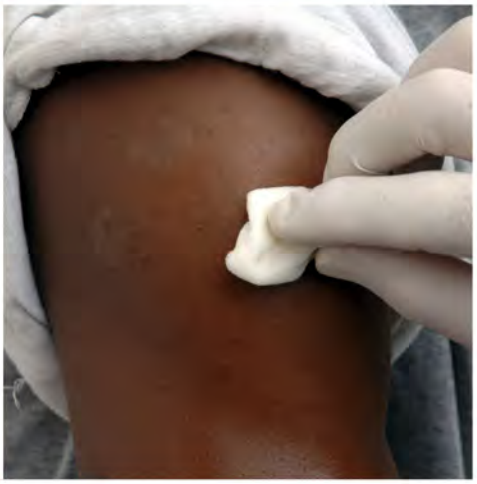

THE JORDAN REPORT

ACCELERATED DEVELOPMENT OF VACCINES **2007**

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health
National Institute of Allergy and Infectious Diseases

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Table of Contents

Introduction

Carole Heilman, Ph.D.

Background

It has been almost 25 years since the first vaccine research and development "state of the science" report, otherwise known as The Jordan Report, was published by the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health. Since that time, significant scientific progress has been made in developing new and better vaccines against a wide array of infectious diseases, including those that are emerging or re-emerging.

Historically, vaccines have led to some of the greatest public health triumphs ever, including the eradication of naturally-occurring smallpox from the globe more than 50 years ago, and the near-eradication of polio. In addition, there has been a significant reduction in the disease burden imposed by measles, mumps, hepatitis, influenza, diphtheria, and many other infections.

This edition of the Jordan Report outlines a number of significant advances made in infectious disease vaccine research and development since the last document was published in 2002. In addition, it offers a variety of perspectives from experts in the field on timely immunization topics, including adolescent vaccine platforms and vaccine supply.

Advances in Vaccine Research and Development

One factor contributing to the rapid expansion of the current vaccine arsenal is the acceleration of technological advances, including those using recombinant DNA. Scientists also continue to improve existing vaccines and identify new vaccine candidates to prevent diseases for which no vaccines currently exist. By increasing their understanding of the immune system and how it fights off harmful microbes, researchers have also made exciting developments in vaccine research methodology, which have resulted in recent clinical trials to evaluate candidate vaccines against malaria, tuberculosis and West Nile virus.

An exciting scientific milestone occurred in January 2004, when a new tuberculosis vaccine entered Phase I clinical trials. NIAID has supported research on this candidate vaccine since its earliest stages. The vaccine was the first to reach human trials in the United States and, in fact, the first new tuberculosis vaccine to be tested in more than 60 years.

Childhood and adolescent vaccines are another area in which significant advances have been made. In March 2005, global health leaders presented new research findings showing that vaccinating infants against *Streptococcus pneumoniae*—a bacterium that causes deadly pneumonia, meningitis, and sepsis—could substantially reduce death and serious illness among children in the developing world. This study, *The Gambia Pneumococcal Vaccine Trial*, was supported in part by the NIAID.

In October 2005, study results were released for the NIAID-supported acellular pertussis trial, which showed that the candidate vaccine was more than 90 percent effective in preventing the transmission of *B. pertussis* in people between the ages of 15 and 65. An important additional benefit of the vaccine may be to decrease transmission of *B. pertussis* to infants, who are particularly vulnerable to severe illness from *B. pertussis*. The illness affects 50 million people worldwide each year.

Challenges and Opportunities

Despite considerable progress in vaccinology, the emergence of new infectious diseases, the re-emergence of old diseases, and the persistence of intractable diseases (sometimes due to drug resistance), make infectious diseases some of the most complex and difficult challenges facing the public health community today.

HIV/AIDS, malaria, and tuberculosis are still among the leading killers worldwide and no licensed vaccines against them currently exist. In addition, new infectious diseases continue to emerge. Since the last edition of *The Jordan Report*, the world has seen an outbreak of a novel coronavirus termed "severe acute respiratory syndrome" (SARS), yearly West Nile virus outbreaks, and most recently, the continuing threat of a potential avian influenza pandemic.

Even as infectious diseases emerge and re-emerge, however, scientists continue to rapidly develop and design novel vaccine approaches. These include developing new adjuvants and novel delivery systems such as oral, nasal, transcutaneous vaccinations, and combination vaccine strategies. In addition, the application of genomic and post-genomic technologies in the development of a new generation of tailor-made vaccines has the potential to provide a stunning opportunity to "customize" vaccines against novel microbes.

There is also great enthusiasm and renewed effort for exploring opportunities for vaccine development in less traditional areas. These areas include therapeutic vaccines for managing chronic diseases, vaccines for controlling autoimmune diseases, and vaccines against diseases of particular public health concern that have implications for global health.

Positive Spin-offs of Basic Research

NIAID's investment in research on biodefense pathogens will have many positive implications for global public health. Studies of microbial biology and the pathogenesis of organisms with bioterror potential will lead to a better understanding of other, more common and naturally occurring infectious diseases and advance future vaccine development pathways and strategies. Furthermore, this research promises to enhance the understanding of molecular and cellular mechanisms (including regulation) of the immune system, which may help in the search for new ways to treat and prevent a variety of immune-mediated diseases such as type 1 diabetes, rheumatoid arthritis, cancers, neurological diseases, and allergic and hypersensitivity diseases.

Conclusion

While the insightful articles composed for this publication are invigorating and thought-provoking, the complexities, intricacies, and unknowns of host-pathogen interactions continue to pose considerable challenges. Researchers continue to address these obstacles with acute scientific curiosity and intensity. With the expanded commitment and resulting synergy from the federal government, industry, and the scientific community, the pace of progress will undoubtedly lead to unprecedented levels of discovery.

Tribute to Maurice R. Hilleman, Ph.D., and John R. La Montagne, Ph.D.

William S. Jordan, Jr., M.D.

It has been five years since the publication of the 20th anniversary issue of this Report. I am pleased to again introduce the current issue of the Jordan Report and to join Dr. Anthony S. Fauci, Director, NIAID, and Dr. Carole Heilman, Director, DMID, in dedicating it to my good and long-time friends, Dr. Maurice R. Hilleman, and Dr. John R. La Montagne. These two scientists, both of whom passed away, exemplified the finest in collaboration and cooperation between industry and government.

I knew Dr. Hilleman since the early 1950s when we were both young investigators, he at the Army Medical Department Research and Graduate School and me at Western Reserve University as a member of the Department of Medicine and Preventive Medicine. The latter was chaired by Dr. John H. Dingle, one of my attending physicians at the Boston City Hospital where I returned to complete my residency after World War II. During that war, Dr. Dingle served as Director of the Commission on Acute Respiratory Diseases (CARD) at its laboratory at Fort Bragg, NC. CARD was under the Armed Forces Epidemiology Board, of which Dr. Dingle was later to become President. These activities introduced him to Dr. Hilleman and led to his suggestion that I call on Dr. Hilleman for assistance.

As the staff member in charge of patient care on the Infectious Diseases ward of University Hospital, I called on Dr. Hilleman for help with the diagnosis of psittacosis. Several members of a family that had recently purchased a pet psittacine bird at a downtown Cleveland store were admitted with pneumonia. Serologic tests performed by CDC at Chamblee, GA, using Lygranum antigen, and by Dr. Hilleman, using a phenol-enhanced lymphogranuloma venereum antigen prepared in his laboratory, confirmed the diagnosis. Years later we learned that the psittacosis was not caused by a virus but by *Chlamydia psittaci.*

I saw Dr. Hilleman many times in subsequent years, and particularly relished the occasion when I could compliment him for demonstrating that an adenovirus (RI67) was the cause of an epidemic disease (later called acute respiratory disease or ARD) amongst military recruits. His considerable contributions to vaccine research and development at Merck became legend before and after I joined NIAID in 1976 as Director of its Microbiology and Infectious Diseases Program (MIDP, later DMID). It was natural for Dr. George Galasso, Chief of the Development and Applications Branch, and me to encourage Dr. Hilleman to obtain the attenuated varicella virus developed by Professor Michiak Takahashi of Japan. We offered NIAID's assistance in conducting clinical trials. Dr. Hilleman nursed the virus through the laboratory until he was satisfied with the product. Dr. Anne Gershon was then recruited to coordinate field trials in young, at risk children, receiving chemotherapy for leukemia. The rest is history. Both science and industry lost a great champion when Dr. Hilleman passed away in 2005.

I first met Dr. La Montagne in 1976, the year both of us arrived at NIAID. In that year he received a baptism by fire as the new influenza program officer in the Development and Applications Branch. An atypical, swine-like influenza virus had been isolated from a soldier at Fort Dix, NJ, with a fatal respiratory illness. It fell to Dr. La Montagne to find companies to produce a univalent swine flu vaccine and to recruit investigators to test it. Pressure was on because President Ford, after having been advised by a group of consultants who recalled that the virus of the 1918-1919 pandemic was also associated with pigs, agreed that universal immunization to protect against this might be desirable. So many questions had to be considered: How much vaccine was needed? What part of the population should be vaccinated?

Dr. La Montagne did his job calmly and thoroughly while respectfully handling the swine flu dilemmas that followed. He approached his later assignments with the same demeanor. His comprehension of issues was always thorough and helpful, as was his ability to bring together those sharing his objectives.

Following my retirement from NIAID in 1987 to continue as a Volunteer, Dr. La Montagne succeeded me as Director of MIDP. The Program

was given Division status (Division of Microbiology and Infectious Disease; DMID) and, to my surprise, Dr. La Montagne named this periodic Vaccine Report for me. The premature loss of his talent is indeed tragic.

I am indebted to the Sabin Vaccine Institute and to Dr. Fauci, Director of NIAID, who prepared and published the following biographies.

In Memory of Maurice Hilleman, Ph.D.

Vaccinologist of the 20th Century

Courtesy of the Sabin Vaccine Institute

A long-time colleague of the Sabin Vaccine Institute, Maurice R. Hilleman, Ph.DSC., passed away on April 11, 2005 at a hospital in Philadelphia where he was being treated for cancer. Hilleman was a microbiologist who developed over three dozen vaccines for diseases including mumps, measles, chickenpox, pneumonia, and meningitis. His discoveries have saved tens of millions of lives and reached into every home.

