced with conjugate versions, many of which are now licensed vaccines.

Thus, a combination of acellular and conjugate based approaches began to fill some of the gaps in the bacterial vaccine repertoire. However, many pathogens did not produce readily tractable polysaccharide antigens or obvious toxins that could induce broad immunity. Thus, new approaches were required to start to target these remaining classes of pathogens.

## 4 THE IMPACT OF GENOMICS ON BACTERIAL VACCINE DEVELOPMENT

In 1995 the first complete genome sequence of a bacterial pathogen, *H. influenzae* Rd, was published signaling the era of bacterial genomics.[38] This genome contains 1,830,137 base pairs, in which ~1749 protein-coding genes are embedded. This remarkable achievement laid bare the full protein repertoire of this pathogenic bacterium, in the form of a simple blueprint that could be browsed to identify many previously unidentified antigens. Many of the genes were completely unknown and some encoded proteins that were only expressed when the bacteria were growing within the human body. Thus, vaccine developers could target new antigens for evaluation as vaccine candidates simply by cloning, expressing, and purifying the proteins. Over the following years, high-quality reference genomes were produced for many of the classical bacterial pathogens, some of which had no licensed vaccine against them.[39] Subsequently, within each bacterial species more and more sequences were generated for different bacteria so in addition to reference genomes, it was possible to build up maps of where variation was occurring within individual genes down to the amino acid level.[22] This facilitated the identification of antigens that were relatively conserved within the circulating bacterial population. Conserved antigens were

considered less likely to evolve under immunological pressure or escape vaccination programmes.

Additionally, by sequencing pathogen populations, it is possible to build up a picture of genes that are present in all isolates (the core genome) compared to genes that are only present in some isolates (the accessory genome).[40] The selection of antigens encoded within the core genome has the advantage that a vaccine based on a core antigen should target all bacteria within a population. By selecting antigens from the accessory genome only those members of the population that express the antigen will be targeted.[41] This might be an advantage if, for example, a pathogenic clade was being targeted while sparing nonpathogenic members of the population. For example, in *E. coli* an antigen from the core genome would target all *E. coli*, including commensal organisms that might bring benefit to the host. In contrast, if an antigen such as intimin (from enteropathogenic *E. coli*) or heat-labile toxin (from enterotoxigenic clades) were selected, then commensal *E. coli* would be spared and only pathogenic clades targeted.[42] Indeed, studies of this type have already been reported for *E. coli*.[41]

In addition to providing a genetic blueprint, whole genome sequences can be exploited in a variety of functional genomic approaches. For example, both RNA sequencing (RNA-seq) and proteomic approaches can be applied to bacteria either growing on laboratory media or within host cells in order to catalog proteins and other antigens that are being expressed.[43] This approach is particularly valuable for identifying proteins that are expressed within the host but such studies can also highlight the cellular location of antigens as well as the time during the growth cycle when they are actually expressed and available for targeting. Other studies such as mutagenesis or over expression, can follow on from these approaches, which are now finding broad utility.

## 5 REVERSE VACCINOLOGY

Prior to the publication of the first bacterial genomes the discovery of potentially protective antigens in bacteria was a relatively hit-and-miss affair. Investigators tended to target either highly immunogenic or relatively abundant surface-associated or secreted proteins such as toxins, adhesins, major membrane proteins or capsules. Most of these antigens were selected "positively" because they exhibited one of these properties and they proved to be protective in some in vitro or in vivo models of protection. This approach was highly successful, but did not work for all pathogens. Shortly after the first reference genomes were published, the concept of reverse vaccinology was developed.[44] In reverse vaccinology, the approach is to work up from the entire genome, considering all antigens as potential targets but use selected criteria to rank and evaluate the candidates from the total pool of potential antigens. A screen then follows to narrow down lead candidates (Fig. 16.1).

The first proof of principle of this approach was undertaken with *N. meningitidis* serogroup B (MenB). The ability to develop vaccines against

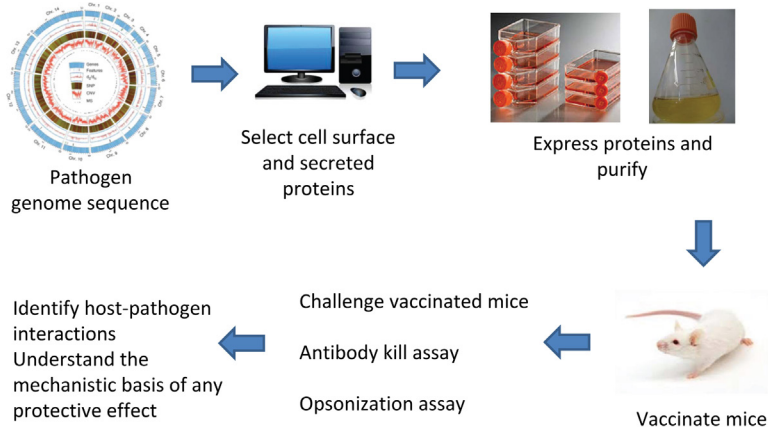


**FIGURE 16.1 Outline of a generalized reverse vaccinology scheme.** A reference genome(s) for a particular pathogen is interrogated bioinformatically for potential surface located or secreted proteins. Candidates are then expressed in a heterologous host (bacteria/mammalian cell etc.) and purified. Candidate proteins are then inoculated into a mouse or other animal and immune assays are performed to identify candidate protective antigens.

MenB had been hampered by the poor immunogenicity of the capsular polysaccharide which cross-reacts with human polysialic acid present in neural tissue, and the fact that the key known abundant antigens were highly variable between strains.[45] Hence, a conjugate vaccine was not feasible and protein-based antigens were compromised by lack of obvious targets.

The genome of the fully DNA-sequenced reference strain *N. meningitidis* MC58 was predicted to encode 2158 proteins (open reading frames) that could be considered as potential targets.[46] Initially bioinformatics approaches were used to narrow down these candidates to 570 predicted proteins that were likely to be secreted or surface-associated. A high throughput protein expression facility was established to synthesis these candidates in *E. coli* and from these, 91 antigens that were highly likely to be surface associated were selected from ~350 expressible candidates. Antigens that were predicted to be highly variable were also discarded as candidates. In order to further narrow down the list of candidates, antibodies were raised to each antigen and these were evaluated for their ability to kill *N. meningitidis* isolates in bactericidal killing assays. These assays were selected as they were an accepted correlate of protection for meningococcal conjugate vaccines based on surface capsular antigens.[47] Other antigens were also evaluated in a rat protection assay. This mammoth project yielded a small number of potentially protective antigens, many of which had never been considered as potential vaccine candidates. Indeed, many were also unknown prior to analysis of the genome.

One of the antigens discovered during the reverse vaccinology work on *N. meningitidis* was the factor H-binding protein (fHbp) that targets the key factor H

component of the complement system.[48] fHbp is relatively conserved and plays an important role in the pathogenesis of systemic meningococcal disease. fHbp became one of the components of the Novartis multivalent MenB vaccine, Bexsero, that has now been licensed in several countries, validating the reverse vaccinology approach. Other antigens that were discovered using the reverse vaccinology approach included Neisserial Adhesin A (NadA) and Neisserial Heparin Binding Antigen (NHBA).[49,50] The discovery of these antigens stimulated intensive studies on their biological and structural properties, highlighting the developing link between studies on the molecular basis of infection and vaccine development.

Variations of reverse vaccinology are potentially applicable to any pathogen with a relatively complex genome including larger viruses, bacteria, and parasites. Indeed, the approach has already been undertaken for several pathogens including *S. pneumoniae* and Group A *Streptococci*.[51–53] For each pathogen, the basic principle of using genomics to identify potential candidate antigens applies. Initially, bioinformatics approaches are applied although these can be linked to other functional genomic data sets such as RNA-seq or proteomic analysis. Once the number of candidates is narrowed down, proteins are expressed in a heterologous system and any purified proteins can be subjected to a biological screen to identify potentially protective antigens. Although bactericidal activity was selected as the principle screen for *N. meningitidis*, other assays have been employed including opsonisation or animal challenge studies.[54]

## 6 LIVE VACCINES AGAINST BACTERIA

Live vaccines based on attenuated variants of pathogenic bacteria have attracted the interest of investigators since the start of vaccine development. An excellent early example of this approach was the development of BCG, based on a passaged and attenuated variant of *Microbacterium bovis*. BCG was originally developed as an oral vaccine but has now found broader utility as an injected vaccine mainly used in children.[55,56] Recent genome analysis of BCG seed vaccine lots has identified the regions of the genome that are likely associated with the attenuated phenotype.[57] However, comparison of BCG vaccines from different companies found significant variation between different vaccine seed lots used for manufacture, highlighting some of the challenges associated with using live vaccines.[58] Historically, the attenuated derivatives used in live bacterial vaccines were isolated using empirical approaches based on laboratory passage or chemical mutagenesis. Although attenuated derivatives could be obtained in this way, the mechanism of attenuation was often left undefined, resulting in problems associated with quality control or even the threat of reversion to virulence.

Nevertheless some useful vaccines were developed based upon such approaches. For example, chemical mutagenesis was used to isolate the live oral typhoid vaccine Ty21a based on an attenuated derivative of *Salmonella* Typhi strain Ty2.[59] This vaccine lacks a Vi capsule, harbors multiple mutations that impact on galactose incorporation into LPS (*galE* mutations) and other mutations in undefined

genes that lead to the attenuated phenotype.[60] Using different vaccine formulations, Ty21a was tested in a series of field studies in different geographical locations and a 3–4 dose regimen consistently gave a reasonable efficacy of ~50–70% protection against typhoid. However, Ty21a is a fastidious microorganism, potentially because of the number of mutations it harbors, and has not found broad utility.

With the development of improved genetics and DNA sequencing, it became possible to take a more rational approach to live vaccine design. With more and more pathogen genes discovered that were required for the full expression of virulence, it is now feasible to build designer bacteria that harbor combinations of stable mutations within such genes. Here the aim is to bring together combinations of attenuating mutations that reduced virulence to a tolerable level but retain appropriate immunogenicity sufficient to elicit protection.[61] Perhaps the first vaccines of this type were also developed in *S*. Typhi, exploiting deletion mutations in genes such as *aroA* (required for aromatic compound biosynthesis) and *purA* (associated with purine biosynthesis).[62] Candidate vaccines of this type were evaluated in volunteers but to date they have not been licensed as human vaccines. Similar approaches have been taken in *B. pertussis*, *Shigella*, and *Vibrio cholerae* targeting virulence-associated genes such as cholera toxin.[63–65]

## 7 VACCINES BASED ON MEMBRANE COMPLEXES

An alternative innovative vaccine strategy is the use of membrane complexes, usually in the form of vesicles of bacterial outer membranes.[66] These have the advantage of delivering multiple membrane components to the immune system, including membrane proteins. Many of these components are B-cell and T-cell antigens. Importantly, the B-cell antigens are present in their native three-dimensional conformation and correct orientation, thereby increasing the likelihood of inducing antibodies with functional activity against a specific bacterial pathogen. Bacterial vesicles can also deliver a number of danger signals to the immune system in the form of pathogen-associated molecular patterns such as the toll-like receptors (TLR) ligands flagellin (ligand for Tlr5) and lipopolysaccharide (ligand for Tlr4).[67] These enhance the antibacterial immune response by conferring an adjuvant effect.[68] Additionally, outer membrane particle vaccines are generally straightforward to produce at high yields indicating potential as an affordable vaccine option.

In 2004, prior to reverse vaccinology delivering the Bexsero vaccine, an epidemic of meningococcal serogroup B emerged in New Zealand that was successfully controlled by a vaccine of detergent-extracted outer membrane vesicles (dOMV) termed MeNZB.[69,70] The extraction process releases outer membrane vesicles from within the bacteria, with outer membrane proteins displayed so that their outer surfaces face into the vesicle. The use of detergent partially removes lipids, including lipid A, thereby reducing potential reactogenicity to levels where the vesicles can be used in humans. However, lipoproteins are also removed and a number of these, for example, fHbp, are known to be important for conferring protective immunity. In fact, there is limited cross-protective

immunity elicited to antigenically heterologous meningococcal group B strains, likely due to protective immunity being primarily conferred through the antibody response to polymorphic protein antigens, particularly PorA. The success of this vaccine in New Zealand was probably in part due to a single clone of group B meningococcus being responsible for the epidemic and the same clone being used to generate the dOMV vaccine. Although no longer produced as a stand-alone vaccine, MenZB is one of the four components of the licenced Bexsero vaccine. A bivalent meningococcal A and C dOMV, for use primarily in the African Meningitis Belt, is currently under development at the Finlay Institute in Cuba, in partnership with the Norwegian Institute for Public Health.[71]

To overcome the problem of limited strain coverage inherent to dOMV vaccines, a biological phenomenon common to gram-negative bacteria has been exploited, whereby bacteria spontaneously shed vesicles from their surface. The exact role of this process is not well understood, but is thought to be involved in host-pathogen interactions with the delivery of toxins and virulence factors to the host. In relation to vaccinology, these native outer membrane vesicles (NOMV) have several advantages over dOMV. Unlike dOMV, in NOMV outer membrane proteins are orientated so that their outer aspects are on the outside of the particles. Additionally, because detergents are not required for their production, lipoproteins such as fHbp are retained. Overexpression of fHbp and other key antigens can be achieved by genetic manipulation of the parent bacterial strain and similarly unwanted antigens, such as the meningococcal group B capsule, which cross-reacts with self-antigens in man, can be removed.[72,73] The lack of the detergent extraction also simplifies the production process thereby reducing cost. However, lipid A is still present and genetic manipulation of the bacteria is required to modify this molecule in order to reduce potential reactogenicity. This is normally achieved by the inactivation of the *lpxL1* acyl transferase gene.[74] Phase 1 clinical trials using meningococcal B NOMV have shown these to be safe and immunogenic.[75]

Natural shedding of NOMV by bacteria is potentially rate-limiting for vaccine production and can simply be too low for some pathogenic species. It has been shown that manipulation of the tol-pal pathway, which is responsible for maintaining the integrity of the link between inner and outer bacterial membranes, can lead to the upregulation of release of outer membrane vesicles from gram-negative bacteria.[76] To distinguish these from dOMV and NOMV, the term "GMMA," generalized modules for membrane antigens, has been adopted.[77] To date, GMMA have been produced for *Shigella sonnei*, [77] meningococcus,[78] and *Salmonella enterica* serovars Typhimurium and Enteritidis.[79] Deletion of *tolR*[80] has been used to enhance shedding of GMMA from *Shigella* and *Salmonella*, while *gna33*[81] can be mutated in meningococcus to achieve the same effect. It has proved necessary to knock out genes encoding other lipid A acyltransferases in order to reducing reactogenicity of *Shigella* and *Salmonella* GMMA, including *htrB, msbB*, and *pagP*.[82] Nevertheless, once the GMMA-producing bacterial strain has been generated, GMMA vaccine production is a straightforward process with large numbers of vaccine doses generated with each fermentation and limited downstream purification required.[77] These

considerations make GMMA a potentially highly affordable approach to vaccinology, a consideration which is key for the sustained implementation of vaccines in the developing world.

## 8 KILLED ORAL VACCINES

Key considerations for a successful vaccine are immunogenicity, protective efficacy, ease of administration and overall safety. However, if a vaccine is to reach areas or economically challenged populations that are difficult to access, cost becomes a key consideration. Currently, almost all vaccines licensed globally and used in either the expanded program on immunization (EPI), or by travelers to prevent regional endemic diseases are administered through a needle-based injection. Even so there is substantial interest in needleless vaccine delivery technologies, with the oral route perhaps the most intensively investigated.[83] There are licensed oral bacterial vaccines against two diseases: typhoid and cholera, which were described above. Additionally, there are two licensed inactivated whole-cell oral vaccines, Dukoral® and Shancol™, that target cholera using a cocktail of different *V. cholerae* strains and cholera toxoid.[84] With live attenuated vaccines there is always a theoretical risk of reversion or genetic drift to an infectious form, although this can be reduced dramatically by using multiple attenuating mutations. On the other hand, killed or inactivated whole cell vaccines cannot revert and are arguably simpler to manufacture and deliver. Killed oral vaccines thus tick many positive boxes as they are painless, easy to administer, potentially safer and likely cheaper to produce.

Many pathogens, invasive or noninvasive, initially colonize their hosts on mucosal tissues. Systemic antibodies generally have a restricted ability to permeate through uninflamed mucosal membranes. For those pathogens that exhibit infectivity largely limited to epithelia, parenteral can be less effective than mucosal immunization, particularly in the immunologically naive. In fact, for noninvasive and noninflammatory infections of intestinal mucosal tissues like cholera and ETEC, the oral route of vaccination is potentially an optimal immune response elicitor because these infections require a local mucosal immune response involving compartmentalized plasma cells and antibody production.[85]

For both cholera and ETEC infections, secretory IgA (SIgA) produced locally at the site of infection shows some correlation with protection.[86] Currently, there is no licensed vaccine available for ETEC and the cholera vaccine Dukoral was the first and, until recently, the only licensed killed oral vaccine on the market. Dukoral, also known as whole-cell-B subunit (WC-BS) vaccine, contains ~1 mg of the nontoxic B subunit of cholera toxin (rCTb) and a total of $\sim 10^{11}$ heat or formalin killed serogroup O1 *V. cholerae* cells covering classical and El Tor biotypes and Ogawa and Inaba serotypes. Dukoral is recommended as a 2-dose vaccine taken 1–6 weeks apart in an alkaline buffer to allow effective passage through the acidic gastric barrier to the small intestine. After reaching the site, inactivated whole cells mimic aspects of a natural cholera infection

resulting in recognition of the *V. cholerae* cell by the immune system, with the nontoxic B subunit eliciting a neutralizing immune response against cholera holotoxin. SIgA are produced in the intestine against vaccine antigens that and are capable of limiting colonization and replication of *V. cholerae* on intestinal epithelial cells and neutralizing the cholera toxin produced. Serum vibriocidal antibodies and antitoxin antibodies are also produced against whole cell components and toxin respectively, resulting in the protective efficacy of the vaccine.[87]

Large field trials in areas of Bangladesh endemic for cholera showed 85% protective efficacy for killed whole-cell cholera vaccines, which lasted for 6 months in 2–6 years old children.[88] However, the efficacy fell to 60% in the second year in adults. This analysis also suggested that 2 doses were as effective as 3. Potentially as a consequence of the significant immune cross-reactivity between cholera toxin and *E. coli* heat labile toxin (LT), a short-lived protection of 67% at 3 months was observed against LT-producing ETEC.[89] Despite this reasonable protection, the overall high cost of ~40$/dose for the Dukoral formulation has meant that the vaccine is currently only extensively used in the "travelers" market within richer countries.

Based on the similar technologies used to develop Dukoral, a low cost whole cell cholera vaccine known as Shancol was produced and recently licensed in India and other countries.[90] Shancol lacks the cholera toxin B subunit component and is administered as a 2-dose vaccine given 2 weeks apart but without any alkaline buffer since it lacks the acid-sensitive B subunit. Manufactured by Shantha Biotech of India, large field trials of Shancol exhibited reasonable protective efficacy,[91] providing >65% protection 3 years after vaccination.

New killed oral whole-cell vaccines are currently under development, including a genetically engineered *V. cholerae* strain, which behaves as a stable Hikojima biotype, that is, it expresses both Inaba and Ogawa serotypes.[92] This vaccine targets the epidemiologically relevant El Tor biotype and O1 serogroup of *V. cholerae*. Mice orally immunized with this vaccine showed an immune response comparable to that induced by Dukoral. Because this will be a single strain vaccine, manufacturing, inactivation and downstream processing should be more cost effective.

There are ongoing efforts to develop killed oral vaccines against other important enteric pathogens including ETEC and *Shigella*. After the unexpected failure of a first generation killed oral ETEC vaccine in a phase 3 clinical trial, a second generation tetravalent inactivated whole cell vaccine has been developed that harbors a genetically detoxified *E. coli* heat-labile toxoid component.[93] This vaccine is currently entering phase 2 human clinical studies. A trivalent inactivated oral vaccine against *Shigella* is also undergoing clinical evaluation.

## 9 BIOCONJUGATES

The development of conjugate vaccines based on polysaccharides chemically linked to soluble carrier proteins to enhance immunogenicity has been extensively exploited by the vaccine industry. However, many polysaccharides

found at the surface of bacteria are highly complex, difficult to purify or impossible to synthesize chemically. These factors have limited some of the utility of the conjugative approach. Studies on the mechanisms of biosynthesis and export of these surface polysaccharides by bacteria have revealed many of the steps involved in these pathways, some of which are generic and potentially exchangeable between systems. Importantly, proteins have been identified in the periplasm of bacteria that capture assembled polysaccharides ready for assembly at the bacterial cell surface. The first of this class of proteins to be identified was the AcrA protein of *Campylobacter jejuni*.[94] AcrA harbors a specific peptide domain that serves as the recipient of the polysaccharide from an oligosaccharide transferase. The general N-linked glycosylation system of *C. jejuni* is broadly functional in *E. coli* facilitating the production of different polysaccharide-protein conjugates in a manner that can be used to synthesize candidate conjugate vaccines.[94] Indeed, different bacterial polysaccharides, including LPS side chains or O antigens, with an *N*-acetyl sugar at the reducing end can be transferred from undecaprenyl-pyrophosphate precursors to AcrA or related capture proteins. The oligosaccharyl transferase PglB of *C. jejuni* targets a consensus sequence D/E-X-N-Z-S/T that can be moved onto other proteins that might be exploited as carriers such as *Pseudomonas aeruginosa* exotoxin A and haemolysin A of *Staphylococcus aureus*. The term "bioconjugate" has been coined to describe these novel conjugates.[95] Since the discovery of AcrA and PlgB many other proteins with similar properties have been discovered, broadening the utility of the approach.

Bioconjugates have several potential advantages over chemical conjugates. Since they are made through a biological process, the integrity of both the polysaccharide and the carrier remain largely intact and may thus be more immunogenic and likely to induce efficacious antibodies. Also, a variety of proteins can theoretically be engineered to act as carriers, including homologous proteins from the same pathogen as the polysaccharide. Additionally, complex polysaccharides can be captured in a relatively nontoxic form, for example, LPS side chains without the lipid A component. Several bioconjugate candidates have now been described. For example, conserved staphylococcal protein antigens such as Haemolysin A have been bioconjugated to capsular polysaccharides from *S. aureus* and evaluated in mice.[95] Other bioconjugates based on *Shigella* lipopolysaccharides have entered Phase I studies in humans and a company based on this technology, GlycoVaxyn, was recently acquired by GSK (http://www.glycovaxyn.com).

## 10 CONCLUDING REMARKS

The field of bacterial vaccine development is very active with many exciting target vaccines still to be developed. New approaches, including reverse vaccinology and bioconjugate production, are opening up routes toward previously intractable vaccines. However, many challenges still remain. New vaccines are required against tuberculosis and despite intensive efforts these remain some

way off. Such vaccines likely require a significant T-cell component and few suitable adjuvants are available for use in humans to promote such responses. Nevertheless, this field is active with several approaches under evaluation. Vaccines against sexually transmitted infections are still required but these have so far proved elusive. Reverse vaccinology may open up this area but the lack of effective animal models is a barrier. Here, direct trials in humans and potentially pathogen challenge studies may be required. Vaccines against enteric pathogens such as *Shigella* and ETEC are not yet available. Live oral vaccines against *Shigella* have proved to be difficult to progress beyond early clinical studies, in part due to poor immunogenicity or reactogenicity. Here, novel routes of vaccination including the use of nonliving oral vaccines, GMMA or bioconjugates need to be explored further. Finally, may bacterial infections occur in the elderly or the immunocompromized, particularly those associated with antibiotic resistance, so extensive rethinking may be required for these health-care-associated infections. Here, novel adjuvants or other adjunct therapies may be required. Whatever, we can anticipate significant progress in these areas in the next years.

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Chapter 17

# Vaccines Against Parasites

**David L. Sacks, PhD*, Nathan C. Peters, PhD**, Jeffrey M. Bethony, PhD†**

**Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States; **University of Calgary, Snyder Institute for Chronic Diseases, Department of Microbiology Immunology and Infectious Diseases, Cumming School of Medicine, Alberta, Canada; †George Washington University Medical Center, Microbiology, Immunology, and Tropical Medicine, Washington, DC, United States*

## Chapter Outline



Infectious diseases caused by parasites are major causes of morbidity and mortality in the poorest countries of Asia, Africa, and Latin America. Among the most prevalent infectious diseases commonly referred to as "neglected," 11 are caused by helminthic and protozoan parasites, which along with malaria affect more than 1 billion people and cause more than 1 million deaths annually.[1] Unfortunately, there is as yet no safe, uniformly effective vaccine against any human parasitic infection. While the absence of strong market incentives remains a barrier to the development of so-called "antipoverty" vaccines,[2] the greater impediments may be the complexity of parasites as immunologic targets. The

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hallmark of parasitic infections is chronicity, achieved by diverse strategies for immune evasion that have evolved to prolong parasite survival and enhance their transmissibility. Thus, for a given antiparasite vaccine to succeed, it will have to elicit a response that outperforms naturally acquired immunity, and this is fundamentally different from the majority of currently licensed human vaccines that are designed to mimic the sterilizing response to natural infection. There are, nonetheless, experiences with selected antiparasite vaccines in humans that are sufficiently encouraging to justify their current application, or to inform the design of future vaccines. The current status and prospects for parasite vaccines are discussed in the context of malaria, leishmaniasis, schistosomiasis, and hookworm infections, as these are the major human parasitic infections for which vaccines have reached more advanced stages of clinical development.

## 1 VACCINATION AGAINST MALARIA

More human death is caused by malaria parasites than by all other eukaryotic pathogens combined, with approximately 584,000 deaths globally in 2014, primarily in young children infected with *Plasmodium falciparum* in sub-Saharan Africa. The malaria disease burden remains unacceptably high despite intensified efforts at malaria control that have halved *P. falciparum* infection prevalence in Africa between 2000 and 2015.[3] Infection with malaria parasites transmitted by mosquitoes generally produce a characteristic set of symptoms, including fever, sweats, chills, nausea, headaches, and general malaise. Malaria is a serious disease: although the case fatality rate is around 2%, particularly in young children, severe malaria has a variety of devastating complications, including cerebral malaria, with impairment of consciousness, seizures, coma, severe anemia due to hemolysis [destruction of the red blood cells (RBC)], acute respiratory distress syndrome, low blood pressure, and kidney failure. Particularly dangerous is malaria in pregnancy that results in high rates of fetal death and accounts for a very high risk of maternal death as well.

The striking reduction in malaria morbidity and mortality has been achieved by implementation of artemisinin-based combination therapies in conjunction with new strategies of vector control. The hope that continued implementation of these control efforts will further reduce the burden of disease is being undermined by the emergence of widespread resistance to insecticides and the most effective drug, artemisinin.[4] Thus, the existing tools are inadequate, and an effective malaria vaccine remains key to achieving the current goals of the Roll Back Malaria partnership to reduce malaria mortality and case incidence by 90% from 2015 levels by 2030 (http://www.rollbackmalaria.org).

The malaria parasite has multiple life-cycle stages within the human host and mosquito vector, each of which expresses a multiplicity of antigens that can potentially serve as targets of an immune response to inhibit various stages of the infectious process (Fig. 17.1). Given these opportunities for immune intervention, why has the development of an effective vaccine remained so

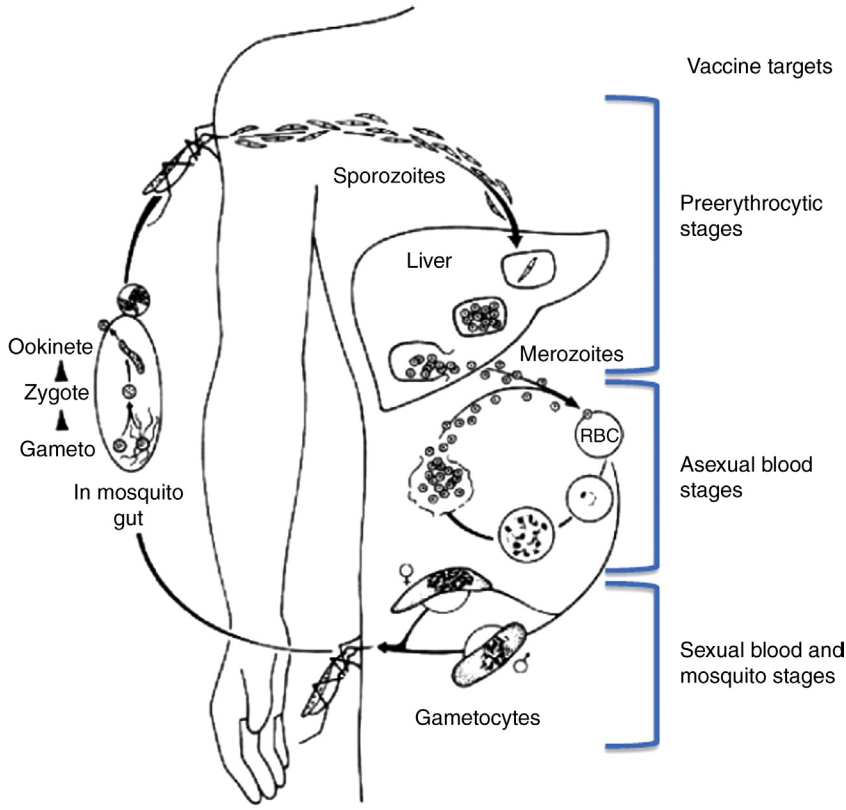


**FIGURE 17.1 The life cycle of the malaria parasite and the stages targeted by vaccines.** Sporozoites contained in the mosquito salivary gland are inoculated into the skin when an infected anopheline mosquito takes a blood meal. The sporozoites travel to the liver where they multiply inside hepatocytes. After about 1 week the hepatocytes rupture and release merozoites that invade red blood cells *(RBC)*. For *P. falciparum*, there is a 48-h cycle of merozoite multiplication and release from ruptured RBCs. Gametocytes develop within RBCs that are taken up by the mosquito where sexual development occurs, leading to the production of sporozoites and their invasion of salivary glands. *(Adapted from Miller LH, Howard RJ, Carter R, et al. Research toward malaria vaccines. Science 1986;***234***:1350, with permission.)*

difficult to achieve? The reasons are framed in the following sections in context of the immune evasion strategies that allow the malaria parasite to avoid elimination by immune responses elicited by natural exposure, and that similarly undermine the efficacy of vaccines.

## 1.1 Naturally Acquired Immunity and Immune Evasion Mechanisms

In endemic regions, young children are highly susceptible to deaths due to malaria. With exposure, older children rarely die due to malaria, and may become

immune to severe disease after only a few malaria episodes.[5] Importantly, older children, despite years of repeated exposures, do not have sterile immunity and can still develop mild febrile illness.[6] Immunity to infection, therefore, is slow to develop, but the reduced prevalence of symptomatic infection with age and lower rates of severe disease indicate that natural immunity does occur. Understanding the nature of this slow developing immunity is thought to be key to vaccine design. Naturally acquired resistance seems not to depend on an immune response directed at the preerythrocytic stages because immune adults are still protected against symptoms when liver-stage infection is bypassed by direct challenge using blood-stage parasites.[7] The absence of a strong protective response directed against the liver stages is thought to be due to the low numbers of transmitted sporozoites, that is, the form injected by the mosquito, that results in too few hepatocytes becoming infected to induce an immune response, or to be detected quickly enough by effector T cells to prevent their release of merozoites into the blood, which infect and damage RBC[8] (Fig. 17.1).

The acquired resistance that does develop in older children after repeated exposure is mainly against blood-stages, directed to antigens that are expressed on the merozoite surface, or on the surface of infected RBC. In each case the target antigens are highly polymorphic due to both allelic and somatic gene variation, and the prevailing view is that development of immunity requires the accumulated exposure to a large number of strains circulating in a community so as to cover the diverse repertoire of blood-stage antigens. The importance of antibody in naturally acquired immunity was directly demonstrated long ago by the passive transfer of gamma-globulin from immune adults into semiimmune individuals that conferred stronger protection against blood-stage infection.[9] Antibodies can function by blocking merozoite invasion of RBCs, or by promoting the effective clearance of parasitized RBCs by the spleen. The best characterized of the variant surface antigens is *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1), which the parasite exports to the red cell surface to mediate binding to vascular endothelial receptors, allowing the parasite to sequester in peripheral tissues and avoid being cleared in the spleen.[10] Adhesion by infected RBCs to the endothelial cells lining blood vessels and capillaries clogs the microvasculature, triggers local inflammation, and gives rise to the cerebral, respiratory, and renal symptoms associated with severe malaria. Antibodies against PfEMP-1 block adherence or sequestration, and the parasite employs a system of clonal antigenic variation to produce chronic infection. There are more than 60 copies of the gene for *PfEMP-1* or *var* genes per parasite, tightly regulated at the transcriptional level and only 1 gene is expressed at a time. PfEMP1 has been implicated as the key target antigen involved in naturally acquired immunity[11] and the extensive antigenic variation of PfEMP-1 poses the most serious obstacle to the development of a blood-stage vaccine.

Antibodies targeting merozoite proteins involved in erythrocyte invasion can also contribute to acquired immunity. Malaria parasites possess apical organelles that contain hydrolytic enzymes required for cell invasion. Several

*P. falciparum* proteins have been shown to be involved in erythrocyte invasion, including merozoite surface protein-1 (MSP-1) involved in the initial attachment, merozoite apical membrane antigen-1 (AMA-1) that mediates the reorientation of merozoites, and erythrocyte binding ligands (EBLs) that bind to sialic acid containing glycoproteins on the erythrocyte membrane to create the tight junction that allows invasion to proceed. The genes encoding each of these proteins display extensive different allelic forms or polymorphisms, and antibody-mediated inhibition of the invasion process is generally strain-specific.[12]

Last, a consideration of immune memory will be critical to the design of any malaria vaccine. Based largely on anecdotal evidence, it seems that immunity to malaria is rapidly lost if an individual leaves an endemic region and then returns, suggesting that there is poor immunological memory, and that continued exposure to malarial antigens is required to maintain the populations of memory and/or effectors cells necessary for protection. Consistent with this continuous exposure requirement, in young children living in low transmission areas, antibody responses to merozoite antigens appear to be short-lived.[13] With respect to memory T cells, Th1 cells producing IFNγ gradually declined in the absence of continued malaria exposure,[14] and rodent models also indicate a rapid decay in the frequency of memory CD4+ T cells in the absence of infection.[15] The requirement for continuous exposure or persisting infection to maintain the threshold number of memory and/or effector cells required for protection has crucial implications for the design of an effective vaccine.

## 1.2 Preerythrocytic Stage Vaccines

Nearly 40 years ago it was observed that sterilizing immunity against *P. falciparum* could be achieved by exposing human volunteers to the bites of irradiated mosquitoes carrying sporozoites in their salivary glands.[16] These trials were inspired by the groundbreaking studies in mice using intravenous inoculation of irradiated *Plasmodium berghei* sporozoites.[17] In each case the radiation-attenuated parasites were unable to develop beyond their liver stages, and live, metabolically active sporozoites were required for the protection. Protection also required a high dose exposure to the sporozoites, with more than 1000 mosquito bites needed to achieve sterile immunity. Studies in the mouse revealed that CD8+ T cells are paramount for protection,[18] and the high-dose immunization, by generating greater numbers of C8+ T cells to more effectively survey the extremely low numbers of infected hepatocytes following natural transmission, likely explains why irradiated sporozoites can achieve far better protection than natural infection.[19]

The inability to grow sporozoites in culture posed a major obstacle to their wide application as live, attenuated vaccines. The cloning of the gene for the major surface coat on sporozoites, called circumsporozoite protein (CSP), and its identification as a major target of the antibody and T-cell response in vaccinated

mice and people, led to a number of clinical trials involving recombinant protein- or DNA-based CSP vaccines. The initial trials, however, showed disappointing efficacy against sporozoite challenge.[20,21] A newer formulation of the *P. falciparum* CSP, called RTS,S, is the most clinically advanced and encouraging malaria vaccine candidate to date.[22] The vaccine is produced by GlaxoSmithKline (GSK) and incorporates the CSP repeat region (R) and T-cell epitopes (T) as fusion proteins with hepatitis B surface antigen (S) that along with unfused hepatitis B surface antigen (S), spontaneously assemble into virus-like particles. The vaccine is given with a new adjuvant system, $AS02_A$, which contains monophosphoryl lipid A (MPL) and saponin in an oil-in-water emulsion. An important private/public partnership between GSK and PATH malaria vaccine initiative (MVI) has carried out the pediatric development of RTS,S. The main evidence for its efficacy derives from a large clinical trial conducted in seven African countries showing modest protection against *P. falciparum* malaria in 56% of children aged 5–17 months, and in 31% of children aged 6–12 weeks, without significant protection from severe malaria after 18 months.[23] Despite these modest results, the benefits of vaccination to reduce mortality among children in high-transmission areas was considered important enough that its use has recently been recommended by a regulatory agency, the European Medicines Agency, an important step toward eventual licensure.

Because irradiated sporozoite immunization still represents the gold standard for sterile protection, there remains a concerted effort to understand and reproduce the essential features of the immunity conferred by this whole sporozoite vaccine. The most direct and ambitious approach has been to scale up the production of irradiated, aseptic, cryopreserved sporozoites manually dissected from mosquito salivary glands, and deliver them by needle.[24] The most recent studies employing this vaccine found that high i.v. doses (>600,000 sporozoites) completely protected six out of six volunteers against infectious sporozoite challenge.[25] Despite the clear disadvantages associated with the need for high doses and i.v. administration, the approach still represents the first innocuous vaccine produced under current good manufacturing practice (cGMP) standards, and delivered by needle to confer sterile immunity against malaria. Further studies are needed to determine if the vaccine confers long term heterologous protection against other strains, and to define the immune correlates of protection.

In the meantime, additional approaches employing whole organism sporozoite vaccines are being pursued, including genetically modified parasites that are arrested at a late stage in liver-stage development, and chemoprophylaxis with infectious sporozoites.[26] The advantage of these approaches compared to nonreplicating, irradiated sporozoites is that a greater repertoire and quantity of liver stage antigens is expressed. Sterile immunity was reported in the clinical trial of 10 volunteers immunized by repeated exposures to infectious mosquitoes under chemoprophylaxis with chloroquine that kills the merozoites as they emerge from the liver.[27] Importantly, all were completely protected against

blood-stage infections following sporozoite challenge, some volunteers for as long as 2 years, and some even against heterologous challenge,[28] suggesting that liver stage immunity may not be strain-specific. While the requirement for large numbers of infected mosquitoes delivering high doses of sporozoites is clearly not practical for large scale vaccination, recent progress in cryopreservation of purified, infectious sporozoites for needle inoculation[29] should enhance the feasibility of this approach.

Because the sterile immunity conferred by whole sporozoite vaccines is associated with strong CD8+ T-cell responses, a number of prime-boost strategies vaccines have been tried to induce CD8+ T cells targeting liver stage antigens in people. In the most recent trial, a heterologous prime-boost vaccine employing a replication-deficient chimpanzee adenovirus vector followed by a modified *Vaccinia virus* Ankara booster induced high frequencies of CD8+ T cells specific for the liver stage antigen ME-TRAP, and lower blood stage parasitemia was observed in 8 of 14 volunteers following controlled human malaria infection (CHMI), but sterile immunity in only three of them.[30] The same vectors encoding CSP showed even lower efficacy then ME-TRAP.[31] Thus, a strong CD8+ T-cell response that targets a single liver stage antigen does not appear to be a sufficient condition to confer sterile immunity, and other attributes of whole sporozoite vaccines, notably the multiplicity and persistence of the protective antigens that they present, may be essential qualities that can only be accommodated by live, and live-attenuated vaccination strategies. The next few years should yield critical new information regarding the immunogenicity of these vaccines in infants and semiimmune children, and the strain transcendence and duration of the immunity induced.

## 1.3 Blood Stage Vaccines

The rationale for the development of a blood-stage vaccine is based on the naturally acquired immunity that is directed against blood stages of the parasite, and the fact that sterilizing immunity is not necessary to achieve a reduction in the severity of illness or the transmissibility of infection. Unfortunately, despite a number of candidate vaccines progressing to clinical testing, in no case have Phase II trials demonstrated significant efficacy to justify further evaluation in a Phase III clinical trial. The main vaccine candidates, including AMA-1[32] and MSP1[33] were selected based on in vitro assays showing that they are targets of antibody responses that inhibit merozoite invasion of erythrocytes. In one of the few trials to show some efficacy, analysis of breakthrough infections showed that the protection was strain specific, with the monoallelic MSP2 component of the vaccine selecting for patent infections with a strain expressing another MSP2 allele.[34] A recombinant AMA1 vaccine appeared to confer a similar strain-specific immunity,[35] and the short-lived protection conferred by a recombinant vaccine in Kenya was thought to be due to polymorphisms in the region of MSP3 covered by the vaccine.[36]

Because sterile immunity is unlikely to be achieved by any blood-stage vaccine, there is widespread agreement that a more achievable vaccination goal is to reduce the incidence of severe disease, including death. The fact that immunity to severe disease develops more rapidly than immunity to mild disease suggests that severe disease might be associated with a restricted set of PfEMP-1 variants, and there is recent evidence to support this possibility. The identification of a particular PfEMP1 subset associated with severe malaria that binds endothelial protein receptor C (EPCR),[37] and the characterization of broadly inhibitory antibodies that bind to a conserved EPCR binding structure on PfEMP1,[38] offer a sound rationale for development of a recombinant vaccine targeting this conserved structure. Similarly, a particular PfEMP1 variant, known as var2csa, binds selectively to chondroitin sulfate A (CSA) and mediates attachment of infected erythrocytes to the syncytiotrophoblasts of the placenta.[39] Pregnancy-associated malaria produces high rates of fetal mortality and accounts for a very high risk of maternal death as well. Naturally acquired immunity to pregnancy malaria is indicated by the reduced incidence of placental infections that is directly correlated with the number of pregnancies and the increased levels of maternal antibodies against var2csa.[40,41] Thus, var2csa is considered a leading candidate to prevent malaria in pregnancy.

## 1.4 Transmission-Blocking Vaccines

Transmission-blocking vaccines (TBV) are designed to target antigens expressed on gametocytes or ookinetes, the rare sexual forms of malaria parasites that are acquired from blood meals by mosquitoes. Antibodies have been shown to reduce oocyst development in the mosquito below the number required to produce a transmissible infection. The vaccinated individuals are intended to be the source of both the sexual stage parasites and the transmission-blocking antibodies, Transmission-blocking antibodies are sometimes referred to as "altruistic vaccines" because although the vaccinees might not be directly protected, they would contribute a potentially important indirect benefit by helping to reduce the level of malaria transmission in their community. One advantage of TBVs is that the target antigens are under little if any immune selection pressure and thus tend to be conserved. This is particularly true of antigens confined to mosquito stages of the parasite, for example, ookinetes. The disadvantage is the absence of natural boosting. Because nonsterilizing immunity induced by other vaccines may not reduce the number of infection reservoirs in a community, TBVs may be an important component of any multipronged effort to control malaria, especially if malaria eradication is the ultimate goal. To date, the only TBV that has progressed to clinical testing is Pfs25 and its ortholog in *Plasmodium vivax*, Pvs25, each of which is expressed on zygote and ookinete stages. A Phase Ia trial involving recombinant Pvs25 given intramuscularly in combination with hydrogel as adjuvant produced modest antibody titers and modest transmission-blocking activity.[42] Efforts to improve the immunogenicity of Pfs25 include

fusion with heterologous proteins and expression by viral vectors or as protein nanoparticles, each of which elicited high anti-Pfs25 titers in mice. Clinical trials of these vaccines have been initiated.

### 1.5 Future Prospects for Malaria Vaccines

The malaria vaccines that have shown some prophylactic benefit in human trials are summarized in Table 17.1. The recent successes involving RTS,S and whole sporozoite vaccines, while modest in their efficacy or practicality, point the way forward. Stakeholders will need to decide what they want from a malaria vaccine. If sterile immunity is the goal, then whole organism vaccines involving attenuated sporozoites or sporozoites delivered under cover of antibiotics are clearly the way to proceed. The key questions here will be how to scale up production of sporozoites and deliver a live vaccine. If the goal is to reduce the burden of severe disease, including pregnancy malaria, then there seems a sound strategy for development of subunit vaccines targeting particular blood-stage variant antigens associated with severe disease. The critical questions here are what is the nature of the protective immunity, and what kind of antigen expression system, delivery platform and adjuvant will elicit the appropriate response. If the goal is eradication in geographically isolated areas, transmission-blocking vaccines targeting mosquito stages could beautifully complement other control measures. A key question here is how to make up for the lack of natural boosting. Ultimately, a multipronged approach involving a multistage vaccine may offer the best chance for success.

## 2 VACCINATION AGAINST LEISHMANIASIS

The failure to generate an effective human vaccine against leishmaniasis is one of the most frustrating challenges in the parasite vaccine field. This is because people often develop highly protective life-long immunity to reinfection following a single primary infection, indicating that protective "natural" immunity exists, thereby providing a blueprint that a vaccine need only emulate. However, while numerous experimental vaccines reproduce certain correlates of the protective response, none have fully replicated it.[43]

Leishmaniasis is a set of chronically neglected tropical diseases, affecting individuals in equatorial and subequatorial regions around the globe. *Leishmania* parasites, which are spread by the bite of phlebotomine sand flies, cause different forms of disease in humans. The most common forms are cutaneous leishmaniasis, which causes skin sores and visceral leishmaniasis, which affects multiple internal organs (usually spleen, liver, and bone marrow). Drugs against leishmaniasis are expensive, toxic, have intensive treatment regimes, and resistance is common.[44] There are 1–2 million new cases of leishmaniasis every year, an estimated 12 million people are infected worldwide, and 20,000–50,000 people die due to visceral leishmaniasis every year.[45] Leishmaniasis

**TABLE 17.1** Malaria Vaccines Demonstrating Efficacy in Clinical Trials

| Target stage | Vaccine | Clinical testing | Efficacy | References |
|---|---|---|---|---|
| Preerythrocytic | RTS,S/AS01 | Phase III field trial | 56% reduction in episodes of clinical malaria; 45% reduction in severe malaria in children 5–17 months, 31% in children 6–12 weeks; no protection against severe malaria after 18 months | [22,23] |
| | Exposure to infectious mosquitoes and chemoprophylaxis | CHMI | Sterile immunity in 10/10 volunteers, 2/13 fully protected, 11/13 delayed patency against heterologous challenge | [27,28] |
| | Heterologous prime boost with ChAd63/MVA expressing ME-TRAP | CHMI | Sterile immunity in 3/14 subjects, delay in patency in 5/14 subjects | [30] |
| | Heterologous prime boost with ChAd63/MVA expressing CS | CHMI | Sterile immunity in 1/15 subjects, delay in patency in 3/15 subjects | [31] |
| | Irradiated cryopreserved sporozoites, i.v. injected | CHMI | Sterile immunity in 6/6 subjects receiving >600,000 irradiated sporozoites | [25] |
| Blood | Recombinant RESA, MSP1, MSP2 in oil-based adjuvant | Phase I-IIb field trial | 62% reduction in parasite density; lower prevalence of parasites carrying the MSP2 allele corresponding to that in the vaccine | [34] |
| | Recombinant AMA-1 in AS02A | Phase IIb field trial | No protection against clinical malaria; 64% strain-specific protection | [35] |
| | MSP3 in alum | Phase Ib field trial | Protection against clinical malaria in the short term; no reduction in cumulative incidence | [36] |
| Mosquito | Recombinant Pvs25 in hydrogel | Phase Ia field trial | Serum antibodies produced transmission blocking in membrane feeding assays in mosquitoes | [42] |

CHMI, controlled human malaria infection; AMA1, apical membrane antigen 1; MSP, merozoite surface protein; RESA, ring-infected erythrocyte surface antigen; ChAd63/MVA, replication-deficient chimpanzee adenovirus vector/modified *V. virus* Ankara; CS, circumsporozoite protein; TRAP, thrombospondin-related adhesion protein; ASO1/ASO2, adjuvant systems containing monophosphoryl lipid A and saponin in an oil-in-water emulsion.

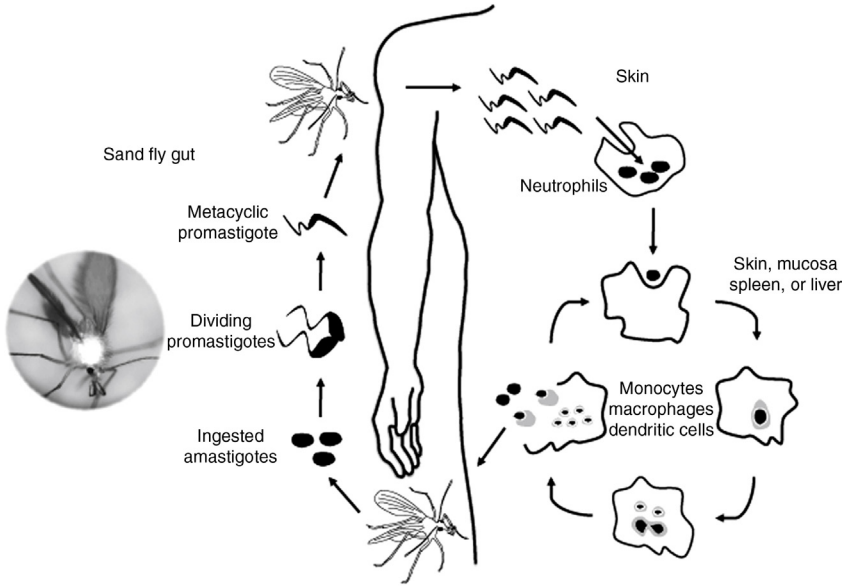


**FIGURE 17.2 The life cycle of *Leishmania* parasite.** Infection in the mammalian host begins when metacyclic promastigotes are inoculated into the skin by an infected Phlebotomine sand fly. Parasites are phagocytosed primarily by neutrophils in the skin. After 12–24 h parasites are released and are taken up by mainly monocytes and macrophages, in which they divide as intracellular amastigotes. Different strains of the parasite remain localized in the skin or disseminate to the mucosa or liver and spleen.

occurs when infected sand flies deposit the protozoan *Leishmania* parasite into the skin of a mammalian host during feeding (Fig. 17.2). Parasites establish infection within phagocytic cells of the immune system, the very cells typically associated with killing invading pathogens. Parasites establish chronic infection that varies depending on the strain from localized cutaneous leishmaniasis resulting in disfiguring skin sores and scarring; mucosal leishmaniasis, resulting in the destruction of the mucosa; or visceral leishmaniasis, resulting in disseminated infection of the internal organs and death in the absence of treatment.

## 2.1 Naturally Acquired Resistance: The Gold Standard of Protective Immunity

Mice and humans with healed *Leishmania major* cutaneous infection maintain robust cell-mediated immunity and are highly resistant to sand fly transmitted secondary *L. major* infection.[46] T helper 1 (Th1) $CD4^+$ T cells are particularly important to naturally acquired immunity by releasing IFN-γ that activates infected macrophages to kill the parasite.[47,48] While Th1 immunity results in recovery from disease, in most instances there is no sterile cure and parasites can maintain chronic but subclinical infection for the life of the host. The

immune state that is maintained after healing of a primary lesion is so effective that intentional, needle inoculation of culture-derived, infectious *L. major* into a selected site in the skin, termed leishmanization, has been employed for years in regions endemic for cutaneous leishmaniasis in the old world, as a highly effective "vaccine" in humans.[49] This is not a proper vaccine per se since virulent organisms that have not been deliberately attenuated are used. The practice has largely been abandoned because of the occasional long-lasting lesions that are produced and difficulties in the quality control of the inoculum and resultant scarring. Nonetheless, mimicking this protective response remains a key objective of vaccines against all forms of leishmaniasis.

Numerous experimental *Leishmania* vaccines generate parasite specific Th1 $CD4^+$ T cells and protect to varying degrees against needle challenge in animal models.[50] However, those vaccines that have been tested employing natural sand fly transmission have failed to prevent disease and none equal the protection observed in animals with a healed primary infection.[46,51] An extensive series of clinical trials carried out in the 1980s and 1990s employing whole cell killed promastigotes with live BCG as adjuvant, referred to as first generation vaccines (Table 17.2), also failed to reduce the risk of developing leishmaniasis following natural exposure.[52] By contrast, the same killed vaccines have been shown to be highly effective against different forms of leishmanial diseases when used as immunotherapy, either alone or in conjunction with drugs[53,54] (Table 17.2). The failure of *Leishmania* vaccines to replicate the prophylactic immune status of leishmanized individuals suggests that the mechanisms of protective immunity are more complex than originally thought. Several lines of experimental evidence support this. First, the $CD4^+$ Th1 immune response at sites of *Leishmania* challenge is significantly faster in leishmanized animals versus animals vaccinated with nonliving, antigen + adjuvant vaccines,[46,51] suggesting that the quantity and/or quality of the Th1 cells primed by these experiences are quite different. Second, the most protective population of $CD4^+$ Th1 cells generated by leishmanization is lost under conditions where the chronic infection is experimentally removed, and possesses functional and phenotypic characteristics of effector cells, not memory cells.[48,55] Because conventional antigen + adjuvant vaccines favor the induction of long-lived memory cells that do not require the continued presence of the antigen(s) for their maintenance, the observation that memory cells on their own fail to mediate the natural immunity induced by leishmanization, suggests that this immunity may not be engendered by conventional vaccination strategies.

## 2.2 Subunit Antigen + Adjuvant Vaccines and Viral Vectored Vaccines in Clinical Development

Several candidate subunit vaccines that employ conventional antigen + adjuvant formulations are in various stages of preclinical and clinical development.[50,56] While these vaccines are unlikely to maintain the short-lived T effector

**TABLE 17.2** ***Leishmania*** **Vaccines Tested in Human Subjects**

| Clinical application | Vaccine | Clinical testing | Efficacy | References |
|---|---|---|---|---|
| Prophylactic | Merthiolate-treated *Leishmania guyanensis* | 2 Field trials[a] | No protection against clinical Leishmaniasis: 1 year follow-up | [52] |
| | Autoclaved *L. major* + BCG[b] (Razi Institute) | 6 Field trials[c] | No protection against clinical Leishmaniasis: 1–3 years follow-up | [52] |
| | Autoclaved *Leishmania amazonesis* + BCG | 1 Field trial | No protection against clinical Leishmaniasis: 1 or 2 years follow-up | [52] |
| | Phenol-treated *L. amazonesis*, *L. brazilliensis*, *L. guyanensis* + BCG | 1 Field trial | 72.4% efficacy 1 year follow-up; no protection 1.5–5 years follow-up | [52] |
| | LEISH-F1: recombinant proteins TSA, STI1, LelF[d] in MPL-SE[e] | Phase II | Safe, immunogenic, and well tolerated in endemic populations | [59] |
| | LEISH-F3: recombinant proteins *L.d.*NH[f] and *L.i.*SMT[f] in GLA-SE[g] | Phase I | Safe, immunogenic, and well tolerated in a nonendemic population | [58] |

(*Continued*)

**TABLE 17.2** ***Leishmania*** **Vaccines Tested in Human Subjects (*cont.*)**

| Clinical application | Vaccine | Clinical testing | Efficacy | References |
|---|---|---|---|---|
| Therapeutic | Autoclaved *L. amazonensis* + half dose meglumine antimony | CL[h] patients | 100% cure versus 8.2% cure with antimony alone after 4 series of treatments | [53] |
| | Autoclaved *L. major* + BCG + sodium stibogluconate | PKDL[h] patients | 87% cure versus 53% cure with antimony alone day 60 posttreatment | [54] |
| | LEISH-F1 | MCL[h] patients | Safe, immunogenic, and well tolerated in endemic populations. No effect on clinical cure | [59] |
| | LEISH-F1 | CL patients | Safe, immunogenic, and well tolerated in endemic populations. Shorter time to cure | [59] |
| | Ad5-KH: recombinant *haspb1* and *kmp11*[i] genes in adenovirus | Phase I | Safe, immunogenic in nonendemic population, recently completed, unpublished data | [61] |

[a]*One trial consisted of two different vaccine and control groups.*
[b]*(BCG) Bacillus Calmette–Guérin.*
[c]*Total of 9942 vaccine recipients.*
[d](L.m.*TSA)* L. major *homolog of eukaryotic thiol-specific antioxidant, (*L.m.*STI1)* L. major *stress-inducible protein-1, (*L.b.*LeIF)* L. braziliensis *elongation and initiation factor.*
[e]*(MPL-SE) monophosphoryl lipid A stable oil-in-water emulsion.*
[f](L.d.*NH)* Leishmania donovani *nucleoside hydrolase, (*L.i.*SMT)* Leishmania infantum *sterol 24-C-methyltransferase.*
[g]*(GLA-SE) glucopyranosyl lipid A-stable oil-in-water nanoemulsion.*
[h]*(CL) cutaneous leishmaniasis, (MCL) mucocutaneous leishmaniasis, (PKDL) post-kala azar dermal leishmaniasis.*
[i](haspb1*) hydrophilic acylated surface protein B1 gene, (*kmp-11*) kinetoplastid membrane protein-11 gene.*

population maintained by leishmanization, it is premature to conclude that they will not confer some protection in people since so far no second generation *Leishmania* vaccines, particularly those employing newer adjuvants, have been evaluated in field efficacy trials. In addition, studies have suggested that a vaccine that was only 50% efficacious for 5 years would still be cheaper than current treatment regimes,[57] and because another important parameter of vaccine efficacy is reduction in infection reservoir potential, even nonsterilizing immunity may achieve a significant community benefit with respect to this endpoint.

The vaccines developed by the Infectious Diseases Research Institute (IDRI) represent the *Leishmania* vaccines that are in the most advanced stages of clinical testing.[58] These vaccines, termed LEISH-F1 and LEISH-F3 are recombinant *Leishmania* proteins formulated in a stable water-in-oil emulsion (SE) plus a TLR4 agonist as adjuvants. LEISH-F1 combines the *L. major* homolog of eukaryotic thiol-specific antioxidant (TSA), the *L. major* stress-inducible protein-1 (LmSTI1) and the *Leishmania braziliensis* elongation and initiation factor (LeIF). LEISH-F1 is safe and elicited an appropriate immune response in volunteers when combined with the adjuvant monophosphoryl lipid A (MPL), a *Salmonella* cell wall–derived TLR4 agonist, MPL, approved for use in people.[59] TLR4 is a T-cell receptor that recognizes microbial products and triggers innate immune responses. LEISH-F3 was designed specifically for use against visceral leishmaniasis and combines two antigens, nucleoside hydrolase from *L. donovani* and sterol 24-C-methyltransferase from *L. infantum* with glucopyranosyl lipid A (GLA), a synthetic TLR4 agonist.[58] These vaccines appear to be safe and immunogenic in Phase 1 and 2 trials and are the most likely candidates to move forward to Phase III intervention trials.