Though he was not as widely known among the general public as some other scientists of note, his achievements match or exceed many of the greats. Hilleman was the fourth scientist to receive the prestigious Sabin Gold Medal, which he was awarded in 1997. He maintained a close association with the Sabin Vaccine Institute since then, lending his expertise to Institute programs as a member of the SVI Scientific Advisory Council.

Raised on a farm in Montana, Hilleman credited much of his success to his boyhood work with chickens, whose eggs form the foundation of so many vaccines. He pioneered the development of eight of the 14 routine vaccines and much of modern preventive medicine is based on his work. He is credited with having developed more human and animal vaccines than any other scientist, helping to extend human life expectancy and improving the economies of many countries. He retired from Merck in 1984 as senior vice president.

Hilleman pioneered the development of numerous vaccines, including measles, mumps, rubella, varicella, Marek's disease, hepatitis A, hepatitis B, and adenoviruses. He also participated in the evolution of vaccines against meningitis and pneumonia.

Another important aspect of his work was advancing the science of combination vaccines. For instance, the combined measles, mumps, and rubella vaccine prevents three diseases with only one vaccination. Children therefore receive fewer painful injections and parents and children face less anxiety. Pediatricians require less storage space for vaccines and less handling is required.

In March 2005, the University of Pennsylvania's School of Medicine and the Children's Hospital of Philadelphia, in collaboration with the Merck Company Foundation, announced the creation of the Maurice R. Hilleman Chair in Vaccinology.

The Hilleman Chair will be occupied by a physician/scientist contributing to vaccinology on the faculty of University of Pennsylvania and the post will serve to accelerate the pace of vaccine research there.

In Memory of John R. La Montagne, Ph.D.

True Public Health Hero

Anthony S. Fauci, M.D.

The infectious diseases community has lost a trusted friend and ally. My colleague, John R. La Montagne, Ph.D., the skilled and much-loved deputy director of the National Institute of Allergy and Infectious Diseases (NIAID), died suddenly of a pulmonary embolism in Mexico City, the city of his birth, on November 2, 2004. He was 61.

John's untimely passing was a heartbreaking shock to all who knew him. I had known John for nearly 30 years; he was a dear friend and one of the finest people I have ever known. In his long career at NIAID, his accomplishments in improving global health—especially with regard to leading vaccine development efforts—were remarkable. On a personal level, his generosity, wit, even-handedness, and kindness made him a friend to all who knew him, from world health leaders to the cleaning lady in his office with whom he spoke Spanish every morning.

John received a B.A. (1965) and an M.S. (1967) in microbiology from the University of Texas at Austin, and went on to receive a Ph.D. in microbiology from Tulane University in 1971 where, for his doctoral dissertation, he characterized a thermophilic bacteriophage of *Bacillus subtilis* named TSP-1. Upon graduation he worked as a postdoctoral fellow in the laboratory of Julius Youngner at the University of Pittsburgh, where his efforts were focused on animal virology. He joined NIAID in 1976 as influenza program officer; of note, his last academic paper, a commentary published in the *New England Journal of Medicine* also dealt with influenza. In the mid 1980s, John organized the NIAID extramural AIDS Program, and in 1987 was appointed director of the NIAID Division of Microbiology and Infectious Diseases Program. I appointed him the NIAID deputy director in February 1998.

Throughout his career, John made significant contributions to the national and international effort against emerging and re-emerging infectious diseases.

Of particular note, he played a central role in the organization of the Multilateral Initiative on Malaria, chaired the World Health Organization Task Force on Strategic Planning for the Children's Vaccine Initiative, advised the Pan American Health Organization on their programs in vaccine research implementation, and led a multinational effort to develop and license acellular pertussis vaccines.

John's career has been lauded by public health leaders around the world, who have expressed their condolences to us by the hundreds. He was, in the words of Tommy Thompson, Secretary of Health and Human Services, a "true public health hero."

EXPERT ARTICLES

The Immunology of Influenza Infection
Implications for Vaccine Development*

Kanta Subbarao, M.D., M.P.H., Brian R. Murphy, M.D., and Anthony S. Fauci, M.D.

Introduction

The "Spanish Flu" pandemic of 1918-19 spread rapidly across the globe, killing an estimated 50 million or more people, many of whom were young and otherwise healthy. Since 2003, highly pathogenic H5N1 avian influenza viruses have caused massive outbreaks in poultry and migratory birds in more than 54 countries. Worldwide, as of early 2007, approximately 270 confirmed cases of human H5N1 influenza in 10 countries, including more than 160 deaths, had been reported by the World Health Organization (WHO) [1]. The H5N1 virus is very poorly transmissible from person to person, but the possibility that the virus could become easily transmissible and spread around the world in a global pandemic similar to that in 1918 underscores the importance of rapidly producing a safe and effective pandemic influenza vaccine. Because a pandemic H5N1 influenza strain has not yet emerged, current efforts, by necessity, focus on developing "pre-pandemic" vaccines against currently circulating influenza strains. Understanding the immunology of influenza virus infection will play a key role in designing effective vaccines against pre-pandemic and pandemic viruses with the maximum potential for protecting against severe disease and death.

Influenza viruses cause repeated infections in humans and are a significant cause of morbidity and mortality annually, accounting for as many as 36,000 excess deaths each year in the United States [2, 3], and 250,000-500,000 deaths globally [1]. Influenza is a winter illness in temperate climates; however, it occurs in two seasons or throughout the year in tropical climates. Three types of influenza viruses, designated influenza A, B, and C, exist in nature and, of these, influenza A and B viruses cause annual epidemics. Humans are the only hosts for influenza B viruses, but influenza A viruses infect a variety of species including birds, humans, and a variety of other mammalian species such as pigs, horses, cats, dogs, ferrets, and mice [3, 4]. Influenza A viruses are divided into subtypes based on the antigenicity of the two major surface glycoproteins: the hemagglutinin (HA) and neuraminidase (NA). These two proteins are the main targets of the protective immune response. The HA is a trimer with a receptor binding pocket on the globular head of each monomer, and the NA is a tetramer with an enzyme active site on the box-shaped head.

Aquatic birds represent the reservoir of influenza A viruses in nature. Viruses of all known (16 HA and 9 NA) subtypes have been isolated from waterfowl and shorebirds. However, influenza infections in waterfowl tend to be asymptomatic, and the viruses are in ecological stasis in these hosts [5]. In contrast, influenza A virus infections in humans elicit an immune response that provides selective pressure and drives the virus to evolve. Influenza viruses utilize two mechanisms, referred to as antigenic drift and antigenic shift, to evade the human immune response. Antigenic drift is a continuous process of change in which mutations occur in and around the antibody (Ab)-combining sites of the HA and NA proteins that allow the virus to escape neutralization by pre-existing Abs. Five Ab-combining sites have been mapped on the HA of H3 subtype human influenza A viruses [6]; however, less is known about HAs of avian influenza A subtypes. Antigenic shift is a rare but epidemiologically highly significant event in which a virus bearing a novel HA, with or without an accompanying novel NA, is introduced into the human population. A virus bearing a novel HA or NA has the potential to cause a pandemic if a large proportion of the population lacks immunity to the novel HA and NA and if the virus has the ability to spread efficiently from person to person. The novel HA and NA genes in pandemic influenza viruses are derived from the reservoir of avian influenza viruses in nature.

Pre-existing Immunity Against Influenza

A redundancy in the immune response to influenza allows the humoral and cellular immune systems to act independently to clear an influenza virus infection. Abs present at systemic or mucosal sites at the time of infection that are directed at the HA or NA protein of the infecting virus are the major mediators of resistance to influenza, whereas the cellular

immune response to influenza works with the humoral immune response in viral clearance [7]. Abs directed at the HA and NA surface glycoproteins of the virus are effective in mediating protection that is long lived in the absence of antigenic drift or shift. This was evident in 1977 when an H1N1 virus that had circulated in the early 1950s reappeared in the human population. Significant disease was only seen in persons born after the H1N1 virus had stopped circulating in 1957, indicating that homotypic immunity is long lived. Because the individuals born after 1957 were infected multiple times with H2N2 or H3N2 viruses that share internal protein antigens (e.g., nucleoprotein) with the H1N1 virus, it was clear that the long-lived homotypic immunity was provided by Abs and that cell-mediated immunity to shared antigens such as the nucleoprotein played a relatively small role in resistance. Thus, homotypic Abs are highly protective and mediate significant protection in humans, whereas Abs to the HA and NA of other subtypes and cell-mediated immune responses are less effective in long-term immunity.

Heterosubtypic immunity, which is protection conferred by previous infection(s) with an influenza virus of a different subtype, is weak in humans, especially in children. Recent analysis of epidemiological data collected before and during the 1957 pandemic suggests that heterosubtypic immunity was observed in adults but not in children [8]. Definitive data regarding the role that heterosubtypic immunity plays in resistance to influenza virus infection in humans are lacking, and the mediators of such immunity in humans have not been identified.

An analysis of genetic and antigenic data on the HA from human influenza A H3N2 viruses led to the conclusions that the HA was under positive selection [9] and that two or more amino acid changes in two or more Ab-combining sites of the HA were sufficient for a virus to evade neutralization by Ab against the previously circulating strain [6]. The mechanism by which Abs protect against influenza virus infection are indicated in Figure 1. Cellular immunity is directed at epitopes on several influenza virus proteins, but this immunity is relatively short lived [7].

Vaccine Development

Several important considerations for vaccine development follow from the interactions between influenza viruses and the host. First, influenza viruses replicate extremely rapidly in the host. Peak titers (which correlate with disease) are achieved before a cell-mediated immune response can be generated *de novo* or from memory to restrict replication (Figure 2). Therefore, the major goal of the currently licensed influenza vaccines is to induce Abs prior to infection that function to dampen virus replication. Second, influenza is a respiratory tract infection and Abs induced by vaccine that restrict replication throughout the upper and lower respiratory tract are desired. Intranasally administered live attenuated vaccines efficiently induce a mucosal as well as a systemic Ab response. Mucosal Abs are more effective than systemic Abs in restricting replication of influenza virus in the upper respiratory tract. In contrast, parenterally administered inactivated vaccines primarily induce systemic (serum) Abs that restrict replication of virus in the lower respiratory tract. Therefore, inactivated vaccines are effective in prevention of severe disease and complications of influenza, but are less effective than previous natural infection and live attenuated virus vaccine in protection of the upper respiratory tract. Third, the ability of the virus to drift and evade immune detection and the paucity of conserved epitopes on the HA that induce cross-reactive neutralizing or protective Abs pose a challenge for vaccine development. Currently licensed human influenza vaccines are updated annually to keep up with antigenic drift that is identified through virologic surveillance. Fourth, clinical studies have established that two doses of currently formulated inactivated vaccine are required to elicit protective Ab titers in immunologically naïve individuals. The live attenuated virus vaccine is significantly more immunogenic than inactivated virus vaccine in naïve individuals. In practical terms, each winter, previously unimmunized children should receive two doses of vaccine one month apart while a single vaccine dose can protect previously primed children and adults.

Pandemic Influenza Preparedness

Recent events in Asia have highlighted the pandemic potential of avian influenza viruses and the need to prepare for an antigenic shift in influenza A viruses. Although antiviral drugs can be effective in prophylaxis, vaccines are the preferred strategy for the prevention of a pandemic, because pandemic viruses might be resistant to licensed antiviral drugs or, even if initially sensitive, can rapidly develop drug resistance. A realistic goal of a pandemic influenza vaccine is to prevent mortality and severe morbidity with acceptance of the fact that infections associated with mild illness will not be prevented. This requires the development of vaccines that, at the least, elicit systemic Abs of sufficient titer to restrict virus replication in the lower respiratory tract,

thereby preventing pneumonia and its associated complications.

Although principles that have been established from basic and applied research in human influenza can be applied to pandemic influenza vaccine development, several critical gaps in knowledge remain. For example, the antigenic sites on avian HAs and the immune correlates of protection from avian influenza virus infections are not known. Additionally, the HA proteins of avian subtypes of influenza A viruses are not as immunogenic as human influenza A HA subtypes for unknown reasons; therefore, approaches to enhance the immunogenicity of the avian HA in a pandemic virus may be needed to achieve a protective level of immunity. Such new approaches will be needed in addition to the two doses of vaccine now required to successfully immunize a naïve population.

Currently two classes of vaccines are licensed for seasonal (interpandemic) influenza in the United States: parenterally delivered inactivated virus vaccines (whole virus or subunit) and a live attenuated vaccine delivered as a nasal spray. Both types of vaccines are trivalent and contain an influenza A H1N1 subtype virus, an influenza A H3N2 subtype virus, and an influenza B virus to protect against each of the co-circulating strains of influenza. Vaccines against potential pandemic strains of influenza are now being developed based on both of these strategies. Seed viruses for inactivated vaccines have been generated against influenza viruses of H5, H7, and H9 subtypes. Pre-clinical data have been generated for all three subtypes, and H5 and H9 subtype vaccines have been evaluated in Phase I clinical trials [10, 11, 12, 13, 14, 15, 16, 17, 18]. The investigational H5 and H9 inactivated vaccines are less immunogenic than interpandemic influenza vaccines (H1 and H3 subtypes). The amount of HA required in unadjuvanted pandemic vaccines produced with inactivated H5N1 influenza virus to elicit a serum Ab response of similar magnitude as the licensed interpandemic influenza vaccine is likely to exceed 15 micrograms (mcg). However, in trials of the whole-virus or split-virion H5N1 vaccines in which an alum adjuvant was also administered, serum antibody responses that correlate with protection in human influenza were observed when patients were immunized with 2 doses of 10 and 30 mcg of antigen, respectively [11, 13]. Results from a Phase I clinical trial of inactivated H9N2 vaccine administered with the MF59 adjuvant indicate an immune response of a magnitude that correlates with protection from human influenza in all volunteers at doses as low as 3.75 mcg [10]. Trials of an inactivated H5N1 virus vaccine administered with the MF59 adjuvant are under way.

In addition to making seed viruses beforehand and evaluating their safety and immunogenicity, several important applied vaccine research issues should be explored to ensure the availability of enough doses of appropriately immunogenic influenza vaccines to protect the population against potential pandemic strains of influenza. These include further exploration of known and novel adjuvants to enhance immunogenicity; exploration of ways to reduce the amount of HA antigen required to elicit protective Ab titers by investigating alternative routes of vaccine administration; and consideration of a strategy of pre-emptive vaccination to prime the population for an Ab response to a novel HA.

Efforts are under way to develop and evaluate live attenuated vaccines against potential pandemic strains of influenza along a track that parallels the development and evaluation of inactivated virus vaccines [19]. The live attenuated pandemic influenza vaccine candidates contain the attenuating genes of the A/Ann Arbor/6/60 cold-adapted virus that is the backbone of the licensed live attenuated influenza A virus vaccine. Candidate vaccines have been generated against H5 and H9 subtype viruses, and vaccines against the other subtypes will follow. Promising data obtained from testing in mice and ferrets suggest that live attenuated vaccines are suitable candidates for evaluation in Phase I clinical trials for safety, infectivity, and immunogenicity and such studies are currently under way [20].

The potential of recombinant subunit DNA, and vectored vaccines to protect against pandemic influenza viruses is also being explored in several preclinical studies. DNA vaccines containing the HA and NA genes of avian influenza viruses and the highly conserved M and NP viral genes have been shown to induce protective immunity in animal models [21, 22, 23, 24]. An NP DNA prime-recombinant adenoviral boost strategy using the NP influenza gene protected mice against lethal infection by H5N1 virus [25]. Recent studies have also demonstrated that mice and chickens immunized with a replication-incompetent human adenovirus vaccine containing the H5 HA gene were protected from infection with H5N1 viruses [26, 27], and a recent report showed that vaccination with plasmid DNA containing HA gene from the 1918 H1N1 pandemic virus protected mice against lethal challenge with the reconstructed 1918 influenza virus [28]. Although DNA vaccines to protect against pandemic and seasonal influenza show great promise and are safe to produce, their safety and efficacy in humans have not yet been determined. However,

a Phase I study of a DNA vaccine containing a modified version of the H5 hemagglutinin gene developed by the NIAID Vaccine Research Center recently began.

Additionally, cell culture substrates are being evaluated as alternatives to embryonated eggs for vaccine manufacture. Unpublished results indicate that an inactivated H5N1 vaccine produced in Vero cells appears to be safe and immunogenic in preliminary studies [29].

Harnessing Immunological Memory

Several decades of experience with human influenza vaccines indicate that the vaccine strain must be closely related to the epidemic strain of influenza in order to be effective. Although the match between vaccine virus strains and epidemic virus strains is generally good because the evolution of human influenza viruses is continuously monitored through careful, global virologic surveillance and vaccine strains are updated based on these data, we have no basis upon which to predict the exact strain of avian influenza that will cross the species barrier and cause a pandemic. This makes it unlikely that the pandemic vaccine strain will exactly match the pandemic virus.

Several recent studies in animal models suggest that a vaccine that is similar, but not identical, to a potential pandemic strain may offer some protection against serious disease and death. For example, in studies in ferrets and mice, live attenuated H5N1 vaccines generated from viruses circulating in 1997, 2003, and 2004 protected against H5N1 viruses isolated over eight years from 1997 to 2005. A single dose of vaccine containing HA and NA gene segments from the 1997 virus protected mice against the lethal infection with H5N1 viruses isolated in 2004 and 2005, and two doses protected both mice and ferrets from replication of challenge virus in the respiratory tract following intranasal administration of 2004 or 2005 H5N1 wild type viruses [20].

Similar results have been observed using an inactivated H5N1 virus that protected ferrets from lethality due to heterologous H5N1 viruses [30]. Further, a 1997 H5N3 vaccine protected ferrets from death when challenged with a 2004 H5N1 virus [31]. If these vaccines induce a broadly cross-reactive immune response in humans, the vaccines may not need to be updated as the H5N1 viruses evolve in poultry.

Clinical studies also offer promise that pre-emptive vaccination with an inactivated H5N1 virus vaccine may generate at least partial, long-lasting immunity that could later be boosted with a pandemic vaccine. Researchers who conducted a trial in 1998 with a vaccine made from an inactivated 1997 H5N1 virus recently re-immunized some of the same patients with a vaccine developed against the 2004 H5N1 virus and found that twice as many of the revaccinated volunteers developed an immune response indicative of protection, compared with volunteers who received only a single dose of vaccine to the H5N1 strain circulating in Vietnam in 2004 [32].

Toward a "Universal" Vaccine

Ideally, a vaccine that provides cross-reactive immunity between the H1-H16 subtypes of influenza would offer a wider range of protection should an influenza virus emerge from a subtype that has not previously infected humans. Further research and development efforts are required to achieve this goal, some of which are discussed below.

The development of cross-subtype HA-based protection requires the identification of conserved sites in the H1-H16 HAs that could induce broadly protective, highly functional neutralizing Abs. It is important to emphasize that such Abs are not regularly induced in humans by infection with influenza A viruses belonging to multiple HA subtypes, an observation that indicates the difficulty of achieving this goal. A recent advance in this area is the determination of the crystal structures of the HA from several additional subtypes of influenza A viruses. The first 15 HA subtypes fall into 4 clades (2 groups of 2), with H9, H1, H3, and H7 being the prototypes of the 4 clades [33]. Perhaps the commonalities within clades of HA subtypes based on HA structure can be exploited to develop immunogens and strategies that can induce cross-reactive Abs effective among HA subtypes. Another approach to inducing broadly cross-protective immunity involves (1) identifying conserved CD8+ T-cell epitopes (Figure 1) that can be induced in most members of the population and (2) maintaining the CD8+ T cells in a highly functional state that can keep an infecting influenza virus from reaching high titer *in vivo*. Maintaining CD8+ T cells in a highly functional state represents a real challenge because the genetic program of the CD8+ T-cell response is to relatively rapidly transition from an activated state to an inactive memory state. The maintenance of CD8+ T cells in a highly functional state will have to happen in the absence of antigenic stimulus. The immunogens capable of inducing this type of response have yet to be identified but could include sequences from circulating H1 and H3 subtype influenza viruses. Immunization with T-cell vaccines could provide varying degrees of resist-

ance to disease following infection with an H5N1 pandemic virus, as well as with circulating H1 and H3 viruses. It is essential to determine the ability to maintain this state of immunity throughout the period of circulation of the first wave of the pandemic virus. The M2 protein of influenza A viruses is highly conserved, and non-neutralizing Abs to the M2 protein protect mice from subsequent challenge (Figure 1) [34, 35]. Clinical studies can be designed to determine whether the induction of anti-M2 Abs prevents disease in humans. If so, efforts can be undertaken to evaluate whether a more robust and protective anti-M2 Ab response can be achieved by immunization than by repeated natural infection in nature. Presentation of the M2 protein to the immune system in a more immunogenic form via vaccination than occurs in natural infection may be important in this regard.