Recombinant viruses that efficiently prime and are cleared by the human immune system and that also express *Leishmania* antigens have been employed to prime strong *Leishmania*-specific CD4+ and especially CD8+ T cells. These vaccines have shown protection against needle challenge in rodent and nonhuman primate models.[60] A Phase I clinical trial of a prime-only adenoviral-based therapeutic vaccine (ChAd63-KH) for visceral leishmaniasis, carrying a novel synthetic gene encoding two *Leishmania* antigens (KMP-11 and HASPB1[61]) has recently been completed in UK volunteers (EudraCT number 2012-005596-14) and results are expected to be published soon. Viral vector vaccines employing prime-boost regimens are an active area of clinical investigation in the malaria vaccine field and the potential exists to synergize these findings with the *Leishmania* vaccine effort.

## 2.3 *Leishmania* Vaccines in Preclinical Development

DNA-based vaccines, designed to target both CD8 and CD4 T cells, rely on the translation of DNA coding for parasitic antigens by host cells and have shown good safety profiles in people. One such vaccine is LEISHDNAVAX, which is unique in its use of DNA encoding *Leishmania* protein antigens known to

elicit T-cell responses in people over a wide range of genetic backgrounds, and a viral protein to enhance immunogenicity.[62] Promising observations have also been reported for a *Leishmania* hemoglobin receptor-encoding DNA vaccine against visceral disease following needle challenge in animal models.[63] Potential concerns with DNA vaccines include integration of injected DNA into the host genome, persistence of plasmid DNA in the environment, and development of anti-DNA antibodies, though these concerns have largely been dealt with through continued refinement of the platform and monitoring. Presently the LEISHDNAVAX is the most likely and the first DNA-based *Leishmania* vaccine to move into clinical trials based on the inclusion of antigens proven to elicit responses in people.

A number of live-attenuated *Leishmania* vaccines are also in preclinical development.[64] The premise of live-attenuated vaccines is that they do not cause disease due to genetic attenuation but they replicate the infectious process and carry the full complement of *Leishmania* antigens and pathogen-associated molecular patterns, all factors thought to contribute to generating strong naturally acquired immunity. These innocuous vaccines have shown protection in animal models following needle challenge, and while they fail to establish long-term chronic infection in the laboratory, the potential for genetic reversion or recombination with naturally occurring parasites makes them perceived as less safe than conventional antigen–adjuvant-based vaccines. These vaccines have not been employed in people.

In experimental models, prior exposure to the bites of uninfected sand flies can result in protection from cutaneous leishmaniasis following exposure to the bites of infected sand flies,[65] presumably via the early activation of infected cells by cytokines that are locally produced by T-cells specific for salivary antigens. Recently, the defined sand fly salivary protein, PdSP15 was employed in a DNA/recombinant protein prime-boost vaccine that was shown to reduce maximal lesion size and time to healing in nonhuman primates following infected sand fly challenge.[66] This approach may represent the type of alternative vaccination schemes that will be required for parasitic diseases and vector transmitted diseases in particular. Presently, these vaccines have not been tested in people.

One novel approach to *Leishmania* vaccination is to harness the efficacy of leishmanization but minimize the lesion pathology by coadministration of infectious parasites with immune-modulators. In animal models, the use of CpG-containing immunostimulatory oligodeoxynucleotides as immune-modulators promoted minimal lesion development and faster healing without compromising the efficacy of leishmanization to provide long-term protection against secondary challenge.[67,68] In unpublished studies carried out in a highly endemic region of *L. major* transmission in Uzbekistan, inclusion of killed *L. major* promastigotes in the live inoculum moderated lesion pathology in leishmanized individuals. The disadvantage of this approach is that it may still establish a chronic infection that may be of concern should the vaccinees

become immunocompromised. It should be noted that despite the historical experience of leishmanization in millions of people, there is no documented case of disease-reactivation in these individuals.

### 2.4 Future Challenges for *Leishmania* Vaccine Development

Due to the financial constraints on developing a vaccine for a neglected tropical disease like leishmaniasis, the best case scenario for *Leishmania* vaccination may be to develop a single pan-*Leishmania* vaccine that provides protection against all clinical forms of the disease in all geographical locations.[43] This would require that the mechanisms and antigen-targets of protective immunity against the different clinical forms of the disease are sufficiently similar to allow their inclusion in a single vaccine.[59] Experimentally, there is evidence to support this.[69] The financial constraints would also benefit from an ability to conduct controlled challenge of vaccinated volunteers using infected sand flies, similar to how malaria vaccines are submitted to preliminary testing using controlled human malaria infection. The scarcity of laboratory-colonized sand flies and the fact that even drug treatment of leishmanial infections may not produce sterile cure, makes this approach difficult for practical and ethical reasons. However, in individuals at low risk of HIV and high risk of cutaneous leishmaniasis, it has been argued that leishmanization can itself be used as the challenge for preliminary evaluation of some experimental vaccines since even if the vaccine confers no protection, the leishmanization will.[56]

## 3 VACCINATION AGAINST HELMINTHS

As with the other neglected tropical diseases, helminths are chronic parasitic worm infections, especially common among the world's poorest people, that is, the bottom billion who suffer from at least one helminth infection.[70] There is currently no effective vaccine against any human helminth infection. The most common helminth infections are the soil-transmitted helminth infections (STH), which include infections by the nematodes hookworm (*Necator americanus* or *Ancylostoma duodenale*), ascariasis (*Ascaris lumbricoides*), and trichuriasis (*Trichuris trichiura*),[71] and the trematodes *Schistosoma* spp. (*Schistosoma mansoni*, *Schistosoma haematobium*, or *Schistosoma japonicum*). The life cycles of *N. americanus* and *S. mansoni* are shown in Fig. 17.3. Helminth infections exhibit several epidemiologic characteristics that distinguish them from viral and bacterial infections for which effective vaccines have already been developed. Individuals are commonly infected with helminths for decades or even an entire lifetime, and while these infections cause relatively little direct mortality, they induce extensive "disability." In the case of the hookworms, which can result in a chronic iron deficiency anemia, there is an effect on overall nutritional status, with sequelae such as lower cognitive ability, poor physical development, and poor birth outcomes.[72] The metric often applied to assess the public health

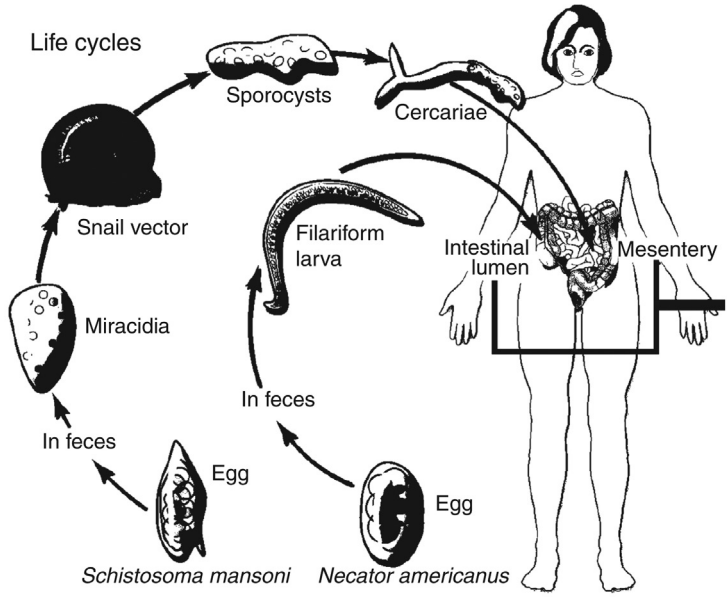


**FIGURE 17.3 The life cycles of the liver fluke *S. mansoni* and the hookworm *N. americanus*.** *S. mansoni* eggs are shed into the water with human feces, transform into a larval stage called "miracidia" that are able to infect an intermediate snail host in which they undergo asexual reproduction. Infective "cercariae" are released that will penetrate the skin of a human host who enters the water. They are then swept up into the vasculature, make their way through the lungs and the gut into the host mesentery where they mature into adult worms, mate, and shed eggs. Some of the eggs become lodged in the liver, where they induce a fibrotic response that accounts for the morbidity associated with schistosomiasis. The hookworm *N. americanus* is also flushed out into the environment with feces. However, unlike the liver flukes, hookworm eggs hatch in the moist ground where the filariform or infective stage larvae find their human hosts, penetrate the skin, often in the extremities, after which they are swept up into the vasculature, making their way through the lungs and into the gut where they mature, mate and expel eggs into the feces. Hookworms move along the lumen of the gut, burrowing into the mucosa and rupturing the capillaries and arterioles. The worms ingest the blood and disease occurs when the constant removal of blood leads to iron-deficiency anemia.

importance of helminth infections is disability-adjusted life years (DALYs) or the number of life years lost from premature disability or deaths. When measured by DALYs, helminth infections increase as a public health concern to the level associated with infections that produce profound mortality such as malaria or HIV/AIDS.[73] Moreover, it is hypothesized that helminth infections are not only most prevalent among the poorest individuals, they are an underlying reason for their poverty.[73] The economic aspects of helminth infections reflect their disproportionate impact on vulnerable populations in resource-limited settings; for example, the growth and development of children are hindered due to the anemia and malnutrition caused by helminth infections, especially hookworm and schistosomiasis.[74–77] In addition, adolescent females and pregnant women represent a highly susceptible population, with anemia and inflammation from

hookworm and schistosomiasis resulting in increased maternal morbidity and adverse pregnancy outcomes.[76,77] Schistosomiasis in the bladder predisposes to cancer and in the genital tract can result in infertility, with evidence that female genital schistosomiasis increases susceptibility to HIV/AIDS.[75] Hence, the term "antipoverty vaccines" is often applied to vaccines developed for helminth infections.[2]

## 3.1 Hookworm Vaccines

STH infections consisting mainly of ascariasis, trichuriasis, and the hookworms are among the most important neglected tropical diseases (NTDs) due to their cumulative disease burden, which is estimated at 39.1 million DALYs.[78] The control of STHs is a single dose inexpensive anthelmintic such as albendazole (400 mg) or mebendazole (500 mg).[79] A single dose of albendazole or mebendazole is most effective in reducing ascariasis, but much less effective for trichuriasis, and only 15% for hookworm infection. Moreover, posttreatment reinfection is common for all the STH targeted by this class of anthelmintic drugs, as they eliminate the adult or established worm infection with no prophylactic effect.[80] As hookworm(s) have the highest DALYs and are the least affected by the standard anthelmintic drugs, much of the financial and intellectual resources for STH vaccine development has been targeted toward this nematode infection.

The initial hookworm candidate antigen was the 21-kDa recombinant protein *Na*-ASP-2.[81,82] The *Na*-ASP-2/Alhydrogel® human hookworm vaccine was also found to be safe and highly immunogenic in a Phase 1 first-in-human randomized, placebo-controlled, double-blind trial using dose escalation among hookworm naive participants in the United States.[83] However, based on the outcome of a Phase 1 study in a hookworm endemic area in Brazil, where immunization with the lowest dose induced generalized hives and rashes (urticarial) in the first three previously infected volunteers, ASP-2 is no longer a candidate for vaccine development.[84]

Research is now focused on candidate antigens targeting the adult hookworm during patent infection to block hookworm blood ingestion.[85] The most promising vaccine antigen is a 45-kDa aspartic protease or *Na*-APR-1, which is a hemoglobin-digesting protease from the hookworm alimentary canal. *Na*-APR-1 is critical for parasite hemoglobin digestion. Vaccination of canines with a recombinant form of *Ancylostoma caninum* APR-1 (or *Ac*-APR-1) elicited a robust IgG response, which significantly reduces patent worm burdens and fecundity in immunized canines compared to control canines challenged with *A. caninum* L3.[86–88] More importantly, immunized dogs are protected from blood loss. The IgG from immunized canines neutralized the catalytic activity of the recombinant enzyme in an in vitro assay and IgG against Na-APR-1 was observed to be bound in situ to the intestinal lining of worms recovered from vaccinated dogs, implying that the vaccine interferes with the parasite's ability to digest blood. However, as it is not practical to immunize humans with an enzymatically

active protease, *Na*-APR-1 was inactivated by site-directed mutagenesis (two aspartic acid residues to alanines). When expressed as a recombinant protein, the mutagenized gene elicited neutralizing antibodies and host protection.[87] Phase 1 clinical testing of *Na*-APR-1 in the United States has shown it to be safe and immunogenic (Diemert and Bethony, personal communication).

A second adult-stage hookworm vaccine candidate antigen is the 24-kDa glutathione S-transferase (GST) or the *Na*-GST-1/Alhydrogel human hookworm vaccine, which also targets parasite blood feeding. Both the human (*Na*-GST-1) and the canine (*Ac*-GST-1) reduced host worm burdens immunized in hamsters with the mechanism of action of both vaccines appearing to be antibody mediated.[89] Hookworm GST-1 molecules belong to a unique Nu class of enzymes, which are involved in heme binding. From the X-ray crystal structure of Na-GST-1,[90] it has been hypothesized that the molecule forms dimers large enough to accommodate heme, hematin, or related molecules. Hence, *Na*-GST-1 may function to detoxify heme. *Na*-GST-1 expressed in the yeast *Pichia pastoris* has completed both process development and cGMP manufacture,[91] and has been shown to be safe and immunogenic in Phase 1 clinical testing in the United States and Brazil. Ultimately, *Na*-GST-1 and *Na*-APR-1 would be used together as a bivalent vaccine.[92]

## 3.2 Future Prospects for Hookworm Vaccines

Hookworm continues to be the target of robust vaccine development (Table 17.3). Due to concerns about the strong elicitation of hookworm-specific and total IgE allergic antibodies by the third larval stage (L3), current development efforts target the "hidden" antigens involved in blood feeding in the hookworm gut, with many having already shown safety in Phase 1 trials in the United States and endemic countries. Much enthusiasm now surrounds the development of controlled human infection (CHI) with the hookworm *N. americanus*. Due to the safe and successful infection of humans with *N. americanus* as a therapeutic for allergic and autoimmune diseases (eg, celiac disease),[93] it is now feasible to develop CHI to immunize humans with hookworm vaccine candidates and then challenge them with L3. This would greatly economize on the number of human subjects, time, and cost needed to conduct an efficacy trial. CHI is currently under development in a dose-ranging study at the George Washington University.

## 3.3 Schistosomiasis Vaccines

Schistosomiasis is caused by trematode *Schistosoma* species that are widely distributed in Asia, Africa, Latin America, and the Middle east. It is arguably the most important human helminth infection in terms of global mortality and morbidity. Infection occurs when skin comes in contact with contaminated freshwater in which certain types of snails that carry the parasite are living. Freshwater becomes contaminated by *Schistosoma* eggs when infected people urinate or

**TABLE 17.3 Hookworm Vaccines in Clinical Development**

| Target stage | Vaccine | Clinical testing | Efficacy | References |
|---|---|---|---|---|
| Larval | *Na*-ASP-2 | Phase 1 | Safe and immunogenic in hookworm naive participants in Phase 1 in the USA; subsequent Phase 1 in Brazil halted due to generalized urticarial reaction to lowest dose | [84] |
| Adult | *Na*-GST-1 | Phase 1 | Safe and immunogenic in hookworm naive participants in Phase 1 trials in the USA and endemic areas in Brazil and Gabon | [91] |
| | *Na*-APR-1 | Phase 1 | Safe and immunogenic in hookworm naive participants in Phase 1 trials in the USA and endemic areas in Gabon | [87] |

*Na*-ASP-2, *N. americanus* Ancylostoma secreted protein 2; *Na*-GST-1, *N. americanus* glutathione transferase 1; *Na*-APR-1, *N. americanus* aspartic protease 1.

defecate in the water. The eggs hatch, and if the appropriate species of snails are present in the water, the parasites infect, develop, and multiply inside the snails. The parasite leaves the snail and enters the water where it can survive for about 48 h. *Schistosoma* parasites can penetrate the skin of persons who come in contact with contaminated freshwater, typically when wading, swimming, bathing, or washing. Over several weeks, the parasites migrate through host tissue and develop into adult worms inside the blood vessels of the body. Once mature, the worms mate and females produce eggs. Some of these eggs travel to the bladder or intestine and are passed into the urine or stool.

Symptoms of schistosomiasis are largely caused not by the worms themselves but by the body's reaction to the eggs. Eggs shed by the adult worms that do not pass out of the body can become lodged in the intestine or bladder, causing inflammation or scarring. Children who are repeatedly infected can develop anemia, malnutrition, and learning difficulties. After years of infection, the parasite can also damage the liver, intestine, spleen, lungs, and bladder, including bladder cancer induced by *S. haematobium*. As argued by King et al.,[94] the public health impact of schistosomiasis as quantified by DALYs should include not only gross organ pathology, but also the "anemia," "pain," "diarrhea," "exercise intolerance," and "undernutrition" during chronic infection. The major approach to schistosomiasis control is treatment with praziquantel (PZQ) integrated into control programs for other NTDs.[95]

Three schistosome antigens have entered into clinical trials (Table 17.4). The oldest is a recombinant 28-kDa GST (glutathione S-transferase) cloned from

**TABLE 17.4** ***Schistosoma*** **spp. Vaccines in Clinical Development**

| Target | Vaccine | Clinical testing | Efficacy | References |
|---|---|---|---|---|
| Extracellular loop 2 (tegument) | *Sm*-TSP-2 | Phase 1 | Currently in phase 1 first-in-humans studies in the USA with subsequent studies planned for *S. mansoni* endemic areas in Brazil | [111] |
| Fatty acid binding protein | *Sm14* | Phase 1 | Safe and immunogenic in Brazilian adults | [98] |
| Calpain (tegument) | *Smp80* | Preclinical | Testing in hamsters and nonhuman primate models | [100] |
| Adult | *Sh28-GST* | Phase 1 and 2 | Safe and immunogenic in Phase 1 trial. Undergoing Phase 2 but still blinded | [96] |

*Sm*-TSP-2, *S. mansoni* Tetraspanin 2; *Sm14, S. mansoni* 14 kDa (fatty acid binding protein); *Smp80, S. mansoni* protein 80 kDa (calpain); *Sh28-GST, S. haematobium* 28 kDa glutathione-S-transferase.

*S. haematobium*, which has undergone clinical testing in Europe and West Africa (Senegal and Niger). The Sh28-GST or Bilhvax from Institut Pasteur together with the French Institut National de la Santé et de la Recherche Médicale is formulated with on aluminum hydroxide adjuvant.[96] Bilhvax has been shown to be safe and immunogenic in healthy adults from France and *S. haematobium* endemic areas in Africa. Another antigen that has undergone recent Phase 1 testing is the 14-kDa fatty acid binding protein known as Sm14[97,98] developed by the Fundação Oswaldo Cruz (Oswaldo Cruz Foundation, or FIOCRUZ) of the Brazilian Ministry of Health. In experimental animals (mice and rabbits), Sm-14 elicits protection against *S. mansoni* as well as *Fasciola hepatica*, a trematode fluke responsible for human and veterinary fascioliasis, infection of the liver. Recombinant Sm14 is being developed as an anthelmintic vaccine for use against both fascioliasis of livestock and human schistosomiasis due to *S. mansoni.* Sm-p80 is another *S. mansoni* antigen at an advanced stage of preclinical development. This antigen encodes the large subunit of a calcium-dependent neutral protease and has been tested in a DNA prime and protein-boost schedule as well as with a more conventional recombinant protein schedule.[99,100] In all cases, Smp80 has shown excellent protection in a variety of animal models, including a nonhuman primates.

Over the past few years several major advances in schistosome molecular biology have occurred: the genome(s), transcriptome, and much of the

tegument proteome of *S. mansoni* have either been completed or mostly characterized.[101,102] These proteomic and transcriptomic analyses point to the importance of the schistosome tegument, the outer coat of the worm, as a vaccine target. The failure to develop an efficacious vaccine against schistosomes is due in large part to the complex immunoevasive strategies by the parasite to avoid elimination from its intravascular environment.[103] Much of this immune evasion is attributed to the dynamic nature of the tegument. Mammalian stage schistosomes have a host-interactive outer surface tegument consisting of a single, contiguous, double-bilayer (heptalaminate) membrane that covers the entire worm. The tegument is thought to be involved in several key physiologic processes: parasite nutrition, osmoregulation, and the evasion of host immunity.[104] The host-exposed capsular surface is the target of the most protective vaccines and includes successful examples of metazoan parasite vaccines, such as the cattle tick *Boophilus*,[105] the gastrointestinal nematode *Haemonchus contortus*,[85] and several species of cestode parasites.[106]

Despite the large number of proteins associated with the tegument structure, few tegument proteins are found in the "outer" tegument of live worms, where they are likely to be exposed to the host immune system.[107] To identify proteins that contain membrane-targeting signals and are putatively expressed in the outer tegument, signal sequence trapping has been used to identify two *S. mansoni* cDNAs of particular interest—Sm-tsp-1 and Sm-tsp-2.[108,109] These mRNAs encoded novel tetraspanins (ie, four-transmembrane domain proteins homologous to surface receptors on B- and T-cells) have two extracellular (EC) domains—the small loop (EC-1) and the large loop (EC-2). In several independent descriptions of the *S. mansoni* adult worm tegument, TSP-2 was one of the few integral membrane proteins to be consistently found on the outer surface of the tegument. A marked and significant reduction (83%) of adult parasites was observed from mice injected with schistosomulae pretreated with Sm-tsp-2 dsRNA compared to control mice injected with untreated schistosomulae.[108] These data suggest that tetraspanins play an important role in maintaining the integrity of the tegument, including its structure and development. Other tetraspanins have served as vaccine candidates: for example, Sj23 is a tegument tetraspanin used in DNA vaccine for water buffaloes, an important reservoir for *S. japonicum* in China.[110]

Because the TSPs may be exposed to the host immune system, sera from individuals putatively resistant (PR) to *S. mansoni* infection from Brazil were screened for antibodies against recombinant versions of these proteins. The PRs had elevated levels of the cytophilic antibodies IgG1 and IgG3 compared with age, sex, and water contact matched individuals chronically infected with *S. mansoni* from the same endemic area.[109] The second EC domain fragment of a schistosome tetraspanin known as *Sm*-TSP-2 has been selected for development as a human vaccine antigen and is currently in Phase 1 first-in-humans clinical testing in the USA.[92,109] When the 9-kDa EC domain was expressed in either *P. pastoris* or *Escherichia coli* and formulated with either Freund's

complete adjuvant, aluminum hydroxide, or aluminum hydroxide together with CpGs,[92,109] it provided high levels of protection in mice challenged with *S. mansoni* cercariae. Of note, in the Brazilian serosurvey, there was the absence of IgE to *Sm*-TSP-2 in both PR and chronically infected individuals, which should permit a *Sm*-TSP-2 vaccine to avoid the recently identified technical challenges for helminth vaccines inducing serious allergic reactions encountered using the hookworm vaccine Na-ASP-2/Alhydrogel, which elicited a generalized urticarial response in previously infected individuals.[92] The *Sm*-TSP-2 vaccine would be intended primarily for school-aged children living in the *S. mansoni* endemic regions of sub-Saharan Africa and Brazil, a population considered at the greatest risk for acquiring schistosomes and suffering the greatest morbidity compared to other age groups. The *Sm*-TSP-2 vaccine would be administered intramuscularly in a prime-boost regimen to prevent the reacquisition of schistosomes in the blood stream following initial treatment with PZQ (vaccine-linked chemotherapy).[111]

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Chapter 18

# Tuberculosis Vaccines

**Willem A. Hanekom, MD, PhD**
*Initiative Lead for TB Vaccines, TB Team Division of Global Health, Bill & Melinda Gates Foundation, Seattle, WA, United States*

## Chapter Outline



## 1 INTRODUCTION

Tuberculosis (TB) usually manifests as a lung disease. Diagnosis is often delayed because of the chronic nature of the disease, while 6 months of treatment is required for a cure. Many diagnostic and antimicrobial tools that are currently used for intervention represent relatively dated technologies.

The causative pathogen, *Mycobacterium tuberculosis* (Mtb), has been remarkably successful in causing and sustaining a global pandemic. One third of the world's population is infected with Mtb. More than 9 million people developed active TB disease in 2014; 1.5 million died.[1] Deaths from TB now exceed those

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from HIV/AIDS.[1] Drug-resistant strains of the pathogen are emerging and are causing disease that is difficult and costly to treat, and increase morbidity and mortality.[1]

Despite reductions in prevalence and mortality, the global decline of TB incidence has been discouragingly slow.[1] Therefore, new diagnostics and antimicrobial regimens are needed, as well as a new vaccine that would effectively prevent adult forms of pulmonary TB—the latter is likely to have the greatest impact among all new tools.[2] Vaccines would also work regardless of drug resistance.

The challenge for developing TB vaccines lies in inducing immunity that would result in protection rather than pathology. It is clear that to propagate and survive in humans because there is no known animal reservoir, Mtb has to cause damage to the lungs, a prerequisite for transmission. It is unclear to what extent lung damage in TB is caused by the pathogen and to what extent by immune inflammatory rather than protective responses of the host.[3]

Epidemiological evidence suggests that development of an effective TB vaccine would be possible. Only 10% of immunocompetent persons infected with Mtb will develop active TB disease in a lifetime, which implies that most humans have immune mechanisms that control the pathogen and prevent disease manifestations. Further, multiple studies from prior to the antibiotic era found that latent (asymptomatic) Mtb infection was in fact highly protective against disease caused by reinfection.[4]

Only one vaccine is licensed to prevent TB—Bacille Calmette-Guérin (BCG)—and is usually given at birth. This vaccine protects infants and young children against disseminated forms of TB (see later). BCG affords variable—mostly poor—protection against pulmonary disease (see later). Although some progress has been made, the world of new TB vaccine discovery and development is in its infancy; new strategies are needed to accelerate the process.[5,6]

## 2 TUBERCULOSIS

### 2.1 History

Mtb was first recognized as the cause of TB in 1882, an achievement for which Robert Koch received the Nobel prize. The pathogen may have coexisted with humans for up to 70,000 years, suggested by a recent report of the global distribution and variability among Mtb strains, which indicated that Mtb migrated out of Africa with humans during the Neolithic period.[7] This also points to the first and perhaps main roadblock in developing effective vaccines against TB: our understanding of the coevolution of the human and Mtb remains limited; in particular, we do not fully comprehend how humans control the infection.

### 2.2 Epidemiology

Although the global public health threat of TB remains significant, some changes in the epidemiology of the disease have been encouraging. TB mortality has

fallen by 47% since 1990; it is estimated that effective diagnosis and treatment of TB has saved 43 million lives between 2000 and 2014.[1] The millennium development goal of halting and reversing TB incidence has been achieved: globally, this incidence has decreased by 1.5% per year since 2000, and in 2014 was 18% lower than the level of 2000.[1] The global TB prevalence rate in 2015 was 42% lower than in 1990.[1]

Regardless, the rate of decline in global incidence is slow, and even if the entire world could achieve a decline of 5% shown in some endemic countries, it is not nearly possible to reach the World Health Organization's (WHO) goal of a global incidence of 20/100,000/year in 2030.[8] This is the major argument for developing a more effective preventive TB vaccine, as a tool to complement current tools for intervention.

The majority of the world's TB cases occur in the Southeast Asia and Western Pacific Regions, while Africa has the highest incidence: southern Africa is the most severely affected.[1] Here, HIV infection is common and the immune compromise caused by this infection has been a driver of the epidemic.[1] TB disease occurs in men at a frequency that is about double of that in women, while $<10\%$ of cases manifest in children.[1] TB is overwhelmingly a disease of the socioeconomically disposed.

Therefore, ideally, a vaccine should be able to target all ages and persons with all comorbidities, but if not possible, an effective vaccine that targets adults without comorbidities such as HIV infection will still have a massive impact (see later).

## 2.3 Bacteriology

Mtb is one of nine mycobacterial species that are collectively classified as the *Mycobacterium tuberculosis* complex: *M. tuberculosis*, *M. africanum*, *M. canettii*, *M. bovis*, *M. caprae*, *M. pinnipedii*, *M. microti*, *M. mungi*, and *M. orygis*. Each can cause clinical TB disease in humans, although Mtb is by far the most common. More than 140 additional nontuberculous mycobacterial (NTM) species are found worldwide, often present in soil and water reservoirs.[9] BCG is an attenuated strain of *M. bovis*. Multiple NTMs, and even modified Mtb, are currently in clinical development as vaccine candidates (see later).

Some of the approximately 3800 proteins of Mtb are immunodominant, meaning that most infected persons' immune systems have developed a detectable immune response to these proteins. Examples include early secretory antigen target-6 (ESAT-6) and culture filtrate protein 10 (CFP-10). New candidate vaccines classically contain immunodominant proteins (see later). This may be a concern, as very limited variation among these proteins has been shown in Mtb strains from across the globe; our assumption would have been that tens of thousands of years of immune pressure from humans on the pathogen would have resulted in significant changes in these proteins.[10] Rather, nonimmunodominant proteins of Mtb show greater variation.[10] These observations suggest human immune responses to immunodominant proteins may hold evolutionary advantage to the pathogen. Overall, it is not known which particular protein our

immune systems should target for protection against TB—a gap in our knowledge that compromises vaccine development.

Mtb has a capsule, the very outer layer of the cell wall.[11] Current procedures for growing Mtb in the laboratory involves detergents in the growth medium, as well as shaking, both of which result in destruction of the capsule. Capsular components are likely to be encountered first by the human immune system following inhalation, and may contain components that should be targeted with vaccination. Multiple efforts are currently underway to grow Mtb in vitro while retaining the capsule; success is likely to enhance vaccine discovery and development efforts.

## 2.4 Pathogenesis

TB is spread by aerosols, following coughing. About a third of exposed persons become infected following inhalation. Infection is detected by a positive tuberculin skin test (TST) or a positive interferon-γ release assay (IGRA). Interestingly, a small fraction of exposed persons appear to resist infection, as demonstrated by persistently negative TSTs or IGRAs following repeated exposure.[12] Studying these individuals may reveal clues for development of vaccines to prevent infection.

Only 10% of untreated persons infected with Mtb will develop active TB disease in a lifetime, which implies that most humans have immune mechanisms that control the pathogen and prevent disease manifestations. Intensive investigation is ongoing to delineate these mechanisms in prospective cohort studies of humans (see later).

Approximately 50% of disease occurs within 2 years of infection, while disease manifests much later in life in the other 50%—so-called reactivation disease. Disease commonly occurs in persons with relative immune compromise, associated with poor nutrition, other conditions that are linked to poverty, or diabetes mellitus, for example. More overt immune compromise, including that caused by HIV infection, increases the risk of progression to TB disease following infection dramatically: Mtb and HIV coinfected persons not on antiretrovirals have a 10% annual risk of developing active TB disease, while the risk decreases significantly on antiretroviral therapy.[13]

Age is also a determinant of progression to active disease following infection. Infants appear to have a 5–10-fold increased risk of progressing to TB disease following infection, compared with adults[14]; their relatively immature immune systems may be responsible. Relative immune compromise in the elderly is also likely responsible for the rise in incidence of TB disease in this age group. Remarkably, prepubescent children between the ages of 5 and 10 years appear to have the lowest lifetime risk of developing TB disease following infection[15]—their host responses to mycobacteria, when compared with other ages, may hold clues to successful vaccination strategies to prevent disease.

The host response to Mtb infection and associated with TB disease is complex.[3,16] Briefly, inhaled Mtb is taken up by cells that patrol the airways, called macrophages. These cells commonly need help to kill or control growth of the

pathogen inside the cell. The immune system provides this assistance by activating other immune cells, called T lymphocytes, which deploy strategies to help the macrophages. Most TB vaccines are designed to induce T lymphocytes, which would then be ready to help fight off the pathogen when infection occurs.[17] Acute TB disease occurs when these immune responses fail, or become excessive, resulting in widespread inflammation. Therefore, a vaccination approach should induce sufficient immunity to protect; a disproportionately large vaccine-mediated response could be detrimental.

Mtb is known to induce a wide range of immune responses beyond those mediated by T lymphocytes.[16] Unfortunately, it is not known what constitutes an essential and sufficient immune response to protect against TB; this lack of information hampers TB vaccine development, and is an area of investigation. This also focuses on many mechanisms that Mtb uses to subvert or avoid the immune response, and on the excessive inflammation that characterizes development of clinical disease.

## 2.5 Clinical Manifestations

As mentioned earlier, the lung is by far the most common site of symptomatic disease.[18] Early symptoms include loss of appetite, malaise, and fatigue, often lasting for weeks. Classical symptoms include chronic cough, low-grade fever, weight loss or failure to thrive, night sweats, and chest pain. Symptoms develop insidiously; patients often present to health-care facilities following an acute bacterial pulmonary superinfection (an infection on top of TB). TB may also affect virtually any organ of the body. Disease manifestations vary between prepubescent children and adolescents/adults,[19] suggesting possible differential mechanisms of protection, which may therefore require differential vaccination approaches. Children with active TB classically have milder lung disease, compared with adults. Pulmonary cavities (holes in the lung) are rare in children, while lymph node enlargement in the thorax is common. In contrast, adolescents/adults often have more severe lung disease with cavitation and much higher bacterial loads, compared with children. Children are at higher risk of disease in other organs, such as neck lymph node disease, or severe disease such as meningitis or miliary disease, when bacteria have disseminated to all organs, compared with adults. HIV-infected persons not controlled on antiretrovirals are also at risk of disseminated TB disease; their lung disease presentation may be atypical.[20]

## 2.6 Diagnosis

Diagnosis of TB disease starts with obtaining a history of exposure, and careful examination for classical clinical features. Chest x-rays are usually performed, and may show consolidation (white changes on the x-ray indicating disease), collapse of parts of the lung, cavitation, and pleural effusions (fluid on the lung). The x-ray

is an insensitive tool, and variability in expert interpretations is common,[21] which may be a problem in clinical trials of vaccines when x-rays findings are outcome measures. High-resolution chest tomography (CT) may afford better sensitivity for diagnosis, but is not commonly available in settings where TB is common.

TB infection is diagnosed by a TST or IGRA (interferon-gamma release assay).[22] In a TST, a small amount of proteins of TB are injected into the skin, and swelling at this site is measured two days later; the swelling is caused by T lymphocytes that had been primed when the person became infected. The IGRA is a blood test that measures production of IFN-γ by immune T lymphocytes. The tests can also be helpful when active, symptomatic disease is present, but cannot differentiate infection from active disease. Both tests may show false positive results, for example, the TST can be positive following BCG vaccination, and the IGRA can be positive following infection of some NTMs.

Sputum, either directly collected following coughing or following induction by hypertonic saline nebulization, or early morning gastric aspirates in children, is usually examined to grow Mtb, to confirm the diagnosis. These so-called cultures may continue for weeks, although most will turn positive within 10 days. Sputum is also examined under the microscope in an attempt to directly visualize the pathogen: this may be up to 90% sensitive in adults, but is usually <5% sensitive in infants and children: an indication of differential bacterial loads in the age groups. The microbiological techniques described above are decades old; newer techniques to directly detect Mtb in sputum by amplifying genetic material of the bacterium (nucleic acids) have emerged relatively recently.[23] These tests are rapid, easy to execute, and perform well: a review of multiple studies indicated that one such test, Xpert, had a pooled sensitivity and specificity of 88 and 98%, respectively, for replacement of smear microscopy in adults.[24] In addition, these tests can determine whether the pathogen is drug sensitive or multi-drug resistant (MDR) within 2 h, but is relatively expensive and not available in low- and middle-income countries at the point of care.

Drug susceptibility testing should be done as a routine, but is often not performed due to limited resources. Conventional culture methods are common for susceptibility testing, but testing is now often incorporated into nuclear amplification testing mentioned earlier.

The relative difficulty in diagnosing TB complicates definition of endpoints of efficacy trials of new TB vaccines, and demonstrates that successful intervention in the TB pandemic would require concerted development of multiple approaches or tools; this was also shown in modeling studies of multiple inventions to control TB.

## 2.7 Treatment

Latent TB infection can be treated by 6–9 months of daily or weekly isoniazid (INH), or by 12 weeks of INH and rifapentine[25]; this will reduce the risk of developing TB disease by >80%. These interventions are effective in high transmission settings only while on therapy, and not thereafter, as reinfection is

common.[26] In the latter settings, it is recommended that HIV-infected persons remain on continuous INH therapy.[27]

A combination of four bactericidal drugs (drugs that kill and not just inhibit the pathogen) are used for 2 months to treat active pulmonary TB disease caused by drug-sensitive strains: INH, rifampicin (RIF), pyrazinamide, and ethambutol. INH and RIF alone is then given for another 4 months. The patient's clinical and diagnostic test profile, drug side effects, and drug resistance may call for modulation of drug choice and of duration of therapy.[28] For example, drug-resistant strains of Mtb require much longer durations of therapy, which is very expensive (often draining large portions of a country's TB treatment budget, even if resistance occurs in a small fraction only) and which is associated with severe adverse reactions to the drugs. Treatment is usually administered within a strategy called DOTS—directly observed treatment, short-course—which means that an independent observer watches the patient swallow his/her anti-TB therapy, at least for the first 2 months.

A rising threat is the emergence of MDR-TB, resistant to isoniazid and rifampicin, the two most effective drugs, and XDR-TB, extensively drug-resistant TB, which requires multiple second-line drugs, often toxic, given over 24 months, with a cure rate of around 60%.[29] It represents a major threat to populations in high burden countries and particularly to health-care personnel.

As stated earlier, it is not prudent to view vaccine intervention into the TB epidemic in isolation—combined and optimal delivery of better vaccines with newer, more sensitive and practical diagnostics as well as shorter, effective, and universally applicable treatment regimens are needed. Finally, the future health crisis of drug resistance calls for urgency in vaccine development, as vaccines would work in preventing all strains of TB, regardless of resistance.

## 3 THE CURRENT TB VACCINE – BCG

This live attenuated vaccine was developed in 1908 in France by Calmette and Guérin, who isolated an *M. bovis* strain from a cow with TB mastitis and repeatedly grew the bacterium in the laboratory, until it was attenuated and no longer virulent (dangerous). The vaccine strain was initially given orally to infants; intradermal (into the skin) and other techniques for administration were developed later. BCG vaccination has been included in WHO's Expanded Program on Immunization since 1974; >4 billion persons have received the vaccine to date, while currently >100 million infants receive BCG annually.

### 3.1 Bacteriology, Manufacturing, and Supply

Multiple substrains of BCG have been generated following decades of subculture (regrowth) of the original strain.[30,31] By examining the genetic material of these substrains, as well as behavior in various laboratory experiments, it is clear that marked variation exists among BCG strains in clinical use today.[30,31] This may be a

concern, as unintended overattenuation (overweakening) of strains, compared with parental BCG, could have developed.[32] Differences in strains are thought to have contributed to variability in BCG efficacy shown in clinical trials (see later). Knowledge of differential efficacy according to vaccine strain remains elusive; however, diverse immune responses induced against different strains in humans strongly suggest that this will be the case.[33,34] An important first step would be to define which immune response induced by BCG is critical for protection against TB.

The most commonly used BCG strains, that is, French (Pasteur) strain 1173 P2, Danish (SSI) strain 1331, Glaxo strain 1077, Tokyo strain 172-1, Russian strain BCG-I, Moreau RDJ, Montreal strain (Canada), and Tice strain (United States), are manufactured according to quality assurance guidelines from WHO, using techniques that in the modern vaccinology age could only be described as archaic and inefficient.[35] This has been responsible, in part, for global shortages of BCG vaccine in recent years. An immediate and urgent priority in the TB vaccine field would be introduction of more modern, and easily feasible, culture techniques. As BCG is supplied in multidose vials, which expire soon after reconstitution, this contributes to significant waste; efforts toward different final formulation are warranted.

United Nations agencies are the main procurers of BCG, and supply over 120 million doses per year to countries receiving vaccines from GAVI (formerly the Global Alliance for Vaccines and Immunization).

## 3.2 Current Use

An atlas of BCG vaccination policy and practices in 180 countries has been published.[36] By far the majority of the world's countries practice universal BCG vaccination, and commonly administer the vaccine by intradermal injection to newborn infants, often on the first day of life.

WHO recommends a single dose of BCG to newborns around the time of birth, which assures high coverage. Evidence of efficacy of revaccination lacks. There may be certain settings where revaccination could hold advantage—likely Mtb-uninfected persons who have not had extensive exposure to NTM, as shown in a large revaccination trial in Brazil.[37] As BCG remains the only available vaccine, appropriate revaccination practice that would have impact is a research question that should be prioritized.

In the past, conversion of the TST has been used widely to assess whether a person has responded to BCG vaccination; revaccination was often advised if no response was detectable. Because multiple lines of evidence now exist that TST reactivity has no relation with vaccination-induced protection,[38] this practice should be avoided.

## 3.3 Immunogenicity

As stated earlier, the qualitative or quantitative determinants of BCG-induced immunity required for protection against TB are not known. To date,

immunogenicity assessment has focused on induced T lymphocytes, presuming an essential role in protection. BCG is potent at inducing this immunity in newborns—in this population, a remarkable feature is variability in the magnitude and character of the response.[39,40] Regardless, a study of nearly 6000 infants vaccinated with BCG at birth showed that these T-lymphocyte responses did not correlate with risk of TB disease in the first 2 years of life.[41] Immunity that is presumed to be critical may therefore not be sufficient for protection against TB, and should be a priority focus for investigation.

## 3.4 Efficacy and Effectiveness

Many studies have been completed to determine how and when BCG protects against TB. Meta-analyses, studies that have examined trials' results together, have taught us the following[42–45]:

- BCG protects against disseminated forms of childhood TB, such as miliary TB (where TB has spread to most organs of the body, causing severe disease) and TB meningitis; overall efficacy is around 80%.
- Overall protective efficacy against active lung disease is in the range of 50%; however, it is important to recognize that this protection is highly variable—studies have shown a range between 0 and 80%. It is clear that protection against lung disease is greater when BCG is used early in childhood and in persons who are TST negative prior to vaccination, and in persons who live farther from the equator. The negative association between NTM exposure and BCG efficacy may explain the latter two observations, in part. NTM may either block induction of immunity by BCG by controlling the live organism in the vaccine (perhaps killing the BCG, preventing induction of immunity), or may mask protection induced by BCG because protection may have been afforded by the NTMs themselves.[46]
- BCG appears to have a modest effect in preventing Mtb infection: overall, ~20% efficacy in retrospective observational studies. As prevention of infection may have a significant impact on the TB epidemic (see later), confirmation of this effect with prospective studies is important.
- Finally, BCG may prevent disease caused by other mycobacteria: efficacy in preventing leprosy is excellent,[47] while the vaccine is poorly protective against Buruli ulcer.[48]

Among controlled clinical trials that have evaluated BCG efficacy in >10,000 participant years of follow-up, three such studies have shown good protection,[49–51] while five have shown a moderate to poor protective efficacy.[52–57] The latter group includes the largest trial, conducted in Chingleput in India, which enrolled 260,000 participants followed for 15 years.[57] Overall, much has been written about the diverse settings, study designs, and procedures, and of the quality of the different trials, all of which could have contributed to the range of findings. Three features distinguish the trials that have shown good

efficacy from the rest[43]: they were completed in geographic regions with a low prevalence of NTMs, suggesting interference by these organisms, the participants were infants or adolescents, and the methodology and statistical precision appeared to be superior. Other reasons for variable efficacy could have been differential vaccine strains used, diverse Mtb strain prevalence and differential host genetic and microbiotic constitution.

It is not known how long protection induced by BCG lasts. Studies of the kinetics of the immune response following newborn vaccination suggest that protective immunity would be short-lived[58]; this was also suggested by a case-control study of BCG efficacy over the first 20 years of life.[59] Regardless, studies of Native American populations suggest that BCG-induced protection may last for up to 60 years.[60]

## 3.5 Safety

BCG has had an excellent safety record over decades of use. Local reactions at the site of vaccination are common but limited in severity and duration; redness, mild swelling, tenderness, a papule and ulceration may occur. Lymph glands under the armpit may also become swollen. The BCG strain and dose used for vaccination, the route of administration and gender and age are covariates for adverse effects.[61–63] A lasting skin scar develops in most patients, although the likelihood is lower when vaccination is given in early infancy.[64] Persons with latent Mtb infection appear to have an accelerated adverse effect response to BCG vaccination.[65]

Disseminated BCG disease—when BCG has caused disease in organs distal to the site of vaccination—is exceedingly rare. This disease occurs in immune compromised persons, such as those infected with HIV or those with congenital immune deficiencies affecting T-lymphocyte function.[66,67] In both settings, BCG may be fatal. In HIV infected infants not on antiretrovirals—a scenario that should not occur anymore—BCG disease incidence may approach that of TB disease itself.[68] Most disease manifests as lymph gland disease, but involvement of multiple other organs, including bone, has been described.

BCG as a live attenuated organism is contraindicated in HIV-infected persons, including infants; however, today, only a very small fraction of infants exposed to maternal HIV will become infected because of widespread use of antiretroviral therapy to prevent transmission. BCG should be not be given at birth to HIV-exposed infants; vaccination should be deferred until the infant has been shown not to be infected with HIV.[69] WHO recommends this practice only in settings where uptake of mother to infant HIV transmission prevention strategies are optimal, and where follow-up and early diagnosis of HIV infection can be guaranteed.[69] Unfortunately, these conditions cannot be met in most health settings where TB is endemic. Here, not vaccinating a significant portion of babies could precipitate an epidemic of disseminated and pulmonary TB in infants; most HIV-exposed infants therefore continue to receive BCG at birth.

Some experts use this dilemma as a rationale for developing a safer BCG, or even attenuated Mtb, for vaccinating babies, although this strategy will not have a significant overall impact on the TB epidemic (see later).

### 3.6 Nonspecific Effects of BCG

BCG may have beneficial effects of morbidity and mortality that extend beyond those caused by TB. For example, some of the large BCG efficacy trials showed a reduction in nonaccidental deaths of about 25%, while studies in Guinea Bissau have shown a 40–60% reduction in all-cause mortality in BCG vaccinated infants.[70–73] Although these results have been controversial, some plausibility was provided by a finding that BCG induces epigenetic modifications of monocytes—the vaccine therefore appears to "train" these cells of the immune system—which may result in protection against other pathogens.[74]

Incomplete data suggest that BCG may also have nonspecific effects that extend to cancer and other chronic inflammatory disorders. BCG is use routinely for the management of bladder cancer. Lessons learned from mechanisms of action of BCG in settings other than for prevention of TB may hold clues to optimal use of this vaccine for TB, or for design of alternate vaccination strategies.

## 4 NEW STRATEGIES TOWARD VACCINATION AGAINST TB

### 4.1 New TB Vaccines in Clinical Development

Twenty new vaccines against TB have entered clinical trials (Fig. 18.1). The vaccines fall into three categories[17,75–77]:

- Whole cell vaccines, where a live attenuated (weakened) bacterium, or an inactivated (killed) whole bacterium, or a lysate of the whole bacterium (fragments after the bacterium has been broken up) is used for vaccination. Vaccination results in an immune response to many components of the bacterium.
- Viral-vectored vaccines consist of a virus such as adenovirus, a cause of the common cold, which has been genetically modified to become safe and to produce up to three proteins of Mtb—this is possible because the genetic code for these proteins has been incorporated into the genetic material of the virus.
- Subunit vaccines contain up to three Mtb proteins, often fused to make them more stable and immunogenic, and formulated together with an adjuvant—a substance that will boost the immune response to the proteins. For both viral-vectored and subunit vaccines, vaccination results in an immune response to the selected proteins only.

All are given by injection. With two exceptions, all modern TB vaccine candidates have shown acceptable safety and reasonable immunogenicity in first trials. Four candidates have entered efficacy trials—studies that assess whether a vaccine may prevent TB, after safety and immunogenicity has been shown.

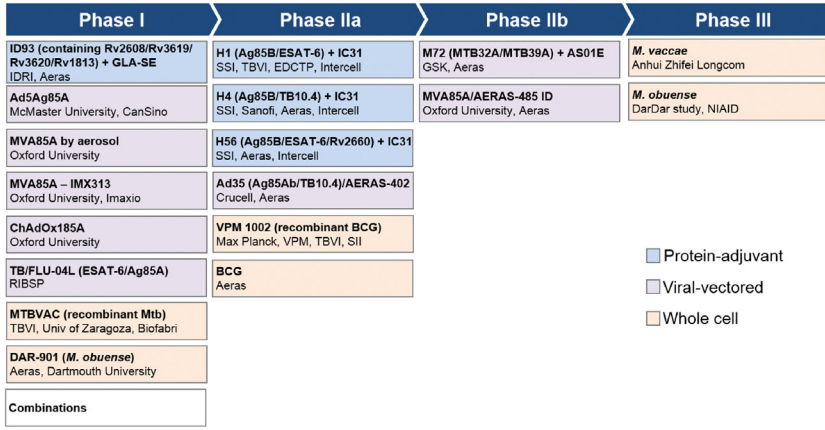


**FIGURE 18.1 The global pipeline of TB vaccines in clinical trials in 2015.** Abbreviations: EDCTP, European and Developing Countries Trials Partnership; ID, Intradermal; IDRI, Infectious Disease Research Institute; RIBSP, Research Institute for Biological Safety Problems; SII, Serum Institute India; SSI, Statens Serum Institute; TBVI, Tuberculosis Vaccine Initiative; VPM, Vakzine Projeckt Management. *(Courtesy: Aeras.)*

The first results reported were those from testing whether 5 doses of a live attenuated whole bacterium, *M. vaccae*—an NTM—could prevent TB disease in HIV-infected persons in Tanzania.[78] Nearly 50% protective efficacy against TB disease was shown, but careful scrutiny has revealed that protection was evident in subgroups of participants only; the vaccine strain has subsequently been shown not to be *M. vaccae*, but *M. obuense*, another NTM. The second trial was of MVA85A, a modified vaccinia virus-vectored vaccine that contains the gene for one Mtb protein.[79] This vaccine was given to 4-month-old infants who had received BCG at birth, and afforded no protection against TB disease beyond that provided by BCG. A third trial, that of a lysate of *M. vaccae*, is currently ongoing in China, where 6 doses are given to persons latently infected with Mtb to prevent disease. A subunit vaccine, M72, consisting of a fusion protein of 2 immunodominant antigens combined with the adjuvant ASO1,[80] is currently being tested for prevention of TB disease in young adults.

Release of the MVA85A trial results constituted a watershed moment in the TB vaccine world. The premise that a boost of BCG-induced immunity with additional T-lymphocyte immunity against a single immunodominant protein of Mtb would result in protection had rarely been challenged. Now, potential efficacy of all subunit and viral-vectored vaccines could be questioned: most elicit immune responses against a limited number of mainly immunodominant proteins (see earlier). Further, all induce T-lymphocyte immunity that is remarkably similar in nature to that elicited by MVA85A; it is not clear whether this kind of immunity alone would be sufficient for protection against TB. Further, because this vaccine was reported to show protection in four animal species

before human trials, the relevance of animal model results used to propose testing in humans is now also challenged.[81] These concerns have led to a reevaluation of discovery and development approaches toward successful vaccination against TB (see next section).

## 4.2 Back to Basics—Which Populations Should Be Targeted by Novel Vaccination Strategies?

Rational TB vaccine discovery and development would be guided by an optimal outcome. Multiple modeling studies have shown that targeting transmission of Mtb is likely to impact the epidemic significantly.[2,82,83] Transmission may be interrupted by preventing infection and by preventing disease. In earlier years of modern TB vaccine development, the focus has been on prevention of disease, predominantly. Currently, it is not known whether one vaccine could result in both effects—until we know, parallel development tracks may be called for.

Globally, TB disease occurs in adolescents and younger adults most frequently.[1] These populations transmit Mtb and should therefore be targeted. Modeling has shown that, compared with an infant vaccine, an adult vaccine would have considerably greater early impact on the epidemic and would be cost-effective, even with a relative low efficacy and short duration of protection.[84] Adolescents and adults should therefore be primary targets of vaccination strategies, while development of vaccines for infants and children (who do not transmit TB), the elderly (a relatively small global population with TB) or persons with comorbidities such as HIV infection (who may not transmit as much as HIV-uninfected persons) and diabetes mellitus (who have higher rates of TB) should be secondary.

The good news is that current clinical trial prioritization follows the lead of impactful intervention suggested by modeling—one ongoing efficacy trial is determining two candidates' ability to prevent Mtb infection in adolescents, while another is testing whether a vaccine can prevent disease in adults—therefore shifting away from investigating prevention of disease only, and from the infant focus of the MVA85A trial.

## 4.3 To Design Novel Vaccines, We Need to Learn More about Protective Immunity

Manufacture of designer vaccines might follow complementation of our inadequate knowledge of protective immunity against Mtb. The largest paradigm shift that has occurred in the TB vaccine world because of the MVA85A results might well be a shift toward basic science, to learn more about how we can be protected against TB.

To delineate protective immunity, human studies are needed, first and foremost. Most animal models of TB disease do not allow complete scrutiny of the complexity of the host response to Mtb (the nonhuman primate may be an

exception)[85]; regardless, animals like mice, guinea pigs and rabbits are still useful to test specific hypotheses about TB pathogenesis or vaccine action.

Human studies to learn about protective immunity fall into two categories:

- Prospective cohort studies that aim to delineate immunity in outlier populations, for instance comparing immunity over time in persons who ultimately will develop TB with those who will not. This study design has, for example, led to description of validated, blood-derived gene expression correlates of prospective risk of TB disease (ie, products of genes measured in blood that are markers of whether a person will, or will not, develop TB disease in the future)—results may offer clues into how we protect ourselves against the disease.
- "Human experimental medicine studies," where a specific hypothesis is tested in great detail in relatively small numbers of human participants, rather than in an experimental animal. An example would be examination of fluid from lungs of healthy persons in whom Mtb proteins were safely installed into the lung, to learn more about how our lungs react—and possibly protect—against mycobacteria.

The nonhuman primate manifests TB disease that closely resembles human disease[86]; it is therefore critical that this model be exploited in investigating protective immunity. This could be completed in parallel with human experimental studies, allowing a more comprehensive dissection of lung immunity, for example, than is possible in the human. Equally important, Mtb challenge studies in the nonhuman primate following vaccination (the animal is infected with Mtb to see if the vaccine would protect) allow an invaluable opportunity to learn about immune correlates (markers) of protection.

Once clues into how we protect ourselves against TB disease, or correlates of protection, become available, vaccines could be designed to elicit these immune responses.

## 4.4 New Approaches to TB Vaccine Discovery

Learning about protective immunity would hold advantage, but might be a near-impossible task in the face of up to 70,000 years of coevolution of Mtb with the human. As mentioned earlier, this time allowed Mtb to develop multiple strategies to avoid immune surveillance, or even to use our immune systems for its own advantage—as exemplified by the concern that lack of variation of immunodominant proteins, used in vaccines, which suggests evolutionary advantage of elicited immune responses for the pathogen (see earlier). This and the role of the immune response in contributing to pathogenesis and tissue damage represent challenges for development of vaccines to engender protective rather pathogenic responses.

An alternate approach to vaccine discovery would be exploiting induction of immune system branches other than classical T lymphocytes to target the

pathogen.[6,87] While it is known that mycobacteria could be targeted by these other immune arms, it is not known whether protection in the natural setting depends on these. Perhaps the approach should be to use these immune branches to induce "unnatural" immunity; that is, immunity that does not necessarily occur in the natural setting, and as such circumvent the effects of thousands of years of coevolution and our inadequate knowledge. In our current repertoire of vaccines that target other infectious diseases, many examples of unnatural immunity exist, for example, the tetanus vaccine induces 10 years of protection, which is not the case when a human is infected with the pathogen that causes tetanus, *Clostridium tetani.*

Proposed approaches to induce alternate arms of the immune system could prioritize mechanisms of action that differ from those used by classical T lymphocytes, for example, using vaccines that induce antibodies rather than T lymphocytes to protect, or that target immune cells that reside in the lung, where they can immediately act against encountered Mtb, for example, by stimulating so-called natural killer cells, and γδ and mucosa-associated invariant T cells, with vaccines, or that would hold advantage over classical T-lymphocyte induction in vaccine development through lesser diversity in how the cells are activated, for example, through stimulating so-called CD1 and HLA-E restricted T cells with vaccines.

Selection of vaccine proteins that show greater diversity among global TB strains, compared with immunodominant proteins mentioned earlier, yet, to which we still develop an immune response, could hold great advantage: these have to be elucidated. Similarly, the role of nonprotein components of the bacterium in protection should also be explored for vaccines, including lipids and carbohydrates.

Finally, TB is primarily a lung disease and our knowledge of immunology strongly suggests that induction of protective immunity in this organ, rather than through an injection, would result in greater protection. Avenues of direct vaccination to the lung should be explored, possibly using inhaler devices similar to those used for treatment of asthma.

## 4.5 Can We Learn About Potential Vaccine Success Earlier Than in a Human Efficacy Trial?

As shown in MVA85A product development, testing of TB vaccines has relied on a linear path toward efficacy testing in humans, with limited contribution of results from animal testing and earlier clinical trials toward decisions to proceed. This expensive approach cannot be sustained for the large vaccine portfolio currently in clinical trials—early learning of potential success would "de-risk" clinical trials.

This is achievable, partially, through rational use of smaller animals—for scientific hypothesis testing—and of nonhuman primates—for gating entry into human clinical trials, by showing efficacy in this model. Ideally, predetermined

gating criteria that are acceptable across the vaccine world should be used for nonhuman primate model gating; efforts are currently underway to institute a global portfolio management entity to coordinate gating activities. There are two NHP models studied in TB, which have different patterns of disease and resistance, so even here there is some uncertainty about the most appropriate model. The gating paradigm will only be successful if capacity for nonhuman primate testing is adequate—a major challenge that is currently being addressed.

As mentioned earlier, nonhuman primate testing should also allow delineation of correlates of protection, or at the very least, a detailed understanding of the immune response induced by the vaccine candidate. If a similar immune response is induced in the human during early testing, the nonhuman primate efficacy results would weight even more toward proceeding with the candidate to later-stage trials.

Community-wide efficacy trials are expensive. Select populations at high risk of TB could be targeted for preliminary efficacy testing, for example, persons who have blood markers that show that they are at risk of TB disease, as mentioned earlier, or health-care workers or even persons who were recently cured from TB disease. If suggestion of protection is strong, the candidate could then be tested in a more definitive community-wide efficacy trial.