In conclusion, two approaches to the development of vaccines for pandemic preparedness can be exploited. The first and more immediately accessible uses existing technology to generate vaccines that induce highly functional and protective Abs. Efforts in this area should focus on pre-emptive preparation of vaccine seed viruses and evaluation of their safety and immunogenicity. Strategies to augment Ab responses with adjuvants and dose sparing immunization regimens are also being explored. The second approach will build on basic research to explore possibilities to induce cross-protective cell-mediated immunity or Ab to conserved epitopes such as those on the HA or M2 proteins but this has a longer lag time than the first approach.

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* This article was adapted with permission from Subbarao K. et al., 2006 [36].

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Figure 1

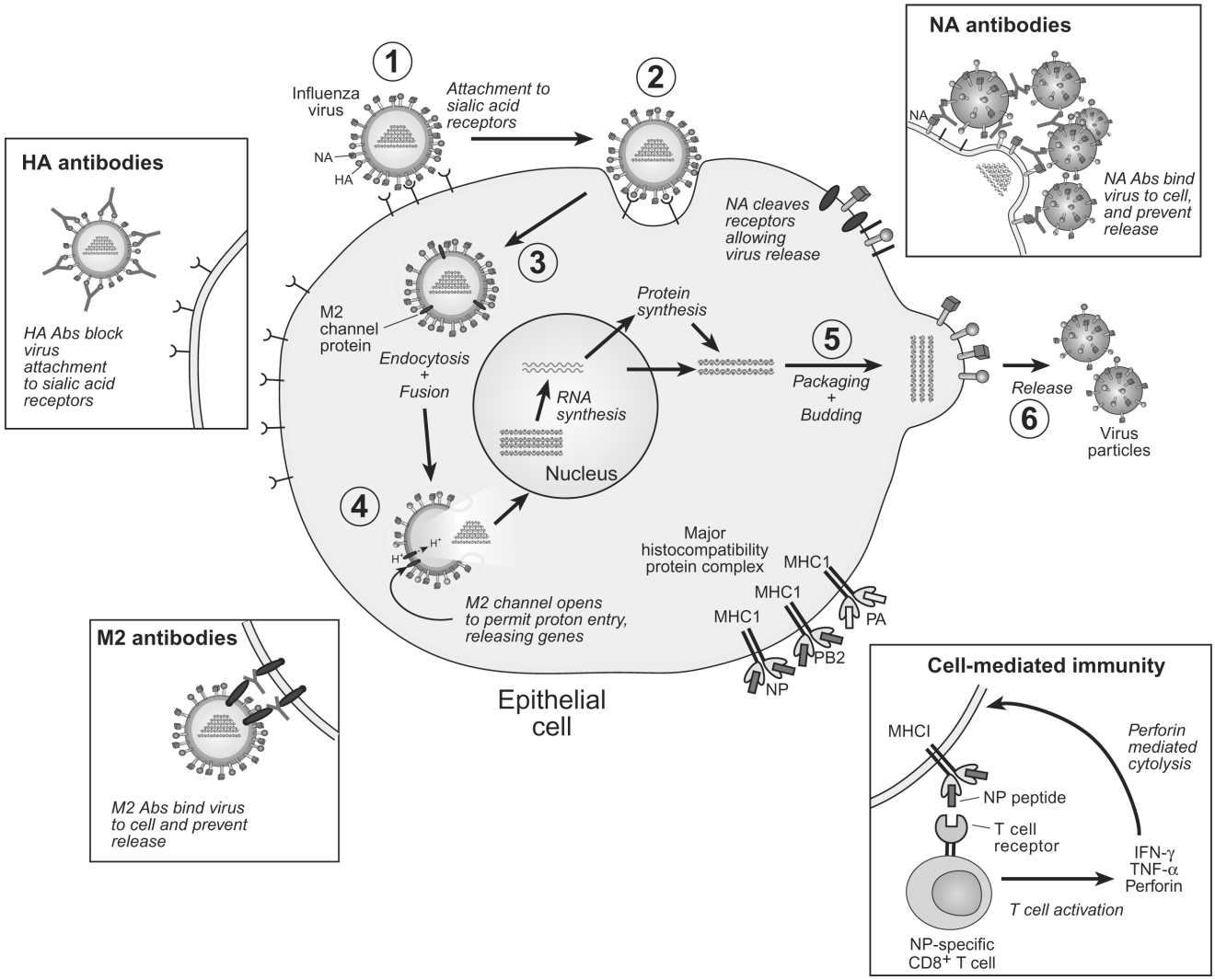


Figure Legends

Figure 1. Life cycle of influenza virus and role of the adaptive immune response during infection. Influenza virus attaches to the epithelial cell surface through binding of the viral hemagglutinin (HA) protein to cell surface sialic acid receptors (1, 2). The virion is internalized through endocytosis and fusion (3). Opening of the M2 channel allows proton flow across the viral membrane (4), triggering release of viral genes into the cytoplasm from where they travel to the nucleus. Viral proteins produced in cytoplasm assemble with viral genes and bud from the cell membrane as progeny virions (5). Release of new virus particles (6) requires the viral neuraminidase (NA) protein, which cleaves sialic acid receptors from the cell membrane. Antibodies (Abs) to the HA protein block virus attachment (inset, upper left), thereby decreasing the number of cells infected. They can also function to prevent fusion (4). Abs to the NA protein (inset, upper right) bind virus to the cell, preventing release of new virions. Abs to the M2 protein bind virus to the cell and prevent release of viral particles into the extracellular fluid (inset, lower left). Cell-mediated immunity contributes to resistance when CD8+ T cells specific for viral proteins such as nucleoprotein (NP) or polymerase proteins (PB2 and PA) recognize viral peptides presented by major histocompatibility complex (MHC) class I proteins, resulting in the release of cytokines with antiviral activity (IFN-γ and TNF-α) and perforins that mediate cytolysis of the infected cell. Lysis of the infected cell decreases the amount of virus released by the cell. The latter three mechanisms, NA Abs, M2 Abs, and CD8+ T cells, operate after a cell becomes infected. Only HA Abs prevent infection; this is likely why they are the most effective *in vivo*.

Figure 2

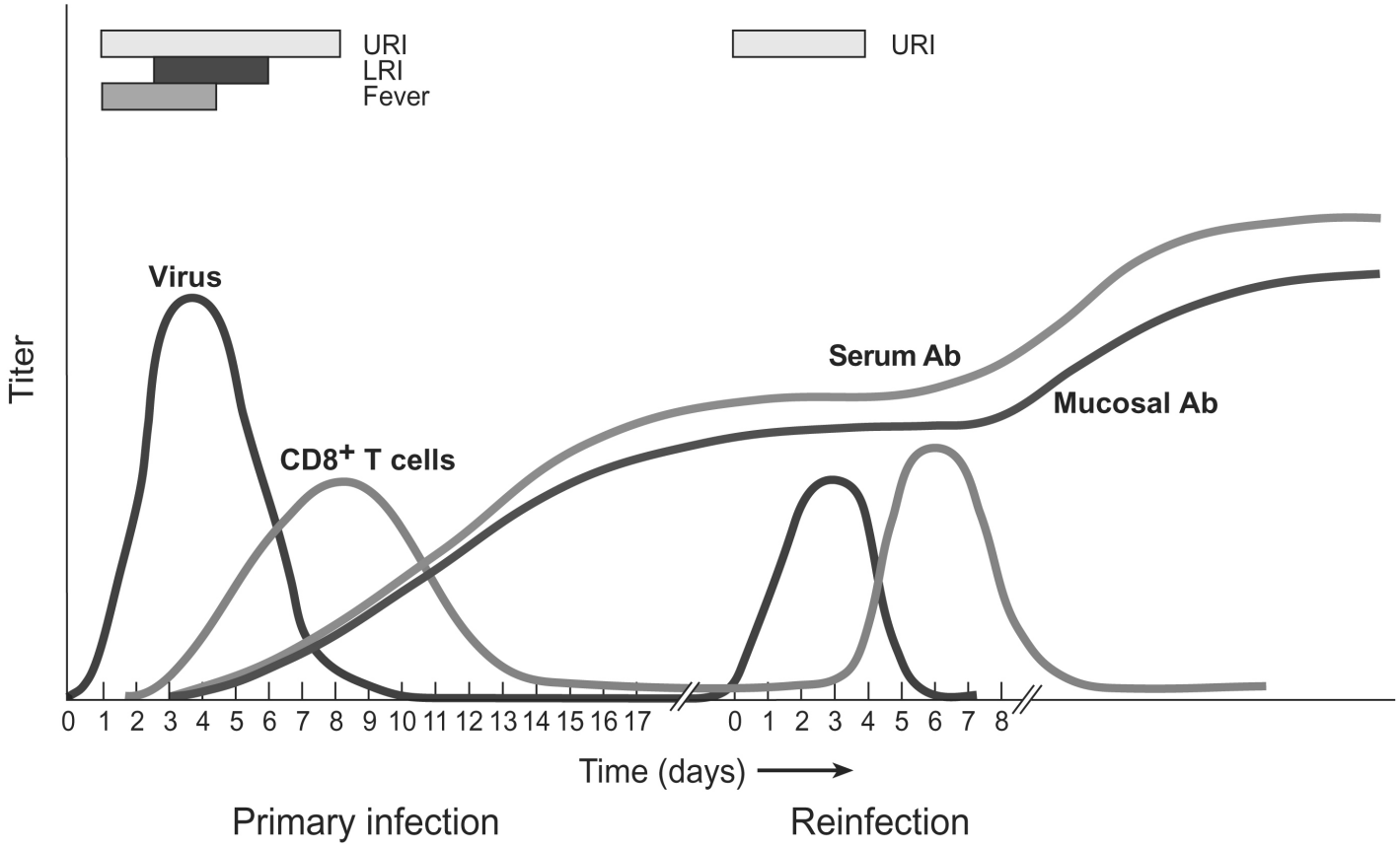
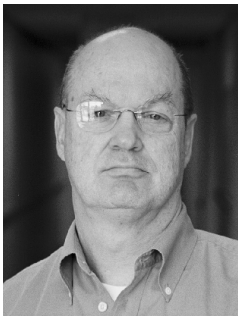


Figure 2. Course of immune response during influenza infection. Influenza virus titers peak at approximately three days post-infection, at which time antibodies (Abs) and T-cell responses begin to appear. Activated T-cell responses peak on days six to nine during the primary infection and then subside into a memory or resting state, whereas serum and mucosal Ab levels are sustained. Abs present at the time of reinfection result in lower viral titers and a reduction in symptoms. (Upper respiratory infection, URI; lower respiratory infection, LRI.)