Finally, the TB vaccine world could learn from other fields about innovative approaches to test vaccines. Vaccines against malaria and enteric pathogens are currently tested by human challenge studies; that is, following vaccination, the human is infected with the pathogen or an attenuated version in a controlled manner that guarantees safety. The vaccine effect on the pathogen's growth and possible clinical manifestations is measured. It is conceivable that Mtb could be engineered into a strain that could be given to a vaccinated person by inhalation, to measure the vaccine effect on growth of the Mtb. Safety would be guaranteed by signals inside the engineered bacterium that results in its death after a defined period.

## 4.6 TB Vaccine Funding, Advocacy, and Working Together

The TB vaccine world is constrained by relatively limited resources. An approximate $171 million was spent on TB vaccine research and development in 2013 (Source: G-FINDER. 2014). The largest contribution to the pool was the National Institutes of Health (NIH)—although activities were not all specific to TB vaccines only; the Bill & Melinda Gates Foundation was the largest specific contributor. This is a fraction of funding available for HIV vaccine discovery and development, for example. Mobilization of funding for TB vaccine research would be an important first step for accelerating the field.

A comparison between HIV and TB efforts for funding advocacy is useful. HIV emerged as an acute and devastating epidemic, initially involving relatively high-income societies and resulting in a highly visible and effective advocacy

effort. In contrast, TB remains a chronic, hidden disease that disproportionately affects lower socioeconomic strata, is associated with significant stigma, and which has very few visible advocates efforts. Experts advise that advocacy for greater funding in TB vaccines is likely to succeed only if presented within a larger bucket, such as TB as a whole, or along the antibiotic resistance path, or within global vaccination efforts. It is critical that the opportunities are recognized and actively pursued.

While resources remain limited, it would be prudent to use these in the most impactful manner possible. Global TB vaccine portfolio management, as mentioned earlier, is therefore critical. Similarly, strategies to learn about vaccine candidates through more rational testing approaches, as shown earlier, are essential. Finally, systematic efforts are currently underway to bring global stakeholders involved in TB vaccine discovery to the table, with the aim of co-leveraging resources through rational collaboration and cooperation, while still allowing healthy competition.

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Chapter 19

# Major Global Vaccine Challenges: Recent Progress in Malaria Vaccine Development

**Johan Vekemans, MD, PhD**
*GSK Vaccines, Brussels, Belgium*

## Chapter Outline



## 1 INTRODUCTION

Malaria is a major cause of human suffering caused by *Plasmodia* parasites, transmitted to humans via the bite of anopheline mosquitoes. While five *Plasmodia* species can infect humans, *Plasmodium falciparum* and *Plasmodium vivax* constitute major public health problems. Access to long-lasting insecticide-impregnated bednets, indoor residual insecticide spraying, use of appropriate diagnostic tools and efficacious artemisinin-derivative based combined therapies led to an estimated 50% reduction in global malaria mortality and over 4 million deaths averted in the last 15 years. Nevertheless, in 2013, there were

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still an estimated 198 million malaria cases and 584,000 malaria deaths. Most fatalities are due to *P. falciparum* and occur in children under 5 years of age in sub-Saharan Africa, where an estimated 1200 children die of malaria every day. The backbone of current control strategies is at risk, with resistance to insecticides in mosquitoes spreading across endemic areas and artemisinin resistance documented in South-East Asia.[1]

The availability of a malaria vaccine is a key goal when considering the sustainability and acceleration of recent progress. In 2006, WHO issued the first version of a malaria vaccine development roadmap, setting the objective to have a first-generation *P. falciparum* malaria vaccine providing at least 50% protection against severe disease and death over at least 1 year, licensed in 2015. The roadmap was recently updated, setting new major objectives for 2030: to reach at least 75% efficacy against clinical malaria for 2 years (allowing boosters), reduce parasite transmission and target both *P. falciparum* and *P. vivax*.[2]

The complexity of the malaria life cycle, stage-specific antigen expression and immune evasion mechanisms are major obstacles to candidate vaccine development (Fig. 19.1). Malaria vaccine development efforts have been greatly facilitated by the availability of controlled human malaria infection (CHMI) models.[3] The classical *P. falciparum* sporozoite challenge model is presented in Fig. 19.2. *P. vivax* CHMI is possible too, but the risk of late relapses upon hypnozoite reactivation and the fact that there is currently no full-cycle laboratory culture system brings additional complexities.[4] Recently, the use of needle injection of sporozoites manually dissected from infected mosquitoes has been proposed.[5] Biological relevance for evaluation of vaccine-induced protection remains to be demonstrated. A challenge model using low-dose infected erythrocytes for blood stage inoculum allowing for longer monitoring of subpatent parasite growth may lead to improved characterization of the biological effect of blood-stage vaccine candidates.[6] Options for the development of experimental models to test vaccines targeting sexual stages are being considered.[7]

Here, we review leading malaria candidate vaccines development strategies.

## 2 PREERYTHROCYTIC VACCINE CANDIDATES

Recent research suggests that natural malaria exposure does not lead to significant preerythrocytic immunity preventing new infections.[8] Vaccine candidates targeting proteins expressed before the blood stage therefore do not aim to reproduce something that happens in nature. Targeting sporozoites and early liver forms before mitotic divisions occur, and targeting conserved antigens under limited selective pressure, may limit the risk of selection of escape variants. The main challenges of preerythrocytic vaccine strategies relate to the very transient passage of sporozoites in the blood stream, and the lack of a clear understanding of the biology of immune effectors against liver forms.

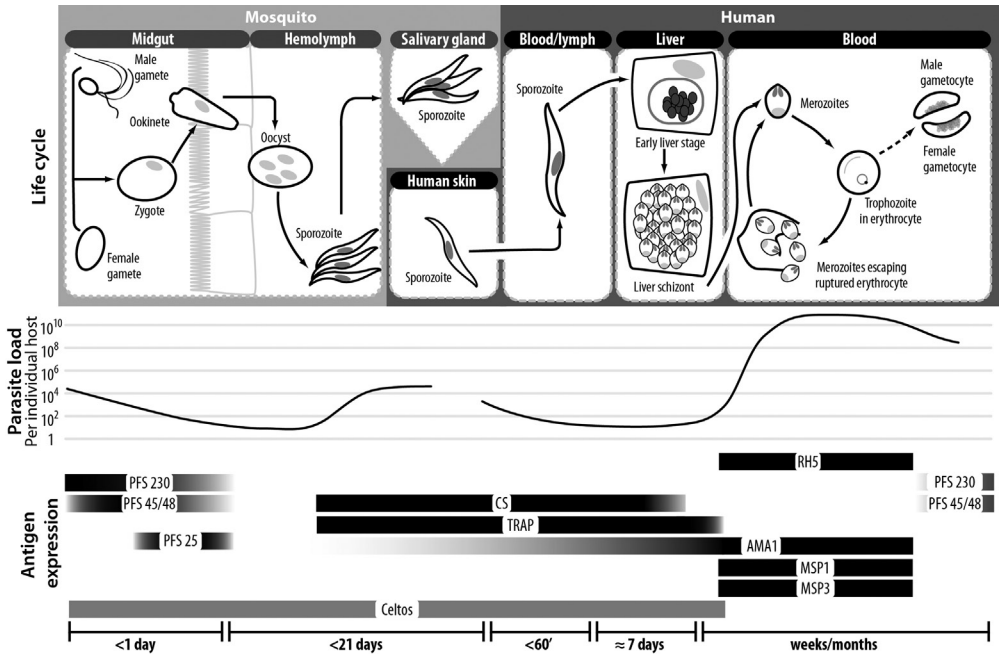


**FIGURE 19.1** **Illustration of the *P. falciparum* malaria parasite cycle, parasite load within the host, stage-specific antigen expression.** Within minutes of being injected in the human host, sporozoites originating from the mosquito salivary glands enter hepatocytes where liver forms will transform and generate thousands of merozoites released in the blood about 6–10 days later, leading to a disease-associated massive asexual multiplication with rapid cycles of erythrocyte entry, destruction, and reentry. Some parasites differentiate into gametocytes which may be ingested by a biting mosquito. Gametes emerging outside of the erythrocytes in the midgut will fuse into a zygote to become ookinetes which traverse the midgut epithelium and differentiate into oocysts. Asexual divisions within the oocysts over the next 1–2 weeks result in several thousands of sporozoites migrating toward the salivary glands. Antigens expressed through the quantitative bottlenecks are potential key vaccination targets. *P. vivax* infection but not *P. falciparum* can generate dormant liver stage "hypnozoites" that can reactivate long after initial infection.

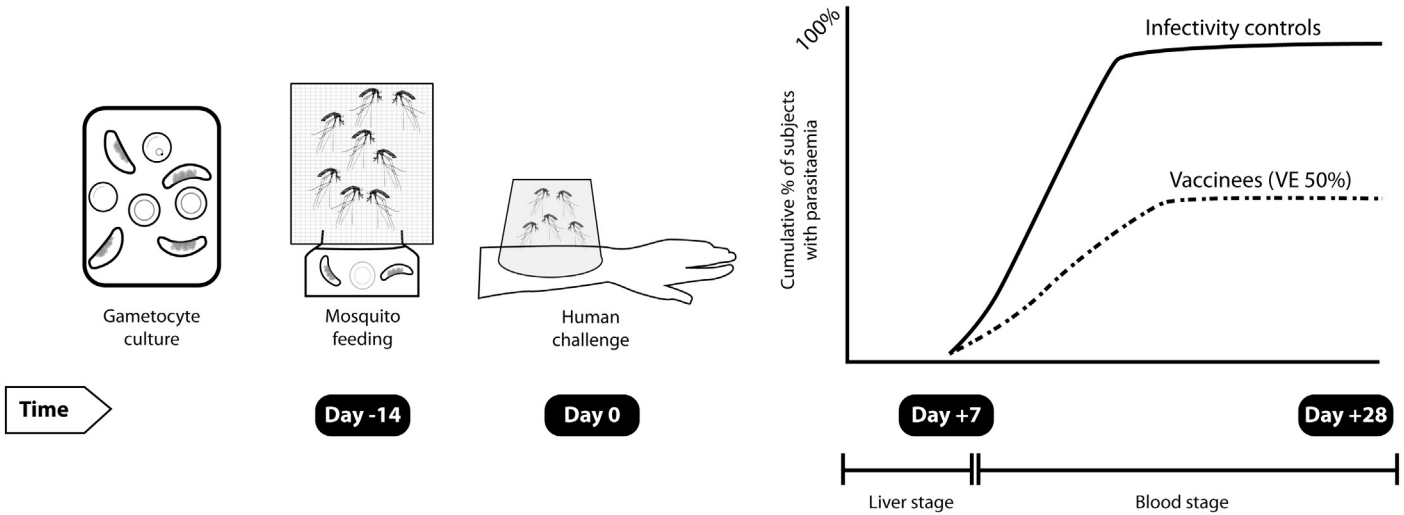


**FIGURE 19.2 The "classical" controlled human malaria infection model.** Infectious sporozoites are administered to healthy human adults via the bite of a small number of lab-reared mosquitoes infected with a well-characterized drug-sensitive strain. Volunteers under close observation are treated as soon as there is evidence of malaria infection. Nonvaccinated volunteers act as infectivity controls. The proportion of vaccinated subjects remaining free of blood-stage parasites through a period at risk, considered to be up to 30 days postchallenge, determines vaccine efficacy (*VE*). In those who become infected, the delay in time to blood stage infection is interpreted as biological evidence of a vaccine-induced reduction in number of parasites successfully reaching the blood-stage of the cycle, hence a measure of preerythrocytic immunity.

## 2.1 Targeting the Circumsporozoite Protein

The *P. falciparum* circumsporozoite protein (CS) is a 412 amino acid (7G8 clone) protein with a characteristic central NANP repeat region and nonrepeat flanking regions. CS is present on the sporozoite surface and early liver forms, and plays an important role in motility, attachment, and entry into hepatocytes, modulating intracellular biochemical pathways.[9] CS was an early target of malaria vaccine research, as passive transfer of CS-specific antibodies or T-cell effectors was shown to protect rodents from experimental infection, but initial constructs targeting the central repeat region of the CS failed to provide conclusive protection.[10]

RTS,S is a chimeric protein including NANP repeats and the C-terminal flanking region of CS fused to the hepatitis B virus surface antigen (HBsAg), coexpressed in yeast with free HBsAg, yielding a spontaneously assembling viral-like particle.[10] CHMI studies showed the critical role of adjuvantation and RTS,S/AS01 emerged as the most immunogenic formulation.[11,12] AS01 is a liposomal suspension and includes the immune-enhancers such as monophosphoryl lipid A, a detoxified lipopolysaccharide derivative, and QS-21 Stimulon® (Quillaja saponaria Molina, fraction 21) (Licensed by GSK from Antigenics Inc.), a saponin molecule purified from the bark of a tree, Quillaja saponaria.[13] While both anti-CS antibodies and cell-mediated responses have been associated with protection, there is no established correlate of protection. Antibodies likely prevent sporozoite entry into hepatocytes, while cell-mediated immunity is assumed to play a helper role supporting the humoral response and possibly an effector role against infected hepatocytes, although there is no robust evidence for the latter.[14]

Since 2001, the RTS,S pediatric development program has been under the leadership of a public-private product development partnership between GSK and the PATH Malaria Vaccine Initiative, in collaboration with multiple academic collaborators. The overall objective of the program is to reduce the burden of *P. falciparum* malaria in young children in sub-Saharan Africa. The vaccine would ideally be implemented through the WHO Expanded Program on Immunization (EPI), in conjunction with other malaria control interventions. Phase 2 RTS,S studies have confirmed partial protection against malaria in children, and demonstrated favorable safety down to infants in the EPI age-range.[15]

In 2009, a multicentre Phase 3 RTS,S/AS01 trial was undertaken in 11 African research centers with different malaria intensity and seasonality. The study included children aged 5–17 months at first vaccination, and infants 6–12 weeks of age immunized together with routine EPI vaccines. Vaccine efficacy against clinical malaria over 1 year was about 50% in the older age category, and about 30% in the younger age category.[16,17] The final results from the study, including evaluation of a booster dose at Month 20, have now been published.[18] Without a booster dose, when considering children in the older age category over the whole follow-up period (median 48 months), primary RTS,S/

AS01 vaccination provided 28% (95% CI 23–33) protection against clinical malaria, and the total number of cases averted ranged in different study sites between 215 and 4443 (1774 overall) per 1000 children vaccinated. No protection against severe malaria was seen when considering the total follow-up period. There was evidence of waning of immunity over time. The reduction of early exposure to blood stage infection associated with vaccination may have delayed acquisition of blood-stage immunity, with a displacement of incidence of severe malaria toward older age. The overall effect was nevertheless favorable, with evidence of a reduction in malaria hospitalizations and all-cause hospitalizations. When a booster dose at Month 20 was given, protection against clinical malaria and severe malaria over the whole follow-up was 36% (95% CI 32–41) and 32% (95% CI 14–47), respectively, and depending on study site, 205 to 6565 cases of malaria were averted per 1000 children vaccinated. When considering other endpoints of public health interest, vaccination with a booster dose provided overall partial protection against incident severe malaria anemia and blood transfusion, malaria hospitalization and all-cause hospitalization.

Lower estimates of efficacy were seen in the younger age category. Vaccine efficacy against clinical malaria over the whole study (median 38 months) was 18% (95% CI 12–24) without a booster dose, and 26% (95% CI 20–32) with a booster dose. There was no evidence of protection against severe malaria. Vaccination with a booster dose reduced malaria hospitalizations by 25% (95% CI 6–40), but there was no evidence of a reduction in other endpoints of public health interest. Overall, approximately 1000 cases of clinical malaria were averted per 1000 infants vaccinated. The reasons for lower protective immunity in young infants relative to older children are unknown, but immaturity of the immune system, passively transferred maternal antibodies, past hepatitis B vaccination, and EPI vaccine coadministration may have played a role.

Safety results were favorable, although vaccination was associated with a risk of febrile seizures when children were vaccinated at a susceptible age. An unexplained increased reporting of cases of meningitis due to a heterogeneous group of pathogens, with no cluster in time-to-event, was reported in children in the older age category, but not in the younger age category.

Results from safety and immunogenicity studies including vaccination of neonates and HIV-infected children will be available in the near future. An RTS,S/AS01 vaccine regulatory application package is currently under European Medicine Agency review for scientific evaluation of the quality, efficacy and safety through the Article 58 procedure, before WHO consideration for recommendation for use and prequalification. If the outcome of these reviews is favorable, submission to national regulatory authorities in African countries will follow.

Other approaches targeting the CS protein are in early evaluation, with the hope that they may represent improvements over RTS,S.[19] The role of the N-terminal region of the protein is being assessed using a full length CS protein antigen. Alternative immunization regimens may generate qualitatively better

humoral responses or protective T-cell effectors. CS is not expressed for very long in the intracellular liver forms and strategies using recombinant viral vectors to generate cell-mediated immunity against CS only conferred limited protection in CHMI studies.[20] Replacement of the first dose of RTS,S/AS01 by a CS-expressing recombinant adenovirus promoting CS-specific CD4+ response failed to increase vaccine efficacy.[21] An alternative approach for qualitatively improved immunogenicity is to use a fractional third dose of RTS,S/AS01 (NCT01857869).

## 2.2 Whole *Plasmodium falciparum* Sporozoite (PfSPZ) Vaccine Candidates

In contrast to natural infection, experimental exposure to large numbers of irradiated sporozoites can protect from subsequent experimental malaria challenge.[22] While irradiated sporozoites can initiate liver stage infection, full differentiation is aborted, leading to protective preerythrocytic immunity which appeared predominantly mediated by CD8+ T cells.[23] Irradiated sporozoites were historically administered through the bites of a minimum of 1000 mosquitoes.[24] Recently, what was a clinical laboratory experiment has inspired a biotechnology company (Sanaria) to develop a candidate vaccine approach based on attenuated whole *P. falciparum* sporozoites (PfSPZ) immunization stored in liquid nitrogen, after dissection of a large number of mosquito salivary glands for sporozoite isolation. In terms of administration route, needle injection had to replace experimental mosquito bites. Only the intravenous route led to protective immunity, while intradermal or intramuscular needle injections failed.[25] A total of $6.75 \times 10^5$ irradiated PfSPZ injected in five doses intravenously protected 6/6 subjects against CHMI. Protection was dose-dependent. Research is ongoing to determine duration of protection, whether cross-strain protection can be achieved, evaluate the role of preexisting malaria immunity and whether more practical storage and injection techniques are possible.[26]

The use of genetically attenuated parasites which are able to initiate liver stage infection but arrested before blood release, or unable to multiply in the blood, is an alternative to irradiation.[26] Exposure to a limited number of PfSPZ under chloroquine coverage, allowing for complete preerythrocytic development but preventing blood stage multiplication, leads to long-standing high protection, suggesting that the longer the preerythrocytic portion of the cycle is allowed to progress, the higher the protection generated.[27] Several candidate gene targets for attenuation have been identified, with the objective to generate the right parasite mutant displaying enough attenuation to ensure no breakthrough clinical infection occurs.[28]

Whatever the PfSPZ approach considered, manufacturing according to regulatory standards for Phase 3 evaluation and commercialization will need to be developed. If successful, field implementation of an immunization program relying on a liquid nitrogen-based cold chain and intravenous injection will be a new challenge.

### 2.3 Targeting the Thrombospondin-Related Adhesion Protein

Building on the notion that T cells can mediate protective immunity against the liver stage of the parasite, researchers at the University of Oxford developed malaria vaccine candidate approaches based on the ability of recombinant viral vectors used in heterologous prime-boost regimens to generate strong T-cell responses. The thrombospondin-related adhesion protein (TRAP), expressed on sporozoites and during the liver stage, plays an important role in motility and cell invasion.[29] ME-TRAP is a recombinant DNA insert which encodes for TRAP and a string of 20 epitopes (ME) selected on the basis of being potentially protective CD8+ targets and HLA promiscuous. Various heterologous prime-boost regimens have been tested. The most advanced approach included ME-TRAP-encoding Chimpanzee Adenovirus 63 (ChAd63) priming followed by Modified Vaccinia Ankara (MVA) boosting, administered 8 weeks apart, which prevented malaria infection after CHMI in 3 out of 14 volunteers, and provided 67% (95% CI 33–83) protection against malaria infection in naturally exposed Kenyan adults over an 8-week period.[30,31] Pediatric evaluation is ongoing.

### 2.4 Combined Strategies Targeting CS and TRAP

Combining two partially effective preerythrocytic vaccines mediating protection through different mechanisms may lead to high efficacy and synergistic effects, as suggested by modelling work and preclinical experiments.[32,33] Recently, the combination of RTS,S/AS01 and ChAd63-MVA ME-TRAP was evaluated but results were inconclusive, as RTS,S/AS01 alone protected 12/16 subjects, and staggered administration of ChAd63-MVA ME-TRAP between RTS,S/AS01 doses protected 14/17 subjects.[34,35] A confirmatory trial is underway (NCT02252640).

## 3 BLOOD-STAGE VACCINE CANDIDATES

Naturally exposed people, upon repeated infections, can develop partial blood stage immunity with a reduced risk of severe disease progression. Antigens shown in sero-epidemiologic studies to be the target of protective antibodies have often been selected for evaluation as candidate vaccines, with the aim to mimic or strengthen partial immunity acquired in nature. Several challenges have however hampered significant progress. The parasite has acquired the ability to evade this selective pressure in nature by displaying high surface protein polymorphism and functional redundancy. The parasite biomass in the body reaches its maximum through multiple cycles of rapid intraerythrocytic asexual multiplication and very transient extra-cellular passage. Expression systems have often been disappointing in their ability to generate conformational antigens.[36]

Among many, only a few (MSP1, AMA1, MSP2, MSP3, GLURP, SERA5, and EBA-17) blood stage proteins have been evaluated as vaccine candidates in human efficacy studies, and none has yet shown conclusively to prevent

malaria. A study in Papua New Guinea showed vaccine-induced allele-specific MSP2 selective pressure leading to a reduction in parasite density,[37] but this was not further explored. Several immunogenic MSP1 constructs were evaluated without success.[38,39] Results from an AMA1 vaccine candidate evaluated in Malian children showed the possibility of strain-specific protection, but a sensitive blood-stage CHMI study using vaccine homologous parasites failed to show any impact on parasite multiplication rate (NCT02044198).[34,40] MSP3 is a blood-stage protein shown to induce in malaria-exposed subjects antibody-dependent cellular inhibition (ADCI) of parasite growth.[41] In a Phase 1b pediatric study, an alum-adjuvanted MSP3 long synthetic peptide showed a reduction in incidence of malaria over 4 weeks after last vaccination.[42] Phase 2b study results are awaited (NCT00652275). GMZ2 is a fusion protein including parts of MSP3 and the blood-stage glutamate rich protein (GLURP). As for MSP3, natural exposure induces ADCI-mediating GLURP-specific antibodies. Short term immunogenicity of GMZ2 in alum has been shown but immunogenicity seems lower in individuals with past exposure.[43]

Renewed interest is emerging from new blood stage antigen discovery efforts. *P. falciparum* reticulocyte binding homolog protein 5 (PfRh5) is essential to erythrocytes entry through interaction with the erythrocyte surface protein basigin, displaying little sequence diversity in nature. Loss of function seems to be associated with minimal sequence alteration, suggesting a low risk of escape variant selection. PfRh5 antibody-mediated in vitro growth inhibition was shown and, building on promising nonhuman primate vaccine studies,[44] the first trial in humans is ongoing (NCT02181088).

The coming years will likely see other newly identified antigens evaluated.

## 4 SEXUAL STAGE VACCINE CANDIDATES

Historical bird malaria studies showed that immunization with killed blood-stage plasmodia could reduce oocyst formation in mosquitoes after feeding. Over the following decades, antigens expressed at various stages between sexual differentiation in the human to any stage in the mosquito have been identified as potential targets of vaccines aimed at reducing transmission through interference with the parasite cycle in mosquitoes. Mosquito midgut proteins have also been identified as attractive cross-species candidates, but sequence homology with human proteins is a concern.

This vaccine development strategy is original in several ways. Vaccinated individuals would not be protected against disease upon pathogen exposure, but would be protected only through a herd immunity effect upon mass vaccination. The site of action of the vaccine-induced immune effectors would be outside the human host, in the mosquito gut. Although it is possible that parasite killing may occur through mechanisms involving antibody interaction with other ingested immune components such as cells or complement, the mode of action would likely be interference with target protein function.[7]

Stage-specific sexual stage antigen expression is illustrated in Fig. 19.1. Antibodies against Pfs230 and Pfs48/45, expressed on gametocytes in the human host, are found in malaria-exposed individuals, and significant sequence polymorphism in field isolates have been demonstrated, illustrating the principle that antigen exposure generates an immune response that exerts selective pressure on the parasite, generating antigenic variants. Very limited sequence variation has been reported for Pfs25 and Pfs28, only expressed in the mosquito. Targeting antigens only expressed in mosquitoes may minimize the risk of escape variants, but no boosting in humans would occur and protection would be dependent on antibody circulating levels rather than memory reactivation. In theory, proteins expressed late in the cycle within the mosquito, such as the *P. falciparum* cell-traversal protein for ookinetes and sporozoites (CelTOS) or even the CS protein, could also be the target of transmission blocking immunity.[45]

Progress in testing of sexual-stage candidate vaccines has been hindered by the difficulty in appropriate production of recombinant proteins.[7] The cysteine-rich structural characteristic of these proteins often led to inappropriate posttranslational folding and the need to explore various expression systems. Recombinant viral vectors are attractive, as host cell posttranslational processing leads to proper antigen folding. Whether viral vectors can generate sufficient antibody levels remains to be determined.

Relevance in conditions of natural transmission remains to be demonstrated. Evaluation in animal population models were encouraging, as even partial reduction of sporogony interrupted transmission.[46] The required clinical development pathway to licensure is under discussion.[7] Laboratory mosquito feeding experiments on donor blood mixed with cultured gametocytes and test antibodies are instrumental[47] but demonstration of impact on transmission in human communities will likely require large cluster-randomized trials.[48]

## 5 MULTIPLE-STAGE VACCINE CANDIDATES

A multistage vaccine candidate is considered by many as the penultimate goal of malaria vaccine development.[49] Vaccines preventing new infections and the appearance of viable gametocytes, whether they target the preerythrocytic or blood stage, would have the potential to interrupt malaria transmission as for vaccines targeting the sexual stage. Combinations of such vaccines would have the benefit of reducing the risk of selecting immune escape variants, and provide vaccinated individuals with a personal benefit in addition to the benefit through herd immunity. Combining a blood stage vaccine capable of reducing the risk to progress to severe disease with a partially effective preerythrocytic vaccine which may interfere with acquisition of blood-stage immunity is also attractive. There is presently no study that has demonstrated the benefit of multiple stage combinations. In one trial in malaria-naïve adults, a DNA vaccine priming followed by an adenovirus vector boost, both targeting CS and AMA1,

led to protection in 4/15 volunteers in a CHMI study, but exploratory analyses suggested protection was mostly mediated by anti-AMA1 immunity.[50]

## 6 VACCINE CANDIDATES AGAINST MALARIA IN PREGNANCY

Women living in malaria-endemic countries and their offspring are at increased risk of adverse outcomes from *P falciparum* (Pf) malaria infection during pregnancy, including severe malaria and anemia in the mother, premature termination of pregnancy, low birth weight, intrauterine growth retardation, congenital infection, and increased rates of infant mortality.[51] Despite current recommendations, the incidence of maternal malaria remains unacceptably high and the development of vaccines for prevention of pregnancy-associated malaria is included in WHO's strategic goals.[2] The distinction should be made between vaccines that would prevent pregnancy-associated malaria only versus any vaccines preventing infection or disease, to be administered to women in reproductive age with or without boosting in pregnancy. Preclinical vaccine development efforts targeting the PfEMP1 variant VAR2CSA shown to adhere to the placental receptor chondroitin sulfate A, is a good example of the former.[52] When considering vaccination during pregnancy, safety expectations will be very high, but the need to overcome unjustified reservations is being increasingly acknowledged.[53]

## 7 *P. VIVAX* VACCINE DEVELOPMENT STATUS

The burden of disease related to *P. vivax* infection is increasingly recognized as being neglected. Preerythrocytic and sexual stage *P. vivax* orthologs of most of the leading *P. falciparum* antigen targets are being considered for development, although at a less advanced stage. The vaccine efficacy of VMP001/AS01, targeting the *P. vivax* CS antigen, has been evaluated in a CHMI study, but failed to induce robust protection.[54] The *P. vivax* TRAP antigen expressed in viral vectors is under evaluation,[55] and a strategy using cryopreserved attenuated sporozoites is also being pursued.[56] The leading *P. vivax* blood stage antigen candidate is the Duffy Binding Protein (DBP), key to *P. vivax* erythrocyte invasion,[57] but the polymorphic nature of DBP constitutes a challenge.

Duration of vaccine-induced immunity will likely matter even more than for a *P. falciparum* vaccine, as the burden of *P. vivax* disease is not as concentrated in specific susceptible groups as that of *P. falciparum*. The dormant liver stage also constitutes a source of added complexity.

## 8 CONCLUSIONS

A first generation vaccine being reviewed for potential licensure constitutes a historical milestone in itself. Several approaches for an improved second generation vaccine are promising. Most progresses are derived from multiinstitutional

partnerships including various academic groups, private and public institutions, working in a collaborative mindset with various sources of funding. A first generation malaria vaccine with moderate efficacy may play a role in disease control in young children, but the malaria-related disease burden is wider. Prevention of malaria in pregnancy should also be a priority, and the role of vaccines in containment of drug-resistant malaria should be considered. The ambitious malaria elimination goal set for the long term future should not derail efforts for the development of vaccines able to further reduce the burden of disease to be implemented alongside existing malaria control interventions.

Malaria transmission is highly heterogeneous geographically and through calendar time. Implementation strategies may need to adapt to local epidemiology. Moving away from EPI-integrated delivery, increasing the number of doses required, antigen combination, high complexity in manufacturing and delivery platforms will increase delivery costs and will constitute important decision factors. Interesting years lie ahead in the field of malaria vaccine development.

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Chapter 20

# AIDS Vaccines

**Bruce D. Walker, MD**
*Ragon Institute of MGH, MIT and Harvard; Howard Hughes Medical Institute, Cambridge, MA, United States*

## Chapter Outline



## 1 INTRODUCTION

In 1984, coincident with the identification of HIV as the cause of AIDS, the US Secretary of Health and Human Services made an announcement indicating that a vaccine should be expected within the next few years. As we enter into the fourth decade of the AIDS epidemic, this has still not come to fruition. Global research efforts have revealed unprecedented obstacles to making a vaccine to this human retrovirus, which have made it such an exceptional scientific challenge.

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Among the challenges posed by HIV is that infection leads to stable integration of the proviral genome into the host chromosome. This establishes a latent reservoir of infected cells that are transcriptionally silent and thus not producing viral proteins that are needed for detection by the immune system.[1] In animal models of AIDS virus infection it is clear that the establishment of the latent viral reservoir occurs within days of exposure to the virus, and thus to prevent a life-long infection, a vaccine-induced immune response will have very little time to act.[2]

Second, HIV exhibits extreme genetic diversity, due to its error prone reverse transcriptase. Even within a single infected individual, the amount of diversity that is generated is in excess of what is observed during a global influenza epidemic. Viruses within clades may differ by over 20%, and differences among clades approach 35%, particularly in the envelope, the most variable of the HIV proteins.[3] This diversity also represents a challenge for the immune system due to rapid evolution of immune escape mutations that represent a problem for both humoral[4,5] and cellular[6,7] immune responses.

Yet another challenge is that the envelope protein, the major target for the humoral immune response, is heavily glycosylated, such that relatively conserved regions targeted by neutralizing antibodies, such as the CD4 binding site which is required for virus entry, are not readily accessible (reviewed in Ref. [8]). As such, effective neutralizing antibodies are not only difficult to generate, but require years of exposure to antigen to undergo mutation that allow proper targeting of the virus, and even then are only generated in a minority of individuals. In addition, most vaccine immunogens tested so far have been composed of monomeric forms of gp120, not the native trimer that is present on the surface of virions, which may be required to generate effective humoral immunity.

Despite these many challenges, there is reason for renewed optimism that an effective HIV vaccine can be developed—something that will be required to bring an end to the HIV epidemic.[9] Here we review the immunology of HIV infection as it pertains to the development of an effective HIV vaccine and discuss advances in understanding the development of neutralizing antibodies and protective T-cell responses, candidate vaccine immunogens including those that are likely to enter vaccine efficacy trials, and new concepts that represent the next generation of vaccine candidates.

## 2 IMMUNE RESPONSES TO HIV: WHAT IS NEEDED?

An ideal HIV vaccine will have to do a better job of generating protective immune responses than is achieved with natural infection.[10] To date there is no evidence that naturally induced immunity can successfully clear HIV infection. Although some rare individuals called elite controllers are able to maintain durable control of infection without the need for medications (reviewed in Ref. [11]), evidence suggests that these persons still have abnormal immune activation and can experience progressive $CD4^+$ T-cell decline despite undetectable levels of

plasma viremia by standard assays.[12] It is for this reason that enthusiasm for a vaccine that would protect from disease progression rather than prevent infection altogether is a less desirable goal.

Despite these caveats, important insights have been gained by studying immune responses in natural infection, and results from these studies provide important insights for vaccine immunogenicity studies. Although following an infection, it is still not clear what the correlates of protection are, the current consensus is that broadly neutralizing antibody responses will be required for protection from infection; but if infection should occur, HIV-specific T-cell responses will be most important in relative containment of infection.

## 2.1 HIV-Specific Neutralizing Antibodies

Early animal model studies with broadly neutralizing monoclonal antibodies showed that these could protect against transmission.[13] However, the extreme genetic variability, particularly in the envelope protein, not only facilitates escape from these responses, but has generated extreme diversity globally which will require the broadly cross-reactive neutralizing antibodies to be generated by vaccines. In natural infection some degree of cross-reactive antibody responses are generated in 50% of infected persons,[14] but only about 1% of infected persons become "elite neutralizers," able to potently neutralize viruses across at least four different clades.[15] Adding to the challenge of generating neutralizing antibodies to HIV is the finding that only a limited number of envelope spikes is present on virions, and these are covered by extensive and rapidly shifting glycans, making antibody recognition of conserved sites very difficult.[16]

Despite these challenges, there is renewed optimism about vaccine-induced generation of broadly neutralizing antibodies from studies of natural infection. Major advances in identification of broadly neutralizing antibodies and their targets initially arose from high throughput neutralization assays that screened memory B cells from chronically infected Africans.[17] Parallel advances in B-cell cloning techniques have led to isolation of ever more potent broadly neutralizing antibodies (reviewed in Refs. [8,18]), and have revealed unique properties of these antibodies and facilitated identification of sites of vulnerability on the envelope glycoprotein. Potent broadly neutralizing antibodies typically take years to develop, require extensive somatic hypermutation,[19] and have unusual characteristics such as long HCDR3 regions and often framework mutations as well.[20]

With the isolation of broadly neutralizing antibodies, at least five sites of vulnerability on the envelope trimer targeted by these antibodies have been identified, with additional ones likely to be revealed: the CD4 binding site, the V3 glycan, the V1V2 glycan, the membrane proximal external region, and a region spanning gp120 and gp41 that is trimer specific.[8] It is clear that responses to each of these regions can be generated by natural infection, but the ability to produce these through immunization remains elusive. Moreover, there may be

genetic limitations since only a subset of variable heavy and variable light chain combinations are able to bind to these sites of vulnerability due to structural constraints.[21] However, adoptive therapy with BNAbs in infected humans has been successful in lowering viral load, giving clear evidence that in the right amounts at the right places these could be effective.[22]

## 2.2 HIV-Specific Nonneutralizing Antibodies

Although broadly neutralizing antibodies are not induced by current vaccines, nonneutralizing antibodies are readily induced.[23] These antibodies bind to envelope epitopes that are not present on the native envelope trimer, but rather target epitopes that are revealed as the trimer dissociates, including gp120 monomers, nonfunctional conformationally rearranged envelope proteins, and gp41 stumps that are left as gp120 dissociates. Nonneutralizing antibodies have been identified against both gp120 and gp41, and are of particular interest since they have been shown to be a correlate of risk in the HIV vaccine trial that showed at least modest efficacy (given later). In the case of nonneutralizing antibodies, antiviral efficacy is linked to antibody-dependent cellular cytotoxicity (ADCC) mediated through the Fc portion of the antibody, or to Fc-mediated phagocytosis, and other potential mechanisms.[24] Monoclonal nonneutralizing antibodies have recently been tested in a SHIV challenge model, and showed a decrease in the number of transmitted founder viruses that was statistically significant compared to controls.[25] However, other animal model studies have shown lack of protection with nonneutralizing antibodies.[26] Evidence for antiviral activity of nonneutralizing antibodies is very weak and not close to the complete protection against infection and suppression of viremia induced with neutralizing antibodies. The extent to which nonneutralizing antibodies contribute to relative control of natural infection or would contribute to vaccine-mediated protection is unclear.[27]

## 2.3 HIV-Specific $CD8^+$ T Cells

Also referred to as cytotoxic T lymphocytes, these cells recognize infected cells through their unique T-cell receptor engaging with the complex of a viral peptide and the cellular HLA Class I molecule on an infected cell. In vitro studies show that infected cells can be recognized before infectious virus progeny are produced, at least under experimental conditions.[28] Even a single viral peptide–HLA complex is sufficient to induce lysis.[29] Genetic studies indicate that certain HLA Class I alleles are associated with protection, implying that there may be genetic limitations on vaccine efficacy.[30] Importantly, the HIV-specific $CD8^+$ T-cell response does not recognize free virus, so it cannot be expected to prevent the initial round of infection, but at best would be expected to contain viral replication once cells are infected.

Much has been learned about these cells from natural infection. In acute infection, there is a massive induction of these cells, representing up to 80%

of circulating $CD8^+$ T cells in some cases, which then contract despite ongoing viremia. The greater the peak magnitude and the more rapidly peak levels are achieved, the lower the subsequent viral set point; antiviral efficacy is also demonstrated by the rapid generation of mutations with the 8–10 amino acid epitopes targeted by these cells.[6,7] Numerous studies indicate that enhanced antiviral efficacy is associated with targeting of Gag,[31] possibly due to the combination of early presentation of Gag peptides on infected cells[32] and constraints on mutations in Gag[33]: $CD8^+$ T cell–induced mutations lead to fitness constraints, which likely contributes to relative immune containment.[34] However, the antiviral efficacy of these responses is limited by escape mutations as well as upregulation of negative immunoregulatory molecules that impair function.[35–37]

## 2.4 NK Cells

NK cells are typically considered to be part of the innate immune response, contributing to antiviral control by killing of virus-infected cells through ADCC. In the case of HIV, these cells kill virus-infected cells following HIV-mediated downregulation of HLA Class I,[38] and HIV-induced upregulation of stimulatory NK ligands cells.[39] NK cells also produce antiviral chemokines CCL3, CCL4, and CCL5.[40] The potential relevance of these cells to vaccine strategies comes from an extension of studies in mice suggesting that NK-cell memory can be induced.[41,42] In a recent study in SHIV- and SIV-infected macaques,[43] it was shown that splenic and hepatic NK cells were able to specifically lyse dendritic cells pulsed with Env and Gag, whereas this was not observed in cells derived from uninfected macaques. Importantly, splenic and hepatic NK cells obtained from macaques immunized 5 years earlier with a recombinant HIV-adenovirus vector lysed antigen-matched but not antigen-mismatched target cells. The demonstration that these memory NK-cell responses can be induced by immunization suggests that they might be exploited by future vaccine regimens.

## 2.5 $CD4^+$ T Helper Cells

Virus-specific CD4 T helper-cell responses are critical for induction and maintenance of both $CD8^+$ T-cell and B-cell responses to viruses. However, these same cells are preferentially infected with HIV.[44] Indeed vaccine-induced $CD4^+$ T-cell responses have in at least one instance been shown to enhance infection and progression in an animal model of AIDS virus infection.[45] In this case, $CD4^+$ T-cell responses were induced in the absence of neutralizing antibodies and HIV-specific T-cell responses. Balanced $CD4^+$- and $CD8^+$ T-cell induction may be critical, as suggested in recent studies in mice in which selective induction of virus-specific $CD4^+$ T-cell responses in the absence of virus-specific CD8 T-cell responses induced massive inflammation and immunopathology.[46] A particular subset of these cells, T follicular helper cells, are critical for providing help to B cells in the process of affinity maturation, and since they correlate

with the development of broadly neutralizing antibodies in persons with chronic untreated infection,[47] are likely to be a key component of an effective vaccine.

## 3 EFFICACY TRIALS OF CANDIDATE VACCINES

To date there have been five efficacy trials of candidate HIV vaccines, but only one showed any measure of protection, albeit modest (Table 20.1). The immune responses induced by vaccine candidates in efficacy trials that have failed have provided insights into what is insufficient for protection. Alternatively, the one trial in which a measure of efficacy was detected has provided important information on what might be required, at least in the context of that particular vaccine.

### 3.1 Recombinant gp120 Vaccination

The first vaccine efficacy trials tested the hypothesis that envelope-specific antibodies would protect from HIV infection. The VAX004 study involved over 5000 volunteers in the United States and The Netherlands.[48] The vaccine regimen involved seven doses over 30 months of a recombinant gp120 envelope glycoprotein vaccine derived from two clade B strains of HIV with alum as an adjuvant. Enrollees were persons at risk for HIV infection, predominantly men who have sex with men. Although binding antibodies were induced in all vaccinees, there was no evidence of protection. In addition, there were no differences in viral load or genetic characteristics of the infecting viral strains in vaccinees compared to placebo recipients, consistent with a lack of vaccine-induced immune pressure. A similar trial (VAX003) using a bivalent B/E recombinant gp120 glycoprotein vaccine with alum was tested in a cohort of over 2500 injection drug users in Thailand, and again there were no differences

**TABLE 20.1** Human HIV Vaccine Efficacy Trials

| Study | Immunogen | Immune response | Outcome | References |
|---|---|---|---|---|
| VAX003, VAX004 | Gp120 protein | Nonneutralizing antibodies | No protection | [48], [49] |
| STEP; Phambili | Ad5 Gag, Pol, Nef | CD4/CD8 T cells | No protection? Increased acquisition | [50], [51], [56] |
| RV144 | Poxvirus Gag, Protease, Env; Gp120 protein | Nonneutralizing antibodies | 31% efficacy | [57] |
| HVTN505 | DNA Gag, Pol, Env, Nef; Ad5 Gag-Pol and Env | Nonneutralizing Ab; CD4/CD8 T cells | No protection | [65] |

observed in infection rates for vaccinees compared to placebo recipients, and vaccination had no impact on rate of disease progression.[49]

Although the results of VAX003 and VAX004 were disappointing, these studies established mechanisms for global HIV vaccine efficacy testing and demonstrated that such studies could be robustly conducted.

## 3.2 Recombinant Adenovirus

The second efficacy trial of a candidate HIV vaccine involved induction of T-cell responses rather than antibody responses. Called the STEP Trial or HVTN 502, it tested whether induction of $CD8^+$ T-cell responses could provide protection.[50,51] Since these cells kill virus infected cells, it was anticipated that a protective effect would be more likely to involve enhanced control of viremia rather than protection from infection.

The vaccine consisted of three adenovirus type 5 (Ad5) vectors expressing Gag, Pol, and Nef, respectively, which had been shown in Phase I studies to preferentially induce T-cell responses.[52] Moreover, in animal models SIV Ad5 prototype vectors had been shown to be immunogenic and to lead to control of viremia in some preclinical studies.[53] The efficacy trial involved 3000 participants who were at high risk of infection, who received a regimen consisting of three injections over 26 weeks. Immunization resulted in induction of T-cell responses by IFN gamma Elispot assay in 77% of vaccine recipients, and 62% recognized two to three of the proteins in the vaccine. However, the magnitude of $CD8^+$ T-cell responses was modest, with intracellular cytokine analysis revealing that only 0.5–1.% of $CD8^+$ T cells were HIV specific, much lower than observed in persons who control HIV spontaneously.[51] Moreover, only 41% of vaccinees had detectable HIV-specific $CD4^+$ T-cell responses, and only 31% had both $CD4^+$ and $CD8^+$ T-cell responses to HIV. The cell-mediated immunity induced by vaccination neither protected from infection nor influenced geometric mean viral load after infection. However, subsequent analysis of breakthrough viruses in vaccinees compared to placebo recipients did suggest some degree immune pressure induced by vaccination, with a sieve analysis showing a signature consistent with vaccine-induced $CD8^+$ T-cell immune pressure seen in the Gag protein.[54] Of concern, the hazard ratio for infection between vaccine and placebo recipients was actually higher in Ad5 seropositive and circumcised men.[55]

While the STEP trial was underway, an additional test of the Ad5 vaccine concept was undertaken in South Africa, in a high-risk heterosexual population where HIV clade C is endemic and Ad5 seropositivity is more frequent. Testing of a clade B vaccine was justified based on induction of cross-clade T-cell immunity observed in initial trials. This trial, termed Phambili,[56] was halted when the results of the STEP trial were announced. This trial, like STEP, resulted in induction of HIV-reactive IFN-γ producing T cells, in this case to both clade B (89%) and clade C (77%) antigens. Also like STEP, despite immunogenicity for cell-mediated immunity, it did not result in decreased acquisition of lower plasma

viral load set point in vaccinees. However, unlike STEP, there was no evidence of increased acquisition due to adenovirus seropositivity or circumcision status.

The results of the STEP and Phambili trials had a major impact on the HIV vaccine field, in turning attention away from T-cell vaccines and back to efforts to generate neutralizing antibody responses.

## 3.3 Recombinant Poxvirus Plus gp120 Boost, RV144

There has been only one efficacy trial of a candidate vaccine that has shown efficacy, and protection was modest. The trial, termed RV144, was conducted in Thailand and involved immunization with four doses of an avian poxvirus (ALVAC) expressing Gag, Protease, and Env, followed by boosting with a bivalent CRF_01AE/B gp120 Env protein in alum. In a trial of over 16,000 persons, 31% protection was demonstrated at 42 months.[57] However, posthoc analysis showed 60% protection at 12 months[58] and in persons who did become infected, the vaccine regimen had no effect on viral load or CD4 T-cell counts.

Since a level of protection was detected, this vaccine provided the first opportunity to define correlates of protection in a human HIV vaccine trial. The vaccine produced T-cell responses in only 19% of individuals. Binding antibody responses were detected in almost all participants, but there were no neutralizing antibodies observed. Subsequent analysis showed that nonneutralizing antibodies to the V1V2 region of the HIV envelope were associated with decreased transmission risk, and were able to mediate ADCC in vitro, suggesting a mechanism of antiviral efficacy.[59,60] Comparison of the antibodies induced by RV144 to the unsuccessful VAX003 study revealed differences in subclass selection, with RV144 inducing highly functional IgG3 antibodies and VAX003 inducing less functional IgG4 responses.[61] A molecular sieve analysis of viruses from those who became infected was also consistent with an antibody-mediated antiviral effect, showing genetic signatures of vaccination involving the V2 region.[62] This trial also suggested responses associated with increased risk of infection, namely induction of Env-specific IgA antibodies, that may function by blocking the binding of ADCC-mediating IgG3 antibodies to the envelope protein, thereby diminishing ADCC effector function.[63]

Although the original decision to move forward with the RV144 trial was very controversial,[64] in the end it provided the first evidence of protective efficacy of a candidate vaccine regimen in humans, and has provided extensive insights regarding potential mechanisms of protection. Whether these mechanisms are unique to this regimen is unclear. A follow-up efficacy trial with modifications is planned (given later).

## 3.4 DNA Prime Plus Recombinant Adenovirus Boost

The most recently conducted efficacy trial of a candidate HIV vaccine also involved an Ad5 vector, but with important distinctions compared to the STEP

and Phambili trials. Termed HVTN 505, this trial tested a combination regimen that was designed to induce $CD4^+$ and $CD8^+$ T-cell responses as well as antibodies to envelop protein of the major clades of virus.[65] This multigene, multiclade regimen consisted of six DNA plasmids individually expressing HIV clade B Gag, Pol, and Nef, as well as clade A, B, and C envelope proteins, given at weeks 0, 4, and 8. This was followed by a boost with Ad5 vectors expressing a Gag–Pol fusion protein and clade A, B, and C envelope proteins. Over 2500 high-risk individuals were enrolled, with entry requirements including circumcision and an Ad5 serum neutralizing antibody titer of less than 1:18, due to concerns raised in the STEP trial.

The vaccine was modestly immunogenic, eliciting CD4 T-cell responses in 62% of vaccinees and CD8 T-cell responses in 64%. Although binding antibodies were induced to the vaccine strains, very little in the way of neutralizing antibodies were induced. However, the final results were again disappointing: the DNA/Ad5 vaccine regimen neither protected from acquisition nor did it impact viral load in those who became infected.[65]

## 4 PLANNED EFFICACY TRIALS OF CANDIDATE HIV VACCINES

Over the years, a number of vaccine candidates have been shown to confer some level of protection in animal models of AIDS virus infection. Most of these have used relatively neutralization sensitive challenge virus stocks,[66] and efficacy in animal models unfortunately has not always predicted efficacy in humans.[65] Having said this, there are a few recent studies of vaccine candidates in animal models that provide strong support for taking these products in human efficacy trials. The two candidate regimens discussed later are now in Phase I testing. Efficacy trials are expected to begin in 2017.

### 4.1 Ad26/MVA/Protein

A major advance in the HIV vaccine field came with the demonstration that candidate vaccine regimens containing adenovirus and poxvirus vaccines could protect against acquisition of neutralization resistant SIV challenge in rhesus monkeys.[67] Immunization with adenovirus–poxvirus and adenovirus–adenovirus vaccines expressing Gag, Pol, and Env antigens resulted in a striking 80% reduction in the per-exposure probability of infection by a neutralization-resistant virus in a repetitive intrarectal challenge model. Protection against acquisition correlated with Env-specific binding antibody responses and tier 1 neutralizing antibody titers, as well as V2-specific antibodies. Indeed, the inclusion of Env antigens was required, since protection was decreased when a Gag–Pol regimen was used that did not include Env.[67] Correlates with virologic control included magnitude and breadth of Gag-specific T-cell responses as measured by Elispot, indicating T-cell-mediated antiviral control was also induced.[68]

Given the clear signals that Env-specific responses were important in preventing acquisition, a follow-up study was designed in monkeys to test whether adenovirus–poxvirus regimens coupled with a protein boost might enhance protection. This study included a nonreplicating Ad26 vector expressing Gag, Pol, and Env, followed by boosting with a recombinant gp140 envelope with adjuvant.[68] Although neutralizing antibodies to tier 2 viruses were only borderline, nonneutralizing antibodies with multiple Fc receptor functions were induced, as determined by high throughput antibody profiling. Upon repeated intrarectal challenge, the Ad26/Env vaccine regimen provided a 90% reduction in per-exposure risk of infection, and 50% of the animals remained uninfected after completing the six serial challenges. Importantly, this was the first test of a trimeric envelope protein in humans.

Given the impressive results of this regimen in the animal model, Phase I trials of an analogous regimen based on HIV are underway in humans, with plans to move on to clinical efficacy trials. One of the compelling features of this approach is the use of mosaic gene inserts that represent the dominant variants that are present in the population.[69] This makes the vaccine potentially suitable not just for US and European populations, but for the rest of the world, including the areas of Africa that have the highest burden of disease.

### 4.2 Pox-Protein Public–Private Partnership (P5)

Given the evidence of modest efficacy of the RV144 Trial, an international partnership was established in 2009 to build on these positive results and expand the development of pox-protein candidate vaccines, with the goal of licensure, should efficacy be shown. In addition to planned efficacy trials that mimic RV144, the P5 Partnership will also test variations on the RV144 trial, to create a better vaccine. This multipronged approach is intended to accelerate vaccine development. In the P5 trial that is essentially a repeat of RV144 to be conducted in Southern Africa, a modified poxvirus expressing clade C will be used, along with an envelope protein boost that is likewise based on clade C virus. This will have important differences compared to RV144, including a poxvirus expressing clade C antigens, a different adjuvant (MF59) for the gp120 clade C envelope protein component, and a booster dose at 12 months. A similar regimen tailored for local circulating virus strains will be tested in Thailand. Phase I trials have already commenced in Africa, and the candidates moving into efficacy trials will be determined based on potency and durability of responses.

## 5 NEW HIV VACCINE CONCEPTS ON THE HORIZON

To date only a few vaccine concepts have been tested, and there is no question that there is a need for more to come to efficacy trials. Some lead candidates are given next.

## 5.1 CMV-HIV Vectors

Given the fact that a latent viral reservoir is likely to be established within days of exposure to HIV,[2] an effective vaccine will have to mediate its effect rapidly at the site of viral entry. For T-cell-based HIV vaccines, this will require persistent effector memory cells at mucosal sites, which are not sustained by conventional vaccine candidates. In contrast, CMV is attractive as a potential HIV vaccine vector because it is a replicating virus characterized by high-level sustained tissue resident effector memory cells.[70] A candidate rhesus CMV-SIV vaccine in rhesus macaques has been shown to confer protection from systemic or mucosal challenge in approximately 50% of immunized animals following intrarectal or vaginal challenge with a highly pathogenic SIV.[71–73] What is extraordinary about this vaccine is that it does not prevent infection, with documented dissemination by lymphatic and hematologic spread.[73] However, animals go on to completely clear SIV infection, as demonstrated by highly sensitive nested PCR and by adoptive transfer of cells to naïve animals.[72,73]

Much progress has been made in defining the immune responses induced in these animals, revealing multiple surprises. Extremely strong SIV-specific $CD8^+$ T-cell responses are induced, which are threefold higher than seen with conventional SIV vaccines or with SIV infection, and these responses are also broader. However, no responses to canonical Class I restricted epitopes are seen; rather, the responses induced are to entirely new epitopes.[74] About two-thirds of these are restricted by HLA Class II, which has been reported for $CD8^+$ T cells but is a rare event.[75] The unique characteristics of the vaccine-induced immune responses are linked to deletion of the equivalent of the human CMV UL128-UL131 gene complex, largely limiting virus replication to fibroblasts.

Exactly what mediates protection in these animals is unclear, with efforts underway to define the antiviral contribution of both the Class I and II restricted $CD8^+$ T-cell responses. Also unclear is why the protection occurs in only 50% of animals, and why the response appears to be binary—animals are either protected or not, and in those in whom infection persists, there is no evidence of partial virologic control.[76] Clinical trials of this approach are being pursued, although safety issues with a replicating virus are confounding variables. Development of an attenuated CMV vaccine vector is one approach being pursued. This approach could redirect the immune response to epitopes that have not been under immune selection pressure, making this an important approach for both therapeutic and prophylactic vaccines.

## 5.2 SOSIP and Other Trimers

Antibody-mediated neutralization of HIV is dependent on recognition of epitopes on the envelope trimer. However, HIV vaccine efficacy trials completed to date have used monomeric forms of gp120, and have largely induced non-neutralizing antibodies. There are now a number of immunogen trimers that are

currently undergoing evaluation, and more are likely to follow now as structure of the envelope trimer has been defined.[77,78] A promising trimer already in animal trials is the BG505-SOSIP.664 trimer, a proteolytically cleaved and stabilized trimeric soluble envelope protein, engineered from a clade A tier 2 virus. The antigenic properties of these SOSIPs are promising—the proteolytically cleaved trimers are recognized by broadly neutralizing antibodies but fortunately are generally not seen by nonneutralizing antibodies, so are less like to skew responses away from neutralizing antibodies.[79] This and SOSIPs derived from other isolates are moving forward in animal trials, and have recently been shown to induce autologous tier 2 neutralizing antibodies.[80]

Although stronger and more broadly directed responses will be required for protection, the demonstration of a tier 2 neutralizing antibody response, even if just to the autologous virus, is an important step forward.

## 5.3 B-Cell Lineage Immunogen Design

The pathway to development of broadly neutralizing antibodies is complicated, requiring induction of a particular germline B-cell response that subsequently undergoes affinity maturation through somatic hypermutation. Immunogen design is thus limited by the requirement that the initial immunogen be able to activate the appropriate germline precursor. A major obstacle to development of BNabs is poor binding of native HIV envelope to unmutated precursors of BNabs. Recent studies using an engineered outer domain of gp120 fused to a protein has been shown to induce appropriate germline precursors and thus may be effective as an initial immunogen, coupled with additional immunogens that would lead the way toward development of the mature broadly neutralizing antibody,[81] an approach that has been termed B-cell lineage immunogen design.[82,83]

## 5.4 eCD4 Ig

Inducing high titer broadly neutralizing antibodies through vaccination is a daunting task, and even the best neutralizing antibodies developed during chronic infection still fail to neutralize a subset of viruses. A novel approach to overcome challenges in de novo induction of broadly neutralizing antibodies and the limited breadth of even the best neutralizing antibodies is the use of bivalent inhibitor that binds to the CD4 binding site and the coreceptor binding site on the virus.[84] By delivering this through an adeno-associated viral vector, persistent high titers are achieved in vivo. This approach involves CD4-Ig to block the CD4 binding site on the virus, while at the same time the CCR5 coreceptor binding site on the virus is blocked by a CCR5-mimetic sulfopeptide fused to the CD4-Ig. This compound binds avidly to the envelope glycoprotein of HIV, and has been shown to inhibit 100% of diverse global viral isolates[84] far better than any broadly neutralizing antibody identified to date. A rhesus

version of eCD4-Ig protected four out of four monkeys repeatedly challenged with SIV.[84] Early studies indicate that transgene-specific antibodies do not develop, but long-term follow up will be important.

## 6 VACCINES AND THE HIV CURE AGENDA

The proof of concept that HIV infection can be cured has apparently been achieved in the "Berlin patient," an HIV-infected person who underwent allogeneic bone marrow transplant from a CCR5 delta 32 homozygous donor[85] and has subsequently remained free of detectable HIV infection.[86] Exactly what is responsible for his "cure" remains unclear, including potential contributions of conditioning chemotherapy and graft versus host disease. Nevertheless, this single case has mobilized a global effort focused on achieving cure in persons with HIV infection. Since infected CD4 cells do not undergo spontaneous death, it is widely believed that the immune system will need to work in concert with approaches designed to activate the latent reservoir in order to eradicate infection. As such, almost certainly there will be a need for immunotherapeutic intervention,[87] and thus candidate HIV vaccines are taking center stage as adjunctive therapy in cure strategies (reviewed in Ref. [88]). Of note, numerous attempts at therapeutic immunization to control viral load in chronic HIV infection have failed, but the more potent vaccines now in development may achieve a degree of success.

## 7 CONCLUSIONS

Despite robust challenges, momentum toward the development of an effective HIV vaccine is growing. Now 35 years into the epidemic, there are clear reasons for optimism. It is now certain that broadly neutralizing antibodies to HIV do exist and the pathways to development of these responses are being revealed. The ability of virus-specific $CD8^+$ T cells to clear systemic infection has been demonstrated in the monkey model. New efficacy trials are about to commence, with candidate vaccines that have achieved impressive protection in animal models. Reasons for failures of past HIV vaccine trials are being revealed, and the antiviral functions of different arms of the immune response are being defined, providing new insights into the correlates of protective immunity. There is no question that there is still a long path ahead, but ample reasons to be optimistic that extensive global collaborative efforts currently underway will meet this challenge.

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Chapter 21

# Influenza Vaccines and Vaccination Strategies

**Kathleen Maletic Neuzil, MD, MPH*, Justin R. Ortiz, MD, MS****

**University of Maryland School of Medicine, Professor of Medicine and Pediatrics, Director, Center for Vaccine Development, Deputy Director, Institute for Global Health, Baltimore, MD, United States; **World Health Organization, Medical Officer, Initiative for Vaccine Research (IVR), Immunization, Vaccines and Biologicals (IVB), Family, Women's and Children's Health (FWC) Cluster, Geneva, Switzerland*

## Chapter Outline



## 1 INTRODUCTION

Influenza is a communicable acute respiratory disease and one of the major infectious disease threats to the human population. Influenza virus affects individuals of all ages, causes repeated infections throughout life, and is responsible for annual worldwide epidemics of varying severity, commonly referred to as "seasonal influenza." Influenza also causes periodic pandemics that are characterized by a novel virus strain to which the majority of the population is susceptible and which have the capability of causing disease and sustained transmission from person-to-person.

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This chapter focuses on seasonal influenza vaccines and vaccination programs. It provides an update on global influenza disease epidemiology and reviews the currently available influenza vaccines, the global recommendations for their use, and the programmatic challenges of delivering them.

## 2 THE INFLUENZA VIRUS

There are 3 types of influenza viruses that infect humans—A, B, and C—which are classified based on immunologic and biologic properties. Influenza viruses are negative strand RNA viruses with a segmented genome—influenza A and B viruses contain 8 RNA segments, and influenza C contains 7 RNA segments. Type A influenza viruses are further classified into subtypes according to the combinations of the hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins. HA is the major envelope protein and is the protein against which most neutralizing antibodies are directed. NA is important for release of virus particles and viral spread from cell to cell.[1,2] As of 2015, 18 different hemagglutinin subtypes and 11 different neuraminidase subtypes have been identified and distinguished structurally and antigenically—H1 through H18 and N1 through N11, respectively. To date, H1, H2, and H3, and N1 and N2 have been found as components of epidemic viruses in humans. The influenza A subtypes currently circulating among humans are influenza A(H1N1) and A(H3N2).[1,2,3]

Influenza B viruses are mainly, although not exclusively, found in humans and form a single antigenic group. Although the antigenic variation is well-established, influenza B viruses are not divided into subtypes. They are, however, further classified into lineages and strains. Currently circulating influenza B viruses belong to one of two lineages: B/Yamagata and B/Victoria. Type C viruses cause disease much less frequently than type A and B, are not believed to cause epidemics, and are not a target for influenza vaccines.[1,2,3]

The influenza virus undergoes frequent antigenic change. When mutations occur in influenza virus HA and NA surface glycoproteins, they are able to evade immunity induced by infection to previously circulating strains. This is the basis for annual influenza epidemics and necessitates the frequent changes in vaccine composition. A more substantial antigenic change can occur through gene reassortment. As a segmented RNA virus, influenza may reassort with other human and nonhuman influenza viruses. When that gene reassortment occurs in such a way that the virus has major changes to the HA and/or NA antigens, yet retains the capacity to cause disease and transmit among humans, a new strain can emerge, for which immunity in the population is lacking. These reassortment events may create new pandemic influenza viruses that could cause substantial disease, including deaths, globally.

Avian, swine, and other animal influenza viruses may also directly infect humans, and to date human-to-human transmission of these viruses has fortunately been limited.[3] Clearly, these viruses in animals need to be closely monitored, as the human population has little immunity to them. If animal influenza viruses

were to ever efficiently transmit from person-to-person, there would be the potential for a severe influenza pandemic. Because there are many animal reservoirs for influenza, eradication of the influenza virus is not a viable control option.

## 3 INFLUENZA DISEASE AND BURDEN OF ILLNESS

Influenza virus infection can result in a spectrum of illness from asymptomatic infection, upper respiratory tract illness with or without fever, lower respiratory tract illness, exacerbation of cardiopulmonary disease, secondary bacterial infection, and progression to severe respiratory failure and death.[2] Classic influenza illness is characterized by a sudden onset of fever, and respiratory symptoms such as cough, sore throat, runny nose, or earache. Systemic symptoms such as headache, muscle and joint pain, and malaise are common. For clinical studies and surveillance purposes, "influenza-like illness" is frequently defined as the sudden onset of fever or feverishness with cough and/or sore throat. Most people recover from influenza illness within a week without requiring medical attention, although the cough may be more prolonged and last for several weeks. However, a subset of individuals develops serious and sometimes fatal disease.[2]

Because influenza symptoms are nonspecific, a definitive diagnosis of infection requires laboratory diagnosis. For example, influenza virus infection could resemble infection by any number of respiratory viruses.[4] Further, influenza may cause nonrespiratory diseases such as nonspecific febrile illness in infants, febrile seizures in children, as well as encephalitis, myositis, and myocarditis/pericarditis in all age groups.[2,3] Clinicians, researchers, and policy makers often underestimate the incidence of severe influenza due to the underutilization of influenza-specific diagnostic tests.[4]

In general, influenza virus infection is most common in children, while severe complications of influenza virus infection are most common among young children, the elderly, pregnant women, persons of all ages with underlying medical conditions (such as chronic heart or lung disease), and persons with immunosuppressive conditions.[2,5–7] While studies conducted in temperate, developed-country settings largely determined these risk conditions, studies have confirmed many of the same factors to be associated with severe disease in Bangladesh and Thailand.[8,9] Furthermore, there may be additional risk factors associated with severe disease particular to developing-country settings, such as crowding, prevalence and spectrum of chronic illness including HIV, malnutrition, and low birth weight, proximity and proportion of the young to the elderly, and environmental exposures.[10,11]

In areas where it has been studied, influenza deaths are most frequent in older adults. From 1976 through 2007, a yearly average of 21,098 influenza-related deaths occurred among United States adults 65 years and older.[2] During the period from 1998 to 2005, age-standardized excess mortality among the elderly in South Africa were even higher than in the United States.[12] Importantly, deaths due to influenza may occur at any age. In South Africa, a substantial burden of

influenza mortality has been estimated in children younger than 1 year of age, and in HIV-positive persons of all ages. In South Africa, 28% of influenza-associated deaths at any age occur among HIV-positive individuals, which has important implications for other parts of Africa with high HIV prevalence.[13]

Deaths can increase during pandemic periods when a population has no pre-existing immunity to the virus. In the United States, between 37 and 171 children in the United States died each year from laboratory-confirmed influenza infection during annual epidemics between 2004 and 2015, as compared to 300 laboratory-confirmed pediatric deaths during the 2009–2010 H1N1 influenza pandemic.[2,14] These are certainly underestimates given the underutilization of diagnostic testing. The 1918 influenza A (H1N1) pandemic is estimated to have caused 50 to 100 million deaths worldwide.[15,16] Even so, the cumulative mortality from seasonal influenza exceeds that of pandemic influenza in the United States, and likely throughout the world.[17]

While countries with temperate climates have conducted intensive influenza surveillance and clinical/epidemiologic research for more than 50 years, influenza in tropical and subtropical climates has been understudied. The World Health Organization (WHO) Global Influenza Surveillance Network has coordinated pandemic planning efforts and influenza surveillance activities since its creation in 1948. During most of this time, influenza surveillance was focused on the collection of virus isolates to inform the influenza vaccine strain selection, with activities mainly concentrated in developed, temperate countries. Since the emergence of avian influenza A (H5N1) in 1996 and the subsequent concern about an imminent pandemic, the global community has strengthened influenza surveillance and research capacity in tropical developed and developing regions around the globe. The increasing availability and use of reverse transcription polymerase chain reaction (RT-PCR) diagnostic techniques has revealed much higher rates of influenza virus infection in developing-country settings than had been demonstrated in prior studies that used less sensitive diagnostic tests. The 2009 influenza A (H1N1) pandemic led to a further intensification of influenza surveillance and research in developing country settings.[3]

In temperate and subtropical regions, influenza spreads in seasonal epidemics that coincide with the winter season. In tropical regions, many countries have reported peaks in influenza activity associated with rainy and cold seasons and either longer epidemics or year-round transmission.[18] While attack rates vary substantially by season and locale, influenza typically infects up to 10% of adults and 30% of children each year in temperate regions.[2,19] Epidemics can result in high levels of worker/school absenteeism and productivity losses. Furthermore, closed populations such as schools, hospitals, and isolated communities may experience much higher attack rates. School-aged children play an important role in the spread of influenza viruses.

Available data are incomplete to estimate influenza incidence in most tropical regions. Recent influenza vaccine studies in pediatric age groups in Senegal and Bangladesh, for example, have revealed attack rates similar or higher than

those seen in the United States.[20,21] Even higher attack rates have been noted in certain circumstances. For example, investigations of seasonal influenza outbreaks estimated attack rates of clinical infection to be 67% in Madagascar in 2001 and 47% in Democratic Republic of Congo in 2002.[22,23] While much is known about influenza transmission dynamics in temperate regions, many factors in developing regions may alter disease activity where household, community, environmental, and host factors may differ.

Worldwide, the WHO estimates 3 to 5 million cases of severe illness, and about 250,000 to 500,000 deaths associated with annual influenza epidemics. A 2011 *Lancet* meta analysis in children younger than 5 years of age estimates 20 million (95% CI 13-32) acute lower respiratory infections (ALRI) associated with influenza, including 1–2 million cases of severe ALRI. This study estimated 28,000–111,500 influenza-attributable deaths annually, with 99% of early childhood influenza deaths occurring in low- and middle-income countries.[24]

## 4 INFLUENZA VACCINES

Immunization against influenza serves as the primary means for preventing influenza illness. An unprecendented number of influenza vaccines are available on the global market. (Fig. 21.1). The currently available vaccines are targeted to the HA and NA glycoproteins of the virus, and thus must be reformulated

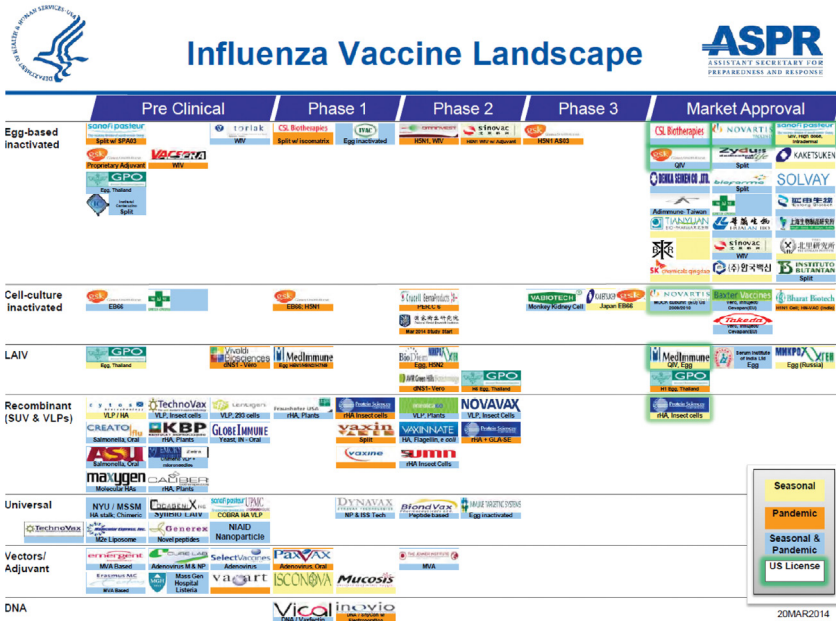


**FIGURE 21.1** **Influenza vaccines on the market and in development, 2014.** Available at: www.who.int/phi/DAY2_10_Bright_PM_SaoPaulo2015.pdf

frequently due to the circulating virus propensity to mutate at key antigenic sites. The strains included in the vaccine are selected based on information derived from globally coordinated epidemiologic and virologic surveillance—WHO's Global Influenza Surveillance and Response System (GISRS). The GISRS monitors the evolution of influenza viruses and the emergence of influenza viruses with pandemic potential. Twice a year, WHO convenes technical consultations (vaccine composition meetings) to recommend the viruses to be included in influenza vaccines that are termed Northern and Southern Hemisphere formulations. All current vaccines are recommended to contain the selected Influenza A (H1N1) and A (H3N2) strains, and either one (trivalent) or two (quadrivalent) influenza B viruses. Quadrivalent vaccines were developed to protect against both B lineages currently in circulation in humans, as it has been difficult to accurately predict the predominantly circulating influenza B virus lineage. Further, in many parts of the world, both lineages have cocirculated.[20,21]

Currently, two general classes of influenza vaccines are licensed for production globally: parenterally administered non-replicating virus vaccines and intranasally administered live attenuated vaccines. The non-replicating vaccines may be further divided into manufacturing substrate (eggs, cell culture, fully recombinant), route of administration (intramuscular, intradermal) type of preparation (whole virus, split virus, and subunit vaccines) and by presence of adjuvant (MF-59) (Table 21.1).

The availability of licensed influenza vaccine products is dependent on the age and health status of the individual. For children younger than 6 months of age, there are no currently approved influenza vaccines anywhere in the world. For children younger than 2 years of age, nonadjuvanted inactivated vaccines are the only approved vaccines in most places, although Canada has approved an adjuvanted inactivated vaccine for children from 6 through 23 months of age. For children 2 years of age and over, both nonadjuvanted, inactivated and live-attenuated vaccines are approved and available. The options increase for adults, as intradermal and recombinant vaccines are licensed beginning at 18 years of age. For adults 65 years and over, a high dose inactivated vaccine is available in the United States, while Europe, Canada, and the United States have approved an MF-59 adjuvanted vaccine for this group (Table 21.1).[25,26]

## 5 NON-REPLICATING INFLUENZA VACCINES

Inactivated influenza vaccines (IIVs) were first licensed for broad use in 1945. The 15 microgram HA per antigen component of IIVs was determined by consensus in the 1970s after improvements in quantification methods.[27] Only recently has the antigen content of such vaccines been altered to optimize immune response in certain populations, for example, higher antigen content vaccines for the elderly, higher antigen content in the recombinant vaccine, and reduced antigen content in intradermal vaccines.

**TABLE 21.1** Categories of Vaccines Licensed for Prevention of Seasonal Influenza Worldwide

| | | Non-replicating vaccines | | | | |
|---|---|---|---|---|---|---|
| | **Live attenuated** | **Standard inactivated** | **High dose inactivated** | **Recombinant** | **Intradermal inactivated** | **Adjuvanted inactivated** |
| Route | Intranasal | Intramuscular | Intramuscular | Intramuscular | Intramuscular | Intramuscular |
| Frequency | Annual | Annual | Annual | Annual | Annual | Annual |
| Approved ages* | 2 through 49 years | ≥ 6 months | ≥65 years | ≥ 18 years | 18 through 64 years | 6 through 23 months, > 65 years |
| HA (mcg/strain) | 15 | 15 | 60 | 45 | 9 | 15 |
| Substrate for production | Eggs | Eggs, cell culture | Eggs | Cell culture | Eggs | Eggs, cell culture |
| Use in pregnant women | No | Yes | No | Yes | Yes | No |

**Approved ages may differ by manufacturer and country.*

## 6 SAFETY OF NON-REPLICATING VACCINES

IIVs have been in use for 70 years, and as a class they have a robust safety profile, as determined in clinical trials as well as large postlicensure surveillance programs. Product-specific information is less available, and the safety databases for the newer products will be more limited until use of the products increase. In general, the most common adverse events associated with IIVs are local injection site reactions.[2,25,27] However, more serious adverse events have been recognized and are described more specifically below.

In children, across multiple large studies, IIVs are generally considered to be safe for all ages and all risk groups. In clinical trials, fever and injection-site reactions are the most common events, and tend to be mild and transient.[2,28,29] In 2010, a IIV-trivalent (IIV-T) formulation from a single manufacturer in Australia was strongly associated with increased febrile seizures in children.[30] This led to varying recommendations in countries precluding the use of this vaccine in younger children, and enhanced surveillance for febrile seizures in the United States and elsewhere. The febrile seizure risk among children in the United States was noted to be elevated in some years and not others, and more so when IIV was coadministered with PCV-13 vaccines. In all cases the risk for febrile seizures in the United States was determined to be substantially lower than observed in 2010 in Australia.[30,31]

Likewise, in adults, IIVs have a strong safety record. In placebo-controlled trials, only injection site soreness is consistently associated with receipt of IIVs.[27] The adjuvanted IIVs and newer high-dose vaccines are associated with increased injection site reactions and mild systemic events, although these are generally mild and transient.[32,33] Safety of IIVs has also been well-studied among pregnant women, again realizing a general lack of product-specific and limited randomized clinical trial data.[34–37]

Safety surveillance in pregnancy is particularly challenging, as the background incidence of rare pregnancy-related adverse events is not well-established, particularly in low resource countries. However, multiple studies to date have not identified consistent, unexpected serious acute events, adverse pregnancy outcomes, or congenital anomalies associated with receipt of influenza vaccine during pregnancy.[25,34–36]

The oculorespiratory syndrome (ORS) is an acute, self-limited reaction associated with bilateral red eyes, facial edema and/or respiratory symptoms such as coughing, wheezing, hoarseness, sore throat, chest tightness or difficulty breathing occurring within 2–24 h of receiving IIV. It is more common in adults and in women. It was first described in Canada in the 2000–2001 influenza season and was strongly associated with one specific preparation manufactured in Quebec. Subsequently, enhanced surveillance did identify lesser associations with other vaccines in Canada, the United States, and Europe. While the pathogenesis is not known, it is not IgE-mediated. Thus, persons with previous ORS may be safely revaccinated if IgE hypersensitivity events can be excluded.[25,38]

Individuals with egg allergy may experience hypersensitivity reactions after receipt of influenza vaccines given the residual egg protein that may exist in most vaccines. While the cell culture-based vaccines do not use eggs in the manufacturing process, influenza seed viruses are passaged in eggs so very small amounts of residual egg proteins may still remain in cell culture vaccines. The new recombinant vaccine, FluBlok, is the only product to be entirely egg-free. Thus, the risk for reaction in egg-allergic individuals will vary based on the product and the individual's history, and the manufacturer's package insert and country-specific recommendations should be consulted.[25,39]

In 1976, there was concern in the United States regarding an imminent swine influenza pandemic, which resulted in mobilization of public health resources and development of a specific vaccine. The swine influenza vaccine was associated with an increased frequency of Guillain–Barre syndrome (GBS), an acute inflammatory polyneuropathy. No subsequent study of influenza vaccines and GBS has demonstrated a risk of the magnitude seen in 1976, which was estimated at one additional case of GBS per 100,000 persons vaccinated. While not consistently noted, studies have identified risks on the magnitude of 1 additional case of GBS per 1 million persons vaccinated, such as the 1992–93 and 1993–94 seasons in the United States. Multiple studies during other seasons have identified no association.[2,25,27,40]

During the 2009 pandemic, several countries demonstrated an increased risk of narcolepsy following receipt of ASO3-adjuvanted influenza vaccine in children, adolescents and young adults.[41,42] The ASO3 adjuvant is in the oil-in-water adjuvant class, and is not approved for use in any seasonal influenza vaccine. MF-59 is also an oil-in-water adjuvant. MF59 adjuvanted seasonal influenza vaccines have not been associated with narcolepsy.[43]

## 7 IMMUNOGENICITY OF INACTIVATED VACCINES

Currently licensed IIV are designed to elicit immunity predominantly against the virus hemagglutinin (HA), the surface glycoprotein critical for virus attachment to host cells. Specific antibodies to the HA are believed to be the best correlate of protection against influenza virus infection, and they are the primary endpoint used by regulatory agencies to evaluate vaccine immunogenicity. However, the influenza virus, and particularly the HA glycoprotein, undergo constant genetic and antigenic change. Thus, antibody elicited by vaccination is generally strain-specific, such that antibody against one influenza virus type or subtype confers limited or no protection against another type or subtype. In addition, nonadjuvanted inactivated influenza vaccines are less likely to confer protection against antigenic variants of the same virus that arise by antigenic drift.[27]

The immunogenicity of influenza vaccines varies with the influenza strain, the age and underlying condition of the recipient, prior exposure to antigenically similar influenza viruses or vaccines, and the vaccine formulation used. In general, young and middle-aged healthy adults, including pregnant women,

have robust antibody responses. Antibody responses may be diminished in young children, the elderly, and those with underlying chronic or immunocompromising condition. Adults and older children require one dose of vaccine each season to induce immunity. Unimmunized young children require two doses of IIV given at least 4 weeks apart to produce sufficient immunity.[25,26,44,45] Based on studies conducted in 1976, children were traditionally administered one-half the standard dose of influenza vaccines, to minimize reactogenicity.[44] More recently, with the use of less reactogenic vaccines, certain countries are recommending full dose vaccines be used even in the youngest children to enhance immunogenicity.[26] Improving the immunogenicity and performance of vaccines has stimulated new product development for specific age groups, including adjuvanted vaccines for young children and the elderly, and high dose vaccines for the elderly.

## 8 EFFICACY AND EFFECTIVENESS

IIVs have demonstrated efficacy and effectiveness across broad age groups and among different populations over many influenza seasons. In general, the term efficacy is used to describe a vaccine's performance to prevent influenza disease in clinical trials, while effectiveness is used to describe a vaccine's performance in observational, nonrandomized settings. Specific efficacy and effectiveness estimates vary considerably from study to study, as they are influenced by many variables—age and underlying health condition of the recipient, the inherent immunogenicity of the vaccine, the match between vaccine virus and circulating strain, and the design characteristics of the studies (eg, surveillance method, outcome measures, placebo versus active controlled trials versus observational studies). Caution should be exercised in comparing efficacy across studies. For example, comparing efficacy among different age groups is problematic unless individuals of different ages are included in the same study season and receive the same study product. Likewise, different vaccine formulations are best compared in head-to-head trials.

A recent metaanalysis of eight United States randomized controlled trials in healthy adults from 2004 to 2008 estimated the pooled efficacy of IIV against culture-confirmed influenza to be 59% (95% CI, 51–67%) among those aged 18 through 64 years.[46] Efficacy estimates are significantly higher in years when vaccine match to circulating strains is higher, and may be lower in years with vaccine-circulating strain mismatch.[47] From the public health context, it is important to consider that even a vaccine with modest efficacy can have important benefits against a disease as common as influenza. Due to the variability in influenza seasons and vaccine match, monitoring for influenza efficacy or effectiveness is best done over multiple influenza seasons.

The now rapidly changing landscape of influenza vaccines, coupled with the unpredictable aspects of influenza epidemics, necessitates a nimble system to monitor and evaluate the impact of individual vaccines and policy decisions. A

growing number of surveillance systems in the United States, Canada, Australia, and Europe monitor influenza effectiveness annually and have the ability to produce early, in-season estimates of vaccine performance.[47,48] Annual estimates of influenza vaccine efficacy are critical to guide policy decisions and public health communications, and should be expanded to allow for more robustly powered investigations of the relative performance of particular vaccines against influenza types or subtypes, across age groups and risk groups, and over multiple years.

## 9 POPULATIONS OF INTEREST

### 9.1 Children

Estimates of the efficacy or effectiveness of IIV among children vary by season and study design. In a randomized, controlled trial in healthy children aged 6–23 months, vaccine efficacy was 66% (95% CI, 34–82%) in the first year, but could not be assessed in the second year due to a low influenza attack rates.[49] Using a case-control design, influenza vaccination was associated with a 75% reduction in the risk of life-threatening influenza illness in children during the 2010–11 and 2011–12 seasons in the United States. In this study, there was no effectiveness demonstrated among children receiving influenza vaccine for the first time who did not receive the recommended 2 doses.[50]

A clinical trial in Europe in 2007–08 and 2008–09 randomized healthy influenza vaccine-naive children aged 6 months to less than 72 months to receive IIV, MF-59 adjuvanted IIV, or a noninfluenza control vaccine. Vaccine efficacy was 43 and 86%, respectively, for IIV and adjuvanted IIV versus the noninfluenza control vaccine against all laboratory-confirmed influenza illness across both influenza seasons. The adjuvanted IIV was 75% better than the IIV comparator vaccines used in the study.[29]

### 9.2 Pregnant Women

The immunogenicity of IIV is generally considered to be similar among healthy pregnant women and nonpregnant women of similar age. In comparison to a noninfluenza vaccine in a randomized trial, IIV reduced febrile respiratory illness by 36% among pregnant women in Bangladesh.[51] Among HIV-uninfected and HIV-infected pregnant women in South Africa, influenza vaccine was 50.4% (14.5–71.2) and 57.7% (0.2–82.1) efficacious, respectively, against laboratory-confirmed influenza illness. Importantly, in both Bangladesh and South Africa, infants born to mothers who received influenza vaccine had fewer laboratory-confirmed influenza illnesses.[37,51]

### 9.3 Older Adults

A single randomized placebo-controlled study of IIV-3 was conducted in persons 60 years and over and demonstrated a vaccine efficacy against

laboratory-confirmed influenza illness of 58% (95% CI, 26–77%).[52] Concerns about suboptimal performance of influenza vaccine are based on immunogenicity studies that demonstrate that older individuals, and particularly those with poor health status, have poor immune responses to vaccines. Thus, new vaccines have been developed to improve performance in older individuals. In Europe, an adjuvanted influenza vaccine has been approved for use in persons 65 and over since 1997, and in the United States the vaccine was approved in Nov., 2015. Regulatory approval was based on immunogenicity, and no head-to-head trials with unadjuvanted IIV are currently available. However, such studies are ongoing, and observational studies have demonstrated superior immunogenicity and effectiveness of the adjuvanted formulation.[53,54]

A high dose inactivated influenza vaccine, with 4 times the antigen content as standard-dose vaccine, was approved in 2009 for use in persons 65 years, based on superior immunogenicity as compared to standard dose IIV.[32] A postlicensure study demonstrated that among nearly 32,000 older adults, most of whom had at least one chronic medical condition, the high-dose vaccine had a relative efficacy of 24.2% against laboratory-confirmed influenza as compared to the standard dose product. Based on the incidence rates reported during the two study seasons, this relative efficacy translated into approximately 1 additional case of influenza prevented for every 200 persons vaccinated.[33]

## 10 LIVE-ATTENUATED INFLUENZA VACCINES (LAIVs)

LAIVs rely on viral replication and immune activation within vaccine recipients. The replication mechanism is altered such that the vaccine virus grows below normal body temperature (ie, cold-adapted), ensuring that virus selectively replicates in the mucosa of the nasopharynx. The vaccine virus is also temperature-sensitive, meaning that replication is hindered at the higher temperatures of the lower respiratory tract. There is no accepted correlate of immunity for LAIVs and in contrast to IIVs, the HA antibody response cannot be used to predict vaccine performance. LAIV is administered intranasally.

There are currently two LAIVs in use worldwide One was developed in the former Soviet Union from an attenuated influenza A/Leningrad/134/57 backbone and has been manufactured and used in Russia for over 30 years.[55] More recently, through a program spearheaded by the WHO, these Russian attenuated seed strains were provided to developing country manufacturers. Serum Institute of India, one of those manufacturers, now has a licensed trivalent LAIV for seasonal use.[56] The second vaccine was developed in the United States from an attenuated influenza A/Ann Arbor/6/60 backbone and has been approved since 2003.[55,57]

## 11 IMMUNOGENICITY

A number of systemic and mucosal immune responses have been routinely assessed following administration of LAIV.[58,59] LAIV immunogenicity data have lacked correlation between efficacy and standard immunogenicity

measures.[27,60] For this reason, there is no correlate of protection recognized by regulatory agencies for LAIV vaccines, highlighting the importance of clinical efficacy studies for licensure and vaccine policy determination.[53] A single study described cell-mediated immunity as determined by ELISPOT assays that measure gamma-interferon as correlating with protection following LAIV in children, however the results have not been corroborated and this measurement is not yet considered to be standard for LAIV vaccine trials.[61]

## 12 SAFETY

LAIV receipt is associated with mild increases in signs and symptoms of upper respiratory tract infection including runny nose, nasal congestion, headache, low grade fever, and myalgia. In clinical trials, an increased risk for wheezing illness was observed in LAIV/Ann Arbor backbone recipients aged <24 months (3.8% LAIV vs. 2.1% IIV). An increase in hospitalizations also was observed in children aged <12 months after vaccination with LAIV/Ann Arbor.[27,62,63] For these reasons, LAIV/Ann Arbor is approved for use beginning at 24 months of age. Postlicensure surveillance data from North America and Europe has not demonstrated an increased frequency of wheezing illness after administration of LAIV/Ann Arbor among healthy children over 2 years of age.[2] Clinical trials of the LAIV/Leningrad in Russia were done prior to the recognition of the wheezing signal, and did not prospectively solicit this event. In Bangladesh, a Phase 2 trial of the LAIV/Leningrad found no imbalance of medically important wheezing events in 300 children aged 2 through 4 years prospectively followed for the outcome.[64]

## 13 EFFICACY AND EFFECTIVENESS

LAIV/Ann Arbor has demonstrated high efficacy in children, including during years with a vaccine-circulating strain mismatch.[62,65,66,67] The efficacy of LAIV/Ann Arbor was superior to IIV in children in 3 randomized efficacy trials.[68] A meta-analysis of these 3 trials and a nonrandomized clinical trials using LAIV/Ann Arbor calculated 46% fewer cases of influenza in children who first received LAIV compared with children first receiving inactivated vaccine. For older children, vaccination with LAIV/Ann Arbor caused 35% fewer cases of influenza compared to children receiving IIV.[69] Based on these results, several countries, including the United States, United Kingdom, Canada, Germany, and Israel preferentially recommended LAIV over IIV for young children. The United States subsequently rescinded this recommendation after effectiveness studies in the 2013–2014 influenza season demonstrated that LAIV did not perform well in young children. The United States currently recommends that either IIV or LAIV be given to young children.[25] Similar to IIV, young children receiving LAIV/Ann Arbor for the first time should be given two doses of LAIV, separated by at least 4 weeks.

In adults up to 50 years of age, most comparative studies suggest either similar efficacy of LAIV and IIV, or that IIV is more efficacious.[27,69,70] As with other influenza studies, the relative efficacy of LAIV and IIV among adults may vary depending on the influenza season and the vaccine match as well as the influenza and vaccine exposure history of the population.

## 14 INFLUENZA VACCINES IN DEVELOPMENT

The development of improved influenza vaccines is a public health priority. The Global Vaccine Action Plan calls for progress toward a universal influenza vaccine by 2020.[71] A universal vaccine that protects against all influenza A virus subtypes would be transformative. In the shorter term, vaccines are being designed to be more effective at preventing influenza illness and severe infection, to be more broadly protective, and to have longer lasting immunity. It is also important to consider ways in which the vaccine production process could be improved, or timelines shortened to permit a more rapid response to an emerging outbreak. Ultimately, an influenza vaccine that illicits broad immunity could preclude the need for the continuous chasing of evolutionary changes seen with influenza. If significant cross-protective immunity could be induced, influenza vaccines would be more effective against novel strains and could be manufactured and delivered throughout the year or even stockpiled for rapid use in the event of an outbreak of a drifted or reassortant virus. These factors could ease the stress placed on vaccine manufacturers and health-care providers in such situations.[72]

The limitations of current influenza vaccines have stimulated an unprecedented pipeline of new vaccine candidates. (Fig. 21.1). Many of these candidates are directed at more conserved regions of the influenza virus, including the stem portion of the HA antigen. While the need for an improved vaccine is clear, and the efforts remarkable, the development pathway is challenging. Substantial investment will be required as multiyear head-to-head trials with currently available products will likely be needed to ensure that enhancing cross-protection, for example, does not diminish vaccine performance during a season in which vaccine is matched with circulating virus strains. Likewise, any new seasonal influenza vaccine will need to have a robust safety record in targeted populations.

## 15 VACCINATION POLICY AND PROGRAMS

Many countries throughout the world have seasonal influenza vaccine policy recommendations (Fig. 21.2). Policies and implementation of such policies will be dependent on the availability of national or regional data, and the capacity, resources, and priorities of the individual country. In temperate industrialized countries with seasonal outbreaks, influenza vaccine is given annually, prior to the influenza season, and generally targeted to individuals with the highest risk of severe disease and to those who may be important in the transmission

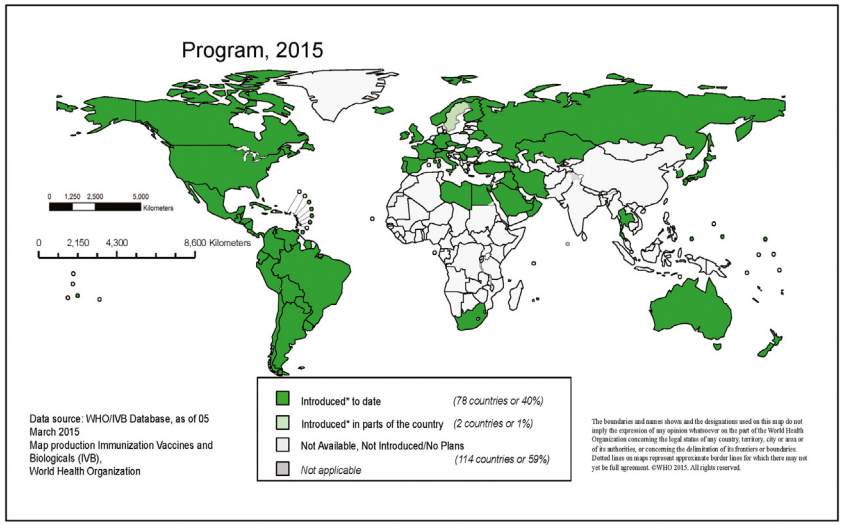


**FIGURE 21.2** **Countries with seasonal influenza vaccine recommendations.** Available at: www.who.int/immunization/sage/meetings/2015/april/Hombach_SAGE_13April2015.pdf

of virus to such high-risk individuals. Health care workers are recommended to receive influenza vaccine in many countries both to limit transmission to vulnerable patients are well as to maintain the health-care work force during influenza outbreaks.

Influenza vaccine programs are more complex in tropical and subtropical countries. Given the varying influenza circulation patterns in the tropics, it is not yet clear if a Southern or Northern Hemisphere vaccine administered in annual campaigns would provide year-round protection against the diverse strains that may be seen in such countries. Further, the optimal formulation or timing of immunization is still uncertain in many countries with limited historical influenza surveillance. In the Americas, for example, some tropical countries use Southern Hemisphere vaccine while others use the Northern Hemisphere formulation. (Fig. 21.3)

Recognizing the complexity of influenza and influenza vaccination programs, and the importance of country-specific data and decision-making, WHO updated its recommendations on use of influenza vaccine in 2012.[18] For countries considering the initiation or expansion of programs for season influenza vaccination, WHO recommends that pregnant women should have the highest priority for vaccine receipt. This recommendation was based on the risk of severe disease, evidence on the safety of the vaccine during pregnancy, the potential for benefit to the women and infant, and the operational feasibility. Additional groups to be considered, in no particular order of priority, are children aged 6 through 59 months, the elderly, individuals with specific medical conditions, and health-care workers. As no vaccines are approved for children

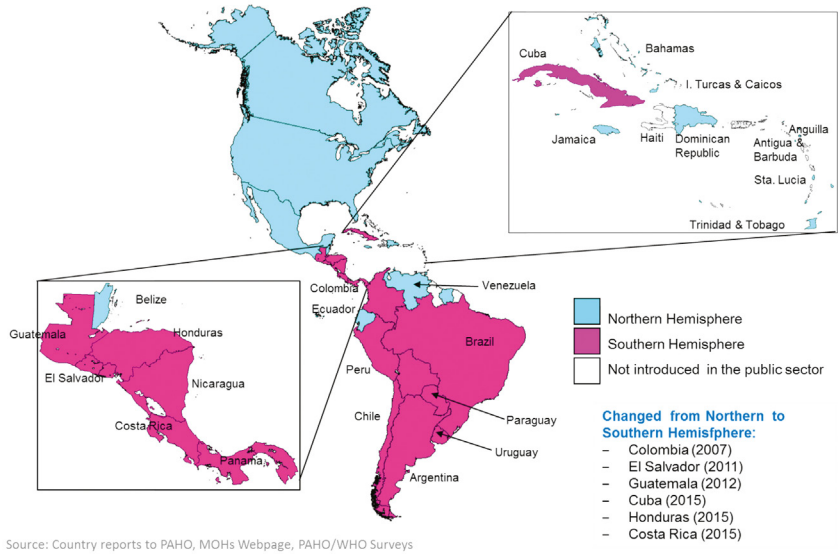


**FIGURE 21.3 Use of seasonal influenza vaccine and formulation in the Americas 2015.** Available at: www.paho.org/hq/index.php?option=com_content&view=category&layout=blog&id=4048&Itemid=4210

younger than 6 months of age, protection of these vulnerable infants can only be achieved through vaccination of the mother during pregnancy and vaccination of close contacts to limit transmission.[18]

## 16 CHALLENGES OF INFLUENZA VACCINE PROGRAMS IN LOW RESOURCE SETTINGS

Most low resource countries have no recommendations for influenza vaccine, and limited capacity to initiate such programs. (Fig. 21.2). Adolescent and adult preventive health services are poorly developed in many countries. Even strategies that target those in the population with regular access to medical care, such as pregnant women or young children, may be logistically difficult. Unlike other vaccines, influenza vaccine formulations change up to twice annually, and may not be available year-round. Young vaccine-naive children require 2 doses of vaccine. Further, due to the nonspecific nature of the clinical illness, health-care providers and patients lack an understanding of the risks of influenza disease. Influenza vaccine package inserts are often vaguely written with regard to the risks and use during pregnancy because pregnant women are seldom included in prelicensure vaccine trials.

Vaccine financing is always an important consideration, and currently, other than the Pan-American Health Organization Revolving Fund for Vaccine Procurement, financing mechanisms do not exist to support influenza vaccine


**FIGURE 21.4 Influenza vaccine coverage for pregnant women, Latin America.** Available at: www.paho.org/hq/images/stories/AD/FCH/IM/Influenza_vaccine/maps/influenza_pregnant_2013.jpg?ua=1

programs for low resource countries.[73] In 2013, GAVI the Vaccine Alliance reviewed maternal influenza for investment in low-income countries worldwide. GAVI chose not to open a funding window due to the logistical challenges, the low country awareness, and the uncertain health benefits of influenza vaccine in comparison to other vaccines.[74] However, GAVI will reconsider opening a funding window for maternal influenza vaccination in 2018, when additional data from clinical trials will be available.[74,75] Further, a number of programmatic initiatives are underway to understand and facilitate maternal influenza vaccine delivery in low resource countries.[76,77] Lessons may be learned from the Latin American experience, as well, where influenza vaccine of pregnant women has been a priority. (Fig. 21.4)

Recognizing that there will be challenges in adding seasonal influenza vaccine to routine childhood vaccination schedules in low resource countries, a better understanding of the burden of severe disease attributable to influenza has emerged as a key area for data generation.[78] Influenza vaccine trials in low resource countries have generally been designed to show efficacy against laboratory-confirmed influenza infection of any severity. In contrast, other recent childhood vaccine introductions, such as rotavirus and pneumococcal vaccines, had supportive data from much larger, randomized controlled trials that demonstrated efficacy of the vaccines against severe disease. Such data have been instrumental in influencing policy and financing decisions on childhood vaccines.[78] Unfortunately, no definitive data exist for the vaccine-preventable

burden of severe influenza illness in low resource settings, nor have studies to date examined the possible causal role of influenza in the progression of other respiratory diseases, such as bacterial pneumonia, to severe respiratory illness and death.[21] This is particularly important for children younger than 2 years of age, for whom acute respiratory illness remains a major cause of morbidity and mortality.

## 17 SUMMARY

Influenza is a common respiratory illness that accounts for substantial global morbidity, mortality and lost productivity on an annual basis. Currently available influenza vaccines are safe and effective, although absolute efficacy varies by year and will be influenced by the virus, the vaccine, and the population. Increasing the use of influenza vaccines can reduce the impact of influenza illness, and high risk groups have been identified that would benefit most from influenza vaccine. Vaccination schedules of the future are likely to be more nuanced in regard to the use of specific vaccines for specific age and risk groups. While HA-based non-replicating and live-attenuated vaccines will be the primary options in the near-term, the influenza vaccine development pipeline is robust. It is critically important that additional data be generated in tropical and subtropical regions to understand how to best deliver influenza vaccines to pregnant women, young children, and other high risk populations. Influenza vaccine probe studies with severe disease outcomes in young children will be important to guide funding priorities and country level decision making on routine pediatric influenza vaccine in low resource settings.

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Chapter 22

# Ethical Considerations in Vaccine Trials in Resource-Limited Settings

**Catherine M. Slack, PhD**
*University of KwaZulu-Natal (UKZN), HIV AIDS Vaccines Ethics Group (HAVEG), School of Applied Human Sciences, College of Humanities, KwaZulu-Natal, South Africa*

## Chapter Outline



## 1 INTRODUCTION

This chapter focuses on ethical concerns early in the life cycle of vaccines, namely trials designed to test safety, immunogenicity, and efficacy of vaccines in human participants. Vaccine trials involve many ethical complexities that stem from several features. These include that vaccine trials are frequently international projects involving organizations in high-income countries collaborating with those in low-resource settings; such trials are often implemented in settings with constrained healthcare systems; they involve multiple sites within and across host countries; and they involve complex trial designs and sometimes highly stigmatized conditions.[1,2]

Furthermore, such trials may enroll participants considered vulnerable, that is, where intraindividual, interpersonal, or contextual factors elevate research

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risks, or undermine consent.[3] Very often, participants are infants or children, recognized as among the most vulnerable of participants.[1] In addition, vaccine trials may be reviewed by research ethics committees (RECs) with variable review capacity and be implemented in host countries with diverse and sometimes weak ethico-legal frameworks.[2] Also, enrolled participants may be "healthy yet at-risk" of acquiring the medical condition that is the vaccine target, at least for late-stage trials, and reside in settings with inadequate health care.[4,5] Last, vaccine trials require participants (sometimes in their thousands) to be retained over several years, to attend many visits and to undergo several procedures, some of which may be burdensome.

## 2 ETHICAL CONSIDERATIONS

The ethics of vaccine trials are concerned with promoting the rights and welfare of participants while participants are contributing to the social good of evaluating vaccines designed to impact public health.[6–8] There are several ethical resources to assist vaccine stakeholders to respond to ethical complexities in vaccine trials. Such resources comprise of broad ethical principles, ethical guidelines, ethical frameworks or models, and, in some instances, empirical data—the latter likely to be ethically relevant if not definitive.[9]

Several broad principles are held to apply to research including vaccine trials. First, respect for persons—researchers should respect people's decisions and actions, and protect persons with impaired or diminished autonomy.[10,11] Second, beneficence—researchers should maximize potential research-related benefits, minimize potential research-related risks, and ensure the potential risks are reasonable in light of expected benefits.[10,11] Third, justice—there should be a fair distribution of research-related burdens and benefits among the collaborating parties, and no single group should bear a disproportionate share of the risks nor access a disproportionate share of the benefits.[12]

Because ethical principles are formulated at a fairly abstract level, they must be specified and applied to actual cases, which can lead decision makers to reach different conclusions about what should be done.[6,10] The ethical principles are generally held to be "universally germane" with expected variations in how they are interpreted, applied, and balanced with each other.[6,12] Ethical standards operate at a less abstract level, such as the ethical standard that informed consent be obtained for trial participation.[13] Several ethical complexities in vaccine trials are outlined here, informed by a popular framework for reviewing the ethics of research protocols,[8] including vaccine trials.[14]

### 2.1 Engaging "Community"

The engagement of representatives from participating communities in resource-limited settings can help offset disparities in power that exist between them and Sponsor-Investigators—as recommended by general guidelines[2,10] And

dedicated guidelines on the topic.[15] Engagement efforts should extend to other stakeholders, for example, media, civil society, advocates, regulatory authorities, and policy makers.[15] Important practices include awareness-raising, research-literacy building, and soliciting views about the research. It may help to set up dedicated structures such as Stakeholder Advisory Mechanisms (SAMs) to get input from diverse constituencies about key issues throughout the vaccine trial.[15,16] Proposed practices should be outlined in a written plan and such efforts should be adequately funded and staffed.[15,16]

Engagement of various role-players can strengthen the ethical conduct of vaccine trials. For example, canvassing the views of participating community members can help identify risks to be minimized that would otherwise have been hidden from vaccine researchers[17] or can help to identify benefits congruent with community priorities[18] or can help communicate complex concepts in the consent process.[19–21] Soliciting inputs from advocacy organizations can help identify how social harms (social adverse events) to vaccine trial participants can be reduced and resolved. Partnering with treatment organizations can ensure participants care needs are addressed by referral.[22] Early engagement of the regulatory authority can help identify concerns about trial conduct or licensure requirements to be addressed. Early involvement of governmental policy makers can increase the likelihood that knowledge or products will be utilized.[23] Rapid responses to social media users can head off myths and misrepresentations about vaccines and vaccine trials.[24] Reaching out to faith-based organizations can help identify beliefs that are unfavorable to the vaccine or research agenda that need to be addressed.[25]

Vaccine trials are increasingly sharing their approaches to community engagement.[26] For example, for HIV vaccine trials and TB vaccine trials, such approaches include the establishment of dedicated structures at sites called Community Advisory Boards (CABs) comprised of diverse community stakeholders who advise vaccine teams throughout the trial; as well as the employment of dedicated engagement staff to involve stakeholders in activities across the trial life span, from protocol development to results dissemination.[27–29] In malaria vaccine trials, engagement for results dissemination included training community-based fieldworkers, soliciting inputs from community members on core messages, monitoring reactions to messages, and requesting feedback on the results-dissemination process.[30] Steady engagement can ensure robust stakeholder responses even in the face of negative trial results.[31]

## 2.2 Considering Social Value and Scientific Validity

Vaccine trials, like all health research, should yield potentially valuable scientific knowledge, otherwise participants are exposed to potential risks for no sound compensatory reason.[8,32] Vaccine development forms part of an effective response to infectious diseases that burden many low-income countries and often the most needy and deserving of their citizens, namely children.[1,33] Because

vaccines are cost-effective they constitute a critical tool against disease burden in resource-limited settings. Despite the value of the overall vaccine research agenda, for each individual vaccine trial the potential risks and burdens to participants should be justified by the potential value of the study question in terms of societal knowledge generated.[14] The potential value of the study conduct for other beneficiaries can also be assessed, such as training and infrastructure development for collaborating health-systems or research institutions.[34] However, it is societal gain in the form of knowledge that most directly justifies potential risks to participants.[8] Social value is enhanced when there is timeous access to knowledge or products from the research by key beneficiaries—set out in a later section. Different conclusions might be reached about the potential social value of a vaccine trial, because—across contexts—there will be differences in disease burden, disease characteristics, population factors, and availability of nonvaccine prevention strategies or treatments, as illustrated in debates about whether developing countries with high rotavirus disease burden should evaluate a rotavirus vaccine with intussusception risks.[35]

It is not sufficient that a vaccine trial explore a valuable research question; the trial design and methods must also ensure that reliable, interpretable, and generalizable data are yielded.[14] This implicates the issue of the most appropriate control against which to compare study vaccines. It is generally accepted that placebo use in vaccine trials is appropriate when where no efficacious and safe vaccine exists—in such instances placebo-recipients are not deprived of the advantages of an efficacious vaccine.[1,33] However, recent discussions in advance of Ebola vaccine trials indicates that even where no efficacious vaccine exists, such designs require robust discussion with affected stakeholders about their merits.[36]

Far more controversial are the circumstances under which placebo use is allowable even when an efficacious vaccine already exists.[33] The World Health Organization (WHO)[33] recommendations, however, outline several such scenarios, including when the existing vaccine is inaccessible in the country's public health system and is likely to remain so and the research aims to develop a new locally affordable vaccine (also alluded to in ethical guidelines[10]). Or, when the existing vaccine may not be sufficiently efficacious in the local context due to epidemiological, demographic, or environmental factors and the research aims to evaluate a new locally appropriate vaccine (also alluded to in ethical guidelines[2]). Or, when the existing vaccine is accompanied by insufficient data on its safety and efficacy for the local context, and the research aims to test its safety and efficacy in the local context. All these scenarios are anchored in the requirement for social value[33] and responsiveness to local problems of the proposed population.[10] The impact of using the existing vaccine as a comparator on sample size, time frames, and interpretability of results—and ultimately eventual vaccine introduction—should be carefully considered.[1,33] As an example, African review authorities approved a vaccine trial comparing nine-valent pneumococcal vaccine to placebo (and not to a seven-valent vaccine available

in the routine immunization programs in many high income countries) because the serotypes on which the existing vaccine was based were of questionable appropriateness in the setting[1,33] and its use as the control would have rendered the trial results so difficult to interpret that the information needed to facilitate introduction of the vaccine would have been compromised.[1]

Also, both trial arms should receive known preventive interventions to reduce their risk of acquiring the disease that is the vaccine target.[2,14] Also, delaying or foregoing the efficacious vaccine should expose participants to an acceptable level of risk—proposed as minimal risk by some guidelines[10] but (in an important shift) as greater than minimal where risks are adequately minimized or mitigated.[33] Also key stakeholders should be appropriately engaged and consensus reached on this issue.[1,2,15]

It has also been argued that vaccine trials should consider various kinds of placebo interventions[33] namely providing control-arm participants with a licensed vaccine against a disease that is not the focus of the study, with health benefit for participants, on the condition that the scientific integrity of the trial endpoints or outcomes will not be affected.[1,33,36] For example, in the RTS, S/AS malaria vaccine trial, control-arm participants received hepatitis B vaccine.[37]

Another key design issue is when trials involve the deliberate exposure of healthy volunteers to infectious agents, so called challenge studies. These trials can provide valuable knowledge about preliminary efficacy, to enable decisions about which vaccines to move into large-scale field trials thereby 'limiting the exposure of thousands of humans in field trials to only the most promising candidates".[32] Research risks must be reduced, for example, the risk of unexpected adverse events from the challenge agents should be reduced by close monitoring, hospitalization, and ensuring the disease is responsive to treatment[4,32] and satisfy a risk-knowledge ratio, that is, where substantial risks or burdens will be imposed, then commensurate potential knowledge value must be offered.[32]

## 2.3 Selecting Participants Fairly

Each vaccine trial should ensure that participants are recruited and selected fairly. There are several historical examples where vulnerable populations were chosen for vaccine testing (prisoners, the institutionalized or the mentally impaired),[4,14] which we recognize today as violating norms for fair selection.

Vaccine researchers should select participants for scientific reasons, that is, because participants will meet the scientific goals and requirements of the trial, not merely because they are vulnerable or unable to protect their interests.[8] If children are to participate in vaccine trials it should be because their participation is indispensable to evaluate safety, immunogenicity, or efficacy for this subgroup[1] and they are the intended beneficiaries of the vaccine. For example, adolescents' involvement in HPV vaccine trials is justified because their enrolment is scientifically indispensable to the development of products designed to prevent HPV acquisition before and over periods of sexual risk.[1]

Vaccine researchers should select participants to minimize risks, that is, from the available pool of scientifically appropriate (scientifically eligible) participants, and those with simultaneous vulnerabilities (or those at increased risk) should have their vulnerabilities addressed or be excluded if this cannot be achieved.[38] For example, challenge experiments may exclude participants with medical conditions that compromise their ability to fight infection.[32] HIV vaccine trials have excluded participants that have a history of mental health problems, because such factors may increase social harms resulting from participation. Community representatives should understand the reasons for selecting participants to avoid perceptions that eligible participants (who may have simultaneous vulnerabilities) are being targeted expressly because they are vulnerable.[15] Vaccine researchers should also ensure that participants and participating communities selected for participation, and therefore likely to assume some research-related risks, burdens, inconveniences, and uncertainties, stand to benefit from the trial.[14,39]. This involves planning for how key groups (participants, the societal groups they represent, and participating community) will access trial benefits in the form of knowledge or vaccine products (see "Considering Post-enrolment issues").

## 2.4 Establishing a Favorable Risk Benefit Ratio

Vaccine trials should present a favorable balance of benefits to risks. This means first, that the potential risks of vaccine trial procedures to participants should be identified and minimized, and secondly that the potential benefits to participants and society should be identified and maximized, and thirdly, that potential risks should be sufficiently outweighed by potential benefits.[8,14] Vaccine investigators should attempt to reduce foreseeable risks of trial participation. For example, in HIV vaccine trials, female participants who could become pregnant are required to use contraception, they are tested for pregnancy before each vaccination, vaccinations are ceased if they become pregnant, and pregnancy outcomes are monitored. These steps are designed to reduce exposure of pregnant women and their fetuses to unknown effects of experimental HIV vaccines.[40,41]

Vaccine trials should have mechanisms for monitoring the physical impact of vaccine products on trial participants, such as reactogenicity and adverse effects, and where necessary for modifying procedures to reduce risks, or taking other steps. Vaccine trials should also have some way to assess social harms or social adverse events. For example, in HIV vaccine trials participants undergo social harms evaluations at regular intervals to identify if their participation has caused negative impacts (stigma, discrimination, relational discord) so that these can be reduced and resolved.[42]

Vaccine investigators and sponsors should carefully consider their responsibilities to ensure participant access to existing modalities to prevent acquisition of the condition that is the vaccine target, especially in late-phase trials

enrolling at-risk participants.[43] The "standard of prevention" issue is accentuated when the condition is incurable (eg, HIV) or stigmatized (eg, TB) and an array of partially effective tools are in existence. For example, in malaria vaccine trials, existing tools to prevent malaria include Insecticide Treated Nets (ITNs) and house spraying[44]; and in HIV vaccine trials, existing tools to prevent HIV include counseling, condoms, medical male circumcision, postexposure prophylaxis, and even preexposure prophylaxis/ PrEP.[45] Vaccine teams should consider whether prevention tools are "proven" to prevent acquisition for the study population, whether the modality is approved for use by relevant authorities,[46] whether participants will be ensured access, and the projected costs, for example, reduced incidence, increased enrolments, and impact on power to detect vaccine effects.[2,44,46,47] Stakeholder input should be obtained, and prevention service providers engaged. Empirical research in HIV vaccine trials found that participants were offered a comprehensive HIV prevention package but the access strategy varied across sites (direct provision versus referral) as did participant uptake of prevention services.[45]

Efforts should be made to maximize the potential benefits of vaccine trials. Various types of benefits are possible namely *aspirational benefit* that may accrue to future persons and society arising from the research results,[48] and *direct benefit* that may accrue to participants arising from the experimental intervention if they are randomized to receive vaccine, sufficiently protected and subsequently exposed to the infectious agent.[48,49] Finally, there are *collateral benefits* that might accrue to participants because of study procedures—tests, examinations, and monitoring required to implement the study safely and scientifically as well as benefits that might accrue to participants arising from care steps taken by researchers[48,50] (more on this issue below).