Kanta Subbarao, M.D., M.P.H.

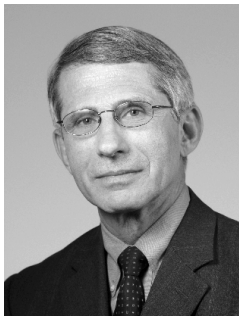
Dr. Subbarao received her M.B., B.S., in 1982 from the Christian Medical College, Vellore, University of Madras, India, and completed a residency in pediatrics at Cardinal Glennon Memorial Hospital for Children, St. Louis University. She completed a fellowship in pediatric infectious diseases and earned an MPH in epidemiology from the University of Oklahoma Health Sciences Center. After postdoctoral training in the Laboratory of Infectious Diseases (LID), NIAID, she was on faculty at McGill University, Montreal, Canada, and subsequently served as Chief of the Molecular Genetics Section of the Influenza Branch at the Centers for Disease Control and Prevention in Atlanta, Georgia. Dr. Subbarao joined the LID as a Senior Investigator in 2002. Her research is focused on the development of vaccines against pandemic strains of influenza and the development of animal models and evaluation of vaccines against the severe acute respiratory syndrome (SARS) coronavirus.



Brian Murphy, M.D.

Dr. Murphy received his B.A. degree from the Wesleyan University, Connecticut in 1964 and his M.D. degree from the University of Rochester School of Medicine in 1969. After an internship in medicine at Stanford University he came to the Laboratory of Infectious Diseases (LID), NIAID in 1970. He became Head of the Respiratory Viruses Section of LID in 1983 and Co-Chief of LID in 2001, a position he holds today along with Dr. Robert Purcell.

His research on the live attenuated influenza virus vaccine from 1975 to 1995 led to the licensure of the live attenuated trivalent influenza virus vaccine (FluMist) by MedImmune in 2003. He also participated in the development of the first monoclonal antibody to prevent/treat an infectious disease—the monoclonal antibody called Synagis, that is used to prevent disease caused by respiratory syncytial virus infection in infants with compromised cardiopulmonary function. In 1985, Dr. Peter Collins joined LID, and he and Dr. Murphy have been working collaboratively to develop live attenuated virus vaccines to prevent hospitalizations caused by a set of six respiratory viruses: two subgroups of RSV, three parainfluenza viruses, and the newly discovered human metapneumovirus. He has recently been involved with the development of vaccines for six flaviviruses that are major causes of disease in humans: the four dengue viruses, West Nile Virus, and tickborne encephalitis virus. Vaccines for three other viruses causing encephalitis are also under development.



Anthony S. Fauci, M.D.

Since 1984, Dr. Fauci has been Director of the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH). He also is Chief of the Laboratory of Immunoregulation at NIAID. Dr. Fauci has made many contributions to basic and clinical research on the pathogenesis and treatment of immune-mediated diseases. He has pioneered the field of human immunoregulation, making a number of scientific observations that serve as the basis for current understanding of the regulation of the human immune response. Dr. Fauci has made seminal contributions to the understanding of how the acquired immunodeficiency syndrome (AIDS) virus destroys the body's defenses.

At major medical centers throughout the country, Dr. Fauci has served as Visiting Professor. He has lectured throughout the world and is the recipient of numerous awards.

Dr. Fauci is a member of the National Academy of Sciences; Royal Danish Academy of Science and Letters; American College of Physicians; American Society for Clinical Investigation; Infectious Diseases Society of America; American Academy of Allergy, Asthma and Immunology; and other professional societies. He serves on many editorial boards and is author, coauthor, or editor of more than 1,000 scientific publications.

Vaccine Production and Supply
Vulnerabilities in the Present System

Walter A. Orenstein, M.D., Alan R. Hinman, M.D., M.P.H., Lance E. Rodewald, M.D.

Few measures compare with the public health impact of vaccines. During the past century, smallpox has been eradicated worldwide, polio has been eliminated in most countries of the world, and measles and rubella are no longer endemic in the United States [1]. In an evaluation by the Partnership for Prevention of 30 clinical preventive services widely recommended by the U.S. Preventive Services Task Force, childhood immunization received a perfect score, based on clinically preventable burden and cost-effectiveness [2]. New vaccines continue to be developed and introduced for widespread use. During the last decade alone, the Advisory Committee on Immunization Practices (ACIP) has recommended that all children be protected against six more diseases (varicella, pneumococcal disease, influenza, hepatitis A, meningococcal disease, and rotavirus) [3-8]. Human papillomavirus vaccine was recently recommended for all 11- to 12-year-old girls [9]. More new vaccines are close to final development and licensure. Translating vaccine development successes into disease reductions, however, rests on a fragile base of vaccine suppliers [10].

Supply vulnerabilities have been highlighted by an unprecedented number of vaccine shortages since 2000. While there is not a single reason for these problems, as a whole they underscore the need to improve the vaccine supply system. Between November 2000 and August 2006, there have been shortages of vaccines against 10 diseases: diphtheria, influenza, measles, mumps, pertussis, pneumococcal disease, rubella, tetanus, varicella, and meningococcal disease [10-13].

Supplies have been inadequate at some times during this period for tetanus and diphtheria toxoids (Td); diphtheria and tetanus toxoids and acellular pertussis vaccine (DTaP); pneumococcal conjugate vaccine (PCV7); measles, mumps, rubella (MMR) vaccine; varicella vaccine; influenza vaccine; and meningococcal conjugate vaccine. Table 1 shows shortages in childhood vaccines between 2000-2006 [10]. The causes of the shortages include manufacturers' unexpected departures from the market, long time frames for remaining manufacturers to gear up production to meet the shortfalls, production problems, failure to keep up with requirements for current Good Manufacturing Practices (cGMP), plant shutdowns to make renovations, and inadequate reserves to cover short-term supply disruptions. In addition, there were unanticipated consequences of the decision to remove the ethyl-mercury containing preservative, thimerosal, from vaccines as a precautionary measure to reduce overall exposure to mercury. Removing the preservative required switching from multiple dose to single dose packaging of DTaP vaccine, which reduced the number of doses produced by one manufacturer.

In a number of instances, the shortages led to transient changes in the routine immunization schedule to defer or drop doses. In the case of influenza, there were marked decreases in routine immunization coverage among some population groups for whom the vaccine was usually recommended [14].

A major reason for the tenuous status of the vaccine supply is the limited number of manufacturers for any individual product. As of August 2006, there were only single licensed manufacturers for vaccines against measles, mumps, rubella, varicella, pneumococcal disease (PCV7), polio (as a single vaccine), meningococcal disease (meningococcal conjugate vaccine, MCV4), rotavirus, human papillomavirus vaccine, zoster vaccine, and influenza (for children less than four years of age). In addition, most other vaccines in the routine child and adolescent schedule have only two manufacturers, including vaccines against hepatitis B, hepatitis A, diphtheria, tetanus, and pertussis. However, having multiple manufacturers does not assure shortages will be prevented. Vaccines with multiple manufacturers can have shortages because the manufacturers seldom have large inventories or unused capacity that can be rapidly brought online to mitigate an unanticipated shortfall. There is very little surge manufacturing capacity because redundant capacity is costly.

Academia, funded primarily by the National Institutes of Health (NIH), and many small firms have played major roles in vaccine discovery and early development [1]. However, few manufacturers have been able to bring a vaccine through late stages of development, clinical testing, submitting the required documentation

for licensure, and establishing the production capacity to supply multiple cohorts of children with vaccine produced under cGMP. The costs of vaccine development have been estimated to range generally from $300 million to $800 million [15]. Costs for development of live attenuated influenza vaccine may have exceeded $1 billion [16]. Few private firms are willing or able to make those kinds of financial commitments.

Vaccine supply would be more secure if there were multiple manufacturers of each vaccine. However, with the notable exception of influenza vaccines, there seems to be little interest in challenging manufacturers of current vaccines with comparable products not yet licensed in this country. This may reflect the hesitancy of other global manufacturers to go through the expensive process of receiving U.S. Food and Drug Administration (FDA) approval only to compete to receive a share of a market defined for the most part by the birth cohort (approximately four million each year) and the limited number of doses needed in the immunization schedule (one to five, depending on the vaccine). Some economists have stated that the current U.S. market would be unable to sustain more than one or two manufacturers for a given vaccine, which is why so few "me-too" products are introduced [17]. In contrast, there may be substantially more interest in the influenza market because current recommendations call for annual vaccination of approximately 218 million Americans and the largest use of vaccines to date has been 83 million doses [8]. Thus, there is substantial potential to increase the size of the market. During 2005, an additional producer was licensed in the United States and another producer from Canada is attempting to enter the U.S. market. As of August 2006, there are three licensed manufacturers of inactivated influenza vaccine and one manufacturer of live attenuated vaccines.

The National Vaccine Advisory Committee (NVAC) has reviewed the vaccine supply situation in the United States and has made a number of recommendations to try to avoid future shortages [11, 12, 18, 19]. These include (1) developing financial incentives, (2) simplifying the regulatory process, (3) developing or enhancing vaccine stockpiles, (4) addressing liability issues, and (5) enhancing communication and collaboration among key stakeholders. Financial incentives are needed to encourage manufacturers to invest in research and development as well as continuing upgrades of their facilities for currently licensed vaccines to meet evolving good manufacturing practices. Such incentives may include setting pricing compatible with reasonable profits, covering the costs of developing products, offering tax incentives for constructing new facilities or upgrading others, and providing rewards for companies that consistently meet federal and other contract requirements. At the moment, multinational vaccine producers must meet a host of different regulatory requirements depending upon the countries for which they seek licensure. Harmonization of requirements could make it easier to get vaccines approved in multiple countries, which could lead to considerable cost savings.