## 2.5 Addressing Ancillary-Care Needs

Vaccine investigators should consider their responsibilities to address participants medical needs identified during vaccine trials, particularly where such responses are not required for science nor safety, but rather represent helping steps.[51] In resource-constrained settings, care alternatives may be unreliable. The "ancillary care" debate has had several facets. First, what needs should investigators address? Should they address conditions that are of interest to the trial such as malaria in a malaria vaccine trial, or should they also respond to conditions of little scientific interest but for which participants need care, such as HIV in a TB vaccine trial?[50] Second, who should be the focus of investigators' attention—enrolled participants, or those screened but not enrolled, or even community members?[5] Third, how far should investigators go to address needs? Should they make only slight sacrifices or should they implement more "costly" steps?[52] Fourth, why do vaccine investigators have responsibilities in this regard? Is it based on reciprocal justice whereby participants assume risks and burdens and therefore are owed something in return[53] or is based on

reducing health inequities across settings and promoting social justice[54] or some other principle? Fifth, what about the consequences of implementing certain responses for participants but not for nonparticipants, such as introducing local inequalities[55] or intracommunity tensions[56] or inappropriate incentives?[47]

Guidance on this vexing issue is provided more clearly in some ethical guidelines[2,15] than others.[10,57] More detailed guidance is emerging in leading ethical frameworks.[51,58] The "partial entrustment" framework would direct investigators to focus on medical conditions identified by trial procedures for which explicit consent is given ("entrusted" conditions), which may be of varying degrees of scientific import to the vaccine trial. On this account, investigators should take costly steps to help where participants assume many risks and burdens, and where interaction with them is likely to be long and intense, however, care steps should not disproportionately consume trial budgets or undermine scientific integrity.[51] Investigators should do pretrial planning, and form partnerships with care providers.[22,59] They should consult stakeholders, aiming for pretrial consensus about the approach,[5] which should be assessed as the trial continues.[2] Robust discussion before controversy develops is recommended.[23]

Consent processes should clarify those responses being implemented for the science (eg, close monitoring) versus those being implemented to help (eg, referral for care). This may offset the so-called therapeutic misconception, where participants falsely believe that specific study procedures intended to generate knowledge are actually intended for their personal therapeutic benefit,[60] for example, a blood draw for lab testing of immune responses might be falsely understood as intended to generate clinical results of value for participants health. Empirical data is increasingly available for ancillary care practices and perspectives in vaccine trials, such as malaria vaccine trials[18] and HIV vaccine trials.[61,62] Findings suggest that vaccine researchers take many extra-scientific, helping steps, and that they recognize that ancillary care is a motivator for enrolment.[18,61] Empirical research in malaria vaccine trials indicated that participants valued ancillary-care benefits[63] and that substantial systems strengthening is associated with trials.[64] A recent article on Ebola vaccine trials underscores the importance of engaging referral sites, constantly assessing the quality of care at referral sites, and strengthening service delivery at referral sites.[65]

## 2.6 Securing Sound Informed Consent

Participants, or their legally authorized representatives in the case of child participants, should give sound informed consent for vaccine trial participation. Factors complicating consent in vaccine trials may include: complex concepts, low educational and research literacy, linguistic barriers, diverse cultural beliefs about health and illness and decision-making norms, impoverishment, power imbalances between investigators and participants, as well as low trust possibly due to past exploitative experiences with research.[26,47,56,66–68] Some have noted

that consent is widely valued, yet "imperfectly realized"[14] and research shows deficiencies in participant understanding in multiple settings.[69]

Ethical guidelines underscore the need for strengthened consent procedures where participants have vulnerabilities.[10,57] Consent should be viewed as an ongoing process of decision making and not as a once-off event.[70] Vaccine trial staff should implement multimethod approaches to secure consent, such as sound written materials as well as staff trained in communication skills, to promote understanding of key trial concepts.[70] They should implement first-person consent albeit with cultural sensitivity.[71,72] They should engage community representatives to improve consent processes, and reflect on adopted consent strategies.[15,66,67,73] For example, malaria vaccine researchers have recommended the use of consent counselors with cultural and linguistic matching to potential participants, ensuring continuous consent discussions, and tailoring information to educational levels.[74,75]

Vaccine investigators should seek the individual consent of enrolled participants or their proxies, in ways that incorporate culturally sensitive procedures, for example, allowing time for consultation with significant others, and seeking permission from community leaders for trial conduct.[67] Some trials may require dual permission-giving from parents *and* enrolled participants, such as in HPV vaccine trials with adolescent participants[76] in order to offset adolescent vulnerabilities, for example, incomplete cognitive development, limited life experience.[1] Other vaccine trials may require proxy consent from parents who give consent for enrolment of infants, such as in malaria vaccine trials.[37] Challenge studies may require special attention to trial procedures in the consent, for example, isolation and impact on rights to leave the research facility.[32]

Vaccine researchers should assess whether key concepts ("deal-breakers") are understood, that is, concepts for which the consequences of misunderstanding may be severe.[72] Vaccine trials for HIV, TB, and malaria have all published data on assessment of participant understanding.[72,77,78] Open-ended questions may yield more accurate appraisals of understanding than scored forced-choice questions.[72] Any such assessment should be implemented sensitively to offset participant anxiety.[78]

Participants or their proxies should decide about participation free from coercion or undue inducement. Coercive influences on decision making must be prevented, namely threat of negative sanction,[79] for example, communicating to a mother that unless she enrolled her child into a vaccine trial, the child's routine treatment would be denied. Vaccine staff should be sensitive to perceptions that refusal to enroll will lead to sanctions as explored in malaria vaccine trials.[66,73] Benefits to participants, in the form of medical benefits (eg, ancillary care) are not coercive, even while they are ethically complex.[13,79] Such offers generally raise concerns about undue inducement, which is best understood as an offer, that is excessive,[11] that distorts decision-making or impairs judgment.[10,13] Steps to address undue inducement include: limiting offers in an ethically justifiable manner, strengthening consent processes to promote understanding of research

risks, reducing risks of trial procedures to acceptable levels[13,32] and consulting community representatives.[15]

## 2.7 Considering Post-enrolment Issues

There should be ongoing efforts to promote participant welfare, after consent to enrolment.[6,8,14] The proposed approach to these concerns should be evaluated when protocols are submitted. Safety of vaccine products should be assessed in an ongoing way (including reactogenicity and adverse events) along with other endpoints. Procedures should be in place to stop trials early in the event of early beneficial or nonbeneficial trends or even futility.[80] Ongoing consent efforts are important, including revisiting critical concepts (consent "booster" sessions) and sharing information relevant to continuing participation.[56]

Vaccine investigators should consider how participants will be paid, bearing in mind objections to payment on grounds that it might disproportionately attract poor participants, or influence participants to conceal facts rendering them ineligible or that it might constitute undue inducement.[68] Reimbursement payments that refund direct costs (travel, meals) and compensation payments that offset burdens such as time should be considered because they may offset barriers to participation and acknowledge contributions.[68] Payment for time can be calculated using an hourly rate, commensurate with other essential but unskilled jobs in the surrounds, rendering payments modest and indexed to locally acceptable standard for similar work.[68] Vaccine investigators should engage community representatives about proposed payment approaches, schedules, and amounts. In malaria research, participating community representatives were consulted using participatory methodologies about payment approaches to incorporate their views.[81]

Finally, posttrial access by appropriate beneficiaries to key benefits should be carefully planned. Key benefits include knowledge and products. Dissemination of knowledge (results) to various stakeholders is important, including to participating community, tailored to their informational and linguistic needs.[15] Planning for access to vaccine products proven safe and effective may seem most relevant in phase III trials, yet early deliberation is important.[1,2] Here potential beneficiaries include participants, and the at-risk groups they represent. Participants who did not receive the vaccine should be assured of access to it, where benefit to them is still likely because they are still at risk of disease.[1] Because participants assume trial-related risks, inconvenience, uncertainty, and invest time and energy, they arguably deserve reciprocal recognition for their essential contribution in the form of posttrial access.[39]

Access by at-risk citizens to vaccines shown to be efficacious also requires early planning and the involvement of multiple stakeholders. Regulators responsible for approval and licensure should be engaged early to ensure that data to support licensure will be yielded.[28] Policymakers responsible for in-country introduction should be engaged early to ensure the vaccine responds

to their risk-benefit concerns.[28] Vaccine manufacturers, development agencies, international health organizations, and multinational funding bodies need to be engaged to ensure vaccines can be manufactured, purchased, and delivered to ensure their successful introduction into the public health system in the host setting.[1] To ensure coverage while support sources are established, sponsors are frequently requested to commit to making the vaccine available for a particular time-frame. For example, Gambian health authorities planning for a trial of a nine-valent pneumococcal vaccine trial argued for a 5-year access commitment should efficacy be demonstrated to allow other support sources to be explored.[1]

Considerations of access to the RTS,S malaria vaccine (Mosquirix) has involved collaborative discussions between sponsors and researchers and regulatory authorities responsible for recommending vaccine use, the WHO responsible for recommending vaccine deployment in affected countries, the UN and other organizations who will make purchasing decisions, GSK who has committed to not profiting from sale of the vaccine, GAVI who will likely fund the roll-out dependent on prior recommendations, as well as implementing-country regulators and health authorities who must decide on domestic licensure and implementation through national immunization programs.[82–87] A recent example of posttrial access is provided by an Ebola vaccine trial conducted in Guinea that showed high levels of efficacy in interim trial results, therefore randomization of participants (to immediate versus delayed vaccination) was immediately ceased to enable participants-at-risk to access the vaccine.[88]

## 2.8 Ensuring Review by Research Ethics Committees

Vaccine trial protocols should be reviewed for their ethical soundness by review bodies with independent, diverse members, who have required competencies including in vaccines or pediatrics[33] who can ideally deliver well-reasoned judgments using efficient processes.[20,89]

Reviewers should assess the declared approach to stakeholder engagement to ensure it is inclusive. In some cases, they may wish to see evidence of engagement, for example letters of support from key organizations, or memoranda of agreement from care referral sites. REC members may need to review complex protocols, and may benefit from written resources to assist them, for example, placebo use for vaccine trials.[33] RECs may require capacity building, or the opportunity to consult with experts. REC members should carefully review justifications for placebo use where an efficacious vaccine exists, insisting on discussion of the relative merits of alternative trial designs, evidence to support empirical claims (eg, about local data for existing vaccines) and reasoned argumentation[33] to establish whether justifications are "compelling."[10]

To review whether fair selection practices will be adopted, RECs should assess proposed sites, and recruitment materials. They should evaluate the prevention package to be offered participants to help them avoid acquiring the target disease, as well as the steps researchers will take to help address participants

ancillary-care needs.[59] Also, consent strategies should be evaluated to assess if they will sufficiently offset participant vulnerabilities.[2] To avoid financial payments constituting undue inducement, RECs should ensure proposed payment amounts and schedules are modest, and well-justified.[68]

To enhance quality and efficiency of multisite review, the following approaches may be useful—across-REC networking can identify joint concerns and appropriate responses,[1] prereview of protocols (or synopses) can sensitize reviewers to relevant ethical concerns[33] and capacity-building might lessen the chance of poorly justified responses, or unjustified variations in judgments across RECs.[20,89] Various models of ethical review may need to be considered, including centralized review where the appropriate balance between efficiency and quality can be struck.[20,89]

## 3 CONCLUSIONS

Vaccine trials in resource-limited settings raise several complex ethical concerns. Vaccine teams should have a good understanding of the norms in international and host country ethical guidelines regarding various concerns. A pretrial audit of ethical–legal norms can identify gaps, and contradictions within and across settings.[2] Vaccine teams should strive for well-reasoned ethical responses to key complexities (and consider empirical data where it illuminates some aspect of the ethical debate) to ensure the welfare of current participants is promoted, while facilitating the conduct of rigorous trials to address critical health problems of deserving future beneficiaries in resource limited settings.

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Chapter 23

# Economic Considerations for the Development of Global Vaccines: a Perspective From the Vaccine Industry

**Daniel Bakken, MBA*, Donna Boyce, MS**, Luis Jodar, PhD†**

**Strategy, Global Marketing, and Commercial Development, Pfizer Vaccines, Pfizer Inc, Collegeville, PA, United States; **Global Regulatory Affairs, Worldwide Safety and Regulatory, Pfizer Vaccines, Pfizer Inc, Collegeville, PA, United States; †Global Medicines Development Group, Medical and Scientific Affairs, Pfizer Vaccines, Pfizer Inc, Collegeville, PA, United States*

## Chapter Outline



In May 2012, the World Health Assembly approved the Global Vaccine Action Plan (GVAP) to achieve the Decade of Vaccines vision by delivering universal access to immunization.[1] One of the ambitious goals set by the GVAP was to unleash vaccines' future potential with the aim of developing and launching two new major vaccines by the end of this decade. In its assessment report from 2014,[2] the GVAP secretariat asked the World Health Organization Scientific Advisory Group of Experts (SAGE) the following provocative question: Are conditions optimal for vaccine research and development to proceed as fast as possible, or is anything other than the inherent scientific challenge standing in the way of progress?

In the same report, a number of bottlenecks were highlighted including lack of sufficient support for research ideas, lengthy clinical trials, delays in the publication of clinical trials results, long and complex development and regulatory pathways, and lack of coordination between different stakeholders.[2] Admittedly, all of these factors are barriers for timely vaccine development and licensure.

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In this chapter, however, we would like to focus on the challenges and barriers encountered by large pharmaceutical companies when making investment decisions pertaining to the development of new vaccines. The reason for focusing on large, multinational pharmaceutical companies is that it has been estimated that over 80% of the global vaccines market sales in 2011 were generated by five pharmaceutical companies[3]; GlaxoSmithKline, Sanofi, Novartis, Merck, and Pfizer. The recently completed acquisition of Novartis' vaccine division by GlaxoSmithKline has only served to further concentrate the vaccine market space.

This level of industry concentration is a relatively recent phenomenon. In 2002, there were more than 10 manufacturers producing vaccines in the United States alone, compared to nearly 40 US vaccine manufacturers in the late 1960s.[3] The reasons for continued industry concentration are multifactorial. From the 1960s to the 1990s, the vaccines industry saw a shakeout due to lower prices in developed markets, lower access to developing markets, higher legal risks (eg, autism), and increasing barriers of entry into the industry.

These barriers include, but are not limited to[3]:

- high capital requirements driven by strict regulatory and manufacturing quality standards
- lengthy development timelines due primarily to regulatory requirements for a large safety database
- the need for vast global distribution and relationship networks targeting governments, tender agencies, and other organizations (eg, GAVI, UNICEF, WHO) to enable effective commercialization of vaccines
- manufacturing complexity driven by long lead times for production, fragility of supply, and demand unpredictability (eg, pandemics) which often drive purchasers to contract with established players
- difficulties for new entrants to obtain the required capital to enable organic growth, as transformational revenue-generating M&A targets no longer exist
- uncertainty of Vaccine Technical Committee recommendations for use of the vaccine within established National Immunization Programs due to evolving epidemiology, changes in healthcare systems, and financial pressures.

Does this mean that the vaccine's business is not a profitable industry? Not necessarily. In recent years, vaccines sales have experienced rapid growth [16% compound annual growth rate (CAGR) from 2005 to 2011][3] due to demand from emerging markets, funding from global vaccine programs such as GAVI and the Bill and Melinda Gates Foundation, improved understanding of the cost effectiveness enabling increased pricing, innovation that has enabled the development of combination vaccines, and increased demand for flu pandemic and biodefense products. Driven by these factors, the global vaccines industry is expected to average compound annual sales growth of at least 8% from 2011 to 2017.[3]

Despite these promising future financial prospects, investing in the development, production, licensure, and launch of new vaccines entails a considerable

risk for large pharmaceutical companies. In this chapter, we seek to analyze some of the factors that vaccine multinational companies need to take into consideration when making investment decisions regarding the development of new vaccines.

## 1 PROGRAM VALUATION AND PORTFOLIO MANAGEMENT

Large pharmaceutical companies are often faced with constrained development budgets and a diverse set of investment opportunities which may include both internal portfolio assets and external business development candidates. Often, these investment options are not limited to vaccines but span across multiple therapeutic areas, geographies, and development phases, adding further complexity and increasing the difficulty of making optimal budget allocation decisions. Given the disparate nature of these investment alternatives, companies often utilize valuation metrics that seek to combine assumptions around key program attributes (revenues, costs, timelines, risk profile, etc.) to quantify the expected value of an opportunity and determine if continued investment is warranted. Valuation metrics typically serve as an initial screening tool to assess the viability of an investment opportunity and, if evaluated properly, can enable some degree of objective comparison to inform investment decisions across a variety of diverse investment options.

In this chapter, we will focus on valuation metrics typically used by the pharmaceutical industry to make informed decisions about investment options and while the forms of valuation metrics may vary from company to company, there are a few essential principles that must be incorporated to maximize the utility of the metric for decision making purposes. We will attempt to capture these key elements through an explanation of one commonly used value metric, the expected net present value, and an illustration of value assessment using real examples for vaccines that have either recently been licensed or are currently in development.

### 1.1 Expected Net Present Value (eNPV)

The expected net present value (eNPV) is a valuation metric that is commonly used across the pharmaceutical industry to analyze the profitability of an investment or project. By definition, it is the risk-adjusted difference between the present value of cash inflows and the present value of cash outflows. To better assess both the utility and limitations of this valuation metric, it is sometimes useful to deconstruct the metric into its constituent parts and rearrange the order to more fully understand the contributions of each component.

#### *1.1.1 Value*

In the context of this usage, value is assessed from the viewpoint of the corporation and must account for all incoming and outgoing cash flows that are

expected to occur during the lifetime of the project. For a typical vaccine development program, this would include items such as clinical development costs, registration fees, commercial revenues, and potential postmarketing commitments, among others, as well as the associated manpower and overhead expenses required to support the vaccine throughout its lifetime.

### 1.1.2 Net

The term "net" refers to the fact that net cash flows are utilized in the valuation calculation. Net cash flow simply refers to the difference between the cash inflows and outflows in a given time period and reflects the amount of cash remaining after all required charges and deductions have been subtracted.

To illustrate this concept, imagine that company A is developing vaccine X. Proof-of-concept clinical studies have shown that vaccine X is safe and immunogenic. Fig. 23.1 illustrates a typical clinical development program for which the net development costs (after-tax) from Phase 2b to approval are estimated to be $650M over 6 years. Cumulative net income (after-tax) from global commercialization of the vaccine is estimated to at $1.5B over an 8 year postlaunch time horizon. At first glance, one might calculate the net value for such a vaccine to be $850M ($1.5B−$650M) making vaccine X a highly attractive investment and supporting continued clinical development of the vaccine. However, this cursory valuation assessment may be misleading. Let us continue to analyze the key components of an objective valuation metric.

### 1.1.3 Expected

Given the inherent uncertainty associated with any vaccine development program, including vaccine X in our hypothetical example, it is critical that valuation assessments appropriately reflect the probability of success (POS) for each stage of development. The term "expected" is used to connote that the appropriate risk adjustments have been incorporated into the valuation calculation. To accomplish this, POS assumptions are estimated for each stage of development to assess the likelihood of progressing from one development stage to the next.

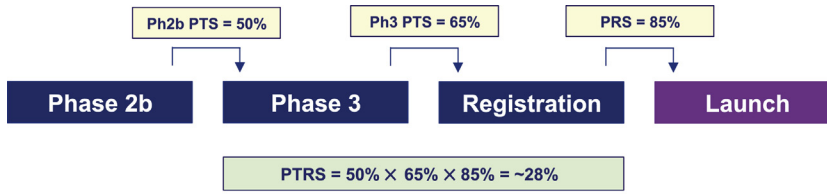


**FIGURE 23.1 Process depiction for estimating the Probability of Technical and Regulatory Success for a hypothetical Phase 2b vaccine development candidate.**

For clinical development stages (eg, Phase 1, Phase 2, Phase 3), a probability of technical success (PTS) can be used to estimate the likelihood of successfully progressing from one phase of clinical development to the next. Success is generally defined as a vaccine having an acceptable safety profile and demonstrated clinical efficacy or immunogenicity against the targeted pathogen. To account for any additional risk associated with the regulatory review process, a probability of regulatory success (PRS) estimate can be used to estimate the likelihood of regulatory approval. The probability of regulatory success value is dependent on the selected regulatory pathway, whether traditional or accelerated, regulatory requirements, as well as regulatory precedent. The more novel the vaccine candidate or regulatory pathway, the lower the PRS value estimate that is assigned. The PRS estimate will evolve as the candidate moves through clinical development and with increased regulatory interactions. The estimates can go up or down, depending on whether the new information is favorable or unfavorable. These individual probability estimates are then combined to produce an overall estimate for the likelihood of product launch, typically referred to as the probability of technical and regulatory success (PTRS). Fig. 23.1 provides a depiction of the overall PTRS calculation process for our hypothetical vaccine X candidate. It is important to note that an individual PTS or PRS estimate should assume success of the prior phases to ensure that program risks are not double counted in the calculation. In our example, the Phase 3 PTS estimate should assume that prior development phases, including the Phase 2b trial, were successful and the PRS estimate should assume regulatory agency agreement with licensure pathway and that the Phase 3 trial will successfully meet the prespecified clinical and safety requirements to support product registration.

Once the PTRS assessment is completed, the cash flows that correspond to each development stage can then be appropriately risk-adjusted to account for the likelihood of occurrence. In our hypothetical example for vaccine X, there is a 100% probability of incurring the Phase 2b development costs and associated cash flows; however, there is only a 50% probability of incurring the Phase 3 development costs and associated cash flows. Therefore, for valuation purposes the cash flows associated with the Phase 3 activities need to be appropriately risk-adjusted to account for the likelihood of occurrence. By similar logic, the calculated PTRS estimate connotes a ~28% probability of product launch and so all cash flows associated with product launch (eg, sales revenues, cost of goods, sales and marketing expenses, etc.) need to be risk-adjusted in a similar fashion. The appropriate risk adjustment of all project related cash flows is the critical element in the calculation of the *expected* net present value.

Returning to our earlier example, let's assume the net development costs are allocated in the following manner across the development phases depicted in Fig. 23.1 (Table 23.1). Given the provided cost distribution from Table 23.1 and probability estimates from Fig. 23.1, we can calculate the *expected* net development costs of ~$366M. Applying similar logic, we can convert the cumulative net income estimate of $1.5B into an *expected* net income using the

**TABLE 23.1** Net Development Cost Distribution and Calculation of Expected Net Development Costs for Vaccine X

| Development phase | Net development cost | Probability of spend occurring | *Expected* net development cost |
|---|---|---|---|
| Phase 2b | $100M | 100% | $100M |
| Phase 3 | $500M | 50% | $250M |
| Registration | $50M | 32.5% (50% × 65%) | ~$16M |
| Total | $650M | — | ~$366M |

overall PTRS estimate for vaccine X. Using the calculated PTRS of 28% from Fig. 23.1, we arrive at an *expected* net income of only $420M ($1.5B × 28%). Subtracting the expected net development costs from the expected net income now results in an *expected* net value for the vaccine of only $54M. In this example, an investment that at first seemed highly attractive has become less valuable once properly risk-adjusted. Admittedly, assigning percentage estimates to the technical and regulatory risk components can be challenging and, while there are some established benchmarks that can be leveraged to inform estimates, a significant degree of subjectivity is inevitable. Later in this chapter, we will provide some real world examples of different PTRS estimates.

### *1.1.4 Present*

The final component of the "expected net present value" valuation metric is the term "present." Given that the time horizon for most investment opportunities can often span many years, it is essential that valuation metrics appropriately account for the time value of money. This notion is predicated on the concept that a certain quantity of money available at the present time is worth more than the same amount in the future due its potential earning capacity. In short, a dollar today is worth more than a dollar tomorrow, provided money can be invested and earn interest. To account for this impact in our valuation assessment, future cash flows must be discounted to appropriately reflect their corresponding value in the present time and we thus we include the term "present" in our description of the valuation metric to connote that the quantified value assessment is expressed in present value dollars.

Fig. 23.2 provides an illustration of this concept. Assuming an interest rate of 5%, a deposit today of $100 in a savings account would be worth $105 in 1 year, $110.25 in 2 years, and $115.76 in 3 years. By similar logic, the present value of $100 earned at some future time point can be calculated by using the appropriate discount rate. The key concept here is that the present value of a given cash flow continues to diminish as the time gap increases, making cash

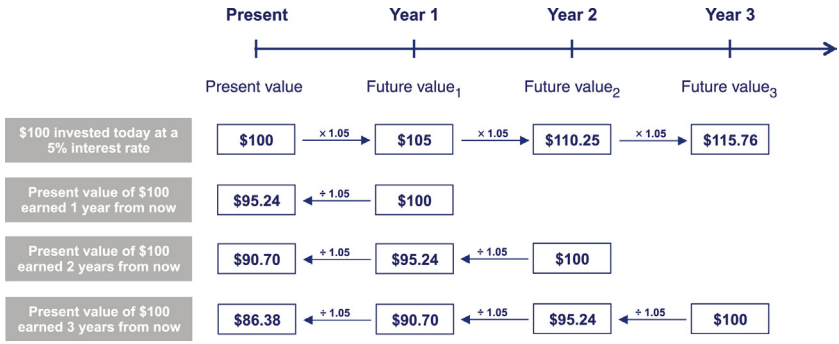


**FIGURE 23.2** **Illustrative example of the time value of money.**

flows in the distant future considerable less valuable than those in the near term. This has especially relevant consequences on the valuation of investment opportunities with long time horizons, such as vaccine development, as cash *outflows* associated with vaccine discovery and development will occur in the near term and cash *inflows* associated with potential sales may not occur for some time.

Returning to our hypothetical vaccine X, if we apply a 10% discount rate to the expected net developments costs that occur over a projected 6-year time horizon we arrive at expected net *present* development costs of $284M (vs. expected net development costs of $366M calculated previously). Similarly, application of a 10% discount rate to the expected net income over an 8-year time horizon (years 7 through 14), results in expected net *present* income of $150M (vs. expected net income of $420M). As described earlier, these calculations account for the time value of money with cash flows in outer years (eg, revenues) affected more significantly than cash flows in early years (eg, development costs).

With the inclusion of this final step, we can now calculate the expected net present value (eNPV) of vaccine X using the following formula:

$$\text{eNPV} = \text{expected net present value (income)} - \text{expected net present value (development costs)}$$

$$\text{eNPV} = \$150\,\text{M} - \$284\,\text{M} = -\$134\,\text{M}$$

Note, this value estimate differs significantly from our original calculation and suggests that, on an expected basis, investment in vaccine X would result in a loss of $134M for company A.

In summary, as we have described earlier, the results of the valuation for a typical vaccine candidate is primarily influenced by four factors; development costs, technical and regulatory risk profiles, commercial opportunity, and program timelines (Fig. 23.3).

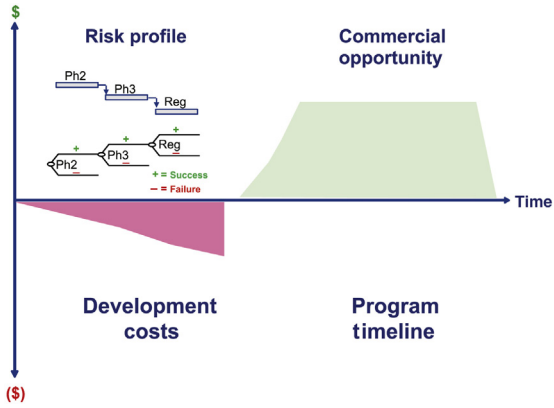


**FIGURE 23.3** **Vaccine candidate valuation framework.**

## 1.2 Key Valuation Pillars

The process as described here has been oversimplified to illustrate how each of the elements of the valuation metric can dramatically influence the attractiveness of an investment opportunity. While the detailed calculations required to complete a thorough valuation assessment for a vaccine candidate are beyond the scope of this chapter, there is value in describing the key valuation pillars with real life vaccine examples so that one can better understand the critical value levers and potential implications of various vaccine development strategies.

### *1.2.1 Development Costs*

Given the high level of costs required to discover, develop, and successfully bring a vaccine candidate to market, it is not unsurprising that the overall development costs can have a significant impact on the valuation of a particular vaccine candidate. Furthermore, these costs are primarily incurred during the initial stages of the overall program timeline and thus impart greater downward influence on the present value metrics than positive cash flows in outer years. As one would expect, higher expected development costs will negatively impact valuation metrics for a given vaccine candidate. More complicated or novel vaccine candidates will require higher development costs. For example, the development costs incurred in the development of monovalent conjugate vaccines against *Haemophilus influenzae* type b or serogroup C meningococcus are disproportionally lower than those to develop multivalent conjugate vaccines against *Streptococcus pneumoniae*.

Production of monovalent conjugate vaccines is highly complex and must take into account the purity of each component, the biological process, the synthetic conjugation process, and the formulation of the final product. Immune responses elicited by a single monovalent conjugate may be affected by different properties including the structure of the polysaccharide, choice of carrier

protein, conjugation chemistry, degree of cross-linking, and the presence of aluminum salts and perhaps amount of free polysaccharide.[4] The complexity and development costs are exponentially higher if we consider the manufacturing process of a 7- or 13-valent pneumococcal conjugate vaccine (which is not just adding 7 or 13 separate conjugates). Each drug polysaccharide must be manufactured and tested separately. The individual 7 (or 13) polysaccharides are then individually conjugated to a protein carrier and subsequently mixed together in a final formulation matrix. Stringent quality control and stability testing occurs throughout the manufacturing process. Quality release of the final vaccine is dependent on satisfactory quality results for each polysaccharide, conjugate, as well as the combined vaccine formulation. Stability of each component, as well as the final formulation, must also be tested and shown to be acceptable.[5] The manufacture of one lot of the 13-valent conjugate vaccines takes approximately 12–18 months. Given the technical development complexities described earlier, it is not surprising that, whereas a large number of manufacturers currently produce Hib conjugate vaccines alone or in combination, only two manufacturers are currently manufacturing licensed pneumococcal multivalent conjugate vaccines.

Clinical development costs and regulatory requirements are also highly variable depending on the vaccine candidate and the disease target. For example, quadrivalent meningococcal conjugate vaccines for use in children below 2 years of age have been licensed globally without the need to conduct large Phase 3 double-blind randomized placebo-controlled efficacy trials which are logistically complex and expensive as they require large sample sizes to meet clinical efficacy primary endpoints due to low incidence of disease.[6] Instead, prespecified antimeningococcal bactericidal antibody titers measured using a human complement source were used for inferring effectiveness in infants and young children.[6] The antimeningococcal bactericidal antibody titer was validated as a correlate of protection to demonstrate vaccine effectiveness. Other multivalent conjugate vaccines are currently being developed against vaccine targets for which a correlate or surrogate of protection has not been validated. For example, conjugate vaccines targeted against group B streptococcus (GBS) for use in pregnant women intended to prevent GBS-related neonatal disease are currently being developed but face a more challenging clinical development path and higher costs. While maternal anti-GBS antibodies are associated with protection from GBS-related early onset disease in neonates, a correlate of protection has not yet been defined, and a Phase 3 efficacy trial in pregnant women (likely in the developing world where GBS incidence is sufficiently high) will likely be required for licensure.[7] Arguably, the overall production and manufacturing costs between a quadrivalent meningococcal conjugate and a tri- or pentavalent GBS conjugate vaccine may not be very dissimilar, but the overall development costs are likely to be highly influenced by the clinical development costs and regulatory pathway required for licensure.

Value maximization strategies in this pillar typically involve cost containment measures to minimize overall development costs or cost deferment strategies to delay spend until various risk milestones have been successfully satisfied (eg, proof of concept, etc.) and thus minimize the quantity of "at-risk" spend.

### 1.2.2 Risk Profile

In similar fashion, the inherent uncertainty associated with the discovery and development processes has a major influence on the valuation for a typical vaccine candidate. PTRS estimates for a preclinical vaccine candidate typically range from 10–35% indicating that more than two-thirds of preclinical vaccine candidates will not result in a commercially available vaccine. As one would intuit, higher levels of risk (lower PTRS), especially risks that are unable to be resolved until later development stages (eg, Phase 3), will negatively impact valuation metrics.

A serogroup C meningococcal conjugate vaccine was licensed in the United Kingdom and European Medicines Agency as a result of Phase 2 immunogenicity studies which compared serum bactericidal assay titers induced by the new meningococcal serogroup C conjugate vaccine to those induced by a licensed serogroup C polysaccharide vaccine, which demonstrated direct evidence of efficacy and accepted correlates of protection.[8] The studies used rabbit complement (rSBA) with the "gold standard" criterion for protection based on serum bactericidal assay titers using human complement (hSBA).[9] Seminal studies conducted in the late 1960s by Goldschneider et al. showed that hSBA titers of $\geq 4$ were indicative of protective efficacy.[10] In the United Kingdom, paired sample studies using both rSBA and hSBA showed that 85% of individuals with rSBA titers $< 8$ had hSBA titers $< 4$, and 93% of those with rSBA titers $\geq 128$ had hSBA titers $\geq 4$. However, for those with rSBA titers between 8 and 128, protection could be assumed if the rSBA titers rose fourfold as a consequence of vaccination. Regulatory authorities accepted the use of SBA as a correlate of protection and the conduct of comparative clinical studies between serogroup C meningococcal conjugate and polysaccharide vaccines were relatively straightforward with limited risks.[8] In this case, both probabilities of technical and regulatory success were estimated to be very high.

Now consider the development of Human Papilloma Virus (HPV) vaccine. Since there is not a known correlate of protection, the interpretation of achieving a certain antibody level in response to HPV vaccination is not clear.[11] As a requirement for licensure, the US Food and Drug Administration (FDA) and the World Health Organization (WHO) required that HPV vaccines demonstrate a reduction in the incidence of premalignant disease.[12] Thus, the primary endpoints used in the clinical trials have included cervical intraepithelial neoplasia of grade 2 or worse and corresponding high-grade lesions in the vulva and vagina, and anal dysplasia of any degree of severity in men.[13] Protection against invasive cancer was not used as an endpoint because the standard of care requires

treatment of premalignant disease, which is recognized as being on the causal pathway to invasive cancer. Furthermore, the time from acquisition of infection to the development of cancer can exceed 20 years. Therefore, the clinical trials of the HPV vaccines were conducted in large numbers of women across the globe with the anticipation that it would take approximately 4 years to acquire enough cases to demonstrate vaccine efficacy. For example, the Phase 3 efficacy studies of quadrivalent HPV vaccine included approximately 17,000 women enrolled across North America, South America, Europe, Australia, and Asia.[13,14] The trials were planned for 4 year duration, but because of the high observed vaccine efficacy, the independent Data and Safety Monitoring Board recommended vaccination of women in the placebo group earlier than planned, thus the total study duration was approximately 3.6 years.[15] Due to the complexity of the clinical studies, the resources required to execute, and the PTRS risk elements highlighted earlier, the overall risk profile for the development of a vaccine such as HPV is logically several fold higher than that for the development of a serogroup C meningococcal conjugate vaccine.

Value maximization strategies in the risk profile pillar typically seek to shift key "derisking" activities earlier in the development process, where possible, with the goal of identifying critical issues and possibly terminating programs prior to major development cost outlays (eg, Phase 3 trial, etc.). Examples might include earlier safety studies or smaller Phase 2b efficacy studies to increase confidence in the mechanism of action prior to initiating and investing in large Phase 3 efficacy trials.

### 1.2.3 Program Timelines

As described earlier, vaccine development timelines are often lengthy with 10–15 years between vaccine discovery and approval not entirely uncommon. The extent of these timelines can have significant ramifications on valuation metrics as cash inflows (eg, revenues), which may not occur until 10–20 years later, will be heavily discounted in the present value calculation. Cash outflows (eg, development costs), by contrast, will have greater impact on the valuation assessment as these costs will be incurred during the near term with less discounting applied in present value calculations.

Value maximization strategies in the program timeline pillar often seek to compress overall development timelines with the goal of accelerating launch timelines to achieve positive cash flows more quickly. For example, the FDA has established several expedited licensure pathways for drugs and biologics (including vaccines) which address serious conditions.[16] One such program, Accelerated Approval, allows for a potential earlier approval of a candidate vaccine intended to prevent serious conditions that has the potential to provide a meaningful advantage over existing therapies and demonstrates an effect on a surrogate endpoint that is thought to predict clinical benefit. Under this program, the clinical benefit of the candidate vaccine is confirmed postapproval. For

example, in 2014, the FDA used the Accelerated Approval regulatory pathway to approve serogroup B meningococcal vaccines for use in the United States.[17] This mechanism enables the FDA to approve vaccines intended to prevent life-threatening diseases, such as meningococcal disease, based on early evidence of the vaccine effectiveness that is reasonably likely to predict clinical benefit, therefore reducing the time it takes for these vaccines to become available to the general public. In the case of serogroup B meningococcal vaccines, evidence of effectiveness was demonstrated by the ability of vaccine recipients' antibodies to kill a number of representative serogroup B test strains. In accordance with the Accelerated Approval regulations, vaccine manufactures are required to conduct studies to confirm the anticipated clinical benefit. These studies are known as confirmatory trials. If the confirmatory trial shows that the vaccine actually provides the anticipated clinical benefit, then the FDA grants traditional approval of the vaccine. If the predicted clinical benefit is not demonstrated, the Accelerated Approval license will be withdrawn.

### *1.2.4 Commercial Opportunity*

The commercial opportunity is often the most speculative element of the valuation assessment and is heavily dependent on a number of factors including the target population, disease epidemiology, burden of disease, clinical profile of the vaccine, and the competitive landscape at the time of launch. Furthermore, the recommendations of National Vaccine Recommending Bodies can have a significant impact on both reimbursement and vaccine uptake and thus are a major driver of the overall commercial opportunity. A vaccine may get licensed globally but will have little to no uptake in the absence of a recommendation due to the lack of reimbursement.

There are a number of Vaccine Technical Committees (VTC) established at the country level that, based on scientific and other evidence, advise their respective governments on the vaccines that should be introduced into their National Immunization Programs. In some countries, the recommendations of the VTC place a duty to the government to implement such recommendations (eg, the Joint Committee on Vaccines and Immunization from the United Kingdom[18]). In other countries, the recommendations emanated from the VTC are merely advisory. In the United States, the Advisory Committee on Immunization Practices (ACIP) provides advice and guidance to the Director of the Centers for Disease Control and Prevention (CDC) regarding the most appropriate selection of vaccines and related agents for effective control of vaccine-preventable diseases in the civilian population. In accordance with Section 1928 of the Social Security Act, the ACIP also establishes and periodically reviews a list of vaccines for administration to children and adolescents eligible to receive vaccines through the Vaccines for Children Program (VFC).[19] The VFC is a federally funded program that provides vaccines at no cost to children who might not otherwise be vaccinated because of inability to pay. A child is eligible for the VFC

Program if he or she is younger than 19 years of age and is Medicaid-eligible, uninsured, underinsured, or American Indian or Alaska Native. VFC covers around 60% of the US population under 19 years of age.[20] The remainder of US children are covered by health insurance plans.

An ACIP recommendation is a prerequisite for a new vaccine to be widely used in the United States. A recent example of this vulnerability has been demonstrated with the recently licensed meningococcal serogroups C and Y and *H. influenzae* type b tetanus toxoid conjugate vaccine (Hib-MenCY-TT). Following many years of technical and clinical development, Hib-MenCY-TT was licensed in the United States in 2012 to prevent invasive disease caused by *Neisseria meningitidis* serogroups C and Y and *H. influenzae* type b for children 6 weeks of age through 18 months of age.[21] In Oct. 2012, the ACIP voted to recommend the use of the Hib-MenCY-TT vaccine only in infants at increased risk for bacterial meningitis. Notably, the ACIP did *not* recommend Hib-MenCY-TT for routine meningococcal vaccination for infants who are not at increased risk for meningococcal disease.[22] In the absence of a routine recommendation, the potential commercial opportunity offered by this vaccine may encounter serious difficulties. As described earlier, the valuation calculations significantly discount these cash inflows to reflect both the likelihood of success (PTRS adjustment) and time value of money such that a sizeable commercial opportunity is often required to justify the large development costs and risk profile of early stage assets.

## 1.3 Value Maximization Strategies

Value maximization strategies in the commercial opportunity pillar can be categorized into two major themes discussed in the subsequent sections.

### *1.3.1 Acceleration Opportunities*

Acceleration strategies are often focused on compression of development timelines to pull forward the anticipated launch timing of the vaccine candidate. For example, the FDA has designated Fast Track designation to two *Clostridium difficile* candidate vaccines to facilitate the development, scientific evaluation, and approval of these vaccines. Similar to the Accelerated Approval program, the Fast Track program is intended to facilitate and expedite the development and regulatory review of new drugs and biologics to address unmet medical need in the treatment of a serious or life-threatening condition. Fast Track designation is granted for a candidate vaccine intended to prevent a serious condition that has the potential to address an unmet medical need. Having a Fast Track designation provides vaccine manufacturers with the possibility to have additional meetings with the FDA to discuss critical development decisions, thereby facilitating the scientific evaluation during the Investigational New Drug (IND) application stage, an organizational commitment involving senior FDA managers, and

the possibility of a "rolling" submission of the Biologics License Application (BLA). This allows sponsors to submit sections of the BLA to FDA for review as they are completed, as opposed to waiting to submit the complete BLA at one time.[16]

Other acceleration strategies typically involve detailed operational planning to streamline processes and minimize any timeline "white space" between the various clinical development and regulatory steps required to approve the vaccine. The conduct of "at risk" clinical studies in high risk populations (eg, HIV subjects), not as a part of postmarketing commitments or life cycle strategies but prior to licensure, may expand the label indication and offer a greater expanded commercial opportunity.

Acceleration of launch timing can have significant implications on overall program value metrics; primarily through competitive dynamics which can greatly increase forecast revenues but also in terms of the time value of money calculations as earlier positive cash flows will be worth more in today's dollars.

Acceleration of vaccine uptake following launch is another strategy that can have significant positive implications to program valuation metrics as a forward shift in incoming cash flows will positively impact the present value calculation. Efforts to improve vaccine uptake are often focused on market preparation activities to improve disease awareness, better understand disease epidemiology, and generate data to support health economic assessments. For example, a 13-valent pneumococcal conjugate vaccine (PCV13) for the prevention of vaccine type invasive pneumococcal disease and vaccine-type pneumococcal pneumonia was licensed in the United States in Dec. 2011.[23] On Jun. 20, 2012, ACIP deferred their decision to recommend PCV13 use among adults aged ≥65 years until data became available on (1) the impact of PCV13 use in children on disease in adults (ie, indirect effects) and (2) the efficacy of PCV13 against noninvasive pneumococcal pneumonia among adults.[24] To answer the second question, a randomized placebo-controlled trial (CAPiTA trial) was conducted in the Netherlands among approximately 85,000 adults aged ≥65 years. Surveillance for suspected pneumonia and invasive pneumococcal disease was conducted from Sep. 15, 2008, several years in advance of the actual licensure, through Aug. 28, 2013. Finally, results were available and presented for the first time in an international conference on Mar. 2014 demonstrating a 45.6% (95% confidence interval [CI] = 21.8%–62.5%) efficacy of PCV13 against vaccine-type pneumococcal pneumonia.[25] On Aug. 13, 2014, the ACIP recommended routine use of PCV13 among adults aged ≥65 years.[24] If executed properly, these activities can be used to support rapid inclusion of vaccines in recommendations by National Vaccine Recommending Bodies and reimbursement by payers.

### *1.3.2 Growth Opportunities*

Growth strategies are often focused on label expansion into new populations, age segments, or geographies. Sometimes referred to as "lifecycle" or "layering" strategies, these initiatives seek to rapidly expand the eligible target population

through additional clinical studies and/or data generation, thus increasing the revenue potential as additional populations are made eligible to receive the vaccine. For example, invasive pneumococcal disease is highest in children below 5 years of age and adults over 65 years of age. In children aged 6–18 years of age, the CDC estimated that the average annual incidence was 2.6 cases per 100,000, with 57% of invasive pneumococcal disease (IPD) caused by serotypes included in PCV13. In contrast, incidence rates of PCV13-type IPD among children in the same age group with sickle-cell disease and HIV infections are substantially higher at 56/100,000 and 197/100,000 respectively.[26] Vaccine recommendations in these high risk populations required the conduct of safety and immunogenicity studies with PCV13. These studies are typically part of life cycle programs after licensure of the vaccine. For every vaccine, each life cycle opportunity is evaluated on an individual basis to determine if the incremental commercial opportunity justifies the incremental costs and risk profile of the initiative. Proper lifecycle management can play a major role in the overall value of a vaccine and should be prioritized accordingly.

## 1.4 Portfolio Management

In a theoretical world with unlimited development budgets, one would seek to invest in any program with a positive expected net present value (eNPV) as these programs would be expected to offer incremental positive value to the company assuming the underlying assumptions of development costs, risk profile, program timeline, and commercial opportunity are accurate. To be clear, a positive eNPV does not guarantee the success of any individual program and many programs with positive eNPVs will ultimately fail without yielding a positive return, however, the number of successes and failures across a portfolio of programs should ultimately balance out if program risks have been assessed appropriately.

Unfortunately, most companies must work within the constraints of limited development budgets and are forced to make difficult decisions regarding which assets to progress further in development. In this context, the utility of using value metrics such as eNPV is somewhat limited as these types of metrics only capture the magnitude of a program's value but do not inherently capture the efficiency or yield of the investment. In these situations, senior decision makers often turn to alternative value metrics such as internal rate of return (IRR) or expected internal rate of return (eIRR) that provide a sense of the annualized return for an individual investment and thus provide an indication of which projects offer the best return for a given dollar of investment.

### *1.4.1 Internal Rate of Return/Expected Internal Rate of Return*

The internal rate of return is a value metric that measures the rate of return for a particular investment project and is commonly used to compare the relative profitability across a portfolio of investment options. By definition, the IRR of

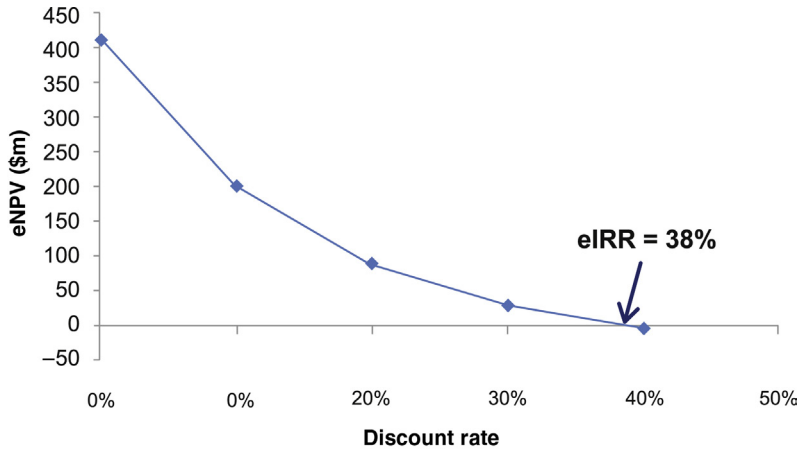


**FIGURE 23.4** **Illustration of the expected internal rate of return (eIRR).**

a project is the annualized rate of return (discount rate) that makes the net present value (NPV) equal to zero. Similarly, the expected internal rate of return (eIRR) is the annualized rate of return that makes the *expected* net present value (eNPV) equal to zero (Fig. 23.4). Since value metrics such as IRR and eIRR are calculated as a rate quantity, they can be used as an indicator of the efficiency of an investment and provide a relative sense of which investments offer the best return on investment. As such, they are commonly utilized when making budget allocation decisions to ensure that funds are being invested in projects which offer the greatest return on investment.

## 2 CONCLUSIONS

Pharmaceutical companies are increasingly facing constrained budgetary environments, complex market and societal forces, and a plethora of internal and external investment opportunities. Given the risk and uncertainties inherent in drug and vaccine development and the large capital requirements, objective decision making processes to discern projects that warrant further investment from those that need to be discontinued is becoming increasingly important. Whereas valuation metrics, as described in this chapter, are valuable tools to inform investment decision, it is important to note that they cannot fully capture all of the strategic nuances associated with a particular investment opportunity and must always be supplemented with management judgment. Examples of these strategic elements that go "beyond the numbers" include corporate social responsibility goals, portfolio balance and diversity objectives, and the necessity to maintain portfolio alignment with the corporate strategy, vision, and mission.

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Chapter 24

# Introduction of Vaccines Into National Programs

**Mark A. Miller, MD**
*Fogarty International Center, National Institutes of Health, Bethesda, MD, United States*

## Chapter Outline



## 1 INTRODUCTION

The decision for a national government to adopt a population-based health intervention such as vaccines is a complex process involving political, economic, operational/logistics, and governance issues. There are as many variations of decisions as there are the number of countries and vaccines. Three important issues predominate: the financing and costs of the vaccine intervention, existing infrastructures on which to add a vaccine component, and the perceived disease burden including the potential benefits of mitigation.[1–5] These and other factors are discussed in detail.

While variolation to protect against smallpox had been practiced in China, parts of Africa, and populations now considered as "developing countries" for hundreds of years, it was not until the completion of global smallpox eradication and the formation of the Pan American Health Organization (PAHO) and World Health Organization (WHO) Expanded Program for Immunization (EPI) that governments, with the aid of UNICEF, began to develop national immunization programs. The EPI introduced a limited number of vaccines against TB, diphtheria, tetanus, pertussis, polio, and measles since 1974 to protect infants and children. With the advent of additional antigens and combined vaccine products with the possibility to protect against numerous diseases, governments have

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**TABLE 24.1 Considerations for the Addition of a Vaccine Into a National Delivery Program**

| Political | |
|---|---|
| Epidemiology | Disease burden-morbidity, mortality, severity, transmissibility, duration of illness, age group, other risk factors |
| Vaccine characteristics | Differential immunogenicity in targeted populations, duration of protection; adverse reaction |
| Regulatory | Vaccines are assured for safety, effectiveness, and quality standards |
| Operational | Delivery to periphery of system storage considerations including temperature requirements, coadministration versus combination versus new schedule |
| Economic | Financial<br>Opportunity Costs<br>Cost of disease<br>Positive/negative externalities—macro-economic considerations on other sectors |

been provided with opportunities to protect individuals or societies through adoption of new vaccines into existing national vaccine programs.

The decision to introduce a new vaccine into any national or subnational program is based on multiple factors related to political- biomedical-, public-health-, regulatory-, financial-, logistical-, and community-acceptance concerns (Table 24.1). All of these factors can be quantified and accounted in various different dimensions to help define the usefulness of a vaccine to either an individual vaccine recipient, local community, or national government. Once the value of prevention from a vaccine is appreciated, it may be supported through the public and/or private market for the whole or targeted subpopulations. While the EPI has supported the addition of yellow fever vaccines for at-risk populations and vaccines for pregnant women to protect against neonatal tetanus, additional vaccines were not added to routine schedules until the 1990s. Vaccines had been developed for global markets including those against hepatitis B, rotavirus, *Haemophilus influenzae* type B and *Streptococcal pneumonia* infections for inclusion into infant and childhood routine vaccine schedules but had limited uptake. More recently, additional vaccines have been developed for various subpopulations within a country (human papilloma virus, HPV) or for certain geographic regions of the world at higher risk (Japanese encephalitis, *Plasmodium falciparum malaria*).

The economics of vaccine development and innovative financing is covered elsewhere in the text. The chapter focuses on the political, epidemiologic, operational, and economic factors that are considered to help decisions to introduce a vaccine into a national program. Additionally, global efforts to help support local decision making processes are covered.

## 2 POLITICAL CONSIDERATIONS

Political decision makers may base the value of a vaccine based on their perception of the impact of a specific disease on their society and the political expediency from a vaccine versus other intervention(s) or doing nothing. A politician and community's perception of disease may be affected by severity, communicability, the degree of acuteness or chronic nature and societal/economic disruption. In general, there is greater political support for interventions against severe diseases with relatively high communicability/incidence occurring in outbreaks compared to those that are perceived as indolent, mild, and chronic in nature. While the social and public health needs may be similar with diseases occurring in outbreaks or not, the immediacy of epidemics frequently demand a rapid political response due to its visibility. A recent example of this is the rapid development and deployment of pandemic influenza vaccine and the response to develop vaccines against the Ebola virus.

While a vaccine intervention, may be an effective tool, there may be other interventions that may also contribute to disease-burden reduction. Alternative interventions may act synergistically with vaccines or offer another option competing for public funds. An example of this may include municipal water supplies versus multiple vaccines against bacterial enteric agents. Vaccines are generally supported politically as they have popular characteristics: they prevent communicable diseases, can be equitably distributed, frequently benefit children, and have historically been low cost. Vaccines have additional positive externalities of offering direct and indirect protection to those unvaccinated. These are all positive attributes that a well-informed community would logically support. Political considerations, though, depend on accountability of national governments to their populations, which are highly variable over time.

## 3 NATURAL HISTORY AND EPIDEMIOLOGIC CONSIDERATIONS

Unlike therapeutic interventions, vaccines are provided to healthy populations to reduce risk of infection and disease. Policy makers should understand the natural history of disease, individual and collective risk based on the characteristics of the infectious organism, including mode and speed of transmissibility, and characteristics of susceptibility in the population. Infections and resulting disease incidence and prevalence may differ among various populations based on age, gender, comorbidities and therefore can potentially identify more specific target groups and strategies for a vaccination to offer direct or indirect protection to those most susceptible.

Additionally, characteristics of the vaccine must be considered, including the age-specific immunogenicity, the duration of protection and resulting effectiveness with various doses and dosages. The ideal vaccine would offer lifelong immunity with the least number of doses, no side effects, and can be

administered in early infancy. Unfortunately, no such vaccine exists given the biological characteristics of vaccines and immune mechanisms during infancy. Knowledge of the natural history, transmissibility, and vaccine characteristics can be used to optimize the incorporation of a vaccine into an existing schedule, either as a coadministered or combined product. Many vaccines are designed to be incorporated as best as possible into existing infant schedules to minimize the burden of extra visits.

While many vaccines may be administered to the entire population, there may be high-risk groups that are more susceptible to infection and/or its consequences that could lead to a more targeted vaccination when resources are limited. Specialized populations may be based on gender, age, socioeconomic status, comorbidities, pregnancy, or engaged in higher risk activities. Differential risk for infection can define strategies to prioritize the direct or indirect insurance afforded by vaccines. For example, while influenza may infect everyone equally, pregnant women and the elderly are more susceptible to severe disease; however, the latter may not respond as well to vaccine. The complexities of heterogeneous response to infection and vaccination has led to vigorous debate to define the best way to protect various segments of the population, especially when potentially targeting populations outside of routine infant schedules. Sometimes the greatest impact may be achieved through the vaccination of those best able to respond to indirectly protect others in the population.

## 4 REGULATORY CONSIDERATIONS

Vaccines are subjected to evaluation through national regulatory bodies for their licensure and use in a country. The demand for transparency has led to the registration of vaccine clinical trials to heighten awareness of positive and potentially negative results. While vaccines may be licensed and evaluated for safety and efficacy by a regulatory agency in a high-income country, epidemiologic conditions may be sufficiently different in populations in certain low-resource countries.

In addition, any new vaccine that would be introduced should not only have demonstrated safety and efficacy, they should also not reduce the effectiveness when used in combination or coadministered with other products. As national regulatory agencies have variable capabilities, the WHO frequently aids to assure that vaccines are qualified for purchase through partnerships with UNICEF procurement processes and regulatory authorities meeting approval standards to offer another level of accountability beyond what an individual nation could provide.

While demand to license vaccines may be highest in the case of epidemics like Ebola, deployment requires clinical safety and efficacy data. While Ebola vaccine candidates were shown to be effective in animals prior to the large 2014–2015 West Africa outbreak, only one had been tested for immunogenicity and safety in humans.

## 5 PROGRAM CONSIDERATIONS

A new vaccine is only useful if administered appropriately to induce the protection to the recipient. There will be different marginal costs if it can be coadministered with existing vaccines either as a combined or separate product. A coadministered product that can be provided at the same visit as other scheduled vaccines, would not result in additional costs of another visit. If the new vaccine can be combined with an existing product, it further has the benefits of no additional injections or storage requirements. A combination product is the ideal new candidate vaccine versus one that requires separate administration and possibly require additional visit(s). The former has no marginal costs of administration from a logistics perspective, while the latter requires resources for storage. Examples of these include the *H. influenzae* B vaccines, which can be combined with the existing diphtheria-tetanus-pertussis (DTP) vaccine and therefore not incur additional costs. Rotavirus vaccine, currently an oral vaccine, may be coadministered with DTP vaccine, but requires an additional large storage capacity as well as time/training to administer. Other vaccines, such as those against HPV or influenza are administered at a different schedule than DPT, so they require additional storage capacity and an additional visit by the recipient and time by the provider. All of these have an impact on costs.

Program considerations also need to account for additional training that may be required for vaccine administration. The simplest are new vaccines that are combination products, which require little to no marginal training. Even so, there may be costs associated with training if additional steps for storage and mixing are required. Finally, the impact on other vaccines need to be carefully considered.[6]

## 6 ECONOMIC CONSIDERATIONS

Economics is the study of the allocation of resources, which by definition are always in finite supply. While the chapter is concerned with introduction of vaccines into low-resourced populations, similar issues apply to wealthier economies with finite budgets for vaccines and personnel. While an appropriate accounting of the cost of potential vaccine programs to understand short-, medium-, and long-term financial commitments, that is only one component. One must also account for the benefits accruing over time through the value of averting the morbidity and mortality from vaccine use, including the "positive externality" benefits to those unvaccinated due to community protection from lowered rates of microbial transmission as well as other benefits such as the potential lowering of antibiotic use and therefore reducing antimicrobial resistance. Frequently, these costs and benefits are expressed as a net cost (benefit) or a benefit to cost ratio. These calculations, frequently form a critical basis for decisions to publicly support vaccine programs. The different types of economic studies are shown in Table 24.2.

**TABLE 24.2 Types of Economic Analysis to Evaluate Vaccine Interventions**

| | |
|---|---|
| Cost analysis: the cost of the program versus inaction; financial commitment (may or may not include savings from prevention depending on perspective, who pays and who benefits | $(C_i - C_0) = \Delta$cost |
| Cost effectiveness: the cost of the program minus the savings divided by the change in health outcome from the intervention | Δcost /Δhealth outcome |
| Cost utility: the same as effectiveness, however, the denominator allows for comparisons across a common health metric, for example, quality adjusted life years | Δcost/Δcommon health metric |
| Cost benefit: a special type of cost utility where the outcome is expressed as a common currency unit as the costs. This requires an ethical consideration as to an economic value to a year of life. | *C–B* or *B*:*C* ratio |

A cost analysis is an accounting of the financial expenditures that must be considered for the introduction of a new vaccine into a program. The types of costs that may be included are listed later. Key to this analysis is perspective—who pays and who benefits. Generally, a societal perspective should account for both public costs and public savings, though government insurance schemes differ widely even in developing countries. Additionally, financial commitments may be required today to institute a program, but savings from disease prevention may accrue at a later date, sometimes decades in the future. Perspective and time accounting for financing and returns on investments (discounting) should be specified in any cost analysis. All economic analyses use a cost analysis and derive from a basic understanding of the accounting of the vaccine program. Cost effectiveness-, cost utility-, and cost benefit-analysis all use cost in the numerator and differ in what outcomes are used in the denominator. Cost effectiveness measures the change in a specific health outcome for a specific strategy; cost utility, a common health metric for comparisons across the health sector and cost benefit, a unit of currency for comparisons across different sectors of financing.

## 7 FINANCING

### 7.1 Vaccine Program Accounting

A vaccine program is made up of various components, each with an intrinsic cost. These include the vaccine itself, delivery device, operational/logistical infrastructure for delivery to clinics/administration sites, including the appropriate temperature storage at various levels, and administrative fees. In addition, as a vaccine is a preventive strategy administered to healthy individuals, communication/advocacy strategies have costs and are critical to promote its acceptance

and usage by the caregiver and individual recipient. Finally, there may be associated adverse events that may occur due to the vaccine use, which must still be determined with appropriate postmarketing surveillance and accounting in addition to the associated education costs to address the perceived risks and benefits of a particular vaccine.

All of these costs may be additive to indicate the possible financial commitments that may be wholly paid for through public sector funds or copaid by the recipient or additional supportive financial institutions such as the Global Alliance for Vaccines and Immunization (GAVI).

### 7.1.1 Costs

In general, the costs of a vaccine program can be attributed to the cost of the purchase of the vaccine and its administration including the transportation/appropriate storage to the site of delivery and the act of actual vaccination to the recipient. Additional costs related to the administration costs including the training and advocacy required to provide the vaccine and achieve demand/uptake as well as any other actions required to document and investigate adverse events and impact of the vaccine program. Historically, an average cost of $15 was ascribed as the cost to fully administer the full schedule of the six EPI vaccines with $1.50 attributed to the vaccine and $13.50 to administration in developing countries. While the cost of the vaccines may have been uniformly purchased through UNICEF or the Pan American Health Organization collective vaccine fund, there was likely to be great variation of administration costs across populations based on many characteristics, such as population density, level of development of health systems, road access, and coverage levels. For many developing countries, vaccines were purchased through UNICEF with administration costs for vaccination being absorbed either by the government, nongovernmental organizations, or multilateral organizations. Better accounting practices and the monitoring of impact in national programs has increased as more expensive externally purchased vaccines require more accountability.

### 7.1.2 Benefits-Potential Savings

The benefits of vaccine programs may also be captured through an accounting of the current expenditures, and therefore potential savings, from the prevention of morbidity and mortality of the diseases in question. These costs may be estimated for different levels of severity of the vaccine preventable disease. Two types—direct and indirect costs—can be quantified. Intangible costs such as deaths can be listed but cannot be readily calculated. The difference of these costs with and without the vaccine intervention, represents the potential savings.