The NVAC also called for review of existing cGMP requirements to assure they are science-based, potentially eliminate or modify those that are not, and allow for flexibility as long as it does not compromise the safety and efficacy of the vaccines. The Centers for Disease Control and Prevention (CDC) has financial authority, through the Vaccines for Children (VFC) program, to establish six-month stockpiles of childhood vaccines [20, 21]. These stockpiles really represent storage and rotation contracts under which new vaccines go into a "bubble inventory," and older vaccines are rotated out so released products have a reasonable shelf life. These reserves can provide security against short-term supply disruptions but may not be adequate to solve major problems such as sole producers leaving the market. Improvements in vaccines, such as combination vaccines, challenge the logistics of stockpiling strategies because a new vaccine may make a stockpiled vaccine obsolete. Nevertheless, the stockpiles have been invaluable and have been used to minimize adverse consequences of supply problems on at least 12 occasions since 1984.

The National Vaccine Injury Compensation Program (VICP) has played a critical role in providing compensation for persons injured by vaccines and for decreasing manufacturer and provider liability for injuries that occur despite production of vaccines in compliance with all federal regulations and use according to existing recommendations. Nonetheless, concerns have been raised regarding a resurgence of litigation that, at a later point, could cause some companies to leave the market or decrease the incentives for new manufacturers to enter it [22]. Strengthening and expanding the VICP would help to safeguard the vaccine supply and benefit all parties—manufacturers, providers, and consumers.

Finally, the NVAC urged greater communication between industry and vaccine end users, including the CDC, so actions could be taken more quickly should supply problems occur. In addition, it felt the need for an educational campaign to emphasize the safety and effectiveness of vaccines and their important contributions to health.

Table 1. **Childhood Vaccine Shortages 2000-2006**

Vaccine	Approximate dates	Immediate precipitating factors
Td	11/2000–6/2002	Decreased production in 2000 by both major U.S manufacturers (Wyeth, Aventis Pasteur) Decision of one manufacturer (Wyeth) to cease production 11-month period required for production led to a lag before increased supplies became available from remaining major manufacturer
DTaP	3/2001–7/2002	Recommendation to eliminate/decrease use of thimerosal-containing vaccines Decision of one manufacturer (Wyeth) to cease production
PCV	9/2001–5/2003	Unanticipated initial demand Several sporadic manufacturing problems at the sole manufacturer (Wyeth)
MMR	10/2001–7/2002	Voluntary renovations at a vaccine filling suite that affected multiple vaccines (Merck)
Varicella	10/2001–8/2002	Voluntary renovations at a vaccine filling suite that affected multiple vaccines (Merck)
MCV4	5/2006–present	Demand for vaccine greater than anticipated and exceeded manufacturer's capacity
Influenza	10/2004–4/2005	One of two manufacturers (Chiron) dropped out because of bacterial contamination

Abbreviations: Td-tetanus and diphtheria toxoids, adult; DTaP-diphtheria and tetanus toxoids and pertussis vaccines, adsorbed; PCV-pneumococcal conjugate vaccine; MMR-measles, mumps, and rubella vaccine; MCV4—meningococcal conjugate vaccine.

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Walter A. Orenstein, M.D.

Dr. Orenstein joined the Emory University School of Medicine in March 2004 as Director of the Emory Program for Vaccine Policy and Development, and as Associate Director of the Emory Vaccine Center. Dr. Orenstein is the former Director of the National Immunization Program at the Centers for Disease Control and Prevention (CDC) and former Assistant Surgeon General of the U.S. Public Health Service. During his 26-year career at CDC he led a $1.6 billion effort with more than 450 staff dedicated to reducing vaccine-preventable disease burdens around the world, including elimination of some of the greatest causes of childhood mortality and disability. He served as the CDC liaison member to the National Vaccine Advisory Committee and the American Academy of Pediatrics Committee on Infectious Diseases, having played a major role in developing critical immunization policy documents. Dr. Orenstein has been a member of the External Advisory Board of the HIV Vaccine Trials Network. He is Director for Strategic Planning of the Hope Clinic, site of a number of HIV vaccine trials. Along with Dr. Stanley Plotkin, Dr. Orenstein co-edited Vaccines, 4th edition, a 1,600-page volume with more than 125 contributors that is the leading textbook in the field.

Alan R. Hinman, M.D., M.P.H.

Dr. Hinman is a Senior Public Health Scientist at The Task Force for Child Survival and Development, a program affiliated with Emory University and The Carter Center. At the Task Force, he works with the Public Health Informatics Institute, a Robert Wood Johnson Foundation-funded program seeking to foster development of integrated preventive health information systems. In addition, he works with the PARTNERS TB Control Program, a program funded by the Bill & Melinda Gates Foundation addressing multidrug-resistant tuberculosis in Peru and globally.

Since 1965 he has been involved in public health programs at state, national, and international levels, primarily working with the Centers for Disease Control and Prevention. In addition, he has worked for the state health departments of New York and Tennessee. He retired from the U.S. Public Health Service in July 1996, having attained the rank of Assistant Surgeon General. Dr. Hinman is the author or co-author of more than 300 scientific publications. He is an Adjunct Professor at the Rollins School of Public Health of Emory University in the Departments of Epidemiology and Global Health.

Lance E. Rodewald, M.D.

Dr. Rodewald is Director of the Immunization Services Division (ISD) in the National Center for Immunization and Respiratory Diseases of the CDC. The ISD division administers the Vaccines for Children program and the Section 317 immunization grants program to state and urban area health departments; conducts health services research to improve delivery of vaccines; provides training and education to health professionals; evaluates effectiveness of program implementation through the National Immunization Survey; and provides technical support and funding for immunization information systems.

Dr. Rodewald received his medical degree from Southern Illinois University School of Medicine and trained in pediatrics at the University of Virginia and the Robert Wood Johnson School of Medicine and Dentistry. He had three-year NIH fellowship training in medical informatics at the University of Illinois where he received an M.S. in computer science, and two-year health services research training at the University of Rochester School of Medicine and Dentistry in the Robert Wood Johnson General Academic Pediatrics Training Program.

At the University of Rochester he was Associate Professor of Pediatrics and Emergency Medicine and Director of the Pediatric Emergency Department. In 1996, he joined the CDC as Associate Director for Science in the ISD. His research interests include structure of the immunization delivery system, and methods to improve immunization coverage and to integrate immunizations into primary care more completely and seamlessly.

Project BioShield A Tool for Developing, Using, and Stockpiling Needed Public Health Emergency Medical Countermeasures

Noreen A. Hynes, M.D., M.P.H.

Introduction

The development and availability of medical countermeasures against chemical, biological, radiological, and nuclear (CBRN) threats are key components of President George W. Bush's biodefense strategy, as outlined in the National Strategy to Combat Weapons of Mass Destruction [1], Biodefense for the 21st Century [2], and the National Strategy for Medical Countermeasures against Weapons of Mass Destruction [3]. On July 21, 2004, the President signed Public Law (P.L.) 102-276, the Project BioShield Act of 2004 (Project BioShield), as part of the broader strategy to defend America against the threat of weapons of mass destruction [4]. The purpose of Project BioShield is to accelerate the research, development, purchase, and availability of effective medical countermeasures against CBRN agents.

Project BioShield: Three Critical Elements

Project BioShield includes three approaches to reaching the goal of having safe, effective, and deployable medical countermeasures to respond to the effects of CBRN threats on the U.S. population.

- *Facilitating Research and Development:* Project BioShield grants the National Institutes of Health (NIH)/National Institute of Allergy and Infectious Diseases (NIAID) authorities to expedite and simplify the solicitation, review, and award of grants and contracts for the development of critical medical countermeasures.

- *Facilitating Medical Countermeasure Use in Emergencies*: Project BioShield establishes the Emergency Use Authorization (EUA) to provide access to the best available medical countermeasures following a Declaration of Emergency by the Secretary of the Department of Health and Human Services (HHS). The Declaration could be based on either the Secretary's determination of a public health emergency with the significant potential to affect national security, or on a heightened risk of a CBRN attack on the public or U.S. military forces (as determined by the Secretary of the Department of Homeland Security (DHS) or the Secretary of Defense, respectively).

- *Funding of Needed Countermeasures*: Project BioShield institutes a secure funding source for the purchase of critical medical countermeasures, including vaccines, biologics, therapeutics, and diagnostics. The legislation authorizes the use of $5.6 billion in funding over 10 years for the advanced development and purchase of priority medical countermeasures. This "Special Reserve Fund" was provided in the Fiscal Year (FY) 2004 DHS Appropriations Act [3] and becomes available to the Secretary of HHS for procurements following interagency and White House approval. Within the HHS, the Office of the Assistant Secretary for Preparedness and Response has procurement authority for Project BioShield acquisitions using this Special Reserve Fund.

Project BioShield: Prioritizing Government Investments

The three critical elements of Project BioShield aim to seamlessly integrate medical countermeasure acquisitions with overall U.S. Government preparedness and emergency response plans. Under Project BioShield, the U.S. Government seeks to make balanced acquisitions of the most urgently needed medical countermeasures, within the limits of the Special Reserve Fund (See Table 1 for 2004-2006 acquisitions). This provides the "pull" that complements the "push" in medical countermeasure development provided by separate funding mechanisms for discovery, research, and development provided to the NIH (Figure 1). In support of this goal, a U.S. Government science-informed, policy-guided, interagency process, under the auspices of the Executive Office of the President, is responsible for developing and coordinating research agendas focused on medical countermeasures; prioritizing the development and acquisition of new medical countermeasures across Federal agencies, including under Project BioShield; and ensuring the existence of a product development pipeline for needed countermeasures.

Specific statutory requirements must be fulfilled prior to proceeding with any Project BioShield procurement (Figure 2). First, the Secretary of DHS determines that a particular biological, chemical, radiological, or nuclear agent poses a "material threat" to the population of the United States. Second, the Secretary of HHS determines if additional medical countermeasures are required. Third, the Secretary of the HHS assesses both fully developed and commercially available medical countermeasures as well as countermeasures in late-stage advanced development, to determine if they are appropriate for acquisition, using the Special Reserve Fund, and for inclusion in the Strategic National Stockpile. A non-commercially available product must be within eight years of licensure, approval, or clearance by the U.S. Food and Drug Administration at the time of contract award. From an operational standpoint, this means that product manufacturers must provide sufficient data to demonstrate that there are no major barriers to approval or licensure of their product. These data may include, but are not limited to, Phase I clinical trial results, toxicology study results, pharmacokinetics or immunogenicity data, animal efficacy studies, and demonstrations of current Good Manufacturing Practices. Fourth, the Secretaries of DHS and HHS jointly recommend purchase of the medical countermeasure to the Director of the Office of Management and Budget (OMB). The OMB Director, under delegated authority from the President, approves the purchase of medical countermeasures as recommended by the Secretaries of DHS and HHS.

Project BioShield Targeted Medical Countermeasures: Successes and Challenges of the First Two Years

Implementation Successes

The first two years of Project BioShield implementation have been guided by the interagency strategy defined in December 2003 and supported by Department of Homeland Security Material Threat Determinations (MTD) for anthrax, smallpox, botulinum toxins, and radiological and nuclear agents. Procurement programs now exist for vaccines to counter anthrax and smallpox, biologics therapies directed against anthrax and botulinum toxins, and therapeutic agents to treat internalized particulate radiation and acute radiation syndrome secondary to near-total or total body irradiation (For more information, visit www.hhs.gov/aspr/ophemc/bioshield/procurement_activities/PBSPrcrtPrjct/index.html). The nature of the CBRN threat is such that the regulatory pathway for most new products includes use of the so-called "Animal Rule," in cases where human efficacy trials would be unethical [4, 5]. Animal models needed to regulate these products often have been undefined at the outset of product development and occur concurrently along with that development. Pyridostigmine bromide, a pretreatment against the effects of the nerve agent soman, is the first drug to be approved using the Animal Rule [6].

Implementation Challenges

The experience implementing Project BioShield over the first two years since enactment has highlighted a number of issues that make acquisitions challenging and unique.

It is estimated that the cost of developing and bringing to market a new drug is between $800 million and $1.7 billion [7]. The Special Reserve Fund for Project BioShield holds $5.6 billion to be expended over 10 years. This amount has not drawn the attention of large pharmaceutical or biotechnology firms to date, possibly because the potential payoff for a breakthrough in medical countermeasures against CBRN threats is modest when compared with other drugs. For example, the global market for a major cholesterol-lowering agent was $10.3 billion in 2003 and exceeded the global market for all vaccines that year [8]. Despite a global vaccine market that is continuing to expand annually at a 10 to 12 percent growth rate, the annual global market for vaccines is expected to be only $17 billion in 2010 [9].

Smaller companies, however, have been attracted to participate in Project BioShield. Successful participation by these manufacturers should result in an expansion of nationally available pharmaceutical manufacturing capacity and expertise. A cost of building this capacity among smaller, less experienced companies is that more intensive technical assistance and oversight to meet the requirements of Project BioShield procurement contracts and mitigate the risk of failure is necessary [12].

As noted previously, for a countermeasure to be eligible for Project BioShield, solid clinical experience and/or research data must support "a reasonable conclusion that the countermeasure will qualify for [FDA] approval or licensure within eight years after the date of a determination [4]." Late-stage research and development funds, apart from the Special Reserve Fund, to support potential candidates before they are eligible for Project BioShield are critical to ensuring full medical countermeasure maturation. To address this, HHS has

proposed $169 million for advanced development in the FY08 budget to support promising candidates.

Funding advanced development to levels that support multiple candidates is key to mitigating risk in the Project BioShield acquisition phase of the product development pathway. The Pandemic and All-Hazards Preparedness Act established the Biomedical Advanced Research and Development Authority (BARDA) [13]. Through BARDA, HHS will promote innovation, reduce risk to both medical countermeasure developers and the Government, and invest in medical countermeasure advanced development in order to bring candidate products to an acquisition-ready stage. HHS anticipates that available funding through these authorities will support the highest priority medical countermeasure development programs.

Finally, although liability issues have not prevented the completion of any countermeasure acquisitions to date, liability protection remains a major source of concern to the biotechnology and pharmaceutical industries. This has been a recurring concern in the Project BioShield acquisition process. In 2005, Congress passed the "Public Readiness and Emergency Preparedness (PREP) Act" as part of the 2006 Defense Appropriations Act (P.L. 109-148). This legislation included liability protections for manufacturers of security and pandemic countermeasures.

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Figure 1

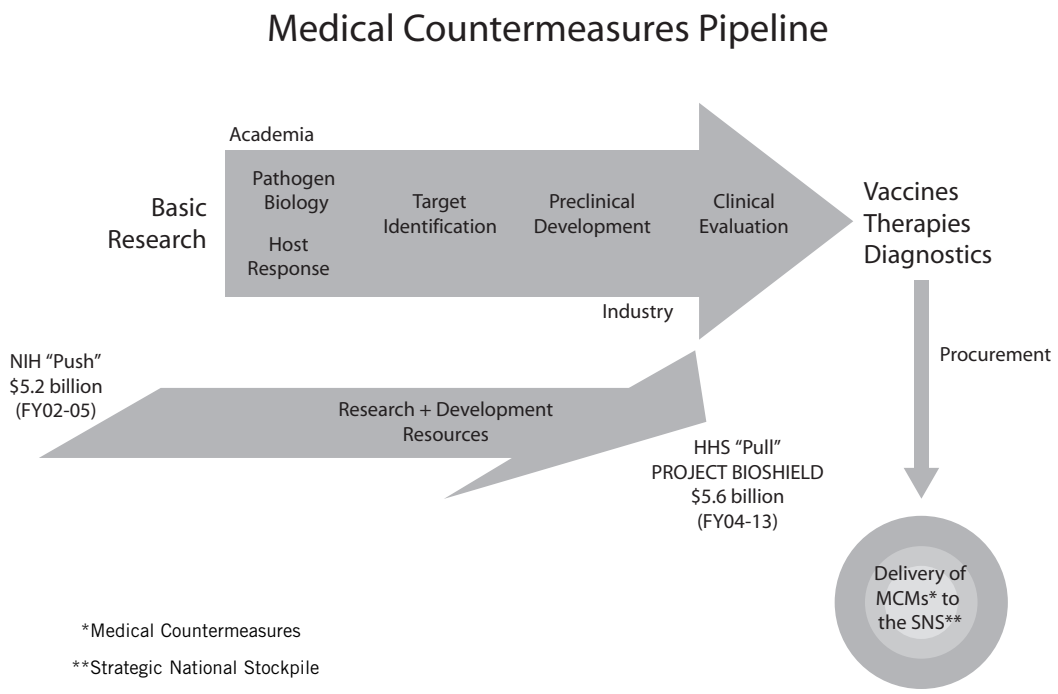

Figure 2

Project BioShield Acquisition Process

Interagency Approval of the Requirement and Acquisition by WMD MCM Subcommittee

Findings by Secretaries of DHS and HHS*

- Determination of material threat
- Determination that countermeasures are necessary to protect public health
- Determination that acquisition of the countermeasure with the Special Reserve Fund (SRF) is appropriate
  - —Numbers of doses required
  - —Production & delivery is feasible within 8 years
  - — Evaluation of commercial market
- Joint recommendation that the SRF be made available for the procurement

Approval by the President* (Delegated to OMB)

HHS Executes Acquisition Program

*Statutory requirements as defined in the Project BioShield Act of 2004 (P.L. 108-276)

Table 1: **Summary of Project BioShield Acquisition Programs, 2004-2007**

Medical Countermeasure	Quantity	Funds Obligated/Status of Contract
Anthrax		
Recombinant Protective Antigen (rPA) anthrax vaccine	75 million doses	Contract awarded by HHS November 2004 to VaxGen, Inc. Terminated December 2006: $1.7M.
Anthrax Vaccine Adsorbed (AVA)	5 million doses	Contract awarded May 2005 to BioPort Corp.: $122.7M. Delivery to the Strategic National Stockpile (SNS) was completed in February 2006.
	Option for an additional 5 million doses	Contract options exercised in May 2006 for an additional 5 million doses: $120M.
Anthrax therapeutics	30,000 treatment courses	Two base contracts (non-BioShield) awarded September 2005 for product testing to Human Genome Science ($1.8M) and Cangene Corp. ($0.4M). Monoclonal Antibody to Protective Antigen Contract options exercised in June 2006 under the Human Genome Sciences contract for 20,000 treatment courses of ABthrax: $165.2M. Human Anthrax Immune Polyclonal Immune Globulin Contract option exercised in July 2006 under the Cangene contract for an additional 10,000 treatment courses of Human Anthrax Immune Globulin: $143.8M.

Medical Countermeasure	Quantity	Funds Obligated/Status of Contract
Botulism		
Botulinum antitoxin	200,000 doses	In FY04 prior to enactment of the Project BioShield Act of 2004, $50M was obligated for support of the botulinum antitoxin program. These funds were used to process existing equine plasma collected by the Department of Defense and to establish the equine plasma program needed to provide new plasma for processing into antitoxin. Contract awarded June 2006 to Cangene Corporation for 200,000 doses of Heptavalent Botulism Antitoxin: $362.6M.
Smallpox		
Modified Vaccinia Ankara (MVA)	10 million—20 million doses	RFP posted August 2005; Proposals received October 2005; Amendment to the RFP released July 2006; Contract award(s) anticipated in May 2007.
Radiological/Nuclear		
Pediatric (Liquid) Potassium Iodide	1.7 million one-ounce bottles	Contract awarded March 2005 to Fleming Pharmaceuticals for 1.7 million bottles: $5.7M.
	Additional 3.1 million bottles	Delivery to the SNS was completed in September 2005. Contract options exercised in February and May 2006, including an additional 3.1 million bottles: $11.8M. Total obligation: $17.5M. Delivery to the SNS started in May 2006.
Medical countermeasures to treat/ mitigate neutropenia associated with Acute Radiation Syndrome (ARS)	Up to 100,000 treatment courses	RFP closed February 2006. RFP terminated March 2007 and will be reissued.

Medical Countermeasure	Quantity	Funds Obligated/Status of Contract
Chelating agents Zn- and Ca-DTPA	~475,000 doses	Contract awarded February 2006 to Akron Inc. for delivery of 390,000 doses of Ca-DTPA and 60,000 doses of Zn-DTPA: $21.9M. Obligation increased by $32,448 to acquire an additional ~5,000 doses of Ca-DTPA and ~19,000 doses of Zn-DTPA (manufacturer overage). Delivery of 395,370 doses Ca-DTPA and 79,369 doses Zn-DTPA to the SNS completed in April 2006.