Direct costs are the medical, therapeutic, and personal expenditures related to an illness. While many vaccine preventable diseases such as respiratory and diarrheal illness may be of short duration, some may have chronic consequences such as hepatitis, cirrhosis, lameness, or not routinely measured sequelae such

as cognitive deficits. Direct costs may also include expenses related to health care seeking such as transportation, which could be substantial in developing countries. Indirect costs are associated with the loss of time and wages from daily labor for either the person afflicted or his or her caregiver. These could be substantial for those diseases requiring chronic care.

In a groundbreaking study of the cost-effectiveness of a wide variety of medical interventions in developing countries by the World Bank, the EPI vaccines were identified as "best bargains," producing enormous savings relative the investment and relative to many other health interventions.[7]

## 8 MODELING THE IMPACT-EFFICIENCY OR OPPORTUNITY COSTS

Models help to articulate the impact of vaccines within a given population and can be formulated based on the local economic and epidemiologic situation on the national or subnational level. There are various complexities of models that can be formulated to help evaluate the impact of a vaccine and multiple formulations can aid the decision-making process. In general, models should incorporate several features that have been outlined earlier, the cost of the program, the epidemiologic impact expressed with various health metrics or a utility that allow a comparison to other expenditures (Table 24.3).

An appropriate accounting of all the factors listed earlier, direct, indirect medical costs and caregiver lost time, as well as a valuation of vaccine, administration, adverse events, advocacy—communication of benefits, public and private programmatic costs—should be included. The cost should indicate the potential required financial commitment that may be assumed by the national government or through external donor support. Accounting of benefits should be made to the individual, community, and national level. Ideally, models can also help to articulate the potential positive externalities of a vaccine program such as the protection of the unvaccinated population, reduction of antibiotics,

**TABLE 24.3** Simple Table That can be Used as Basis for Modeling and Comparing Vaccines for Possible Incorporation Into National Schedules

| | Human papilloma virus | Acellular pertussis | Streptococcal pneumoniae |
|---|---|---|---|
| Disease burden | | | |
| Vaccine program costs | | | |
| Prevented disease | | | |
| Treatment savings | | | |
| Cost effectiveness | | | |

Comparisons can be made relative to what is currently in use.

or macroeconomic impact on tourism and agriculture through the demonstration of a nation free of a disease.

Additionally, models help to look at various strategies for targeting vaccine interventions at various subpopulations, and can account for uncertainty in the data and assumptions.

## 9 GLOBAL EFFORTS TO SUPPORT VACCINES INTRODUCTION

The WHO along with numerous partners including GAVI and NGOs have added support to countries to allow them to evaluate the impact of vaccines for cross-national comparisons of experience. Forum such as international meetings and support for National Immunization Technical Advisory Groups have allowed the discussion of disease burden incidence and evaluation of the potential utility of vaccine interventions. Demonstration projects in a number of developing countries provide various levels of evidence of impact. Surveillance and the dissemination of disease-burden incidence, and cost-effectiveness estimates help facilitate national vaccine introductions. Finally, collective action on supporting the regulatory environment and public–private partnerships to ensure vaccine supply and funding are critical to meet the potential public health demand for new vaccines. GAVI has facilitated discussions of these issues and has backed it up with guaranteed purchase funds to greatly hasten the adoption of new vaccines, especially in resource-poor environments.

In summary, vaccination programs with the original EPI vaccines represent one of the most cost-effectively known interventions in financial and human terms in preventing death and disease. National programs can provide existing infrastructures to which newer vaccines may be added at a nominal marginal cost, especially if incorporated as coadministered or combined products. Yet introduction of new vaccines presents multiple levels of challenges to governments and the international community in terms of cost, distribution, equity, and trade-offs that require a carefully multilevel analysis to fully justify.

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Chapter 25

# Transitioning Immunization Into the Health Care System: Strengthening Routine Immunization in India

**Ramanan Laxminarayan, PhD, MPH**
*Public Health Foundation of India, New Delhi, Delhi, India; Center for Disease Dynamics, Economics & Policy, Washington, DC, United States; Princeton University, NJ, United States*

## Chapter Outline



## 1 INTRODUCTION

India has the largest birth cohort in the world (27 million children) but lags other countries of similar gross national income per capita on immunization coverage (Exhibit 25.1).[1] A third of the world's roughly 27 million unimmunized children live in India. The proportion of children under 2 years of age who are fully immunized has increased by 1% a year and is estimated at 64% nationally, based on the latest Annual Health Survey.[2] Coverage varies significantly, from 45% in Uttar Pradesh, a poorly performing large state, to more than 85% in Kerala and Telangana. Although rural India has traditionally lagged urban India in vaccination, recent evidence suggests that for most states, the rural–urban coverage gap is closing, possibly because of the National Rural Health Mission.[3]

The past 3 years have seen significant change in the routine immunization program along three dimensions—system strengthening, coverage improvement, and introduction of new vaccines. Here we report on these

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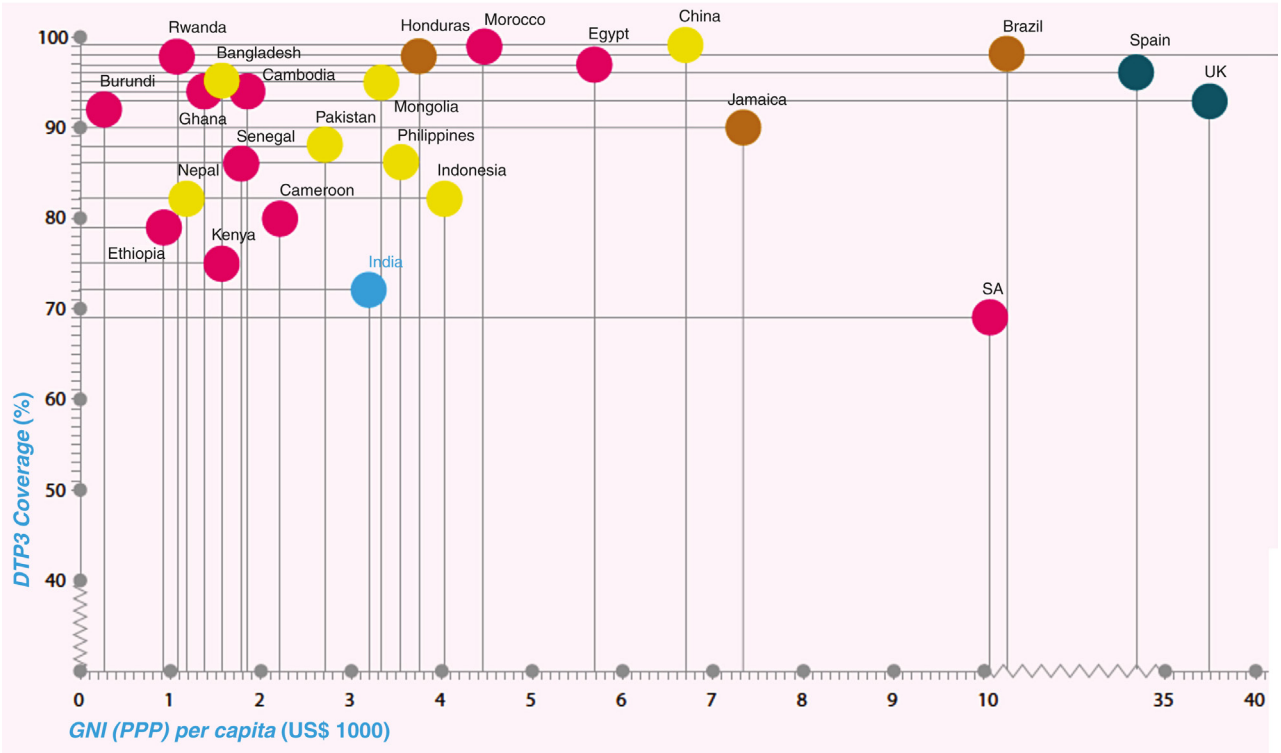


**EXHIBIT 25.1** DTP3 coverage in India and other countries, by gross national income (PPP adjusted). (Source: WHO/UNICEF Data.)

improvements and the lessons learnt by the Immunization Technical Support Unit (ITSU), a public–private partnership established to assist the Universal Immunization Programme (UIP).

## 2 BACKGROUND: INDIA'S UNIVERSAL IMMUNIZATION PROGRAMME

The Expanded Programme on Immunization was launched in India in Jan. 1978 to reduce mortality and morbidity from vaccine-preventable diseases. Gradually, over the next few years, newer vaccines such as the tetanus toxoid vaccine for pregnant mothers, the polio vaccine, and the measles vaccine were added.[4] As a signatory to the UNICEF declaration on the occasion of the United Nation's 40th anniversary, India launched the UIP in Oct. 1985. The UIP's goal was to extend immunization services to cover 85% of all children and 100% of pregnant women by 1990. Yet nearly two decades later, full immunization in India has reached only 64%.[5] In contrast, Bangladesh and Nepal have achieved coverage rates of routine immunization of 80% or more.

The UIP has suffered from several challenges over the years. Foremost was a severe human resource deficit, both in quantity and quality. The immunization cells at both national and state levels were poorly staffed and lack adequately trained personnel.[6] The program also needed strengthening and reevaluation in tracking coverage, monitoring and evaluation, estimating vaccine demand, vaccine logistics, and preventing and handling adverse events following immunization.

However, the program has had important successes, notably the elimination of polio in 2012 and maternal and neonatal tetanus in 2014. The Pulse Polio program, which was coordinated by the World Health Organization's National Polio Surveillance Programme, was funded entirely by the Ministry of Health and Family Welfare (MoHFW) of the Government of India, and in 1990–2010, the budget allocated to polio was nearly twice what was spent on routine immunization. The success against polio has made it possible to raise political awareness about immunization and confidence in the health system, both at the center and in the states. Although many had predicted that India would be the last country to eliminate polio, the Indian experience is now an exemplar of how to run and maintain a high-quality immunization and surveillance effort. However, the campaign strategy to tackle polio came at the cost of building ongoing systems to deliver routine vaccines and is poorly suited to an effort in which front-line health workers must track immunizations and deliver them every week without high-intensity demand generation and community mobilization.

The list of antigens covered by the UIP has remained largely unchanged since it was introduced in 1985, including BCG, inactivated polio vaccine (IPV)/oral polio vaccine (OPV), DPT, MMR, and rotavirus. The only two additions since 1985 were antigens to protect against hepatitis B and *Haemophilus influenzae* type b (Hib), introduced as part of the pentavalent vaccine (which

also covers diphtheria, pertussis, and tetanus) in 2011 and now being scaled up nationwide in a phased manner.

Cold chain capacity has been a challenge as well. The basic infrastructure for the procurement, supply, and delivery of vaccines in the UIP system has been largely unchanged in 25 years. Cold chain infrastructure and logistics management capacity are limited in many states, even for routine UIP vaccines. Despite systematic efforts to identify gaps and address vaccine logistics management,[7] problems persist. Vaccine logistics are a particular challenge in a large program like the UIP. There are no data systems with information on the quantity of vaccines kept in the 27,000 cold chain points across the country or the temperature at which vaccines are stored. Freezing is a persistent problem. A recent study reported that at state and regional vaccine stores, 11% and 26% of the test boxes were exposed to subzero temperatures, respectively.[8] The percentages were greater for peripheral stores and during transportation, indicating that maintaining vaccine temperatures remains a challenge. Freezing of a vaccine can lead to loss of potency[9] and cause lower immunogenicity and greater likelihood of local reactions.[10]

As part of the effort to address these challenges, ITSU was launched in 2012, designated the "Year of Strengthening Routine Immunization" by the Government of India. ITSU began as a partnership involving MoHFW, the Public Health Foundation of India, and the Bill & Melinda Gates Foundation. Its objectives were to strengthen UIP efforts to improve routine immunization coverage by providing support and technical assistance in the following areas: human resources, monitoring and evaluation and data support, cold chain and vaccine logistics management, bringing evidence to inform policy, and strategic planning and coordination and strategic communications. In 2013, ITSU was designated as an arm of the MoHFW[11] and tasked with providing the technical and management expertise required to design, create, implement, and institutionalize a stronger immunization program. ITSU also serves as an in-house think tank and strategic planning unit at MoHFW to innovate, demonstrate, and document best practices to the states for further scale-up and oversee the full execution of program improvement measures, using program management best practices rooted in a sustainability strategy. The 60-plus staff at ITSU have augmented the limited technical capacity at the UIP.

## 3 IMPROVING COVERAGE

Full immunization coverage in India has stagnated at roughly 64% since the 1980s, which nevertheless represents the formidable achievement of about 17 million children receiving some vaccines. According to WHO/UNICEF (Exhibit 25.2), the proportion of newborns who were "left out" of the UIP, as indicated by coverage with the BCG vaccine (against childhood tuberculosis), was only 9%. However, the drop-out rate is 27%, which means that roughly

**EXHIBIT 25.2 WHO/UNICEF estimates of national immunization coverage, by antigen, 2014.**

| Antigen | 2013 (%) | 2014 (%) |
|---|---|---|
| BCG | 91 | 91 |
| DPT-1 | 90 | 90 |
| DPT-3 | 83 | 83 |
| Polio-3 | 82 | 82 |
| Hep B-3 | 70 | 70 |
| Hib3 | 88* | 88 |
| Measles—(MCV-1) | 83 | 83 |
| Measles—(MCV-2) | 51 | 51 |

*Hib3 coverage in 23% of national target population in 10 states where vaccines introduced up to 2013.
**Source:** Joint Reporting Form of WHO/UNICEF estimates of national immunization coverage, 2014.

7.29 million children who received a BCG vaccine do not complete the full immunization schedule.

Mission Indradhanush (MI) was developed by ITSU and MoHFW, with the assistance of partner agencies, as a strategy for rapidly increasing immunization coverage. The name Indradhanush (which means "rainbow" in Hindi) was meant to convey the idea that UIP protected against seven diseases. It was formally announced by the Government of India in Dec. 2014 and began operations in Apr. 2015. Translating India's success with polio into a strengthened routine immunization program has necessitated a strategy that is partly campaign-based but has the long-range objective of transitioning to a routine program. MI identified 201 high-focus districts across the country (details of full immunization coverage in these districts are provided in Exhibit 25.3). MI Phase 1 districts were identified based on a composite indicator, which incorporated full immunization coverage, and the number of partially vaccinated and unvaccinated children. It is estimated that nearly half of India's unvaccinated or partially vaccinated children in India live in these 201 districts. MI runs for 1 week every month in a 4-month cycle. For that week, auxiliary nurse midwives are redeployed from their home locations to areas with large unimmunized populations, including migrant workers and socioeconomically disadvantaged groups.

A major component of the MI program has been a shift in emphasis to "fully immunize every child." Previous programs promoted immunization, and large numbers of India's children did receive vaccines but not the complete set. The MI program makes available resources to states for focused communication campaigns to drive home the message that every child should be fully immunized, and it leverages learning from the polio experience in community mobilization.

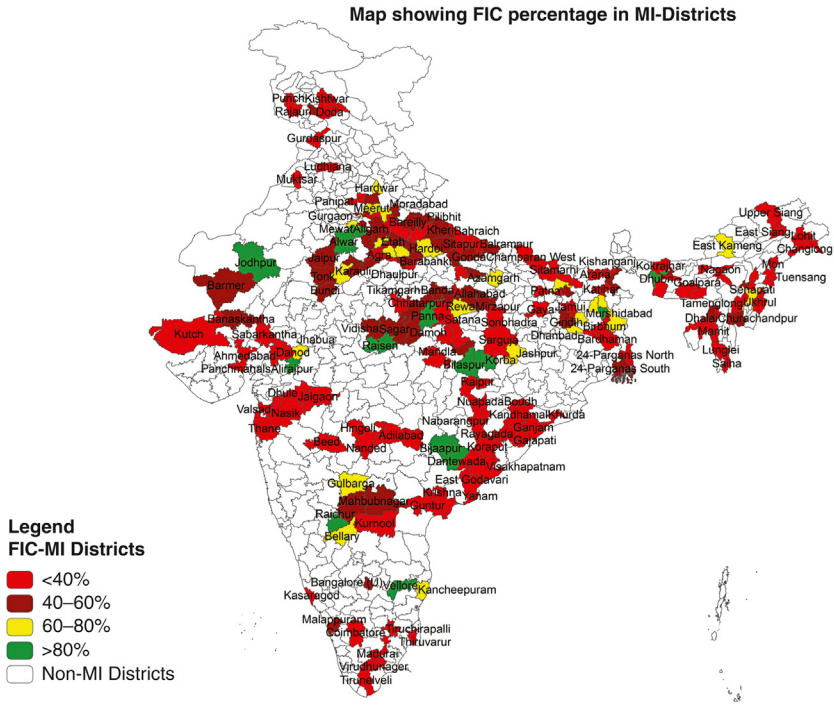


**EXHIBIT 25.3** Percentage of children under 24 months fully immunized in MI, Phase 1. (Source: Program data from Mission Indradhanush, ITSU.)

A second distinguishing feature of MI is its emphasis on strengthening the system and creating a platform for delivering other interventions for reproductive, maternal, and child health.

MI has also worked to strengthen the commitment of the 207,578 auxiliary nurse midwives who deliver the routine immunization program and the 907,918 accredited social health workers[12] who as community mobilizers encourage mothers to bring their children to vaccination sessions. The communications program, which included a letter from the Minister of Health and Family Welfare to each and every front-line health worker across the country, emphasized their importance to the program and reiterated that a monetary bonus of roughly $2–3 was available to them for every fully immunized child.

Exhibit 25.4 shows the number of session sites and children immunized under MI Phase 1, which concluded in Jul. 2015. These estimates are based on regular reports provided by district and state immunization officers to ITSU and are cross-validated with other data sources. A total of two million children were fully immunized, representing roughly a quarter of the children who were unimmunized or partially immunized. A total of 7.6 million vaccines were delivered during the 4 months of Phase 1. The routine program would likely

**EXHIBIT 25.4** MI, Phase 1 results.

| | Round 1 (Apr. 2015) | Round 2 (May 2015) | Round 3 (Jun. 2015) | Round 4 (Jul. 2015) | Total (In millions) |
|---|---|---|---|---|---|
| Total no. of sessions held | 0.21 | 0.23 | 0.26 | 0.25 | 0.95 |
| Total no. of antigen administered | 5.26 | 4.76 | 4.58 | 4.43 | 19.03 |
| Total no. of pregnant women immunized | 0.54 | 0.56 | 0.5 | 0.49 | 2.09 |
| Total no. of pregnant women completely immunized | 0.24 | 0.32 | 0.28 | 0.27 | 1.11 |
| Total no. of children immunized | 2.1 | 1.87 | 1.83 | 1.8 | 7.6 |
| Total no. of children fully immunized | 0.48 | 0.48 | 0.51 | 0.51 | 1.98 |
| Total no. of vitamin A doses administered | 0.27 | 0.44 | 0.71 | 0.6 | 2.02 |
| Total no. of ORS packets distributed | 0.13 | 0.5 | 0.57 | 0.47 | 1.67 |
| Total no. of zinc tablets distributed | 0.28 | 1.64 | 1.92 | 1.81 | 5.65 |

**Note:** ORS, oral rehydration solution.
**Source:** Program data from Mission Indradhanush, ITSU.

have captured some of the children who were immunized under MI, but data from Uttar Pradesh indicate that 80% of children at the MI session sites were receiving vaccines for the first time, indicating that the overlap between MI populations and those covered under functioning subcenters was minimal. Even under a conservative estimate—that half of the children fully immunized under MI would have been covered by the routine program—the gains under MI represent an increase in full immunization coverage of 4.5% points, the largest increase ever recorded in India, and the largest increase in the number of fully immunized children in a short time in any country. For comparison, the incremental one million fully immunized children during 2015 represent more than the birth cohorts of most countries in the world and about a quarter of the birth cohort of a large country like the United States.

MI contact opportunities were used to immunize pregnant women (1.1 million fully immunized against tetanus), deliver vitamin A (2 million doses delivered), and distribute oral rehydration salts and zinc tablets (1.67 million packets and 5.65 million tablets, respectively).

## 4 NEW VACCINE INTRODUCTION

As described earlier, until the introduction of the pentavalent vaccine in 2011, the lineup of antigens delivered under the UIP had remained largely unchanged. However, the burden of disease avertable through new childhood vaccines against pneumococci and rotavirus and new adult vaccines against the human papillomavirus (HPV) was growing. A major barrier to the UIP's adoption of new vaccines was both the confidence in the system to reliably deliver vaccines (addressed in part by the success against polio) and by the program's capacity to evaluate, adopt, and fund new vaccines.

On Jun. 25, 2013, the Government of India reconstituted the National Technical Advisory Group on Immunization (NTAGI), which was established in 2002 as the central government's primary technical advisory group on vaccines.[13] The NTAGI secretariat was moved to ITSU, and a subset of NTAGI was designated as a standing technical subcommittee that would deal exclusively with scientific questions; it was composed of experts from epidemiology, pediatrics, health economics, and other fields deemed necessary to evaluate new vaccines.[14]

In 2014, after deliberations by the technical subcommittee, NTAGI approved the introduction of vaccines against rotavirus in the UIP. It also approved the replacement of the measles vaccine with a measles–rubella vaccine to help eliminate rubella from India. NTAGI also approved the scale-up of the vaccine against Japanese encephalitis to adults in areas of high endemicity, a recommendation that would open the door for the UIP to address vaccination for adults. Finally, NTAGI approved the introduction of the IPV in the UIP. The decision supports the country's commitment to implementing the global polio endgame strategy, which involves a switch from trivalent OPV to bivalent OPV and the eventual risk-free phaseout of OPV. In India, IPV will be administered at OPV3 contact to boost immunity of children against poliovirus during and after the planned global withdrawal of OPV and the switch from trivalent to bivalent OPV. The announcements about new UIP vaccines were issued in Jun. 2014 and were the first on health by the Narendra Modi government.

The IPV is being introduced nationwide in Nov. 2015. Procurement of the rotavirus vaccine is underway and is expected to commence in two states with a progressive rollout, as recommended by NTAGI. Introduction of the measles–rubella vaccine is on hold pending a request to the GAVI Alliance for funding. In Sep. 2015, NTAGI approved the introduction of the pneumococcal conjugate vaccine in the UIP and recommended the technical subcommittee to consider making the HPV vaccine part of the UIP.

## 5 HEALTH SYSTEM STRENGTHENING

The lack of sustained investment in system strengthening has been a challenge for India. The availability of robust data systems to ensure accountability remains the single largest challenge in improving the UIP. ITSU has

sought to improve the quality of immunization data reported through the Health Management Information System and provides monthly feedback to all states reporting into this system. Although the quality of data is improving, much remains to be done.

There have been systematic efforts to improve the Mother and Child Tracking System, a web-based application for improving delivery of health care services to pregnant women and children up to 5 years of age through name-based tracking of each beneficiary and monitoring service delivery. The national system was developed by the National Informatics Centre team that developed Gujarat's e-Mamta software in Dec. 2009. Started in late 2009, the system became fully operational in Apr. 2010 and has become more widely used by states and union territories. Its initial aim was to track (by name and a unique ID) the antenatal, postnatal, and immunization services administered or due to be given to mothers and children identified and registered with the health system, but data quality assessment indicators from a recent study in Rajasthan and Uttar Pradesh show that the system is not fully functional.[15]

The Integrated Child Health and Immunization Survey (INCHIS) includes four components: (1) monitoring and evaluation of targeted immunization campaigns in high-focus districts, (2) cross-sectional surveys of child health, (3) longitudinal cohort studies, and (4) serology surveys. The first round of INCHIS was implemented in Mar. 2015 as a baseline survey of immunization coverage in selected states prior to the launch of MI. INCHIS used a multistage stratified cluster sampling design to survey 593 clusters in 80 districts across 12 states to ensure representativeness at the national level and also at the state level (for selected states). Early results from INCHIS are now available and are being published elsewhere. The follow-up study was conducted in Sep. 2015.

The introduction of vaccine vial monitors has made it easier to monitor high storage temperatures for vaccines, but freezing remains a major concern. Temperature monitoring at a cold chain point depends only on the staff at that place, who are expected to record the temperature of the equipment twice a day, but this practice is not uniform everywhere, and the reliability of the data is questionable. Some states, with the assistance of development partners, have used temperature sensors with data loggers at certain cold-chain points. The recorded data can be used for retrospective monitoring at the central level. Other efforts involve web-based real-time temperature monitoring at some state stores but require infrastructure for net connectivity.

An automated mobile network-based device, which sends real-time temperature text messages to designated program managers and generates temperature graphs and indicators for analysis, has been introduced in both state vaccine stores and at roughly 150 cold chain points. The data are also linked to an online program with GIS coordinates for all cold chain points. The program helps program managers at all levels to monitor storage conditions and plan interventions.

In 2013, India was the recipient of a $107 million GAVI Health System Strengthening grant that has been used to upgrade cold chain infrastructure, improve preparation of microplanning to improve targeted coverage improvements, strengthen data and accountability systems, and establish a research network on vaccines and immunizations.

## 6 CONCLUSIONS

Although rates of routine immunization coverage and pace of adoption of new antigens in India has lagged other countries of similar economic capacity, India is rapidly catching up. However, challenges remain.

Funding for the UIP has not kept pace with increased programmatic needs, and transitioning the pentavalent and IPV programs from external funding (from GAVI and other partners) to the Indian government will require more resources. Moreover, India is set to graduate from GAVI in 2015 as its gross national income per capita exceeds the eligibility threshold, although it will continue to receive threshold support until 2019.

GAVI investments in India have had and will continue to have high payback. The current pentavalent vaccine stock financed by GAVI is estimated to avert over 700,000 deaths, or more than 15% of GAVI's 2016–20 impact goal of averting 5–6 million deaths. Delivery of the rotavirus and PCV in India could achieve roughly 450,000 deaths averted before 2020, or about 10% of GAVI's 2016–20 goal. The measles–rubella vaccine and health system strengthening beyond the current phase could also have high returns per dollar invested. It is expected that UIP budgets will have to increase by multiples of the current allocations to fully meet the immunization needs of India's children and adults.

Challenges to improving coverage also lie on the demand side. Poor education levels, which are consistently correlated with incomplete vaccination status, pose a major barrier to expansion of coverage in rural areas.[16] Adverse events following immunization, even when shown to be unrelated to a vaccine, have been widely reported in the media and are responsible for hostility to vaccination in certain communities. Adverse drug reaction centers were set up by the Drug Controller General of India and the Indian Council for Medical Research in the 1980s but were subsequently discontinued. Better communication about the benefits and potential side-effects of vaccines—for example, the National Pharmacovigilance Program of India provides web-based reports on adverse reactions—could substantially boost confidence in vaccines and the immunization program.

India stands at a threshold of remarkable increase in vaccination coverage, antigen adoption, and delivery system capabilities. These changes are likely to greatly benefit health status in India and lower the burden of disease at a global scale. Recent improvements offer hope but must be sustained to achieve the goal of minimizing the burden of vaccine-preventable disease.

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Chapter 26

# Vaccine Acceptance

## Barriers, Perceived Risks, Benefits, and Irrational Beliefs

**Eve Dubé*, Noni E. MacDonald****

**Institut national de santé publique du Québec and Centre de recherche du CHU de Québec – Université Laval, Québec (QC), Canada; **Department of Paediatrics, Dalhousie University and Canadian Center for Vaccinology, Halifax (NB), Canada*

### Chapter Outline



## 1 INTRODUCTION

Vaccination is recognized as one of the greatest public health achievements of the last century, likely saving more lives in the last 50 years than any other health intervention.[1] For continued success, however, high population vaccination coverage rates need to be attained and sustained. Immunization not only protects the individual, but also, in many instances, provides community protection against vaccine-preventable diseases through herd immunity.[2] Sadly, reported uptake rates are falling short of national and international targets.[3] An increasing number of parents are choosing to delay and/or refuse some or all vaccines leading to faltering community protection.[4–7] Clusters of unvaccinated individuals have provided fertile ground for recent major outbreaks of vaccine preventable diseases such as measles,[8–10] mumps,[11] rubella,[12] poliomyelitis,[13] and pertussis.[14]

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For more than a decade, public health experts have been concerned about the growing resistance to immunization.[15–18] Widespread acceptance of vaccines can no longer be taken for granted.[19,20] To address this problem of vaccine hesitancy and refusal, a better understanding of the underlying dynamics is fundamental.[20,21] In 2014, the World Health Organization (WHO) underlined the urgent need for effective interventions to address vaccine hesitancy and increase vaccine acceptance.[22]

The chapter examines the complex interplay among factors that influence vaccine acceptance such as knowledge, risk perception, past experiences, and personal context, as well as the impact of broader sociocultural, historical, and political landscapes that "gives shape to ideas and ideals" about health, prevention and what a good citizen does about vaccination.[23] Of course, access to vaccines and vaccination services—the "supply side"—is a crucial determinant affecting vaccine uptake rates,[24] but the focus here is on the "demand side" of vaccination. Vaccination hesitancy concepts are described, followed by the broader factors influencing vaccine hesitancy and the examination of the drivers of vaccine acceptance, with a special focus on risk perception and risk communication.

## 2 FROM VACCINE HESITANCY TO VACCINE DEMAND: MAKING SENSE OF TERMINOLOGY AND CONCEPTS

"Vaccine acceptance," "vaccine confidence," "trust in vaccines," "vaccine hesitancy," "anti-vaccinationism," "vaccine demand"; a plethora of terms and concepts are used—sometimes interchangeably—to describe both individuals decision making about vaccination as well as broader societal support of vaccination programs.[25] A common understanding is important as ambiguities make it difficult to describe, compare, and monitor the different factors implicated in vaccination decisions as well "hampering both research and intervention" work.[26]

The dichotomous perspective on vaccination attitudes and behaviors is no longer tenable. Instead, a spectrum of vaccine beliefs and associated behaviors between complete refusal of all vaccines and full vaccine acceptance must be recognized.[22,25] People can occupy different (or many) places along this continuum of attitudes and behaviors and this may vary by time context, place and vaccine (Fig. 26.1).[22]

Vaccine hesitancy is a concept now frequently used in discussions of vaccine acceptance.[27] The WHO Strategic Advisory Group of Experts (SAGE) Working Group on Vaccine Hesitancy defined vaccine hesitancy as "delay in acceptance or refusal of vaccines despite availability of vaccine services."[28] According to this group, the scope of vaccine hesitancy includes instances where "vaccine acceptance in a specific setting is lower than would be expected, given the availability of vaccination services."[28] A vaccine-hesitant person can delay, be reluctant (but still accept), or refuse one, some or all vaccines.[29] Even

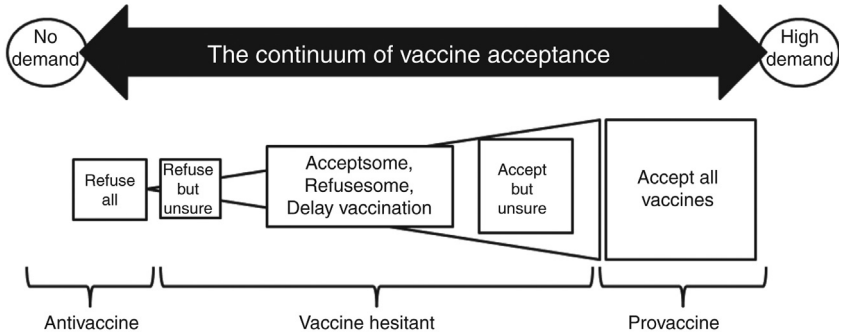


**FIGURE 26.1** **The continuum of vaccine acceptance**.

people who accept vaccination or have their child vaccinated can still have serious doubts and worries and be considered as vaccine-hesitant.[30,31] This latter group is an important target for vaccination promotion interventions in order to encourage resiliency as they are "at-risk" of delaying or refusing vaccination. They are more receptive to public health and health-care providers' messages than outright vaccine refusers.[32,33]

Vaccine hesitancy has been criticized as being an "ambiguous notion with an uncertain theoretical background."[26] Nevertheless, it is useful as the term is becoming increasingly used, in the literature and in practice, to encompass this heterogeneous group of individuals with diverse vaccine attitudes and acceptance behaviors. Application of this concept can also be challenging.[29]

The WHO SAGE Working Group on Vaccine Hesitancy recognized that more efforts are needed to improve the ability to describe, measure, and assess vaccine hesitancy at the country and regional levels.[34] Because research has mainly focused on the metrics of vaccine uptake (coverage rates, delays, refusals), the degrees to which vaccine hesitancy influences vaccination behaviors remains an important, but complex, domain for investigation.[27] Validated tools that can identify patterns of vaccine hesitancy in individuals, subgroups, and populations over time, differentiating outright refusers from the hesitant, are much needed.[21] However, because the factors influencing vaccine acceptance and hesitancy not only vary within and between populations and subgroups, but also according to context, time, and vaccine; diverse types of data and measurement approaches are needed to capture, quantify, and describe hesitancy.[19]

The concept of vaccine hesitancy has also been criticized as being negative, misleadingly implying that the number of people strongly opposed to vaccines is on the rise, which could then negatively impact the provaccination social norm.[26,35] The more commonly used positive alternative for vaccine hesitancy is vaccine confidence.[22,25] However, the WHO SAGE Working Group on Vaccine Hesitancy's review of the evidence highlighted that confidence is too narrow, as it is only one of the three major factor groups that can contribute to vaccine hesitancy (Table 26.1).

**TABLE 26.1 Three Key and Interrelated Factor Groups Influencing Vaccine Hesitancy and Acceptance**

| Factor group | Definitions |
|---|---|
| Confidence | Vaccine *confidence* is defined as trust in (1) the effectiveness and safety of vaccines, (2) the system that delivers them, including the reliability and competence of the health services and health professionals, and (3) the motivations of the policy makers who decide which vaccines are needed when and where. |
| Complacency | Vaccine *complacency* exists where perceived risks of vaccine-preventable diseases are low and vaccination is not deemed a necessary preventive action. Complacency about a particular vaccine or about vaccination in general is influenced by many factors including other life/health responsibilities that maybe seen to be more important at that point in time. |
| Convenience | Vaccine *convenience* is measured by the extent to which physical availability, affordability, and willingness-to-pay for, geographical accessibility, ability to understand (language and health literacy), and appeal of immunization services affects uptake. The quality of the service (real and/or perceived) and the degree to which vaccination services are delivered at a time and place and in the cultural context that are convenient and comfortable also affects the decision to be vaccinated. |

Adapted from Ref. [28].

A further term that needs to be differentiated is vaccine demand and its relationship to hesitancy. The second strategic objective of the Global Vaccine Action Plan (GVAP) states that "individuals and communities understand the value of vaccines and demand immunization as both their right and responsibility."[36] Nichter has differentiated *active demand for* vaccinations—adherence by an informed public—from *passive acceptance of* vaccinations—compliance by a public which yields to recommendations and social pressure and pointed out that "demand is often low, even among populations having impressive immunization rates."[37] Hence, demand is clearly a step further than just acceptance of vaccines by a population (Fig. 26.1).

## 3 DETERMINANTS OF VACCINE HESITANCY, DRIVERS OF VACCINE ACCEPTANCE

Vaccine hesitancy has a long history dating back to the very first smallpox vaccination program.[38] While the concerns and factors driving vaccine hesitancy may have evolved, many are similar to those in the past. The arguments used by the antivaccination activists in the 1800s are echoed in currently voiced concerns (eg, vaccines are ineffective or cause diseases; vaccines are used to make

**BOX 26.1 Trust and attitudes to vaccination: insights from a critical literature review**

Yaqub and coworkers have highlighted the complexity of factors impacting confidence/trust in vaccination based on a literature review of studies on public and health-care professionals' vaccination attitudes in Europe coupled with an analysis of market research data.[46] They concluded that:

1. The general population exhibits high levels of vaccine hesitancy.
2. Vaccine hesitancy is not due to being uninformed or misinformed, but reflected general distrust (of doctors, of government sources, of pharmaceutical companies).
3. Credibility of the institutions delivering vaccination information mattered more than the information itself.
4. The increasing rhetoric around patient-choice and empowerment has resulted in a negative perception of "those who trust (with blind faith) generalised advices from authority"—a "good parent" is a parent who critically appraised health services and products before making an health decision.[46]

profit; vaccines contain dangerous substances; harms caused by vaccines are hidden by the authorities; natural immunity is better than vaccine induced immunity, etc.).[39] A number of more current factors have also been specifically associated with increased vaccine hesitancy and decreased acceptance including: a declining trust in science and state institutions (Box 26.1)[29,40]; an increasingly consumerist orientation to health care[21,41]; a greater influence of social norms with more trust in experiential knowledge, a mother's "natural" instinct and other advice given within the parents' social network rather than from those with professional qualifications and expertise[42,43]; as well as the negative influence of controversies around vaccines in the media, especially the wider diffusion of vaccine-critical messages in the Internet and social media.[44,45]

Like many other health behaviors, vaccination decisions are complex and multidimensional. As illustrated in Fig. 26.2, vaccine acceptance is an individual behavior, but is also part of a "wider social world."[47] Different factors (past experiences with health services, family histories, feelings of control, conversations with friends, etc.) can influence the decision-making process.

Large-scale social forces, such as socioeconomic status, education, gender, or ethnicity, can also affect an individual's vaccination behaviors.[50] For instance, in some settings, the low status of women prevents them from accessing child vaccination services because they lack of decision-making power and/or have restrained mobility in public.[51,52] Education and socioeconomic status are also related to vaccine acceptance, but not as usually seen for other health issues where higher level of education and socioeconomic status are associated with better health conditions or better adherence to public health recommendations. Instead, increased vaccine hesitancy has been associated with both high and low education and high and low socioeconomic status, highlighting the complex


**FIGURE 26.2 Determinants of vaccine acceptance: an adaptation of the Socio-ecological model.** According to the socioecological model, the likelihood of vaccination behaviors is shaped by a complex interaction between factors operating at different levels: individual (ie, psychological, biological, and personal history factors that influence the likelihood of vaccinating); interpersonal (ie, person's closest social circle greatly influences their behavior); organizational (ie, the way that vaccine are deliver and the interaction with health-care providers influence promote or discourage vaccination); community (ie, broad sociocultural factors that help create a climate in which vaccination is either encouraged or inhibited); and public policy (ie, national, regional, and local agencies' support for policies that facilitate vaccine acceptance).[48,49]

array of interrelated factors at play.[53] Religion and religious beliefs have also often been linked with vaccine hesitancy and refusals.[54] The unwillingness to interfere with divine providence or religion-based concerns regarding vaccines' components can negatively affect vaccine acceptance.[55] Interestingly, a review of vaccine-preventable diseases outbreaks within religious communities concluded that, in multiple cases, "ostensibly religious reasons for vaccine refusals actually reflected concerns about vaccine safety or personal beliefs among a social network of people organized around a faith community, rather than theologically based objections per se."[54] Indeed, religious communities are a powerful social force that public health needs to learn how to effectively work

with to resolve objections and enable vaccination programs to continue and be supported by the community.[54] UNICEF has highlighted the importance of proactively working with religious leaders in building trust for vaccination among their followers.[56]

In postindustrial "risk societies," people are increasingly encouraged to stay continuously aware of risks and benefits in order to make their future more secure.[57] In high-income countries, notions of empowerment and individual choices are predominant current health themes. Health is framed as "lifestyle choices" and the state's interventions, as a violation of individual freedom.[29] Public health recommendations run counter to this consumerist and empowered vision of health. High- and middle-class parents, who have the "privilege of choice,"[58] want to decide about vaccines, based on their perceptions of their "children own mix of risk and vulnerabilities" rather than accept the generic "one-size-fits-all" country vaccination recommendations.[47,59–61] Protection of the community is not a concern of this "personalized" decision equation.[58] This reasoning presents a major challenge for public health especially in a context where risk-benefit ratios of vaccination are less apparent due to the very success of vaccination programs.[62,63]

Social norms are also powerful drivers of vaccine acceptance.[16,64–66] Vaccination is a social norm when parents accept vaccination of their children mainly because everybody in their social setting is also doing so or because they are prompted by the system to do it: vaccinating is the "normal" action.[42,64] Conversation with—and advice from—people in a parent's social network can also trigger the development of vaccine hesitancy.[67] In a study of parents of young children in Washington State, the percent of parents' network members recommending nonconformity (ie, delaying vaccination, partially vaccinating, or not vaccinating at all) was more predictive of parental vaccination decisions than any other variable including the parents' own perceptions of vaccination.[43]

The media also contribute to vaccine hesitancy. The negative influence of vaccine controversies communicated by the traditional media (eg, newspapers, magazines, television) on vaccine uptake has been well documented.[68–70] Two-sided news messages with claims both for and against vaccines can lead readers to erroneously infer the state of expert knowledge regarding vaccine safety and negatively impact vaccine intentions.[71] It may also reinforce negative social norms, by implying that there are no problems with vaccine refusal. Routine media coverage of celebrities declining vaccination or questioning the safety of vaccines has also been shown to have a detrimental effect on vaccine acceptance in the public.[72–74] Internet and social media content also can influence acceptance (see assimilation bias below).[31,75–77]

At the individual level, recent reviews have focused on factors associated with vaccination acceptance or refusal in high resource countries, identifying determinants such as: fear of side effects, lack of a provider recommendation to be vaccinated, perceptions around health, and prevention and a preference for "natural" health, low perception of the efficacy and usefulness of vaccines,

negative past experiences with vaccination services, and lack of awareness or knowledge about vaccination.[30,47,78–82] Fear of pain at immunization and fear of needles are also drivers of hesitancy or refusal. Studies in both high- and low-resource countries have shown that more than one-third of parents are concerned about pain at the time of childhood vaccination and most parents would be less anxious if vaccines were given in a non painful way.[78–80] In a Canadian survey, 24% of parents and 63% of children reported a fear of needles and these fears lead to vaccination noncompliance for 7 and 8%, respectively.[81] While some health-care professionals might think that pain is trivial, not a problem worth addressing, early research in Canada has shown that parents are more comfortable and more accepting of infant vaccination when pain is controlled.[82] Implementation of the evidence-based 2015 Canadian immunization pain mitigation guidelines,[83] and the WHO global recommendations derived from these,[84] could help decrease pain related hesitancy and improve vaccine acceptance.

## 4 VACCINATION DECISIONS AND RISK PERCEPTION

Risk perception is a well recognized determinant in vaccine decision making.[61,85–89] Based on the Health Belief Model,[90] two dimensions are usually emphasized: perceived likelihood of harm if no action is taken and perceived seriousness of the consequences if harm was to occur. These risks are viewed as being balanced against the perceived costs and benefits of an action to prevent this harm. Risk perceptions can influence vaccine decision making in two ways: perceived risks of vaccine-preventable diseases can foster vaccine acceptance and perceived risks of vaccines can contribute to vaccine refusal.[86,87,91,92] Sadly, this rational approach to risk perception and health decision making does not reflect reality as other factors come into play. For example, many adults, even in high-income countries such as the United States, have low numeracy and are unable to interpret mathematical concepts such as probabilities.[93] The way in which the information is presented also influences risk perception: frequency formats (eg, 1 out of 10 infants will have fever after the vaccination) *feels* more risky than the same information conveyed in probability terms (10% of infants will have fever after the vaccination),[94] as emotions play a role in how people interpret numerical information. As well, individuals are "cognitive misers," collecting only as much information as they think is needed to reach a decision.[95] Choices are much more linked with how people *feel* about the facts than to the facts themselves—even if they do correctly understand these facts.[96,97] Judgments about risks are intuitive, automatic, and often unconscious. Risks to children *feel* more serious than risks to adults because children are vulnerable; risks from the vaccine *feel* more real because they seem more proximate than the actual disease being prevented.[98]

Cognitive biases, or heuristics, are mental shortcuts that allow people to solve problems and make judgments quickly and efficiently, also impact decision making about vaccines (See Table 26.2 for examples). These rule-of-thumb

**TABLE 26.2 Heuristics and Vaccination Decisions**

| Heuristics | Definition[a] | Quotes[b] |
|---|---|---|
| Omission Bias | Actions more harmful than inactions | I am under the impression that natural is better. I think it's better to develop immunity through diseases rather than induce artificial immunity with vaccines… When I have decided about vaccination, I really thought that if my child catches a disease, well… it will be better for him in the long term (31 years-old mother of partially vaccinated child). |
| Coincidence dragon | After this therefore because of this | She had a cold, and the cold was almost over and we went to get the vaccine then, wow, it started again and lasted a long time, and she had otitis and a runny nose all the time. So, was it that or not that, except that I've heard from others that after getting the vaccine, the same thing happened, and that's why I thought it could be because of that (35 years-old mother of a partially vaccinated child). |
| Availability bias | Judge an event as frequent or likely to occur if can easily imagine or recall it | I have read a lot of things about vaccines… about what vaccines are made of… on money that pharmaceutical companies make from vaccines… on the fact that all research are biased because they are financed by pharmaceutical companies and how government are brought into this because... they get some financial benefits from it... on long term effects from vaccines… Well at a certain point, you have to make a decision and you asked yourself: What do I do? I have realized that there were much more chances that my children get an adverse effect from vaccines than chances that they catch an infectious disease (33 years-old mother of an unvaccinated child). |
| Ambiguity bias | Known or common risks are more acceptable than unknown or unfamiliar risks | Chickenpox, nobody dies from chickenpox, it's a disease that kids catch and it gives them spots and it itches for a week, which is no fun, but it goes away and after that, the body is immunized for life. Now we know that someone who takes the vaccine, well, in fact, we do not how long the vaccine stays active, we just do not know what the long term effects of the vaccine are… (33 years-old mother of an unvaccinated child). |

[a]*These definitions were extracted from Ref. [100].*
[b]*Theses citations are from mothers who were interviewed in a qualitative longitudinal study done in Quebec in 2011–2012. This study was funded by the Canadian Institute of Health Research (MOP-115012). More information regarding the methodology and results can be found in Ref. [67].*

strategies shorten decision-making time and allow people to function without constantly stopping to think about their next course of action.[99] Heuristics are used by everyone (health professionals, the general public, pro- and antivaccine advocates) when faced with complex decision making and are helpful in many situations, but they can also lead to biases.[100]

As noted earlier, the Internet has likely increased vaccine hesitancy as it plays a role in increasing the availability bias.[31,75–77] Individuals who delayed or refused vaccines are significantly more likely to have looked for vaccine information on the Internet.[43,44,76,101] This is an important point as the Internet has become an essential health information source, especially for parents.[102–106] The Internet and social media give the small, but very vocal, minority of firm vaccine opponents a much wider audience for their fringe views.[60,107,108] Studies have shown that viewing antivaccination websites and reading personal stories about negative consequences of vaccination increases users' risk perceptions about vaccination.[109–111] For the vaccine-hesitant parents, this could lead to increased negativity toward vaccines. This exemplifies the "assimilation bias," that is when faced with varied and inconclusive information on a complex issue, people will interpret the information in a way that supports their initial position or beliefs.[112] Instead of stimulating people to question their initial position, it is in fact *reinforced*.[33,113] Sadly, reading provaccination stories has little to no effect on risk perceptions and vaccination intention.[110,111]

Finally, social sciences research has demonstrated that risk perception among lay people, contrary to experts, are grounded in past experiences (such as those with other vaccines or health services) as much as on numerical information.[61,85] Popular interpretation of risk is based on an "uncertainties and ambiguities" approach where doubts remain even in the face of empirical evidence.[47,61] Individuals perceived risk of vaccines in different and unique ways that reflect their cultural, emotional, social, and political worlds.[29,114,115] In the context of a globalizing mass media, the awareness of certain risks may have changed, but people continue to understand and negotiate risks in localized contexts.[116] Risk perception can become institutionalized and collectively reproduced. For example, in the first wave of the A(H1N1) pandemic influenza in 2009, Aboriginal populations in Canada experienced higher rates of infection and were prioritized to receive the A(H1N1) vaccine when it first became available. Research revealed that Metis participants' own collective colonial experiences, histories of racism, and social exclusion mediated their vaccine risk perceptions.[114,117] They believed "their lives were less valued in the eyes of the government, and rationalized that they were being prioritized in order to test the safety of the vaccine before it was more broadly distributed."[114] In polio campaigns in Nigeria, India, Pakistan, and Afghanistan, resistance to vaccination circled around a wide range of "tactical narratives," for example, fears that the OPV deliberately or inadvertently carries the risk HIV, concerns OPV contains fetal tissue or materials derived from pigs. However, these risk perceptions were also a way for economically and politically deprived communities

to express dissatisfaction with wider socioeconomic conditions and noncompliance to vaccination was an effective strategy for leveraging greater response from the state in return.[118]

## 5 COMMUNICATION ABOUT VACCINATION AND RISK PERCEPTION

### 5.1 Communication About Vaccination at the Program Level

The critical role that emotion plays in the interpretation of vaccine risks and benefits is being increasingly recognized in the public health community.[119] Evidence statements on statistics and probabilities are not nearly as powerful as emotive anecdotes. Recognition of these psychological dynamics has prompted calls for the use of emotionally evocative materials in interventions to enhance vaccine acceptance.[119] However, research on science communication indicates that this is not as simple as it may look.[35,94,120]

A summary of the findings from 15 published literature reviews or meta-analysis that have examined the effectiveness of different interventions to reduce vaccine hesitancy and/or to enhance vaccine acceptance has shown that simply communicating evidence of vaccine safety and efficacy to those who are vaccine-hesitant has done little to stem the growth of hesitancy-related beliefs and fears.[121] Sadly, most public health interventions to promote vaccination are designed with the assumption that vaccine hesitancy is due to lack or inadequate knowledge about vaccines ("knowledge-deficit" approach).[35,61] However, as discussed previously, parental decision making regarding vaccination is far more complex.[29,61,64] People are complicated, with different underlying values and priorities that can compete with public health recommendations.[117,122] Buried under an avalanche of (often contradictory) information, individuals use their value predispositions (cultural identity, core beliefs, experience, and interests) as "perceptual screens" to select the information whose outlooks match their own, that is, assimilation bias.[35] Research has shown that people are more drawn toward, and accepting of, information and its sources that share their worldview.[113,123] In contrast, individuals, when faced with information that contradicts their values, can feel threatened, react defensively and their initial beliefs may become even more strongly held. This back-fire effect is well-illustrated in a study in which use of four interventions to refute claims of a link between the measles, mumps, and rubella (MMR) vaccine and autism increased resistance among the very hesitant.[33] The study also showed that the interventions did reinforce the decision of the parents who were already intending to vaccinate, that is, promoted resiliency among provaccine parents.[33] A similar study done using two interventions to correct the false belief that it is possible to contract the "flu" from the influenza vaccine resulted in similar findings: messaging that too strongly advocates vaccination may be counterproductive for those who are already hesitant.[124,125]

Changing risk perception through communication means that messages need to be tailored and targeted to account for the realities of community-driven knowledge systems, and the unique information needs and preferences of particular communities.[126,127] Successful communication is "a two-way process, an equal measure of listening and telling. Understanding the perspectives of the people for whom immunization services are intended, and their engagement with the issue, is as important as the information that experts want to communicate."[128] As highlighted, knowledge is important but not sufficient to change people's risk perception toward vaccines. How people interpret the vaccination information they receive is complex and effective risk communication strategies need to capitalize on heuristics rather than try to fight against it. People process the *gist* of information (the subjective interpretation of information) more than its verbatim representation, highlighting the importance of communicating more than numerical information.[115] Successful public health interventions should be developed using a planning framework, such as the WHO *Guide to Tailoring Immunization Programmes* that provides tools to identify vaccine hesitant population subgroups, to diagnose the barriers and enablers for vaccination in these subgroups, and to design evidence-informed responses to vaccine hesitancy appropriate to the setting, context, and hesitant subgroup, including tailored communications.[129,130]

The question whether the public health community should respond to antivaccination activists is regularly raised.[131] Leask suggests that adversarial approaches against such activists can in fact refresh their battle and contribute to a false sense among the public that vaccination is a highly contested topic.[132] Most of the time, provaccine advocates should be "playing the issue, not the opponent."[132] Only when antivaccination activists' advice could lead to direct harm, should efforts be made to stop them. Future public health vaccine promotion efforts need embrace Internet and social media possibilities to proactively promote the importance and safety of vaccines rather than adopt a reactive approach to the endless antivaccination activists' arguments.[38,128,133]

Educating children and adolescents through school-based programs about the importance, safety, and benefits of vaccines and about the risks of vaccine preventable diseases is another potentially effective strategy. This could lead to an adult population with more provaccine attitudes and with more "immunity" to vaccine hesitancy in the face of antivaccination information.[121] Such child/youth education strategies have been successful in changing behavior around bullying, in stimulating environmental activism, and in improving earth science literacy.[134,135]

## 5.2 Communication About Vaccination at the Provider's Level

Health-care providers have a key role to play in risk communication about vaccination as their recommendations are a major driver of vaccine acceptance.[136–138] Risk communication about vaccines can be emotional for both

parents and health-care providers as ideological positions that may not be in sync.[139] Studies indicate that health-care providers should be well-informed to address parents' questions that arise, as ambiguous responses increase vaccine hesitancy.[140] Health-care providers should make clear recommendations to vaccinate, but should also be careful not to "oversell" vaccination, as this can also increase hesitancy.[141] Given the time required to discuss with vaccine-hesitant parents, providers need support for their risk communication and education on the most effective strategies to increase timely vaccination.[6] While many tools and tips have been presented in the literature,[142–147] few approaches have been evaluated for effectiveness. Although approaches vary, there are common characteristics, such as the importance of maintaining a trustworthy patient–provider relationship and of tailoring the communication to specific patients' concerns and doubts. Simply providing health-care workers with talking points may not be enough, however. A recent randomized trial to test a physician-targeted communication intervention resulted in no detectable effect in reducing maternal vaccine hesitancy or in increasing physicians confidence in communicating with vaccine-hesitant parents.[148,149] Whereas many of these communication frameworks suggest discussing vaccines in a participatory manner, research has shown that more firm, presumptive discussion styles are more effective in improving vaccine acceptance among the hesitant.[140]

Many studies have found that health-care providers' knowledge and attitudes about vaccines are an important determinant of their own vaccine uptake, their intention to recommend the vaccine to their patients, and the vaccine uptake of their patients.[150–153] However, some providers are themselves vaccine-hesitant, for example, despite strong recommendation for influenza vaccination in health-care settings, low uptake continues among health-care workers.[151,154] Hence, more research is needed to understand vaccine hesitancy among health-care providers and how best to address it. Health-care providers need more undergraduate and continuing education on vaccines given the key role they play in patient vaccine decision making.

## 6 CONCLUSIONS

Despite growing recognition of the complexity of vaccine decision making,[114,155,156] many studies have failed to examine the interplay between the different drivers of an individual's decision. It is not enough to identify correlates of vaccine acceptance or refusal, we need to understand *how* and *why* these factors link to different positions on the vaccine acceptance continuum.[53] In addition to the factors affecting vaccine acceptance at the individual level, a thoughtful understanding of vaccine acceptance needs to be grounded in the particular context in which vaccination occurs. Consideration should be given to broader influences on vaccine hesitancy such as the role of public health and vaccine policies, communication and media, and health-care providers.

Context is key and is ever changing. In recent years there has been an explosion in the number of new vaccines licensed and commercialized.[157] In the United States, publicly funded vaccines from birth to 18 years of age more than tripled between 1990 and 2012.[157] In low-resource countries, the Global Alliance for Vaccines Initiative (GAVI) has added many new vaccines; some into multivalent preparations, others standing alone. Oral polio vaccine will be replaced by intramuscular vaccine as polio comes under worldwide control. The increase in the number of diseases prevented is exhilarating, but this also means more injections, whether in a high-, middle-, or low-income setting. The consequent decline in vaccine-preventable illnesses also changes the risk perception: the focus shifts to the risks of the vaccines rather than the risks of the diseases.[158–160]

Understanding and addressing the specific concerns of those along the vaccine hesitancy spectrum is crucial in order to ensure and sustain the success of a country's vaccination programs. Vaccine acceptance and risk perception are complex. There is no one-size-fits-all approach to hesitancy and no strong evidence for a single best strategy. A better understanding of the root causes of vaccine hesitancy and refusals—including the broader sociocultural, political, and historical determinants outside the scope of vaccination programs—is essential to develop effective tailored strategies to fit each context.

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Chapter 27

# Trust and Confidence in Vaccines: Tales of Three Vaccines, Lessons for Others

**Heidi J. Larson, PhD*, Seth Mnookin****

**Department of Infectious Disease Epidemiology, London School of Hygiene & Tropical Medicine, London, United Kingdom; **Graduate Program in Science Writing and Comparative Media Studies/Writing Program, Massachusetts Institute of Technology, Cambridge, MA, United States*

## Chapter Outline



Public trust in vaccines and immunization programs is a dynamic and changing phenomenon. Seeming success in an immunization program can be disrupted by a confidence crisis more quickly than it can be rebuilt.[1] Constant care and vigilance are needed to detect and address waning trust and confidence before they become a crisis. Fixing a crisis once does not mean that another one may not erupt—sometimes driven by the same underlying trust issues, but other times due to new factors which also need to be understood.[2]

This chapter looks at a range of different vaccines and different settings where trust issues emerged for a variety of reasons, ranging from politics to socioeconomic marginalization to genuine safety concerns, and offers examples of how trust issues have been successfully addressed and overcome. It recognizes the importance of an ongoing process of building and sustaining trust to ensure the success of any immunization effort.

Antivaccine sentiment has been around for as long as there have been vaccines: A famous 1802 editorial cartoon from Great Britain titled "The Cow-Pock" showed a group of patients waiting to receive smallpox inoculations while surrounded by people with cows growing out of their bodies.[3]

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Then, as now, vaccine reluctance and refusal stemmed from a confluence of factors—some political and some cultural; some based on specific misinformation and some on anxiety about health and medicine more generally.[4] The flourishing of panic about the safety and efficacy of vaccines is almost always followed by confusion and misunderstandings about the proper political and public health responses—and the proper way to craft these responses.

In this chapter, we discuss the importance of understanding the specific cultural, political, religious, and social forces that lead people to reject facts in favor of feelings. While evidence-based medical science plays an important role in confidence building, it is often insufficient to change views that can be driven by emotions, cultural connections, or politics. The following case studies tell stories where a complicated mix of factors drove vaccine hesitancy and distrust, many well beyond the usual scope of the immunization program.

## 1 THREE CASE STUDIES OF VACCINE HESITANCY AND DISTRUST

### 1.1 The Disneyland Measles Outbreak

November 2014 brought bitter cold to much of the United States, with record low temperatures recorded from Colorado to North Carolina.[5] Even locales with typically temperate climates such as Florida dipped below freezing. However, Southern California was spared from the winter's arctic blasts. This must have made Disneyland, located in Anaheim, California, an especially appealing vacation destination for families looking for some relief from the winter weather. And indeed, more than 1.3 million people visited the 160-acre "Magic Kingdom" that December.[6]

On any given day, thousands of those visitors likely had runny noses, nagging coughs, or the beginnings of a fever. Out of those thousands, perhaps a few hundred had recently traveled out of the country; of those, there might have only been a few dozen who hadn't been vaccinated, and maybe only a handful who traveled to countries where measles is still endemic. Out of that small handful, a single person came to the resort with an active measles infection. It probably took several more weeks for the family of that still-unidentified patient to learn what was making him sick—and by that time, dozens of other children in California had been infected. By mid-January, the Centers for Disease Control and Prevention had linked 50 new measles cases to what had already become known as the Disneyland outbreak.[7] By the time the outbreak ran its course, that lone case had led to an additional 113 infections; dozens of those had led to expensive and frightening hospitalizations.[8] With the cost of containing each individual infection running as high as US$11,000,[9] this was a public health catastrophe.

At the time, California was just emerging from a years-long budget crisis that had seen the state with billions of dollars of shortfall. What made this situation all the more painful was that it was entirely avoidable: The measles vaccine that is administered as part of the trivalent measles–mumps–rubella (MMR) vaccine is close to 100% effective. Unfortunately, California is one of the states that had seen a growth in the number of communities where vaccine uptake rates are so low—in some cases, as low as 60%—as to put herd immunity at risk.[10] The parent of a child with leukemia highlighted an ethical issue created by this reality by making the point that her child's life could be threatened by other children in his school who were unvaccinated. The Disneyland outbreak of 2014–15 prompted the state to pass a law that did away with religious and "personal belief" exemptions for vaccines. "The science is clear," California governor Jerry Brown said at the law's signing ceremony. "Vaccines dramatically protect children against a number of infectious and dangerous diseases."[11]

## 1.2 The Nigerian Polio Boycott

More than a decade earlier and close to 8000 miles away, a dramatically different incident gave another stark illustration of the public health impacts of rumors and broken public trust in vaccines. In July 2003, five states in Nigeria's predominantly Muslim north initiated a boycott of the polio vaccine; one of those boycotts lasted for close to a year. The boycott started with a rumor—that the oral polio vaccine was being used as part of a Western conspiracy to sterilize Muslims—and was fueled by Muslim leaders, including Yusuf Datti Baba-Ahmed, the president of the Supreme Council for Sharia in Nigeria,[12] who claimed "modern-day Hitlers have deliberately adulterated the oral polio vaccines with anti fertility drugs and contaminated it with certain viruses which are known to cause HIV and AIDS."[13]

It was not just the rhetoric of local religious leaders that fueled the boycott. In some areas, locals were suspicious as to why they were receiving free

polio vaccinations despite having extremely limited health services and suffering from outbreaks of other diseases they considered more severe. There were also the ongoing wars in Iraq and Afghanistan and the heated rhetoric of many American politicians, which gave credence to the view that the West was at war with Muslims. Finally, the memory of child deaths suspected to be connected to Pfizer's 1997 trials of an antimeningitis drug, Trovan, heightened the plausibility of claims that Western medicine was killing children.

The cumulative effect was severe: Between 2002 and 2006, polio incidence in Nigeria rose by 400%. Since 2003, polio virus of Nigerian origin has been imported into 25 countries on three continents previously free of the disease.[14] These infections ultimately cost the international effort to eradicate polio more than US$500 million.[15]

## 1.3 The HPV Vaccine in Japan and the United States

In the early 1980s a German virologist named Harald zur Hausen identified the human papillomavirus (HPV) as the cause of up to 75% of all cases of cervical cancers. This research, which held out the promise of potentially creating the world's second cancer vaccine, was of such fundamental importance that it led to zur Hausen winning the Nobel Prize in Physiology or Medicine. (The hepatitis B vaccine, which can protect against liver cancer, was approved in 1981.)

It was not until 2006, when the United States' Food and Drug Administration approved Gardasil, a preventative HPV vaccine manufactured by Merck, that this promise was fulfilled. Three years later, GlaxoSmithKline's Cervarix was also approved. Looked at through one lens, the rollout of the HPV vaccine, which was introduced in 63 countries around the world by 2014,[16] was a success. A closer examination, however, shows how the vaccine's mixed reception in many places put millions of women unnecessarily at risk for cervical cancer.

Take Japan, where, in June 2013, media reports highlighted accounts of young women who were experiencing joint pain and convulsions after receiving the vaccine. Because no causal effect could be found between the young women's symptoms and the vaccine, the Japanese government continued to provide the access to the vaccine, but simultaneously withdrew its proactive recommendation. In other words, the vaccine was available for those who demanded it in spite of the government's silence regarding the public's vocal concerns. The effect was startling: in a very short amount of time, coverage went from more than 70% to less than 1%. In Sapporo, Japan's fourth largest city, coverage plummeted to 0.6%.[17]

In the United States, following the FDA's approval of Gardasil in 2006, the HPV vaccine provoked immediate controversy when 25 state legislatures moved to make immunization mandatory for girls attending school. Because the HPV vaccine prevents a sexually transmitted infection, it touches on sensitive issues around sexual behavior, making both parents and doctors sometimes uncomfortable raising the topic. In addition, some were concerned that the vaccine

would promote promiscuity while others saw the mandate as a governmental intrusion on private autonomy. As a result of these complications, the great majority of state-level attempts to mandate HPV vaccination ultimately failed: out of approximately 200 pieces of HPV-vaccine-related legislation that were proposed in state houses between 2006 and 2015, 43 would have imposed a mandate of some kind, and only 2 of these mandates were ultimately passed into law (in Virginia and Washington, DC), while the remainder were withdrawn, voted down, vetoed, or left to die quietly in committee. A third mandate, instituted by executive order, remains in place in Rhode Island, although a similar executive action by the governor of Texas was subsequently *reversed* by that state legislature due to popular outcry. Hence public health policy has been profoundly affected by the political valences attached to the HPV vaccine.[18,19]

## 2 LESSONS LEARNED

The three previously mentioned examples, drawn from different corners of the world and involving a diverse set of factors, together illustrate the challenges and some of the opportunities for the public health community when dealing with issues of confidence and trust in vaccines.

Vaccine refusers in the United States do not share a single unifying outlook or ideology. One commonality, however, is that they have been subject to a confusing mix of sometimes contradictory information and misinformation about vaccines and vaccine safety. This confusion and anxiety stems from two distinct incidents that occurred in the late 1990s. The first was the 1998 publication in *Lancet* of a small case series in which the British gastroenterologist Andrew Wakefield speculated about a potential link between the measles component of the MMR vaccine and autism.[20] The *Lancet* paper and Wakefield's full-throated promotion of his theories were met with mixed responses in the medical community: Some thought it best to ignore him, some thought he should be confronted head-on, and others were unsure what to do.[21] The lack of a unified response enabled Wakefield to manipulate the media into providing him with free publicity to air his views. By the time it was revealed that he had lied about conflicts of interest and possibly committed fraud, he had already been established as a vaccine "expect" and turned the discussion about the MMR vaccine into the type of he-said/she-said debate more typical of political squabbles that issues of public health.[22]

The second was the decision in 1999 by the US Food and Drug Administration and the American Academy of Pediatrics to recommend the immediate removal of thimerosal, a mercury-based preservative, from standard pediatric vaccines as a precautionary measure given emerging public concerns.[23] The FDA and the AAP made this decision without a clear explanation as to why they were doing it, and their confusing public statements eventually fueled a grassroots, parent-led movement of "Mercury Moms" convinced that thimerosal was the cause of their children's autism. Over the years, the Mercury Moms and Wakefield's acolytes coalesced into an antivaccine movement that has benefited

from celebrity endorsements, unskeptical media reporting, and anxiety-based profiteering by unscrupulous doctors and snake-oil salesmen. Even before the Disneyland outbreak, the effects of this were clear: In 2008, San Diego had suffered a memorable measles outbreak that was caused by an unvaccinated patient of "Dr Bob" Sears, a popular proponent of "alternative" vaccine schedules and a frequent vaccine critic.[9]

One result of the increased visibility of the effects of vaccine refusal is an apparent change in attitudes in favor of vaccination. The 2015 measles outbreak offers a perfect example of this: When polled several months after the outbreak had run its course, 34% of parents said they viewed vaccination as being more beneficial than they did a year earlier, while only 5% reported feeling vaccines were less beneficial.[a] Perhaps not surprisingly, this has meant more and more parents feel comfortable speaking out about the importance of vaccines. There are several illustrations of this new reality: Beginning around 2010, when *Lancet* formally retracted the Wakefield paper, growing numbers of pediatric practices enacted policies that refused admittance of families who would not vaccinate. This was based both on patient desires and on sound medical science: unvaccinated children could have infected infants too young to be vaccinated or children with medical conditions that left them unable to be vaccinated with serious consequences.

The California law doing away with nonmedical vaccine exemptions is another example of a newfound outspokenness from vaccine proponents: While state legislators stressed that this was a move they were making to protect the state's citizens, they were also receiving pressure from the vast majority of parents who do vaccinate. Actions emboldened by the support of provaccine parents may well represent a new front in addressing these strains of vaccine skepticism.

The response to the Nigerian boycott of polio vaccination in 2003–2004 shows a different approach to rebuilding and maintaining public trust—one that requires sustained, directed efforts. When the Global Polio Eradication Initiative tried to confront the "mistrust, resentment, fatigue and complacency"[24] it was facing, it began its efforts by trying to identify individuals or institutions that *did* have the trust of the public. The initiative soon identified Ibrahim Gambari, a United Nations senior advisor for African affairs, as a potential collaborator: Gambari, who had a northern Muslim father and a southern mother, already bridged the country's main political divide. Before long, Gambari had approached Ibrahim Shekarau, the governor of the Kano State and vocal opponent of the polio vaccine, and conveyed the cost that a continued boycott would bring to Shekarau's reputation.

Other responses to the boycott were careful to show sensitivity to local concerns. UNICEF drew attention to the fact that the polio vaccine was being procured from an Indonesian producer, allowing Shekarau to save face by reporting

a. Shute, Nancy. After measles outbreaks, parents shift their thinking on vaccines. *Shots – Health News From NPR*; July 6, 2015. http://www.npr.org/sections/health-shots/2015/07/06/420513540/after-measles-outbreaks-parents-shift-their-thinking-on-vaccines

that the vaccine was sourced from a Muslim country.[25] The Indonesian manufacturer also helped by opening its facilities for inspection by Nigerian delegations.

The combined effect of efforts such as these resulted in vaccination resuming in Kano in July 2004. Significant damage, however, had already been done. In order to continue to build and sustain the confidence of the public in the years to come, immunization activities were modified to include a variety of incentives beyond polio vaccine, ranging from sweets and hygiene kits to vitamin A drops and additional vaccinations such as DTP and measles. These incentives, which were incorporated into called "Immunization Plus Days," responded to the popular sentiment that polio eradication, despite its high international priority, was just one of many health needs and concerns of Nigerians. As a result, local citizens no longer felt the resentment and suspicion that arose when vaccination teams came to provide free vaccines to communities with few or no other health services, and many other health needs.[26]

Time and energy were also dedicated to building and maintaining relationships with leaders in Northern communities through outreach and engagement with traditional and religious leaders. One of the mechanisms for this was the creation of a Northern Traditional Leaders Committee on Primary Health Care,[27] which meant that trusted local leaders became part of the polio program. Another effort to include local voices led to the organization of groups of polio survivors, who would talk about the value of vaccination through the lens of personal experience.[28] More than a decade later, it is clear that the fruits of all of this labor have paid off. In January 2014, India was finally declared polio-free, and, in September 2015, the World Health Organization removed Nigeria from the list of polio-endemic countries,[29] leaving Afghanistan and Pakistan as the only two countries in the world that continue to have native transmission. Somalia reported the last case of polio in August 2014 in all of Africa—an historic achievement that was only possible because of years of work.

If the work of the Global Polio Eradication Initiative shows the importance of developing multipronged, sustained efforts to address the fracturing of trust, the HPV vaccine story illustrates the need for the public health community to anticipate concerns that are likely to arise. In retrospect, it is not hard to see why the HPV vaccine became controversial: Its administration touches on sensitive issues surrounding sexuality and sexual behavior. A survey of physicians and parents in Korea, Malaysia, Taiwan, and Thailand, for example, found that many mothers felt the vaccine was inappropriate because their daughters were unmarried or simply too young to have sex. Elsewhere around the world, physicians acknowledged not promoting HPV vaccination because they felt unprepared to deal with the uncomfortable questions it might prompt.[30,31]

The difficulties with the HPV vaccine also show the importance of responding to concerns promptly when they arise. In 2010, the government of India suspended an HPV vaccine demonstration project being conducted in two states. The suspension occurred after sustained pressure over several months from a broad coalition of civil society groups that wanted a larger role in introducing

new vaccine programs and questioned why the vaccine was prioritized over cervical screening. The government's nonresponse to the early appeals from civil society for a public forum did more harm than good, heightening perceptions that the government was more interested in listening to pharmaceutical companies and international NGOs than to its own people.[32]

In Japan, the Ministry of Health did provide information on the vaccine's safety but the government's ambiguous policy fostered confusion, especially when combined with antivaccine sentiment being spreads by both local patient groups and international social media networks.[33] As of March 2016, the HPV vaccination recommendation was still suspended in Japan.

One notable success in the HPV vaccine's rollout has been Australia, which reported a three-dose coverage rate of 73% in 2014.[34] This is not because the country was devoid of controversies regarding the vaccine: In 2007, 26 girls at a Melbourne school had an episode of mass psychogenic illness following HPV vaccination.[35] There, however, a prompt and transparent response preempted any potential crisis of confidence.[36]

Another instructive example can be found in England, which achieved 86% three-dose vaccination coverage in 2014.[b] This reflects the prompt response and management of public anxieties following the death of a 14-year-old girl following her HPV vaccination in 2009, 1 year after HPV vaccination was included in the national immunization program. Health officials expressed sympathy and concern following the news of the girl's death, while making clear that they were investigating the case. When the investigation indicated that the death was unrelated to the vaccine, rapid engagement with the media helped quell concerns and negative media coverage despite efforts by antivaccination groups to capitalize on the incident.[33]

## 3 THE NEED FOR EVIDENCE-BASED RECOMMENDATIONS FOR THE FUTURE

Vaccines are one of the safest and most cost-effective medical interventions in history, and their success has been a main driver of increased human life span since the beginning of the 20th century. For much of that time, public health officials did not need to worry about specious fears derailing a vaccine program. After all, when a dangerous infectious disease is prevalent, people are understandably more focused on prophylactics than on those prophylactics' potential pitfalls. An illustration of this can be seen in the Cutter incident, which occurred in 1955 when Cutter Laboratories, one of a small handful of companies licensed by the United States to manufacture the newly introduced polio vaccine, produced a batch with incompletely inactivated polio virus. The

b. Public Health England. Annual HPV vaccine coverage 2013 to 2014: by PCT, local authority and area team; December 2, 2014. https://www.gov.uk/government/statistics/annual-hpv-vaccine-coverage-2013-to-2014-by-pct-local-authority-and-area-team

result was 56 cases of paralysis and 5 deaths; despite this, the polio vaccination campaign continued apace.

Today, 60 years after that tragedy, the visceral fear once provoked by diseases like diphtheria, *Haemophilus influenzae* type b, polio, and rubella has, in many developed countries, become more of a notional concern. As a result, many parents view these scourges as relics of a bygone era. Unfortunately, when it comes to infectious diseases, years of progress can be undone in a relatively short amount of time. Take France, which had just 40 cases of measles in 2007. Four years later, there were 15,000 cases and 6 deaths. What's more, the polio vaccination boycott in Nigeria shows that vaccine hesitancy is not unique to higher income countries where vaccine preventable diseases are less visible. There are other complex determinants of public anxieties around vaccines, ranging from personal and sociocultural reasons to historical and political ones.

This is why it is so crucial for public health officials and vaccination campaigns to pay close attention to vaccine concerns and to be responsive as soon as there is evidence of public anxiety and distrust of vaccines and vaccine programs. Listening is key both to understanding what is driving concerns and to devising effective responses. The Global Polio Eradication Initiative's work in Nigeria and Australia and the United Kingdom's handling of scares regarding the HPV vaccines are positive examples of where listening and responding to public anxiety helped quell concerns and restore confidence. Too often, however, the response to concerns about vaccines is based on hunches and intuition—and there is a growing body of evidence that these hunches and intuition are often incorrect.

One striking exemplification of this can be seen in recent study led by Dartmouth College's Brendan Nyhan and published in *Pediatrics*.[37] Nyhan and coworkers tested four interventions designed to reduce misperceptions about vaccines and increase MMR vaccination rates: Parents were given either information about the lack of evidence that the MMR vaccine causes autism; information about the dangers of measles, mumps, and rubella; images of children with measles, mumps, and rubella; or a story about an infant who almost died of a measles infection. Incredibly, none of the interventions had the intended effect. Even more surprising was the fact that several had the *opposite* effect: Refuting claims of a link between the MMR vaccine and autism, for example, made parents already skeptical of vaccines even *less* likely to vaccinate their children, while pictures of children sick with vaccine-preventable diseases increased parents' belief in dangerous vaccine side effects.

Research led by the University of Washington's Douglas Opel also highlights the challenge of devising effective strategies for discussing vaccines. Opel focused his work on provider–parent communication, comparing providers using participatory language (ie, let's talk about the vaccine options) to presumptive language (ie, we are going to vaccinate your child today).[38] Parents whose providers included them in a discussion about vaccines reported higher rates of provider satisfaction. Their children, however, had lower rates of vaccine uptake, an uncomfortable finding that raises thorny questions about whether a

health-care provider's primary purpose is to make patients feel comfortable or keep them healthy.

While Nyhan's and Opel's work adds important data points to our understanding of vaccine communication, it also calls attention to how our overall lack of knowledge in this area has led to well-intentioned efforts that have likely had negative results. Strategies to communicate with vaccine-wary parents *cannot* be devised through hunches and intuition. The nature of public health, policy, and patient communication around vaccines and immunization needs to move from being didactic to dialogic, from feeling coercive to becoming conversational. The questions and reasons for vaccine hesitancy need to be listened to, not guessed. Public health needs public trust, built through genuine engagement.

One of the key overall lessons across the case studies presented in this chapter is the importance of recognizing the multiple levels of confidence that effective immunization programs depend on—public confidence, provider confidence, and political confidence in vaccination. Hesitancy around vaccination by members of the public, their health providers or politicians who make key policy and funding decisions, must all be addressed early to preempt potential disruptions to immunization programs and their public health impacts.

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Chapter 28

# Vaccines for Emerging Viral Diseases

**Adam DeZure, MD, Barney S. Graham, MD, PhD**
*Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States*

## Chapter Outline



## 1 INTRODUCTION

Infectious diseases continue to plague mankind and evolve to keep pace with the efforts to control them. Sir William Osler captured the ongoing fear of infectious pathogens when he said, "Humanity has but three great enemies: fever, famine, and war; of these by far the greatest, by far the most terrible, is fever." Despite the significant impact of antimicrobials and vaccines on public health, there has only been one major human pathogen eradicated—variola virus, the agent of smallpox. In its place have been a series of new and reemerging microbes responsible for isolated infections, regional outbreaks, and global pandemics. Bacterial, fungal, and parasitic pathogens have the capacity to cause widespread epidemics such as the "Black Death" caused by Yersinia pestis in 14th century Europe. However, bacteria, fungi, and parasites are less likely to cause widespread human pandemics at this point in history and are less amenable to vaccine strategies than viral diseases. Focusing on viruses, a catalogue of newly discovered human pathogens from the beginning of the 20th century shows a predictable and nearly linear rate of new agents discovered over time.[1] However, of the more than 100 virus families, only 22 have been associated with human infections, a number that seems to have plateaued.[1] In this chapter we will concentrate on vaccines for emerging viral diseases.

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Experiences over the last 3 decades with HIV, SARS, and Ebola have taught us the potential consequences of viral infectious threats on global health, and economic and political stability. Emerging viral infections with pandemic potential can be chronic, persistent, or acute in nature. They can be disseminated by respiratory droplet or other bodily fluids. They can emerge from animal reservoirs and spread via insect vectors, and can be precipitated by changes in climate, animal habitats, human population dynamics, and other ecological events.[2] And they can emerge as a consequence of human activity in the context of deliberate viral modification as a form of biowarfare. We are faced with important questions including what can be done to anticipate these events and how can we best prepare to intervene when new or changing viral threats arrive?

Most current licensed antiviral vaccines utilize live-attenuated or whole-inactivated viruses, although there are now a few examples of effective virus-like particle (VLP) vaccines. In the setting of a new pandemic viral threat and without the advantage of a preexisting understanding of its pathogenesis, growth or attenuating features, it would be difficult to quickly develop traditional live-attenuated or whole-inactivated vaccine approaches due to uncertainty about the safety of attenuating mutations or the production of replication-competent virus in bulk. Therefore, it is more likely that developing vaccines for emerging infections will involve new technologies, some of which have not yet been licensed for human use. Using technologies that can provide a candidate vaccine based on information derived entirely from target gene sequences is safer and more expeditious than procedures requiring virus isolation and growth that require a high level of containment. Therefore, even for virus families for which there are currently licensed vaccines, additional approaches beyond live-attenuated and whole-inactivated products should also be pursued.

Historically, decades have elapsed between when a new virus is discovered and when a relevant vaccine becomes available for human use (Fig. 28.1). In the setting of an epidemic, such protracted vaccine development timelines are incompatible with rapid deployment of a vaccine intervention and therefore not a practical consideration for immediate control of the outbreak. In part because of the 2014 West African Ebola outbreak, emergence of new viral threats to public health are becoming more of a global concern and have more media and political visibility. Fortunately, this is a time of remarkable technical advances in human monoclonal antibody discovery, structure-guided antigen design, and nucleic acid sequencing—making rapid development of biologics more feasible. Therefore, defining new approaches and pathways to efficiently deploy vaccine interventions for emerging infections is a priority for public health agencies, commercial entities, government officials, and nonprofit organizations. However, the key to a rapid vaccine response is advanced preparation.

Several steps are needed to improve preparedness for emerging viral infectious diseases. These can be divided into 4 broad categories: (1) surveillance and discovery, (2) reagent, assay, and animal model development, (3) vaccine

| Viral pathogen | Virus discovered | Vaccine developed for human use | Years to vaccine |
|---|---|---|---|
| Yellow fever virus | 1900 | 1935 | 35 |
| Polio | 1909 | 1954 | 45 |
| Measles | 1911 | 1957 | 46 |
| HSV | 1919 | Not available | >96 |
| Influenza | 1933 | 1945 | 12 |
| RSV | 1956 | Not available | >59 |
| Dengue virus | 1960 | Not available | >55 |
| Hepatitis B | 1967 | 1984 | 17 |
| Rotavirus | 1973 | 1998 | 25 |
| Hepatitis A | 1973 | 1995 | 22 |
| HPV | 1974 | 2007 | 33 |
| HIV | 1983 | Not available | >32 |
| HCV | 1989 | Not available | >26 |

**FIGURE 28.1 Time from identification of a viral pathogen to vaccine availability.** Vaccine development is a lengthy process often measured in decades. Many steps are required even for a traditional empirical approach including identification of target antigen; assay development; defining seroprevalence and incidence in relevant populations; understanding transmission dynamics and whether there is an intermediate animal host; developing animal models; exploring pathogenesis and defining immune mechanisms of protection; designing vaccine antigens; determining formulation, delivery route and method; preclinical evaluation for safety and immunogenicity; manufacturing; and several years of clinical evaluation and clearing regulatory hurdles. Vaccines can fail at any of these steps, and it is rare for the first attempted concept to become the final product.

design and product development, and (4) manufacturing, clinical evaluation, and an appropriate regulatory framework.

Depending on features of the virus structure, transmission dynamics, entry requirements, tropism, and replication strategy, a vaccine approach should be proposed, designed, and evaluated in small animals for immunogenicity and protection against challenge. Manufacturing a candidate vaccine for which there is no immediate market poses a significant dilemma because most stages of advanced vaccine development are carried out by large pharmaceutical companies that need to make profit to stay in business. While emerging infectious diseases pose a public health threat, they rarely present a compelling commercial opportunity. Vaccines require a large investment and historically have a relatively low probability of being successful without an extensive iterative process of evaluation and redesign. Therefore, in addition to new biological tools, there needs to be political will to prioritize public funding of advanced vaccine development and new business models for managing this process.[3]

In this review, we describe the vaccine development efforts for three distinct viruses that collectively capture many of the challenges faced when developing vaccines for emerging viral threats. Ebola, a member of the Filoviridae family, is spread by body fluids and secretions with a relatively low attack rate but causes a systemic disease with high mortality. Chikungunya, an alphavirus in

the Togaviridae family, is transmitted by mosquito vectors with a high attack rate and causes a systemic disease with low mortality but high frequency of chronic disabling arthritis. Middle East Respiratory Syndrome (MERS CoV), a beta-coronavirus and member of the Coronaviridae family, is spread by respiratory droplets and causes a relatively high mortality in persons with underlying disease. It has a reproductive rate ($R_0$) of $<1$ for person-to-person spread, but occasional "super-spreaders" can infect multiple people. The MERS reservoir, dromedary camels, will continue to be a source of new human infections.

Ebola, Chikungunya, and MERS CoV are representative infectious pathogens with pandemic potential. Use of new technologies to arrive at a more comprehensive understanding of viral structure and pathogenesis has paved the way for rational vaccine design for each of these viruses. Herein we elaborate on the iterative path taken to develop and evaluate candidate vaccines for Ebola, Chikungunya and MERS CoV and the factors that propel or delay progress. We focus our discussions primarily on the candidate vaccines developed at the NIAID Vaccine Research Center, not because they are necessarily the most promising or advanced, but because we are more familiar with the events associated with their development, and the factors impacting advancement and implementation.

## 2 EBOLA

Ebola is a highly virulent pathogen from the family Filoviridae. Ebolavirus is an enveloped, negative-strand RNA virus whose genome encodes 7 structural proteins including a transmembrane glycoprotein that mediates viral entry into host cells.[4] The surface glycoprotein (GP) mediates viral attachment and entry and is the primary antigenic target for vaccine development. Five species of Ebola have been identified including Zaire (the cause of the 2014 West African epidemic), Sudan, Bundibugyo, Tai Forest, and Reston. Ebola virus disease (EVD) was first recognized in two distinct outbreaks in the Ebola River Valley of the Democratic Republic of Congo (formerly Zaire) and in Sudan in 1976.[5,6] EVD emerged again in 1994 in Gabon and in 1995 in an outbreak involving 315 people in Kikwit, Democratic Republic of Congo (DRC). Since then, sporadic outbreaks have occurred in equatorial Africa, especially the DRC, Republic of Congo, Gabon, Uganda, and Sudan with fatality rates averaging over 50%.[7,8]

After an incubation period of 2–21 days, onset of EVD is manifested by fevers, chills, malaise, and myalgias with onset of gastrointestinal symptoms including nausea, vomiting and diarrhea by days 3–5.[8,9] When fatal, death typically occurs by days 7–12 and can be characterized by hypovolemic shock and multiorgan failure.[8,9] The disease is transmitted human-to-human through direct contact with infected bodily fluids through mucosal surfaces and breaks in the skin.[8]

In Mar. of 2014, Guinea's Ministry of Health was notified of a highly pathogenic, febrile illness circulating in Gueckedou and Macenta. An epidemiologic evaluation ensued and samples from hospitalized patients were sent to BSL4

labs in France and Germany. Ebola was confirmed by either polymerase chain reaction (PCR), electron microscopy, or from isolation in cell culture.[10] Viral RNA was extracted, sequenced, and compared to available Ebola sequences in GenBank enabling phylogenetic analysis. An Ebola strain was identified with 97% similarity to previously collected Ebola strains in the DRC and Gabon. In turn, the outbreak was traced to a single index case, a 2-year-old boy who died in Dec. 2013 in Gueckedou. The epidemic of EVD that followed has accounted for more cases than all prior EVD outbreaks combined. As of Jul. 2015, over 28,000 suspected and confirmed cases and more than 11,000 deaths have been reported with the majority of cases occurring in the West African countries of Guinea, Liberia, and Sierra Leone.[11] The World Health Organization (WHO) declared the epidemic a public health emergency of international concern in Aug. of 2014. And as the outbreak emerged, the international community responded with an unprecedented effort to accelerate Ebola vaccine development.[12] Prior to 2014, the largest single Ebola outbreak was 425 infections leading to 224 deaths.[13]

The Vaccine Research Center within the National Institute of Allergy and Infectious Diseases performed a series of phase I clinical trials between 2003 and 2009 to evaluate the safety and immunogenicity of GP antigen constructs. These included a DNA vaccine encoding a transmembrane-deleted, secreted version of the glycoprotein, a recombinant human adenovirus serotype 5 (rAd5) vectored vaccine encoding the Ebola GP with one amino acid mutation, and subsequently a DNA vaccine encoding the full-length, wild-type (WT) GP.[14–16] These phase I studies showed the WT full-length GP was safe and well tolerated. In parallel, studies to define the immunological correlates of protection and optimal antigen delivery approaches were evaluated in nonhuman primates (NHP). In addition to the important role for antibodies targeting GP, it was found that CD8 T cell-mediated immunity was found to be critical for vaccine efficacy in NHP.[17] It was also found that antivector immunity would diminish vaccine potency particularly for the induction of CD8 T cells. Because of the high seroprevalence of Ad5, rare serotype adenovirus vectors were explored including human rAd26 and rAd35 vectors, and chimpanzee-derived rAd vectors.[18]

Chimpanzee adenovirus serotype 3 (abbreviated as ChAd3 or cAd3) encoding the wild-type glycoproteins from both the Ebola Zaire and Sudan species was ultimately chosen as the candidate vaccine vector because of its low seroprevalence and its similar potency and pattern of innate immune response induction to Ad5. This vector was originally produced by Okairos which is now owned by GlaxoSmithKline (GSK). Vector potency comparable to rAd5 was considered to be important for rapid induction of both antibody and CD8 T cells with a single dose. This would facilitate use of the cAd3-Ebola GP vaccine in an outbreak setting using a ring vaccination strategy to achieve rapid short term protection for those at highest risk of infection. Both Zaire and Sudan GP were included in the initial vaccine to protect against both Zaire and Sudan, the most common species responsible for EVD. This replication-defective vaccine, now

called cAd3-EBO, provided 100% protection to nonhuman primates 5 weeks following vaccination in an otherwise lethal Ebola challenge model, and partial protection (50%) to lethal challenge 10 months following vaccination. The cAd3-EBO was also shown to effectively prime for a modified Vaccinia virus Ankara (MVA)-vectored vaccine boost encoding the same glycoprotein inserts, improving survival from lethal Ebola challenge to 100% at 10-months post-boost.[19] The combination of cAd3-MVA prime-boost produces a much higher magnitude response and might provide more durable protection to health care workers, ambulance drivers, burial workers, and others with ongoing risk of Ebola exposure.

The first quarter of 2015 was targeted for phase I clinical evaluation of cAd3-EBO at the NIH Clinical Center and a Pre-Investigational New Drug (IND) application was submitted to that end in Aug. of 2013. However, in response to the epidemic, the NIH, FDA, IRBs, and others coordinated efforts to consolidate timelines. An IND application was submitted to the FDA on Aug. 15, 2014 and a phase I trial began 18 days later. The cAd3-EBO vaccine was found to be safe and immunogenic in early phase 1 testing of two doses, $2 \times 10^{10}$ and $2 \times 10^{11}$ particle units (PU), and the day 28 postvaccination results were published 3 months later.[20] All vaccine recipients developed glycoprotein (GP)-specific antibodies; however, GP-specific antibody responses as well as GP-specific T cell responses were greater with the $2 \times 10^{11}$ PU dosing. Antibody titers with the higher dose were in the range associated with protective immunity in the NHP challenge model.

The Zaire GP antigen encoded by cAd3 was derived from the original Mayinga strain of Ebola isolated in 1976. Compared to strains circulating in West Africa it differed in very few GP residues outside the glycan cap, which is cleaved prior to virus entry. Mayinga was also more related genetically to the outbreak strain than the Kikwit strain that had been used in the NHP challenge studies. Therefore, the available cAd3 Ebola Zaire construct was thought to be antigenically relevant to the outbreak strain and suitable for testing. To directly address the West African crisis and to accelerate manufacturing timelines, additional phase I studies of the cAd3 vaccine in monovalent form (encoding the GP from Zaire, not Sudan species) were performed in the United States, United Kingdom, Mali, and Switzerland at doses ranging from $1 \times 10^{10}$ to $1 \times 10^{11}$.[21] These studies, done in collaboration with GSK, supported advancement of the monovalent vaccine into an on-going phase II/III study in Liberia (NCT02344407) as well as US evaluation of a prime-boost regimen consisting of cAd3-EBO followed by MVA-EBOZ to evaluate durable immune responses (NCT02408913). The monovalent cAd3-EBOZ has also been evaluated as a prime for boosting with a recombinant MVA vector provided by Bavarian Nordic expressing the GP from Zaire, Sudan, and Marburg, and N from Tai Forest at sites in the United Kingdom and Mali. A similar vaccine approach using rAd28 priming and MVA boosting developed by Crucell (now owned by Janssen as part of Johnson & Johnson) has been evaluated in subsequent clinical trials.

In parallel with cAd3-EBO development, a replication-competent, recombinant vesicular stomatitis virus (rVSV) vaccine expressing GP from Ebola Zaire (Kikwit strain) showed promising results in preclinical NHP challenge models.[22,23] The vaccine was developed by the Public Health Agency of Canada, licensed to BioProtection Systems (a subsidiary of NewLink Genetics), and subsequently licensed to Merck. The donation of 800 vials of this vaccine by the Canadian government to WHO enabled initial evaluation of this candidate vaccine in 150 people at doses ranging from 300,000 to 50 million PFU in phase I trials in Gabon, Kenya, Germany, and Switzerland. Although no life-threatening adverse events were observed, there was evidence of unexpected viral seeding of joints and transient arthritis in addition to vaccine virus positive skin vesicles in some participants. A lower dose of rVSV-ZEBOV (300,000 PFU IM) did not diminish the likelihood of rVSV infecting peripheral tissues. Thirteen of 51 participants developed arthritis and 2 participants developed cutaneous lymphocytic vasculitis with rVSV established as the etiology based on synovial fluid and skin lesion analysis.[24,25] The vaccine was immunogenic and all vaccine recipients evaluated developed GP-specific antibody responses.[26] An additional evaluation of this candidate vaccine in two phase I trials in the United States (WRAIR and NIH) supported advancement of the vaccine at 20 million PFU dosing. Compared to 3 million PFU dosing, 20 million PFU resulted in higher IgG and neutralizing antibody titers.[27] The higher dose vaccine is currently under evaluation in the NIAID-sponsored PREVAIL trial in Liberia, the CDC-sponsored STRIVE study in Sierra Leone, and the WHO-sponsored Ebola ça suffit study in Guinea. Notably, the skin and joint complications were not seen during active follow-up of individuals in the United States or African studies.

Interim results from the Ebola ça suffit phase III trial evaluating the safety and efficacy of rVSV-ZEBOV in an unblinded, cluster-randomized trial in Guinea are encouraging and consistent with vaccine efficacy. The trial utilized a ring vaccination design in which individuals at high risk of infection (contacts and contacts-of-contacts of a lab confirmed case of EVD) were randomized as a cluster to receive either immediate vaccination with rVSV-ZEBOV or delayed vaccination 21 days later. There were no cases of EVD with symptom onset $>10$ days following randomization in the group receiving immediate vaccination (48 clusters with 2014 subjects) whereas the authors reported 16 cases of EVD in subjects randomized to delayed vaccination (42 clusters with 2380 subjects).[28]

The pathway to licensure of new vaccines requires evidence of vaccine safety and efficacy in clinical trials. For infectious pathogens that emerge sporadically and with low incidence, such as Ebola, the ability to perform randomized, placebo-controlled, double-blinded efficacy trials remains a limiting factor. The hard fought decline in EVD cases in West Africa is welcome and will hopefully lead to complete control of the epidemic without rebound or reemergence. However, the decline will likely preclude efforts to evaluate vaccine efficacy

of other vaccine candidates during this outbreak. For the cAd3-EBO vaccine, it remains unknown if a path to licensure is feasible in the absence of human efficacy data. Regulators will need to consider if supportive evidence from preclinical NHP challenge models, safety data in on-going human trials, and use of alternative surrogate immunogenicity endpoints is adequate to move forward with licensing.

Therefore, several challenging questions remain for regulators and vaccine developers alike. These include determining which Ebola vaccines will be made available for the next Ebola epidemic and what trial design will be used (ie, ring vaccination strategy with delay or community randomization; a step-wedge design with staged vaccination; or a placebo-controlled, randomized study) or whether the initial results from the recent trial will preclude further evaluation of experimental (unlicensed) products.[28] Nonetheless, several important lessons can be extracted from the recent development of Ebola vaccine candidates. Perhaps the most important is that the rapid development and testing of candidate vaccines in 2014 and 2015 was made possible because of years of prior investment into the study of Ebola basic virology and pathogenesis, in part driven by biodefense concerns. Other factors that enabled accelerated vaccine development include: (1) preexisting established animal models, (2) preclinical data from NHP challenge studies, (3) the presence of cGMP vaccine product, and (4) global concern, extensive media coverage and political visibility that helped foster coordination between funding agencies, regulatory authorities, governments, clinical trial sites, laboratories, commercial partners, and publishers.

## 3 CHIKUNGUNYA

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus of the family Togaviridae transmitted primarily by *Aedes aegypti* and *Aedes albopictus* mosquitoes.[29] Three CHIKV clades have been identified and include West African, Asian, and East/Central/South African, each sharing significant amino acid homology. Chikungunya infection manifests as abrupt onset of fever, myalgias, rash, headache, nausea, and arthralgias with illness onset typically 3–7 days following viral transmission.[30–32] The hallmark arthritis of CHIKV infection can be relapsing, incapacitating, and may persist for months.[30] Severe manifestations of Chikungunya can include myocarditis, hepatitis, and neurologic complications including encephalitis.[33] And although rare, deaths have occurred particularly in the elderly and infants. There are currently no FDA licensed vaccines or treatments specific for Chikungunya.

CHIKV has an 11.8 kB, single-stranded, positive-sense RNA genome that encodes 4 nonstructural proteins involved in virus replication (nsP1-4) and 5 structural proteins including the capsid and envelope glycoproteins E1 and E2.[30,32,34] The E1 glycoprotein mediates cell fusion and the E2 glycoprotein interacts with the host receptor. The mature virion diameter is 70 nm and the external surface exhibits trimeric spikes consisting of 240 E2/E1 heterodimers.[32]

Chikungunya virus was discovered at the East African Virology Research Institute in Entebbe, Uganda, now the Uganda Virology Institute (UVRI) and was isolated from a member of the Makonde tribe in Tanzania in the early 1950s. CHIKV has been responsible for outbreaks in Africa and Asia since the 1960s.[31] CHIKV is endemic in tropical and subtropical regions of Africa where it exists as part of an enzootic cycle. However, intermittent epidemics emerge characterized by human-mosquito-human transmission with attack rates that can exceed 50%.[32] CHIKV reemerged in 2005 in an epidemic infecting >272,000 people across several islands in the Indian Ocean. Genetic mutations in the virus including a substitution (A226V) in the E1 glycoprotein that enhanced viral infectivity of the *A. albopticus* vector contributed both to the 2005 epidemic as well as widespread dissemination of CHIKV into new and temperate climates.[34,35] The first case of autochthonous transmission in the Americas was reported in 2013 and local transmission has now been reported in over 43 countries or territories in the Americas. A CHIKV epidemic continues in the Caribbean and as of Jul. 2015, >1.5 million suspected CHIKV cases have been reported in the Caribbean, Central America, South America, Mexico, and the US. The CHIKV in the Americas is most similar to the Asian strain and is almost exclusively transmitted by *A. aegypti*. An East/Central/South African (ECSA) strain has more recently been detected in Brazil, which may make adaptation to *A. albopictus* more likely. If this occurs, broader spread into North America is possible.

CHIKV viremia peaks on the day of symptom onset with titers reaching $10^9$ viral RNA copies/mL.[36] Both neutralizing activity and induction of IgG3 antibody isotype early in the course of infection are associated with lower risk of chronic disease and persistent arthralgias.[37,38] Research to date supports an antibody-mediated mechanism of protection from CHIKV infection[39] and passive protection was shown in an otherwise lethal mouse model of CHIKV infection following IgG administration from NHPs who had received a Chikungunya virus-like particle (VLP) vaccine.[34]

Several promising candidate vaccine platforms have been evaluated including formalin-inactivated CHIKV vaccines,[40,41] recombinant MVA and measles vectored vaccines,[42,43] chimeric alphavirus vaccines,[44] insect cell-produced VLP vaccine candidates,[45] and DNA vaccine candidates.[46,47] A live, attenuated CHIKV vaccine was advanced into phase II testing and induced neutralizing antibodies to CHIKV by day 28 in 98% of recipients but was also associated with arthralgias in 8% of subjects.[48] Due to our familiarity with the limitations and obstacles for advancement of a candidate vaccine developed at the NIAID Vaccine Research Center, we will focus the discussion primarily on a mammalian cell-produced VLP vaccine. This candidate vaccine was evaluated in a phase I clinical trial and is currently being advanced into phase II clinical testing. To produce the VLP, Akahata and coworkers transfected 293T HEK cells with expression vectors encoding C-E3-E2-6K-E1 proteins from the West African CHIKV strain 37997. Electron microscopy revealed production of VLPs with

the same morphologic appearance as wild-type CHIKV, characterized by a 65 nm external diameter, 40 nm core diameter, and a structure with E1 and E2 glycoproteins organized into 240 heterodimers and 80 glycoprotein spikes. In preclinical assessment, all NHPs receiving this VLP developed neutralizing antibodies to both heterologous and homologous CHIKV strains. In a challenge model, all NHPs were protected against viremia as well as the postinflammatory sequelae of infection when exposed to intravenous CHIKV challenge 15 weeks after VLP administration.[34]

The VLP vaccine candidate was found to be safe and immunogenic in a phase I, dose-escalation clinical trial evaluating a dose range of 10–40 μg over a 3 dose regimen (weeks 0, 4, and 20) in 25 healthy adults. Neutralizing antibodies against an outbreak strain from the ECSA clade were identified in all participants 4 weeks following the second vaccination revealing both robust immunogenicity and evidence of cross-reactive neutralizing activity.[49]

Several advantages of this VLP vaccine include its highly symmetric exterior that resembles wild-type virus, induction of high titer neutralizing antibody, safety profile as a nonreplicating candidate vaccine with low containment manufacturing. Factors contributing to the delay in advanced development of this vaccine include difficulty in establishing a commercial partnership, resources needed for process development and scale-up, and difficulties in establishing clinical trial infrastructure for defining efficacy and immune correlates of protection. Due to the ongoing Chikungunya epidemic in the Americas and the Caribbean, there is an opportunity to obtain an efficacy result in a field trial. Therefore, advancing clinical evaluation of candidate vaccines should be a public health imperative. If field trials are delayed until the outbreak has saturated the region and the disease becomes more sporadic, it will be difficult to ever obtain an efficacy outcome or establish an immunological correlate of protection. This will complicate achieving licensure for general use, and is another example of how public options for manufacturing would facilitate the development of vaccines for emerging infectious diseases.

## 4 MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS

Coronaviruses are enveloped, single-stranded, positive-sense RNA viruses with very large genomes (~30 kb). Endemic human coronaviruses are widespread and include coronaviruses HCOV-229E, OC43, NL63, and HKU1 which generally cause mild respiratory infections including the common cold. However, several features of coronaviruses allow adaptation to new hosts and ecological niches.[50] These include large RNA genomes, high frequency of RNA recombination, and infidelity of the RNA-dependent RNA polymerase. Following the 2002 emergence of severe acute respiratory syndrome coronavirus (SARS CoV), a highly pathogenic lineage B betacoronavirus, concerns arose that novel coronaviruses could represent a major public health threat. This was further validated by the emergence of Middle East Respiratory Syndrome coronavirus (MERS CoV).

The first case of MERS CoV was reported in 2012 in a 60-year-old male in Saudi Arabia with acute pneumonia who subsequently died from progressive pulmonary and renal failure.[50] The subsequent rapid international response to the emergence of MERS CoV included genomic sequencing, development of diagnostic assays, surveillance for a zoonotic reservoir, development of animal models, and evaluation of viral pathogenesis to enable rational design of candidate vaccines.

The complete genome sequence of the implicated coronavirus was rapidly identified and made available in GenBank. Genomic sequencing enabled phylogenetic and taxonomic analysis and MERS CoV was identified as a lineage C betacoronavirus, distinct from previously known human coronaviruses and most closely related to HKU4 and HKU5 coronaviruses previously isolated from bats.[50,51]

Clinical presentation of MERS CoV can range from asymptomatic to a severe respiratory illness that culminates in death. Following an incubation period of 5.2 days (1.9–14.7 days), presenting symptoms can include fever, cough, and dyspnea. Rapid deterioration of respiratory status can occur within just a few days of symptom onset and fatality rates are estimated to be about 35%. Since 2012, there have been 1595 lab-confirmed cases of MERS CoV infection and at least 571 deaths globally.[52] This includes an outbreak of 186 individuals in South Korea initiated by a single index case, a man returning from a trip to Saudi Arabia. The outbreak in Seoul was primarily restricted to hospital settings where 5 individual "super-spreaders" accounted for the majority of infections of other patients and health care providers.[53]

MERS CoV expresses a membrane-anchored trimeric spike (S) protein that consists of a S1 subunit that engages the receptor on the host cell and a S2 subunit that mediates membrane fusion.[54] The receptor binding domain on S1 has served as the primary antigenic target for vaccine development. Similar to the angiotensin-converting enzyme 2 (ACE2) peptidase receptor used by SARS CoV, the MERS CoV target cell receptor is CD26, dipeptidyl peptidase 4 (DPP4), which is a 766 amino acid, type II transmembrane glycoprotein expressed on epithelial and endothelial cells of several organs including lung and kidney. DPP4 has been shown to be a biomarker of IL-13 expression in asthma and is associated with glycemic homeostasis and microvascular complications of diabetes.[55,56] Transfection of human DPP4 into otherwise nonsusceptible cells from feline, murine and canine species enables MERS CoV infection.[57] Lu et al. subsequently solved the crystal structure of the receptor binding domain of S1 in complex with CD26.[58]

Comparative serologic studies emerged shortly after MERS CoV was first identified to look for zoonotic reservoirs. Evaluation of serum-specific IgG targeting the receptor-binding S1 subunit was performed by protein microarray and confirmed by virus neutralization testing and revealed high-titer neutralizing antibodies were prevalent in camels.[59] Experimental support for the camel as a zoonotic reservoir was performed by Adney and coworkers who inoculated

camels with MERS CoV. The camels subsequently developed upper respiratory symptoms and showed evidence of upper respiratory tract viral shedding in nasal secretions for 7 days postinoculation.[60] Evidence of zoonotic transmission from camel to humans was further supported from a patient in 2013 who died of MERS CoV following exposure to camels with rhinorrhea. Nasal swabs for both the patient and camels were positive for MERS CoV RNA and subsequent genomic sequencing revealed the isolates to be identical[61] However, not all cases of MERS CoV infection have been linked to camel exposure. Additional studies are needed to further define transmission dynamics and intermediate hosts.

Low herd immunity in humans, a highly pathogenic virus and evidence of both zoonotic and human-to-human transmission suggest that outbreaks of MERS CoV will continue to occur and may have pandemic potential. There are currently no FDA approved vaccines for MERS CoV but previous vaccine development efforts for SARS CoV created a foundation for MERS CoV vaccine design. The SARS spike protein is similarly responsible for receptor binding and membrane fusion and is the primary antigen target for neutralizing antibody and vaccine development.[62]

Candidate MERS CoV vaccines also target the spike protein. Approaches have included subunit proteins, DNA, and gene-based vectors. A recombinant modified vaccinia virus Ankara vaccine expressing the full length MERS CoV spike protein produced neutralizing antibody in immunized mice.[63] Subsequently, Ma and coworkers focused on the receptor binding domain (RBD) of the MERS CoV spike protein to develop a subunit protein vaccine. Five different receptor binding domain fragments from S1 were individually fused with the Fc of human IgG and each was evaluated for receptor binding affinity, antigenicity, immunogenicity, and neutralizing potential. The S377-588-Fc fragment that contains the stably folded RBD had the highest affinity for DPP4 and induced the highest titer neutralizing antibodies in both mice and immunized rabbits while minimizing exposure to nonneutralizing epitopes.[64] In addition, two recombinant vaccine candidates have been developed using either a baculovirus-based expression system or a Venezuelan equine encephalitis replicon particle approach.[65,66]

Potent neutralization was induced in mice and NHPs following immunization with DNA expressing the full-length spike protein followed by a boost with S1 subunit glycoprotein. This strategy elicited responses against both RBD and non-RBD neutralizing epitopes which may decrease the chance of escape mutations. This DNA prime-protein boost regimen provided protection from computerized tomography (CT) defined pneumonia in a NHP viral challenge model.[67] This work highlights the importance of developing mAbs against the vaccine target antigen in order to define mechanisms of viral neutralization, protein structure, and antigenicity to guide rational vaccine design.

A DNA plasmid-based vaccine encoding full length consensus MERS spike protein was constructed based on available S protein genomic sequences in the

GenBank-NCBI database. The vaccine induced polyfunctional T cell and humoral responses in mice and NHPs, including antigen-specific neutralizing antibodies in mice, macaques, and camels. The vaccine also provided protection from pneumonia in a NHP viral challenge model.[68]

There are no clinical trial data yet for candidate MERS CoV vaccines. MERS CoV is likely to continue to cause sporadic outbreaks, fueled by camel exposure and the occasional super-spreading event. However, the relatively low $R_0$ suggests that MERS CoV has a relatively low probability of causing a widespread pandemic. Therefore, the target populations for a vaccine when available is not the general population, but groups at high risk of exposure to animal reservoirs, health care workers in endemic regions, and possibly at-risk travelers. With this relatively small market, it will be difficult for commercial organizations to invest in MERS CoV vaccine development. With such low incidence, a field study to evaluate efficacy will be large and difficult to complete. Therefore, questions again are raised about how to achieve advanced development and licensing of a vaccine for this type of emerging virus.

## 5 CONCLUSIONS

The activities needed to prepare for future pandemic viral threats include a broad spectrum of disciplines and skill sets ranging from logistics and communication to epidemiology and ecology, clinical trials, and highly technical biomedical research programs. Fortunately, relatively recent technological advances have made the prospects of a comprehensive program to systematically prepare for emerging infectious diseases more feasible. If done with forethought, the development of such a program would strengthen existing health care programs, build research infrastructure, and significantly improve the status of global public health. In particular, we should take advantage of new technologies such as high throughput sequencing, isolation of human monoclonal antibodies, structural biology, atomic-level antigen design, molecular biology, and vector biology. These tools can provide the information needed for rapid achievement of optimal expression, immunogenicity, production, and delivery of vaccine antigens for new emerging threats like the current crisis caused by Zika virus (Fig. 28.2).

Due to the new technologies available for surveillance, assay development, vaccine design, animal modeling, and manufacturing, the scientific aspects of vaccine development are not limiting our ability to prepare for emerging viral diseases. The major factors that need to be addressed include (1) the political will to provide the resources necessary to conduct the epidemiology and laboratory work needed to support vaccine development, (2) new business models to create an infrastructure for advanced vaccine development that does not require profit motive, and (3) creative regulatory processes and clinical trial designs to evaluate products for efficacy during outbreaks that are by nature sporadic and causing social chaos. Importantly, achieving solid efficacy data that can support licensure is critical so products can be more readily available during future epidemics.

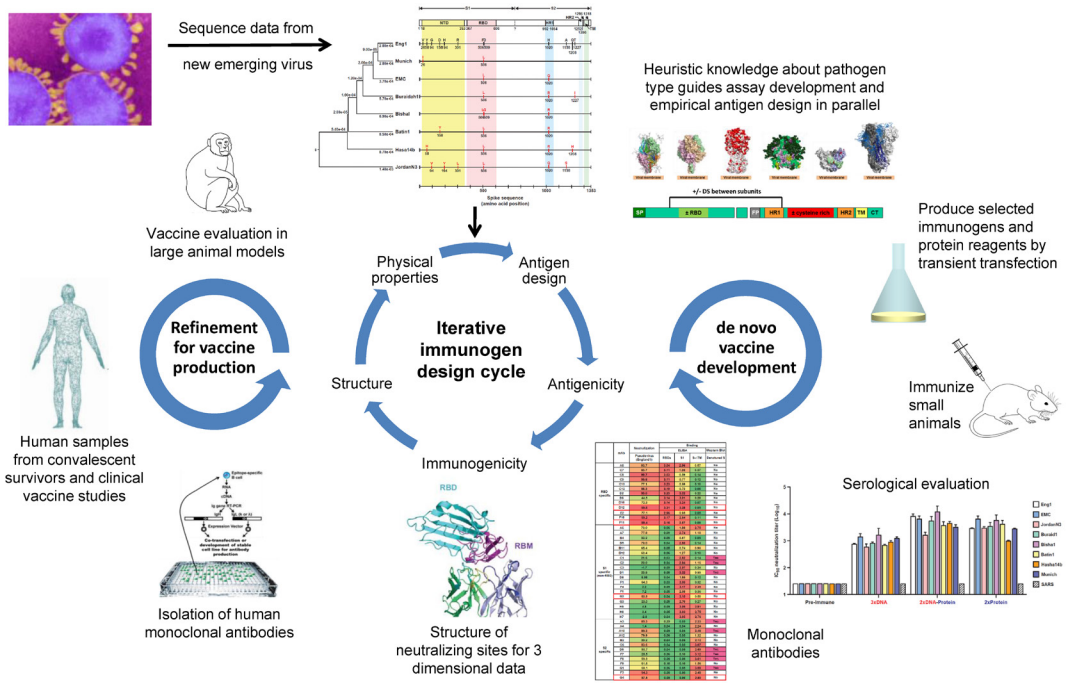


**FIGURE 28.2** **A new paradigm for vaccine development.** Novel approaches to vaccine design may significantly shorten vaccine development timelines and increase the frequency of success. New technologies that have rapidly evolved over the last 5–10 years make atomic level antigen design feasible and provide mechanisms for rapid, iterative improvements in antigenicity and immunogenicity of candidate vaccines. In particular, high throughput sequencing provides a starting point for vaccine antigens and helps to define the extent of genetic variability. Relatively inexpensive gene synthesis, the ease of isolating and characterizing human monoclonal antibodies (mAbs) against target antigens, the ability to define structures of vaccine antigens in complex with mAbs with desirable functional properties, and the development of assays that evaluate immunogenicity of vaccines in animal models, provide the foundation for rapid development of highly characterized candidate vaccines and their advancement into clinical evaluation. The figure illustrates the steps taken in the case of MERS CoV to rapidly develop the tools needed to generate candidate vaccines. Advanced knowledge of effective vaccine approaches for a particular virus family together with these modern development and design approaches may significantly shorten the time needed to prepare vaccines for field testing in the setting of a new pandemic threat.

## ACKNOWLEDGMENTS

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Chapter 29

# Cancer Immunotherapy by Checkpoint Blockade

**Nagahiro Minato, MD, PhD, Tasuku Honjo, MD, PhD**
*Graduate School of Medicine, Kyoto University, Department of Immunology and Cell Biology and Department of Genomic Medicine, Kyoto, Japan*

## Chapter Outline



## 1 INTRODUCTION

*In the last decades there has come into being, without either flourish of trumpets or serious controversy, a general current of belief in what I have come to call 'immunological surveillance'. – One can therefore picture a form of surveillance by which the body is being continuously patrolled, as it were, for the appearance of aberrant protein patterns.*

—M. Burnet[1]

The idea that immune responses may be involved in the survival or destruction of emerging malignant cells has risen as a sort of intuition rather than a solid scientific theory. This optimistic belief arose partly from clinical observations in humans; there are rare but well-documented instances where an established, well-proven malignancy has retrogressed on its own, as in the spontaneous remission of sarcoma in patients who develop erysipelas.[2] Somatic mutations occur constantly in the mammalian body, particularly in malignant cells, and an immune response against these new proteins was considered likely, at least theoretically. In 1960s the observation that a considerable range of systemic

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manifestations, apparently autoimmune in character, occur in various malignancies[3] led to the hypothesis that malignancy-related immune responses may also involve lymphocyte clones that react to autoantigens.[1]

Since then, a large body of evidence has accumulated for adaptive immune responses against cancers in both experimental animal models and humans. The gene encoding the first human tumor antigen specifically recognized by autologous cytotoxic T lymphocytes (CTLs) was cloned in 1991, followed by the identification of a number of tumor antigens in several types of cancer.[4,5] Some of these are "neoantigens" derived from viruses or somatic gene mutations, which might or might not be directly related to the oncogenesis. However, many of the tumor antigens, including differentiation antigens, overexpressed/amplified antigens, and germ (testis)-related antigens, show no detectable mutations,[6] and the responses are thus considered self-reactive in principle. Such adaptive immune responses, mostly detected in optimized culture conditions, do not automatically indicate their actual contribution to tumor eradication in the body, nor do they constitute axiomatic evidence for cancer immunosurveillance. However, a more recent study demonstrated a marked increase in tumor development, whether in response to a chemical carcinogen or occurring spontaneously with age, in mice with a completely defective adaptive immune system.[7] Most humans infected with the Epstein–Barr virus (EBV) remain asymptomatic throughout life despite EBV's strong oncogenic potential for B cells; however, in patients with genetic mutations in T-cell signaling, EBV causes aggressive X-linked lymphoproliferative diseases, including malignant lymphoma.[8] There are a number of case reports of the robust development of otherwise occult tumors in organ-transplantation recipients under continuous immunosuppressive regimens,[9] supporting the importance of the immune surveillance "flagship" in cancer prevention.

## 2 CANCER IMMUNOTHERAPY

Despite the reassuring evidence of adoptive immune responses against cancer cells, the incidence of cancer in humans increases with age. Cancer continues to pose a major threat to human life. It is apparent that immunosurveillance mechanism is not perfect, and some cancer cells may evolve mechanisms of escape from it. Indeed, cancers that develop in immune-sufficient hosts are far more resistant to the immune system than those in immune-deficient hosts; this phenomenon is called cancer immunoediting.[7] There have been numerous attempts to potentiate cancer immunity in humans in the last few decades. Historically, cancer immunotherapy has been based on two approaches—active immunotherapy to reinforce any endogenous adaptive cancer immunity in the host and passive (adoptive) cell therapy, in which immune effector cells are developed ex vivo and supplied back to the original host.

One form of active immunotherapy is therapeutic cancer vaccination (as opposed to prophylactic vaccination for infection), in which likely tumor antigens

are injected into the patient to boost immune responses. There have been clinical trials of cancer "vaccines" consisting of crude tumor cells, recombinant tumor antigen proteins/peptides, genetic vaccines (DNA), or antigen-presenting dendritic cells (DCs) loaded with cancer antigens ex vivo.[10] Cytokines in the IL-2 family (IL-2, IL-15, and IL-21) have also been tested extensively in attempts to boost immune responses to cancer.[11] IL-2 was the first cytokine to be approved by the FDA for treating metastatic renal cancer and melanoma; however, the therapeutic effects are modest, and its highly pleiotropic biological activities can cause severe toxicity.[12]

In adoptive immunotherapy, autologous immune effector cells are activated and expanded ex vivo via procedures that involve anti-CD3 antibodies, IL-2 and other stimulants, or autologous cancer cells, after which the cells are returned to the patient. Although one of the most efficient sources of immune cells has proven to be tumor-infiltrating lymphocytes (TILs) isolated from surgically resected tumor tissues,[13] the activated cells often contain heterogeneous effector populations, including nonspecific cytotoxic cells. In more recent studies, T cells have been genetically transduced with cancer antigen–specific T-cell receptors (TCRs) or chimeric receptors of antibody-variable fragments and TCR-signaling domains (CARs), which enables the T cells to recognize cancer antigens in a non-MHC-restricted manner.[14] Some of these approaches have been effective in patients with certain leukemia, but they are not established as standard therapies yet because of the technical laboriousness involved in treatment and the severe adverse effects such as cytokine-release syndrome.[15]

Recently, a third novel concept of cancer immunotherapy emerged, based on a molecular understanding of immune regulation. Acquired immune responses consist of two distinct phases: an initiation phase, in which naive antigen-specific T-cell clones are robustly expanded to form a sufficient population of effector cell progenies, and an effector phase, in which the differentiated T cells exert such effector functions as antibody production, inflammation, and target-cell destruction. In each phase, the immune responses are inherently and tightly controlled, with checkpoints to prevent excessive immune responses and to prevent attacks on normal tissue cells. The immune receptors CTLA-4 and PD-1, which play crucial roles in cancer immunity, have emerged as promising targets for cancer immunotherapy.

## 3 REGULATION OF IMMUNE RESPONSES

### 3.1 Costimulation Signals in T-Cell Activation

Although TCRs initiate immune responses by recognizing specific antigens, this mechanism alone hardly produces a noticeable response. Measurable T-cell clonal expansion and differentiation into effector progenies are achieved when costimulatory receptors engage, amplifying the TCR signal more than a hundred fold. The major costimulatory receptor, CD28, is activated when it engages

the ligands CD80 or CD86, which are exclusively expressed on professional antigen-presenting cells (APCs) such as DCs.[16,17] Defective CD28 engagement in T cells upon antigen stimulation may rather cause a long-lasting unresponsiveness called anergy, ensuring that productive immune responses are initiated only via professional APCs in lymphoid tissues.[18] Therefore, cancer cells, which usually lack CD80 or CD86 expression, are unlikely to directly induce T-cell responses in tissues; it is more likely that cancer antigens are "cross-presented" by professional APCs either via active shedding or by passive release from damaged cancer cells undergoing immunogenic cell death.[19] In contrast, functionally differentiated effector T cells are much less dependent on the CD28 costimulatory signal, and cancer cells that do not express CD80 or CD86 can induce the proliferation and activation of effector T cells. Thus, cancer cells can be directly recognized and killed by specific CTLs if the CTLs can migrate into the cancerous tissue to encounter the individual cancer cells.

## 3.2 Immune-Initiation Checkpoint via CTLA-4

Immune responses induce diverse collateral biological effects in the body. Therefore, the initiation as well as the magnitude of the responses is tightly controlled. The self-restriction of immune response initiation is mediated by CTLA-4, a structurally related CD28-counterpart that binds CD80 and CD86 with a much higher affinity than CD28.[20] While CD28 is constitutively expressed on resting T cells, CTLA-4 is expressed only after antigen stimulation. Once induced, CTLA-4 counteracts the TCR-costimulatory signal by physically sequestering CD80 and CD86 to prevent them from engaging with CD28 and also possibly by delivering a negative signal, thereby determining the initiation of any response.[21] In essence, engaging the CTLA-4 receptor preempts the CD28 costimulatory signal required for T-cell activation, switching off the response threshold (Fig. 29.1A). The importance of CTLA-4 in immune response is dramatically illustrated by *Ctla4*-deficiency. *Ctla4*$^{-/-}$ mice develop massive lymphoproliferation and die within 5 weeks after birth; this effect is attributed to uncontrolled, sustained T-cell activation.[22,23] It is apparent that not all antigens in the environment cause measurable immune responses, and a normal immune system's default responses against many inert environmental antigens may be produced through the CTLA-4 checkpoint to avoid unnecessary collateral damage. CTLA-4's function becomes particularly crucial in cancer immunity, since many cancer antigens appear to be subliminally immunogenic in nature.

## 3.3 Effector-Phase Checkpoint via PD-1

The immune system is equipped with inherent mechanisms at multiple stages to prevent attacks on normal tissues. T cells that are potentially reactive to specific self-tissue antigens are deleted during T-cell development in the thymus.[24] However, this central self-tolerance is by no means complete, and the immune

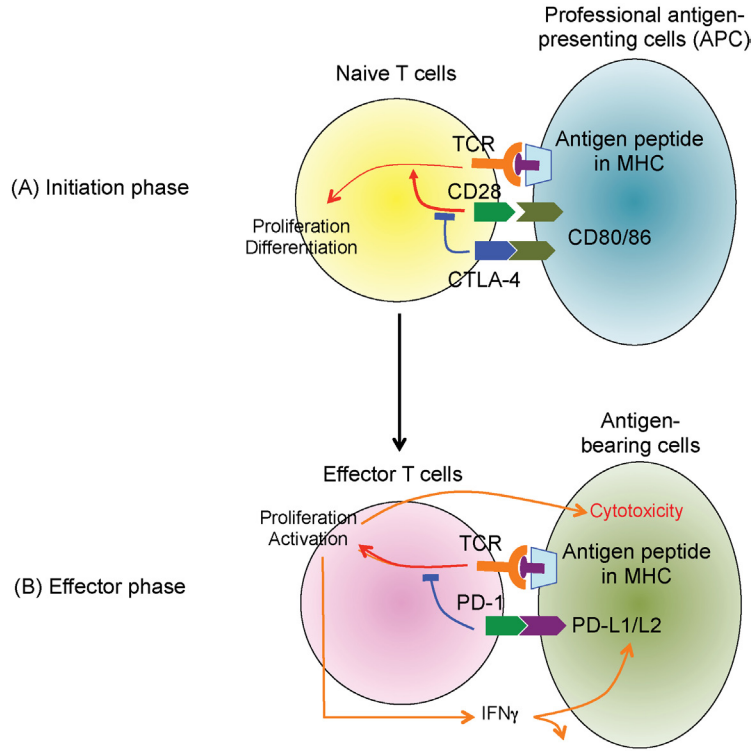


**FIGURE 29.1 Two checkpoints in the immune response, CTLA-4 and PD-1.** The immune response consists of an initiation phase (A) and an effector phase (B). In the initiation phase, naive T cells recognize specific antigens associated with MHC molecules via TCR, but observable activation and proliferation require an additional costimulatory signal from CD28. CD28 is activated by engagement with the ligands CD80 or CD86, which are expressed exclusively on dendritic cells (DCs) and other professional antigen-presenting cells (APCs). CTLA-4, which has a higher binding affinity to CD80 and CD86 and is induced after the initial TCR recognition, inhibits the CD28-mediated costimulatory signal by sequestering CD80/CD86 ("off" signal). CTLA-4 is a checkpoint molecule that determines whether a particular antigen should produce measurable T-cell proliferation and activation. In the effector phase, effector T-cell progenies developed from the initiation phase execute immune functions such as cytokine production and the killing of target cells. At this stage, the activation of effector cells via specific TCRs no longer requires a CD28-mediated costimulatory signal, and thus cytotoxic T cells (CTLs), for example, can affect any target cell bearing the antigen, even in the absence of CD80/CD86. However, the activated effector T cells are induced to express PD-1, which can inhibit TCR signaling if engaged with the ligands PD-L1 or PD-L2. PD-Ls are induced on normal tissue cells in response to IFNγ as on many cancers, thereby inhibiting effector T-cell activation and proliferation via PD-1. PD-1 is a checkpoint molecule that prevents potentially self-reactive effector T cells from attacking normal tissue cells.

system has additional checkpoints in the periphery to prevent detrimental autoimmunities. Self-reactive T cells that have encountered self-antigens on normal tissue cells that do not express CD80 or CD86 may fall into long-term anergy.[18] Even if these reactive T cells produce effector T cells, normal tissue

cells may still be protected from attack. Recent studies indicate that PD-1 is a crucial molecule in the self-tolerance checkpoint at the immune effector phase. PD-1 is a coinhibitory TCR-signaling receptor that is induced when T cells are activated by antigen. The engagement of PD-1 with its unique ligands, PD-L1 and PD-L2, results in the tyrosine phosphorylation of its intracellular domain and the recruitment of the protein tyrosine phosphatase SHP-2, thereby directly interfering with protein tyrosine kinase–based TCR-signaling.[25] Thus, PD-1 functions as a rheostat of effector T-cell activation.[26] Importantly, PD-L1/PD-L2 expression is not restricted to professional APCs; these molecules are rapidly induced in normal tissue cells by inflammatory cytokines such as IFNγ, which are secreted by nearby effector T cells upon activation. Unlike *Ctla4*-knockout mice, *Pdcd1*$^{-/-}$ mice remain healthy into later stages of life; however, they eventually develop overt autoimmune-mediated tissue damage, although the specific target tissues depend on the genetic background of the mice.[27,28] The expression of PD-L proteins in target tissues is crucial for preventing autoimmune damage to tissues.[29] Thus, the PDCD/PD-Ls system serves as an important checkpoint at the effector phase, protecting normal tissue cells from immune attack (Fig. 29.1B).

The PD-1/PD-Ls checkpoint function appears to be important in tumor immunity as well, since PD-Ls are upregulated in various cancer cells. This effect may be due to tumor cell–intrinsic signals related to oncogenes, or to adaptation and selection by adaptive immunity. On the other hand, most tumor-infiltrating effector T cells express PD-1 at high levels.[30] Thus, PD-L expression may effectively allow cancer cells to escape from host immune surveillance, regardless of the nature of the tumor antigens, by inhibiting the activation and cytotoxic activities of effector T cells. Indeed, in a retrospective analysis of patients with various cancers, PD-Ls expression in tumor tissues was found to correlate inversely with prognosis.[31] A similar situation may occur during viral infection, in which the destruction of virus-infected tissue cells by immune effector T cells is crucial for eventually eradicating the virus.

## 4 APPLICATIONS FOR CANCER THERAPY

The elucidation of immune-response checkpoints has led to the application of cancer immunotherapies that block the checkpoint receptors CTLA-4 and PD-1.

### 4.1 Animal Models

In 1996, Allison and coworkers tested CTLA-4 blockade as a cancer therapy in a mouse model that used a colon cancer cell line (51BLim10) transfected with CD80.[32] Although the CD80$^{+}$ 51BLim10 cells were less tumorigenic than the parental 51BLim10 cells as expected, blocking CTLA-4 by injecting an anti-CTLA-4 antibody strongly suppressed tumor growth regardless of whether the tumor cells expressed CD80. All of the mice that received three anti-CTLA-4

injections over the 6 days after tumor-cell inoculation remained tumor-free for more than 80 days. The anti-CTLA-4 antibody was also effective against highly aggressive fibrosarcoma cells. Importantly, the mice developed immune memory for the specific tumor cells, indicating that CTLA-4 blockade can elicit an effective adaptive immune response against cancer. Injections of anti-CTLA-4 also suppressed tumor development in a therapeutic setting as well. A series of preclinical studies demonstrated that anti-CTLA-4 treatment provided a significant benefit in animal models with strongly immunogenic tumors such as lymphoma, prostatic, and renal cancers.[33] In contrast, anti-CTLA-4 treatment had little or no effect against the growth of tumors with poor inherent immunogenicity, such as B16 melanoma and SM1 mammary cancer cells.[33] However, therapies that combined an anti-CTLA-4 antibody with tumor vaccines such as GM-CSF-transduced B16 melanoma cells produced a marked synergism that inhibited tumor growth.[34] The eradication of B16 melanoma in mice was often associated with systemic depigmentation, suggesting that self-reactive T cells are involved in the antitumor effect.

On the other hand, Iwai et al. reported that P815 mastocytoma cells expressing exogenous PD-L1 were more resistant to specific CTLs than the parental PD-L1$^-$ P815 cells, and when transferred into recipient mice, produced highly aggressive and invasive tumors compared with those produced by parental P815 cells.[35] Treatment with an antibody against PD-L1 strongly inhibited the aggressive growth of PD-L1$^+$ P815 tumor cells. Although all of the control mice inoculated with PD-L1$^+$ P815 tumor cells died within 45 days, nearly 40% of mice that were treated with an anti-PD-L1 antibody for the first 7 days after tumor-cell inoculation remained tumor-free for more than 100 days, indicating a complete cure. Anti-PD-L1 treatment had a similar beneficial effect on mice inoculated with J558L myeloma cells that endogenously expressed PD-L1. This was the first report to indicate that the expression of PD-L1 on tumor cells profoundly affects their susceptibility to the host adaptive immune response at the effector level. Importantly, no tumors developed in *Pdcd1*$^{-/-}$ mice after inoculation with J558L cells, whereas control mice rapidly developed tumors and died. These results confirmed that the potent tumor-suppressive effects of anti-PD-L1 are actually due to the blockade of PD-1/PD-L interactions between tumor cells and effector T cells.

Chen and coworkers identified B7-H1, which later turned out to be identical to PD-L1, by a database homology search with human CD80 and CD86.[36] Although B7-H1 did not bind CD28, CTLA4, or ICOS, they reported that the treatment of T cells with a B7-H1-Ig fusion protein enhanced the proliferation and IL-10 production via TCR-stimulation. The authors thus concluded that B7-H1 is a costimulatory rather than coinhibitory molecule. They also found that B7-H1 is frequently expressed on human cancer cells, and that B7-H1$^+$ cancer cells induced the apoptosis of specific CTLs independently of PD-1.[37] Forced B7-H1 expression in CD80$^+$ P815 cells, but not in the original CD80$^-$ P815 cells, increased tumorigenesis in recipient mice, leading to the proposal that

B7-H1 (PD-L1) on tumor cells induces effector T-cell apoptosis via a costimulatory effect through unknown receptors other than PD-1. However, no B7-H1 (PD-L1) receptors other than PD-1 have been reported so far, and the proposed mechanism remains to be verified.

As with the blockade of CTLA-4, *Pdcd1*-deficiency did not significantly affect the ability of poorly immunogenic B16 melanoma cells to form tumors in subcutaneous primary sites. Interestingly, however, the hematogenous spread of B16 cells from the spleen to the liver was markedly inhibited by a genetic *Pd1* deletion or by anti-PD-1 antibody treatment.[38] B16 myeloma cells expressed little PD-L1 in culture, but expressed PD-L1 at significant levels in vivo in the spleen, and a blockade of PD-1 was associated with a marked increase in the expansion and accumulation of $CD8^+$ effector T cells in metastatic tumor tissues. Blocking PD-1 induced similar effects on the hematogenous metastasis of CT26 colon cancer cells to the lungs, indicating that the remote metastasis of tumor cells may be even more susceptible to PD-1 blockade than their growth at primary sites. The profound effect of PD-1 blockade on cancer immunity at the effector phase was further elucidated by Blank et al.[39] in experiments with mice expressing a TCR transgene (2C) specific for a model antigen peptide (SIY). Although B16 melanoma cells expressing SIY antigen (SIY-B16) did not activate T cells from 2C transgenic mice to any significant degree, T-cell effector functions such as IFNγ production and cytotoxic activity were strongly activated in the T cells from $Pdcd1^{-/-}$ 2C mice. The presence of an anti-PD-L1 antibody also restored the activation of 2C effector T cells, indicating that the default response was due to PD-1 engagement with PD-L1 expressed on SIY-B16 melanoma cells. Furthermore, $Pdcd1^{-/-}$ 2C T cells completely suppressed tumor development in immunodeficient mice upon transfer, whereas the original 2C T cells or $Ctla4^{-/-}$ 2C T cells failed to do so. These results indicate that blocking PD-1 enhances cancer immunity far more effectively than blocking CTLA-4 in the effector phase.

## 4.2 Clinical Studies of Checkpoint Immunotherapy

The first clinical trial of a humanized anti-CTLA-4 antibody (ipilimumab) in combination with gp100 melanoma-associated antigen, conducted in melanoma patients, was reported in 2003.[40] Of 14 patients, 3 (21%) showed objective cancer regression with two complete responses. Six patients had severe autoimmune manifestations, including dermatitis, enterocolitis, hepatitis, and hypophysitis. Subsequently, a series of clinical trials were conducted to test ipilimumab's safety and efficacy.[41] A large-scale Phase III trial of ipilimumab and gp100 in 676 patients that had previously been treated for metastatic melanoma confirmed that ipilimumab significantly extended survival[42]; the median survival period was 10 months in the ipilimumab-only and the ipilimumab plus gp100 groups, compared with 6.4 months in the gp100-only group. This was the first convincing evidence of the benefits of checkpoint immunotherapy for cancer in human patients. Another Phase III trial examined the effects of dacarbazine (a standard

chemotherapy agent) plus ipilimumab (10 mg/kg) in 502 patients with previously untreated melanoma.[43] The overall survival was significantly longer in the group receiving ipilimumab plus dacarbazine (11.2 months) than the group receiving dacarbazine (9.1 months). Based on these results, the FDA approved ipilimumab for unresectable or metastatic melanoma in 2011. The FDA recommended an intravenous infusion of ipilimumab (3 mg/kg) every 3 weeks for a total of four doses, with a caution regarding serious adverse autoimmune effects. The FDA approval of ipilimumab for human use was not achieved until 15 years after the first demonstration of an effective CTLA4-blockade therapy in animal models.

The clinical application of PD-1 blockage took a slightly different path. The first hint that PD-1 blockade might be effective against human malignancies was found in a reverse correlation between the survival rate after radical tumor resection and PD-L1 expression in the tumors. Thomson et al. reported that the risk of death after radical nephrectomy was 4.5 times higher for patients with renal cancers with high PD-L1 levels than for those with cancers with low PD-L1, based on a retrospective analysis.[44] The survival rates 3 years after the operation were 63.2 and 88.4% for patients with renal cancers expressing high and low PD-L1, respectively. Similar findings were reported for esophageal cancer, gastric cancer,[45] ovarian cancer,[46] urothelial cancer,[47] pancreatic cancer,[48] melanoma,[49] and other cancers.

Clinical trials of a humanized anti-PD-1 antibody (nivolumab), modified to reduce the antibody-dependent cell-mediated cytotoxicity (ADCC) activity, began in 2006 in the United States and in 2009 in Japan. A comprehensive Phase I study of nivolumab at doses of 0.1, 0.3, 1.0, 3.0, and 10.0 mg/kg was conducted with 296 patients with late-stage nonsmall cell lung cancer (NSLC), melanoma, or renal cell cancer (RCC).[50] Among the 236 patients in whom the response could be evaluated, the cumulative response rate at all doses was 18% for patients with NSLC, 28% for melanoma, and 29% for RCC. Notably, the response lasted for more than 2.5 years in the responsive patients, with far fewer side effects than seen with ipilimumab. A similar study using an anti-PD-L1 antibody showed a comparable efficacy and duration of response.[51] In a recent double-blind test of nibolumab and dacarbazine for previously untreated melanoma without BRAF mutation,[52] 418 patients were randomly divided into two groups; one group received nivolumab (3 mg/kg) every 2 weeks plus a dacarbazine-matched placebo, and the other group received dacarbazine plus a nivolumab-matched placebo. The overall survival rate at 15 months was 70% in the nivolumab group and 20% in the decarbazine group, and the objective response rate was 40% in the nivolumab group and 14% in the decarbazine group. Another study used combined anti-PD-1 and anti-CTLA-4 therapies to treat advanced melanoma,[53] and found that the objective response rate was dramatically enhanced to 53% at the maximum doses, the duration of efficacy lasting at least nearly 2 years. A surprising effect of PD-1 blockade was reported in relapsed or refractory Hodgkin's lymphoma, in which chromosomal alterations at 9p24 increase the PD-L1 and PD-L2 expression[54]; of 23 patients treated with

nivolumab, an objective response was reported in 20 patients (87%), including a complete response (17%), a partial response (70%), and stable disease (13%). In colorectal cancers, anti-PD-1 antibody was particularly effective in patients with mismatch-repair deficiency of cancer cells, which probably contributes to the increased cancer neoantigens.[55] Reports of major clinical trials of PD-1 blockade are summarized in Table 29.1.[56–62] Overall, the efficacy of PD-1 blockade in various types of cancers is quite unprecedented among cancer therapies. At the beginning of 2015, PD-1 blockage has been approved for melanoma, and nonsmall cell lung cancer in United States, European Union, and Asia, and several types of antibodies against PD-1 and PD-L1 have been introduced to clinical trials. We expect a rapid expansion of anti-PD-1 tumor immunotherapy in a near future.

## 5 APPLICATION FOR INFECTIOUS DISEASES

Because a checkpoint blockade elicits the potential immune response, it seems reasonable to apply this strategy also to infectious diseases. A CTLA-4 blockade has not been tried for infectious diseases, probably due to the high risk of severe autoimmunity. Iwai et al. was the first to demonstrate the involvement of the PD-1/PD-L system in viral infection through experiments with a lacZ-expressing adenovirus, which is easily visualized by a blue color in tissues.[63] After acute infection, the virus was cleared much earlier in *Pdcd1*$^{-/-}$ mice than in control mice, and the virus completely disappeared from the liver by day 30. Although *Pdcd1*$^{-/-}$ mice showed transient liver damage on day 7, there was no histological evidence of hepatitis on day 30. It was subsequently reported that chronic lymphocytic choriomeningitis virus (LCMV) infection in mice was associated with the accumulation of PD-1$^{high}$ CD8$^{+}$ effector T cells that were unresponsive to viral antigens, or were exhausted. Treatment with anti-PD-L1, but not anti-CTLA-4, restored antiviral immunity and decreased the viral load.[64] An increase in anergic PD-1$^{high}$ CD4$^{+}$ T cells was also observed with age in normal mice.[65]

The possibility of applying a PD-1 blockade for HIV infection was tested in macaques infected with SIV.[66] In macaques treated with a partially humanized PD-1 antibody, SIV-specific CD8$^{+}$ effector T cells increased and viremia was drastically reduced. Four out of five animals treated with anti-PD-1 survived, although all of the animals treated with control antibody died. Blocking PD-1 reduced the virus load in chronically HIV-infected humanized mice.[67] Clinical trials of a PD-1 blockade have not been pursued for HIV patients, probably because of the recent development of combination chemotherapy. However, it may be beneficial to combine chemotherapy and PD-1 blockade to improve the efficacy of HIV therapy. The PD-1 antibody has also been shown to effectively suppress sepsis induced by polymicrobial infection after gut puncture in an animal model.[68] Altogether, PD-1 blockade therapy appears promising for controlling intractable infectious diseases.

**TABLE 29.1** Summary of Clinical Trials for PD-1 Checkpoint Blockade Immunotherapy

| Patients | Regimens | Clinical response | Side effects | Conclusion | References |
|---|---|---|---|---|---|
| Advanced Mel, NSLC, RCC | [Phase I/II] anti-PD-1 | Objective response rate: Mel 28%, squ. NSLC 33%, non-squ. NSCL 12%, RCC 27%, PD-L1(+) cancers of all 36%, PD-L1(−) cancers of all 0% PFS rate at 6 months; Mel 41%, squ. NSLC 33%, non-squ. NSCL 22%, RCC 56% | Grade 3–4, 15% | Anti-PD-1 antibody produced objective responses of long duration in approximately in patients with Mel, NSCL, or RCC. A relationship between PD-L1 expression on tumor cells and objective response is suggested. | [50] |
| Advanced Mel, NSLC, OC, RCC | [Phase I/II] anti-PD-L1 | Objective response rate: Mel 17%, squ. NSLC 8%, non-squ. NSCL 11%, OC 6%, RCC 12% PFS rate at 6 months; Mel 42%, squ. NSLC 43%, non-squ. NSCL 26%, OC 22%, RCC 53% | Grade 3–4, 9% | Anti-PD-L1 antibody induced durable tumor regression and prolonged stabilization of diseases in patients with advanced cancers. | [51] |
| Advanced Mel | [Phase III] anti-PD-1 (nivolumab) + anti-CTLA-4 (ipilimumab) | Objective response rate: 40% aggregate clinical activity (CR + PR + SD); 65%, >80% tumor reduction at 3 months; 31% in all doses and 100% in optimal dose | Grade 3–4, 53% | Concurrent therapy with anti-PD-1 and anti-CTLA-4 provided a distinct clinical activity from monotherapy, with rapid and deep tumor regression in a substantial proportion of advanced melanoma patients. | [53] |
| Metastatic bladder cancer (BC) | [Phase I/II] anti-PD-L1 (MPDL3280A) | Objective response rate: PD-L1 med–high in IHC 40–50%, PD-L1 neg–low in IHC 8–13% | Grade 3–4, 4.4% | Anti-PD-L1 antibody has noteworthy activity in metastatic urothelial BC and tumors expressing PD-L1+ tumor-infiltrating immune cells had particularly high response rates. | [56] |

(*Continued*)

**TABLE 29.1** Summary of Clinical Trials for PD-1 Checkpoint Blockade Immunotherapy (*cont.*)

| Patients | Regimens | Clinical response | Side effects | Conclusion | References |
|---|---|---|---|---|---|
| Relapsed or refractory Hodgkin's lymphoma | [Phase II] anti-PD-1 (nivolumab) | Objective response rate: 87% (CR 17%, PR 70%, SD 13%, PD 0%) PFS at 6 months, 86% | Grade 3–4, 22% | Anti-PD-1 antibody had substantial therapeutic activity in patients with relapsed or refractory Hodgkin's lymphoma, in which alterations in chromosome 9p24.1 increase the abundance of PD-1 ligands. | [54] |
| Previously untreated Mel w/o BRAF mutation | [Phase III] anti-PD-1 (nivolumab) randomized control study | Objective response rate: (A) nivolumab 40.0%, (B) dacarbazine 13.9% OS rate at 1 year; (A) 72.9%, (B) 42.1% median PFS; (A) 5.1 months, (B) 2.2 months | Grade 3–4, (A) 11.7%, (B) 17.6% | Anti-PD-1 antibody was associated with significant improvements in overall survival and progression-free survival as compared with a standard chemotherapy with decarbazine. | [52] |
| Advanced, refractory squ. NSLC | [Phase II] anti-PD-1 (nivolumab) | PFS at 6 months; 25.9%, 12 months 20.0% OS at 12 months; 40.8% objective response rate; PD-L1(−) tumors: PR 14%, SD 20%, PD 49%, PD-L1(+)tumors: PR 24%, SD 24%, PD 44% | Grade 3–4, 17% | Anti-PD-1 antibody had clinically meaningful activity and a manageable safety profile in previously treated patients with advanced, refractory squamous nonsmall-cell lung cancer. | [57] |
| Advanced, refractory Mel | [Phase III] anti-PD-1 (nivolumab) randomized control study | Objective response rate: (A) nivolumab 31.7%, (B) ICC 10.6% PFS at 6 months; (A) 48%, (B) 34% | Grade 3–4, (A) 5%, (B) 9% | Anti-PD-1 antibody led a greater proportion of patients achieving an objective response and fewer toxic effects than with alternative chemotherapy regimens for patients with advanced melanoma that has progressed after ipilimumab. | [58] |

| | | | | | |
|---|---|---|---|---|---|
| Advanced NSLC | [Phase II] anti-PD-1 (pembrolizumab) | Objective response rate: in all 19.4%, in PD-L1(+) 45.2%; median duration of response: in all 12.5 months, PFS in all 3.7 months, in PD-L1(+) tumors 6.3 months, median OS: in all 12.0 months, in PD-L1(+) tumors >16 months | Grade 3–4, 13.8% | Anti-PD-1 antibody had an acceptable side-effect profile and showed antitumor activity in patients with advanced nonsmall-cell lung cancer, and PD-L1 expression of tumor cells correlated with improved efficacy. | [60] |
| Advanced Mel | [Phase III] anti-PD-1 (pembrolizumab) randomized control study | Objective response rate: (A) pembrolizumab every 2 weeks 33.7%, (B) pembrolizumab every 3 weeks 32.9%, (C) ipilimumab every 3 weeks 11.9%, PFS rate at 6 months: (A) 47.3%, (B) 46.4%, (C) 26.5%, OS rate at 12 months: (A) 74.1%, (B) 68.4%, (C) 58.2% | Grade 3–5, (A) 13.3%, (B) 10.1%, (C) 19.9% | Anti-PD-1 antibody prolonged progression-free survival and overall survival and had less high-grade toxicity than did ipilimumab in patients with advanced melanoma. | [59] |
| Advanced CRC w or w/o mismatch repair deficiency | [Phase II] anti-PD-1 (pembrolizumab) | Objective response rate: (A) mismatch-repair deficient: 40%, (B) mismatch-repair proficient 0%, PFS rate at 6 months: (A) 78%, (B) 11%, median PFS: (A) >12 months, (B) 2.2 months, median OS: (A) >12 months, (B) 5.0 months | Grade 3–4, 41% in all | Mismatch-repair status predicted clinical benefit of immune checkpoint blockade with anti-PD-1 antibody. | [55] |

(*Continued*)

**TABLE 29.1** Summary of Clinical Trials for PD-1 Checkpoint Blockade Immunotherapy (*cont.*)

| Patients | Regimens | Clinical response | Side effects | Conclusion | References |
|---|---|---|---|---|---|
| Advanced, metastatic Mel | [Phase III] anti-PD-1 (nivolumab) randomized control study | Objective response rate: (A) nivolumab: 43.7%, (B) nivolumab + ipilimumab 57.6%, (C) ipilimumab 43.7%, median PFS: (A) 6.9 months [PD-L1(+) tumors 14.0 months], (B) 11.5 months [PD-L1(+) tumors 14.0 months], (C) 2.9 months | Grade 3–5, (A) 16.3%, (B) 55.0%, (C) 27.3% | Anti-PD-1 antibody alone or combined with anti-CTLA-4 antibody resulted in significantly longer progression-free survival than anti-CTLA-4 alone. In patients with PD-L1-negative tumors, the combination of PD-1 and CTLA-4 blockade was more effective than either agent alone. | [62] |
| Advanced, refractory squ. NSLC | [Phase III] anti-PD-1 (nivolumab) randomized control study | Objective response rate: (A) nivolumab: 20%, (B) docetaxel: 9%, Median PFS: (A) 3.5 months, (B) 2.8 months, 1 year survival rate: (A) 42%, (B) 24%, OS: (A) 9.2 months, (B) 6.0 months | Grade 3–4, (A) 7%, (B) 55% | Overall survival, response rate, and progression-free survival were significantly better with nivolumab than with docetaxel, regardless of PD-L1 expression level in tumor. | [61] |

Abbreviations: Mel, melanoma; NSLC, nonsmall-cell lung cancer; squ., squamous; OC, ovary cancer; RCC, renal cell cancer; BC, urothelial bladder cancer; CRC, colorectal cancer; PFS, progression-free survival; OS, overall survival; IHC, immunohistochemistry; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable; ICC, investigator's choice of chemotherapy.

## 6 SUMMARY AND FUTURE PERSPECTIVES

Cancer immunotherapy based on an immune-checkpoint blockade is one of the most effective cancer treatments currently available (Table 29.2). Immune-checkpoint blockade therapy can be applied to a broad range of cancer types and stages with an effect of long duration, and its adverse effects may be less serious than with other current treatments. With these strong advantages, we expect that immune-checkpoint blockade therapies will become the first line of treatment for many types of cancer. The PD-1 blockade, in particular, is preferable to chemotherapies and radiotherapies that have similar efficacy since its side effects tend to be less severe. Any newly developed cancer drugs will have to show a comparable or higher efficacy than the PD-1 blockade to be approved. It may be possible to increase the effect of immune-checkpoint blockade therapies by combining them with lower doses of chemotherapeutic drugs or γ-ray irradiation to enhance cancer-cell immunogenicity. The immune-checkpoint blockade strategy is likely to be a turning point that will significantly change our present views and strategies for treating cancer. Nonetheless, there are several problems with current checkpoint immunotherapies that must be addressed. First, the response rates are still limited; the responses are 10∼20% for CTLA-4 blockade and 20∼30% for PD-1 blockade in late-stage melanoma. This is not surprising, considering the inherent individual variations in immune response to various infectious pathogens, allergens, and vaccines. In this respect, it is desirable to find biomarkers that can predict responses to a checkpoint blockade. The original checkpoint-blockade experiments in mouse models as well as many clinical trials indicated that the tumor expression of PD-L1 might be a good marker for responsiveness to a PD-1 blockade. Other potential biomarkers have been suggested by several clinical studies, including unique genomic changes in cancer cells such as *PD-L1/2* locus rearrangements/amplification and mismatch-repair deficiency. A recent study indicates that high circulatory levels of soluble CTLA-4 may correlate with responsiveness to anti-CTLA-4 in melanoma patients.[69]

**TABLE 29.2 Comparison of Checkpoint Immunotherapy With Other Cancer Treatment Strategies**

| Strategies | Duration of effectiveness | Side effects | For multiple metastasis | Types of tumors |
|---|---|---|---|---|
| Checkpoint immunotherapy | Long | Low | Applicable | Broad |
| Chemotherapy | Short | High | Applicable | Specific types |
| Radiotherapy | Medium | Medium | Difficult | Limited |
| Surgical resection | Long if complete | Low | Difficult | Solid tumors |

The identification of predictive biomarkers should be of great help in selecting patients who will respond to the treatment. Second, although the frequency of severe adverse effects is relatively low, there are significant adverse effects, mostly due to autoimmune reactions. These adverse autoimmune effects are apparently more striking in a CTLA-4 blockade than a PD-1 blockade, as anticipated from the greater severity of phenotypes in *Ctla4*$^{-/-}$ mice than in *Pdcd1*$^{-/-}$ mice. It may be possible to strategically optimize combination checkpoint-blockade therapies to reduce the dosage of each component, thereby lowering the risk of adverse effects, without compromising efficacy. In summary, we now have a novel means for cancer treatment with an unprecedentedly promising therapeutic potential. Further studies to improve the efficacy and reduce adverse effects should lead to a paradigm shift in cancer therapies for humans.

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Chapter 30

# Adoptive Cellular Therapy With Synthetic T Cells as an "Instant Vaccine" for Cancer and Immunity

**Carl H. June, MD**

*Center for Cellular Immunotherapies, Abramson Cancer Center; University of Pennsylvania, Department of Pathology and Laboratory Medicine, Perelman School of Medicine, Philadelphia, PA, United States*

## Chapter Outline



## 1 INTRODUCTION

The fields of vaccinology and adoptive cell transfer (ACT) converge in the shaping of the acquired cellular and humoral responses of the B- and T cells of the immune system toward desired antigens on pathogens or self-targets. However, the approaches differ markedly, with vaccines often requiring immunizations and boosting over a period of months, whereas ex vivo culture of T cells and gene transfer can generate sufficient T cells in less than a week (Fig. 30.1). The process of ACT is more complex but the power of the resultant T cells may justify the expense and effort in some areas such as cancer and chronic infection, where vaccines often fail.

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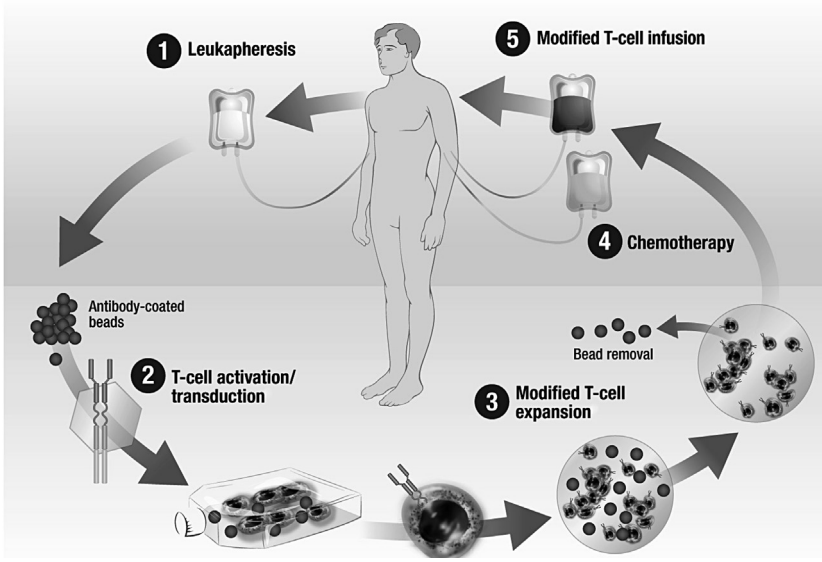


**FIGURE 30.1 General approach for trials with ACT.** The patient donates lymphocytes by phlebotomy, and the cells are genetically modified and expanded in numbers in the manufacturing facility. In an optional step, the patient may receive chemotherapy before intravenous infusion of the CAR T cells.

## 1.1 History of Adoptive Cell Transfer

A scientific and technological revolution in the war against cancer has taken place in the past century. Paul Ehrlich was probably the first to envision targeted therapies with "magic bullets,"[1] an idea that was actualized by the invention of monoclonal antibodies and has inspired generations of scientists to devise powerful molecular cancer therapeutics. In this review the potential uses of chimeric T cells, modified to express antibody fragments is discussed.

The idea that passive transfer of primed immune cells can generate immunity in the recipient of this transfer is a relatively old idea in the history of immunology. As first proposed by Billingham, Brent, and Medawar in 1954, who coined the term "adoptive immunity,"[2] numerous animal studies have demonstrated the effectiveness of adoptive transfer of immunity toward cancer and infectious disease. Immunity has remarkable specificity toward its targets, and specificity can be controlled through strategies such as in vivo and ex vivo priming and genetic engineering to install receptors of defined specificities. Moreover, it has the potential to induce longstanding effects via the establishment of immunologic memory.

## 1.2 Lymphocyte Cell Transfer

In early studies, the transfer of pig and nonhuman primate lymphocytes were tested and found inadequate for ACT due to transient engraftment.[3,4] However,

with the advent of efficient tools for genetic editing, it is not inconceivable that the use of xenogeneic cells for ACT might be reconsidered. Currently, human T cells with alpha beta T-cell receptors (TCRs) are currently the cell of choice for most clinical trials, reviewed in Ref. [5]. Gene transfer of MHC class I-restricted TCRs can "convert" a population of polyclonal CD8+ T cells to cytotoxic T-lymphocytes (CTLs) of monoclonal TCR specificity.[6] The adoptive transfer of engineered CD4+ T cells has promise for adoptive therapy of cancer and HIV, reviewed in Refs. [7,8].

Recent advances in the understanding of the biology of γδ T cells suggest that these cells have promise for ACT. For example, human γδ T cells can prime αβ T cells with an efficiency similar to that of dendritic cells.[9] Human γδ T cells can be engineered to express chimeric antigen receptors (CARs) and αβ TCRs.[10,11] The use of γδ T cells may provide a significant safety feature over the use of TCR-engineered αβ T cells, taking advantage of the finding that α and β TCR chains cannot pair with γ and δ TCR chains.

Natural killer T (NKT) cells are functionally related to γδ T cells since they also bridge innate and adaptive immune responses and can enhance or suppress immunity.[12] The best characterized human NKT cell subpopulation, referred to as invariant NKT (iNKT) cells, expresses CD161 and an invariant Vα24Jα18 TCR that recognizes α-galactosylceramide presented by the MHC class I-like molecule CD1d. After activation, iNKT cells have MHC-independent cytotoxic activity against various tumors and secrete high levels of interferon-γ, although this function becomes impaired in patients with cancer.[13] Compared with mouse NKT cells, human NKT cells are rare and comprise $<1\%$ of total lymphocytes. However, human NKT cells, unlike mouse NKT cells, can undergo substantial expansion in vitro.[14] The first ACT studies of iNKT cells have been conducted and show safety and some evidence of antitumor activity.[15]

## 1.3 Emergence of Immuno-Oncology

Targeting of disease through the adoptive transfer of lymphocytes was first reported more than 50 years ago in rodent models.[16] However, it is only recently that the widespread use of immune-oncology has been accepted into the daily practice of medicine. This is best illustrated by the striking success of immune checkpoint blocking antibodies, which have achieved the complete and sustained remission of advanced solid tumors,[17] and the use of genetically engineered T cells for melanoma and leukemia.[18] The field of ACT has been made possible by improved understanding of T-cell biology, including the mechanisms for T-cell activation and recognition of targets, the role of accessory surface molecules and signal transduction pathways involved in the regulation of T-cell function and survival, as well as the identification and cloning of soluble T-cell growth factors, has facilitated the ability to expand ex vivo large numbers of highly potent T cells for adoptive immunotherapy. There are several excellent reviews of the experimental basis for ACT therapy of tumors and chronic infections.[19–21]

## 2 DISCOVERY OF T CELLS WITH STEM-CELL-LIKE QUALITIES

Mouse syngeneic tumor models have been essential for the identification and preclinical optimization of many tumor therapies. However, mouse tumor models in general have not been a good predictor of responses to ACT for cancer although they have been quite useful for chronic infections. In part this is likely due to the fact that most tumor models in mice don't mirror the mutational loads and chronic inflammation found in humans, and due to the fact that the onset human T-cell senescence is quite different from mice, reviewed in Ref. [20]. The seminal discovery of telomerase[22] and the developing field of telomere biology identified fundamental differences in mechanisms of immune senescence between mice and humans[23–25] that have important implications for ACT. In human T cells, a decrease in mean telomere restriction fragment length was shown for increasing age.[26,27] As one consequence of the appreciation of the differences in biology between mouse and human T cells, the duration of culture for human T cells used in adoptive transfer has decreased from a month or longer to days to a few weeks, resulting in preservation of telomere length in infused T cells.

More recent studies in the mouse indicate that a memory stem cell subset of CD8+ T cells exists.[28] Similar T cells with stem cell-like qualities have been identified in humans.[29,30] In elegant and technically demanding studies, these memory stem T cells were shown to have plasticity and that a single naive T cell can reconstitute diverse effector and memory T-cell subsets in mice.[31,32] Ongoing studies in human allotransplant patients indicate that infusions of very low numbers of CMV-specific T cells are able to provide protective immunity.[33]

Efficient systems for the growth of human T cells were developed based on principles of costimulation.[34,35] When these culture systems were employed in patients with HIV infection, efficient engraftment and long-term persistence of T cells was demonstrated after ACT.[36,37] CD4+ T cells rendered CCR5-deficient by zinc finger nuclease (ZFN) editing also have been shown to persist for at least 5 years after ACT in patients with chronic HIV infection, consistent with the existence of central memory T cells in humans with long life spans.[38]

## 3 APPROACHES TO ENGINEER LYMPHOCYTES USING SYNTHETIC BIOLOGY

In contrast to promising results in patients with chronic infection, ACT with natural T cells was not generally shown to be beneficial in cancer patients. A major reason for this is that tolerance to tumor antigens is induced by a variety of mechanisms, including deletion of tumor reactive cells and the induction of adaptive resistance mediated by checkpoint molecules such as CTLA-4 and PD1.[39]

Synthetic biology, an emerging discipline aimed at reprogramming living organisms through the combined use of genetics, engineering principles, and systems and computational analysis, is able to enhance T-cell behavior.[40–42] Cells are inherently capable of carrying out complex computations and responses, and CTLs of the immune system in particular are composed of cells poised to kill tumor cells after through careful assessment of targets. Approaches to genetically engineered lymphocytes have been reviewed previously.[5] Using the principles of gene transfer it is possible to change "at will" the specificity of T cells using CAR and TCR of known specificity and affinity. Additional strategies have involved the overexpression in T cells of prosurvival signals such as telomerase,[43] antiapoptotic genes,[44] and the downregulation of proapoptotic molecules such as Fas.[45] Yet another approach to enhance T-cell survival involves the expression of dominant-negative receptors for inhibitory molecules such as dominant-negative receptors for TGF-beta.[46]

Over the past decade a number of tools including ZFN, transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 system (CRISPER/Cas9) have been developed to permit gene-specific disruption and site-specific insertion of novel DNA sequences.[47] To date only ZFNs have been used for human trials.[38] However, in vitro these tools are enabling the discovery of genes that increase the function by T cells by loss of function or gain of function mutations. For example, Zhou and coworkers[48] reported a novel RNA-interference based screen conducted in vivo using ACT with transduced TCR transgenic T cells, with enrichment of shRNAs occurring in T cells whose numbers in the tumor bed increased upon knock down of relevant genes. Others used a CRISPR library approach for the in vitro analysis of LPS signaling in dendritic cells.[49] Both screens identified known as well as unknown targets that affected the response being assessed. Using CRISPR/Cas9 technology, Konermann and coworkers reported a discovery method testing selective genome-wide gene activation to achieve desired phenotypes.[50] Together, these new tools are overcoming a key challenge facing the cancer immunology field in the discovery of the most suitable targets to synthetically "hack" T cell to enhance T-cell antitumor functions.

The diverse approaches to effectively engineer T cells combined with recently acquired mechanistic insights into T-cell biology and tumor immunity have converged to the point where the rational engineering of potent antitumor T-cell immunity is a practical and clinically testable reality. The majority of clinical approaches have employed T cells engineered to stably express transgenes via virus-based transduction. Virus-mediated gene transfer approaches typically employ vectors that are derived from gamma retroviruses or more recently lentiviruses, principally because of their ability to integrate into the host genome and drive long-term transgene expression, as well as for their low intrinsic immunogenicity. Gammaretrovirus-based transduction requires replicating cells for viral integration into genomic DNA, whereas lentiviral vectors

can also integrate into nondividing cells; lentiviral vectors also appear to be less susceptible to silencing by host restriction factors and can deliver larger DNA sequences than retroviruses.[51] Although virus-based approaches result in reasonably efficient transduction of primary T cells, they have considerable limitations in terms of cost to manufacture clinical-grade material, the total size of DNA that can be included in the virus vectors, and the potential, principally for retroviruses, for the integration events to result in insertional oncogenesis. A new virus-based system that has not yet entered clinical trials is based on foamy virus vectors, which possess favorable integration properties and are not pathogenic in humans.[52]

Nonvirus-based approaches benefit from lower manufacturing cost and are in principle less immunogenic than viral approaches. Although such approaches are theoretically safer because they are not dependent on viral elements integrating into host DNA, their safety record is shorter than that of virus-based vectors. Nonviral approaches to introduce transgenes into T cells involve the utilization of transposon elements such as sleeping beauty and piggybac as well as ZFN, TALEN, and CRISPR/Cas9 based-technologies, which allow for the ability to engineer T-cell populations with transgene insertions into specific chromosomal loci or that are biallelically disrupted for specific genes.[53–57] Such technologies offer significant potential to engineer T cells in a manner that allows for the ability to interrupt or otherwise modulate expression of particular proteins that may be deleterious to therapeutic function.

## 4 DESIGN OF CAR T CELLS

CARs are synthetic, engineered receptors that can target surface molecules in their native conformation.[58,59] Unlike TCRs, CARs engage molecular structures independent of antigen processing by the target cell and independent of MHC; recognition features that are desirable as an approach to overcome resistance mechanisms found in tumors and chronic infection. CARs typically engage the target via a single-chain variable fragment (scFv) derived from an antibody, although natural ligands have also been used.[60] Individual scFv's targeting a surface molecule are derived either from murine or humanized antibodies, or synthesized and identified by screening of phage display libraries.[61] Unlike TCRs, where a narrow range of affinity dictates the activation and specificity of the T cell, CARs typically have a much higher and perhaps broader range of affinities that will engage the target without necessarily encountering cross-reactivity issues. Varying the affinity can increase the therapeutic index of CAR T cells, by increasing the discrimination of normal tissue expression from levels of surrogate antigen expressed on tumors.[62,63] The length, flexibility, and origin of the hinge domain is also an important variable in the design of CARs.[62,64,65] A major challenge to the field is that it is currently necessary to empirically test these design variables as there are no general rules guiding CAR design for selected target molecules.

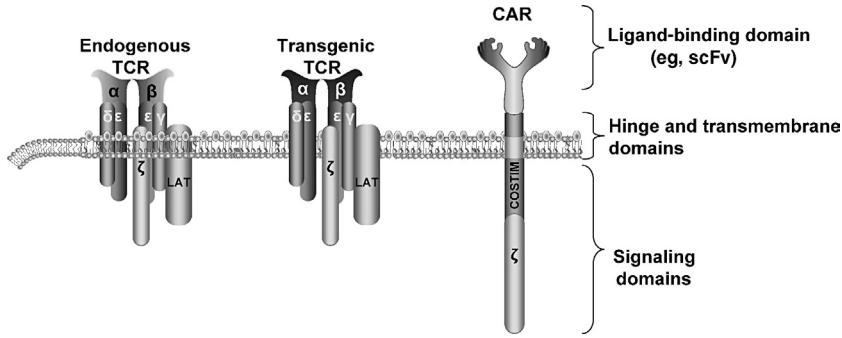


**FIGURE 30.2** **CAR design.** In the natural immune system, B cells display antibodies on their surface and T cells have TCRs on the surface. A synthetic CAR T cell is a chimera of a B- and T cell, displaying both an antibody fragment (scFv) and an endogenous TCR.

The "generations" of CARs typically refer to the number and composition intracellular signaling domains (Fig. 30.2). "First-generation" CARs include only CD3zeta as an intracellular signaling domain whereas second-generation CARs also include costimulatory domains. Most investigators are currently using the hinge and transmembrane domains of CD8alpha or CD28. Hinge domains derived from Fc regions have also been investigated and modified in length,[62] and in some instances these hinges have also been reported to engage Fc receptors and activate innate immune cells.[65]

## 5 LESSONS FROM CLINICAL TRIALS ON PATIENTS WITH CHRONIC INFECTION

Adoptive immunotherapy began in humans shortly after the discovery and production of IL-2 (Table 30.1). The initial trials in cancer patients were largely unsuccessful[66]; the reasons for the failure of the initial trials have only recently become apparent and include quantitative and qualitative deficiencies in the repertoire of the infused TCRs, infusion of cells that had reached replicative senescence, and susceptibility to checkpoint pathways in vivo that prevented responses. However, in patients with chronic viral infections, ACT was shown to have promise in early trials as ACT with natural CTL clones specific for cytomegalovirus and Epstein–Barr virus infection were shown to be effective in immunosuppressed individuals.[67,68] Substantial data exist to indicate that CD8+ T cells can affect the outcome and viral load in HIV-1 infection. Naturally occurring gag-specific CTL responses are inversely associated with viremia.[69] In contrast, adoptive CTL therapy for HIV/AIDS, though demonstrating safety and promising engraftment and trafficking of cells to sites of viral replication, has not been clinically effective,[70] and is accompanied by rapid emergence of viral escape mutants.[71] Mathematical modeling suggests that adoptive transfer of CTLs should augment HIV-1 immunity and control viral replication, but only

**TABLE 30.1 Potential Indications for Various Forms of ACT Therapies**

| Indication | Cell type | References |
|---|---|---|
| Metastatic melanoma | Tumor infiltrating lymphocytes | [76] |
| B-cell lineage tumors | CD19 CAR T cells | [77] |
| Synovial cell sarcoma<br>Multiple myeloma | NY-ESO-1 TCR T cells | [78]<br>[79] |
| Lymphopenia/immune reconstitution | Polyclonal T cells | [36] |
| Donor lymphocyte infusions | Allogeneic T cells: | [80] |
| Type 1 diabetes | Autologous Tregs | [81] |
| Prevention of organ transplant rejection | Inducible and natural Tregs | [82] |
| Graft versus host disease | Cord blood derived donor Tregs | [83] |
| HIV: increase immunity | CTL clones | [84] |
| HIV: increase immunity | CAR T | [85] |
| HIV: increase resistance | CCR5-deficient T cells | [38] |
| CMV: therapy for drug-resistant virus | CTLs | [33,84] |
| EBV: therapy for drug-resistant virus | CTLs | [68] |

when the replicative capacity of the genetically modified CTLs is preserved and functional CD4+ T cells are present.[72,73] Thus, an attractive strategy is the use of genetically enhanced TCRs to facilitate immune-mediated control of viral replication.[74] Ultimately, a two-pronged approach involving gene-modified T cells comprising CD4+ T-cell protection and CTL augmentation therapy might be optimal.[75]

## 6 CLINICAL TRIALS WITH CAR T CELLS DIRECTED AGAINST B-CELL MALIGNANCIES

Maus and coworkers reviewed the literature and listed all CAR T-cell trials up to late in 2013.[86] A search of the clinicaltrials.gov data base reveals that there are 88 trials testing CAR T cells that are currently registered.[a] Geographically most of the trials are being conducted in the United States, although China is now the second most prevalent site of investigation with CAR T cells. Although the earliest trials of CAR T-cell therapy were performed in patients with solid tumors,[87,88] these trials were disappointing. It is actually in trials of patients with B-cell malignancies that the most exciting results have recently been obtained.[77,89–92]

a. Search terms “chimeric antigen receptor,” Dec. 10, 2015.

Many groups including those at Memorial Sloan Kettering, Seattle Children's and the Fred Hutchinson Cancer Research Center, the National Cancer Institute, and others, have reported clinical responses in relapsed refractory leukemia and lymphoma. At our center, we have treated more than 200 patients with CAR T cells targeting B-cell malignancies including chronic lymphoid leukemia, acute lymphoid leukemia, non-Hodgkin's lymphoma, and myeloma. B-cell malignancies are particularly amenable to be targeted using CAR T-cell therapy, due to the presence of the CD19 and CD20 antigens on most B-cell malignancies from the most immature B-cell acute lymphoblastic leukemia (B-ALL) to the most mature lymphomas[93] and the fact that patients can tolerate prolonged periods of B-cell aplasia.

Treatment with CAR T cells specific for CD19 has resulted in complete responses in B-ALL and chronic lymphocytic leukemia. However, one recurrent observation among trials in B-ALL is the emergence of CD19-negative blasts at relapse in a substantial minority (approximately 15%) of patients.[91,92,94] Loss of CD19 is rarely observed in CLL, suggesting that CD19 escape is more difficult, or that CD19 negative precursor cells for CLL do not occur. Fewer published studies are currently available for comparison of response rates against mature B-cell neoplasms and non-Hodgkin lymphoma and no examples of relapse attributable to CD19-negative escape variants have yet been reported. Myeloma has been successfully targeted in a pilot trial with CD19 CAR T cells,[95] an event that was unexpected since myeloma typically does not express CD19.

After treatment with B cell–directed CAR T cells, the response rates vary by disease. In CLL we observe an approximate 60% overall response rate in relapsed/refractory patients.[96] In contrast, in young adults and children with ALL who have already failed a stem-cell transplant, there is a remarkable 90% complete response rate.[94,97] There are some late relapses, largely attributed to either loss of functional CAR T cells in the patient, or to an emergence of a leukemia clone that does not express the CD19 target antigen that is recognized by the CAR T cell.[98] The emerging solution to this is to have another "CAR in the garage" targeting other markers on B cells, a strategy that we have just begun testing with CD22-specific CAR T cells.

## 7 TOXICITIES AND MANAGEMENT

In most cases the toxicities associated with CAR T cells are on-target and reversible, a characteristic setting this treatment apart from nearly all previous cancer therapies comprised of cytotoxic chemotherapy and radiation therapy.[99] These toxicities include B-cell aplasia, tumor lysis syndrome, cytokine release syndrome, and macrophage activation syndrome.[89,100] Hyperferritinemia and elevations in serum C-reactive protein are observed in patients with cytokine release syndrome and macrophage activation syndrome.[91,97] The occurrence of B-cell aplasia and duration of persistence of CAR T cells in patients with leukemia is a predictive biomarker of response to therapy.[94,96] Cytokine release

syndrome has been reported by all groups after treatment of B-cell malignancies, and has also been observed after the treatment of multiple myeloma,[95] which is typically considered a CD19 negative malignancy. Cytokine release syndrome is now effectively managed with tocilizumab and other reagents that block IL-6 signaling.[91,101] The use of cytokine blockage may be preferable to immunosuppression with corticosteroids due to enhanced therapeutic effects and less systemic immunosuppression.[102]

CNS symptoms and signs consisting of expressive aphasia, confusion, and seizures have been observed after CD19 CAR therapy.[103] This syndrome is reversible and the cause of these syndrome remains unexplained because there is no obvious correlation with symptoms and tumor in the CNS. This may be a drug class effect because the syndrome is also observed after treatment with blinatumomab, a CD19 directed bispecific single-chain antibody.[104]

## 8 CONCLUSIONS AND FUTURE CHALLENGES

Adoptive immunotherapy using autologous T cells endowed with CARs has emerged as a powerful approach to treating cancer. However, a current limitation of this approach is that autologous CAR T cells must be generated on a bespoke basis. The development of automated culture technologies employing robotics will be required to scale out this therapy for general incorporation into the routine practice of medicine.[105] Further, the potential development of third party cells through large-scale manufacturing of T cells deficient in expression of their TCR and other molecules will likely ameliorate this issue through the use of "off-the-shelf" CAR T cells.[106] There are now dozens of cell therapy companies and it is likely that this emerging industry will solve issues of manufacturing feasibility.[18]

The major scientific issue in the field is whether engineered T cells will have substantial impact in adenocarcinoma and other solid tumors; at this point efficacy following adoptive transfer is limited and most routinely observed with natural tumor infiltrating lymphocytes and TCR T cells rather than CAR T cells.[107] Finally, the ability to mass produce genetically modified T cells with desired specificities has the potential to enable "instant vaccines" as a form of immunotherapy that can be effective rapidly and in immunosuppressed patients, where standard vaccines are often ineffective.

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