Noreen Hynes, M.D., M.P.H.

Dr. Hynes served as the Deputy Assistant Secretary for Public Health Emergency Medical Countermeasures, Office of Public Health Emergency Preparedness, Office of the Secretary of Health and Human Services. She has held various positions in government and academia. During her 30 years of government service, Dr. Hynes has focused on research, medical, regulatory and public health aspects of infectious diseases, especially potentially vaccine-preventable diseases including dengue, simian immunodeficiency virus, sexually transmitted diseases, anthrax, and other biological threat agents. Following the terrorist attacks of 2001, she served as senior advisor for medicine and public health in the Office of the Vice President of the United States and played a role in the development of Project BioShield.

Using Genomics to Identify Novel Vaccine Candidates Against Pathogens

Hervé Tettelin, Ph.D., Rino Rappuoli, Ph.D., and Claire M. Fraser-Liggett, Ph.D.

Introduction

The Genomes Online Database (www.genomesonline.org) lists more than 2,000 genome sequencing projects, including more than 1,000 bacterial projects that are either completed or ongoing. The vast majority are accessible to the public. This wealth of genome sequence information enables a sizeable repertoire of large-scale analyses geared at understanding the biology, phylogeny, and genetic diversity of organisms of interest. In the case of pathogens, critical insights into their potential to interact with their host and cause disease can be gleaned from comparative and functional genomics approaches. In particular, genomics can be used extensively to accelerate the development of vaccines by enabling the *in silico* identification of promising vaccine candidates prior to undertaking any experimental step of classical vaccine development.

Reverse Vaccinology

Reverse vaccinology is an approach that reverses the steps of classical vaccine candidate discovery and alleviates the need to grow the causative organism. A gene list derived from the complete genome sequence of a pathogen of interest is used to predict gene products (proteins) that are likely to be accessible to host antibodies. The potential candidates are then expressed and characterized experimentally, including serological tests against the pathogen itself as a final step prior to clinical trials.

The reverse vaccinology approach was pioneered in 2000 using the genome sequence of serogroup B *Neisseria meningitidis* [1, 2]. *N. meningitidis* is a Gram-negative bacterium that causes life-threatening invasive infections, meningitis and septicemia, especially in young infants. While vaccines were available against four of the five pathogenic serogroups of *N. meningitidis*, none existed against serogroup B. The serogroup B capsular polysaccharide is a polysialic acid that could not be used for vaccine development because its structure is identical to carbohydrates widely distributed on human glycoproteins such as N-CAM. In addition, candidate proteins identified through classical approaches consistently provided high levels of protection against their homologous strain but failed to confer general protection due to their high degree of sequence variability across isolates. Application of the reverse vaccinology approach [2] identified several highly immunogenic and highly conserved (non-variable) vaccine candidates, and a subset of these is currently being tested in human clinical trials [3].

In Silico Vaccine Candidate Prediction

For many bacterial pathogens, human immunity is mediated by raising antibodies against epitopes accessible at the surface of the pathogen. *In silico* analysis, which uses computer-based modeling in conjunction with bioinformatics, enables systematic identification of proteins that are likely to be exposed at the surface of the bacteria. Surface-exposed proteins are predicted based on the combination of several pieces of evidence including proteins known to carry out functions at the surface of the cell, exclusion of proteins known to be cytoplasmic, and exclusion of proteins likely to be embedded in the cell's membrane and inaccessible to antibodies. Additional evidence contributing to predictions is based on amino acid motifs characteristic of targeting to the membrane (signal peptides), anchoring in the lipid bilayer (lipoproteins), anchoring in the outer membrane of Gram-negative bacteria or the cell wall of Gram-positive bacteria (like streptococci), and interacting with host proteins or structures (e.g., integrin binding domain) [4]. This analysis typically identifies several hundreds of predicted surface-exposed proteins. In the case of *N. meningitidis* serogroup B, 570 candidates were identified.

Experimental Characterization of Vaccine Candidates

The next step in reverse vaccinology is to experimentally characterize the predicted candidates. The potential surface proteins are expressed in *Escherichia coli* and the purified recombinant proteins are used for immunization of mice. Antisera raised against the injected proteins are recovered and assayed for specificity by Western Blot. Accessibility of the candidate proteins on the surface of pathogen is also tested by flow cytometry or immunoprecipitation using the antisera. Finally, the antisera can be combined *in*

vitro with human complement to assay bacterial killing (bactericidal activity) [5] that often correlates with protection in humans. Each experimental step of the process reduces the number of potential vaccine candidates to a refined set of proteins that satisfies all the criteria and warrants high probability of success for the development of a vaccine. It is important to note that candidates are not required to be directly involved in virulence to confer protection when used in a vaccine; indeed, surface exposure and antigenicity are sufficient.

For *N. meningitidis* serogroup B, 350 of the 570 candidate surface proteins were successfully expressed as recombinant proteins. Of these, 85 were strongly positive in at least one of the experimental tests listed above. The seven best candidates that satisfied all criteria were selected and sequenced across a panel of diverse strains of *N. meningitidis* representing all serotypes and spanning the phylogeny of the species [2]. Five of the seven candidates were completely conserved across the entire panel of strains. Thus, for the first time in decades of classical vaccinology, five extremely strong vaccine candidates likely to confer general protection against serogroup B strains of *N. meningitidis* were identified. These were combined and tested in infant rats challenged intraperitoneally with lethal doses of *N. meningitidis*. The cocktail, when formulated with adjuvants suitable for human use, conferred protection in rats against 90 percent of a panel of 85 *N. meningitidis* strains representative of the global population diversity [3]. The cocktail is currently being tested in human clinical trials [3].

Streptococcus agalactiae or Group B Streptococcus

S. agalactiae is the leading cause of bacterial sepsis, pneumonia, and meningitis in neonates in the United States and Europe [6]. It is also an emerging cause of infection in the elderly [7, 8]. The reverse vaccinology approach was applied to serotype V strain 2603V/R of *S. agalactiae*, which is a representative of an emerging serotype responsible for one-third of clinical isolates in the United States [9]. Mining of the 2603V/R genome sequence predicted 650 surface-associated proteins, 291 of which were successfully expressed. Fifty-five proteins were accessible to antibodies tested against the pathogen. A number of strong candidates were selected for vaccination in the animal model. Unfortunately, no candidate or combination thereof conferred general protection against a diverse panel of *S. agalactiae* isolates. Microarray-based comparative genomic hybridizations revealed that *S. agalactiae* is an extremely diverse species [9, 10]. Most of the diversity was restricted to several large genomic islands whose presence and absence varied among strains, but several individual genes also varied. This degree of diversity indicated that multiple genomes of this species should be used to enable the identification of broadly protective vaccine candidates. It was thus decided to generate the complete genome sequence of six additional strains of *S. agalactiae* representing the major disease-causing serotypes of *S. agalactiae* [11].

Bacterial Species Diversity

Comparing Multiple Genome Sequences from the Same Species

The availability of the complete genome sequence of a single representative strain of a pathogen is useful in revealing its core machinery and comparing it to other sequenced species. However, it does not provide information about the diversity encountered across multiple strains of the species. In order to carefully assess the expected degree of diversity among disease-causing strains likely to be encountered in the field, it is preferable to have access to the genome sequence of multiple strains and perform up-front genome comparisons prior to undertaking the development of vaccines based on genome data.

Unfinished genome sequences (no gap closure) are of interest because they are much cheaper to produce than completed genomes. Indeed, much of the effort and costs of a genome project are dedicated to the finishing phase because it is time consuming, less amenable to automation, and less predictable. However, it is important to have at least one, but preferably several, complete genome sequences of the strains of interest in order to understand the structure of their chromosomes and identify regions that are not obtained by shotgun sequencing without targeted gap closure [12].

The Pan-Genome Concept

In order to assess the genetic diversity of the *S. agalactiae* species, the gene content of eight whole genome sequences (three finished and five unfinished) was compared to identify genes shared among all or some of the isolates studied as well as genes that are specific to individual strains [11, 13].

The genes shared by all the strains constitute the *core genome* and represent the machinery that enables *S. agalactiae* to achieve its basic life cycle and encode its major phenotypic traits. *S. agalactiae* has a core genome made up of 1,806 genes that represent about 85 percent of any individual genome. This core genome encodes a wide variety of cellular functions mostly dedicated to housekeeping. Analysis of strain-specific genes resulted in 13 to 61 genes unique to any individual strain, most of which are clustered in

genomic islands, confirming microarray experiments. The flanks of the islands are conserved across the genomes, making them potential hot spots for recombination, possibly with DNA of foreign origin acquired through lateral gene transfer. Indeed, many of the islands display an atypical nucleotide composition when compared to the rest of their respective genomes [11]. The 358 strain-specific genes plus the genes that are present in some strains but absent in at least one of them constitute the *dispensable genome*, which includes functions that enable a subset of strains to adapt to specific conditions, colonize particular niches, or resist certain antibiotics.

The combination of the core genome and the dispensable genome constitutes the *pan-genome* of *S. agalactiae*. The pan-genome contains a large number of genes that exceeds that of any given strain and delineates the entire pool of gene functions that are accessible to this species. Analysis of the *S. agalactiae* pan-genome reveals that new genes are added to the pan-genome every time a new genome is sequenced. The first genome sequence provides approximately 2,200 genes, addition of a second genome provides approximately 100 new genes, a third genome brings about 60 new genes, and so on. Surprisingly, mathematical extrapolation of the observed trend indicates that every new genome will contribute an average of 33 additional new genes to the pan-genome no matter how many genomes are sequenced, possibly expanding the pan-genome to infinity [11]. This led to the concept of an open pan-genome indicating that the *S. agalactiae* species has access to a very large and possibly unlimited number of genes. Similar results were obtained by analyzing the available genome sequences of *Streptococcus pyogenes* (Group A streptococcus) and *Staphylococcus aureus*. In contrast, the analysis of 11 *Bacillus anthracis* genome sequences revealed that 4 genomes are sufficient to identify the entire gene repertoire of this species, indicating that it has a closed pan-genome.

Pan-Genome and Reverse Vaccinology

Vaccine candidates encoded by the core genome are more likely than non-core candidates to be present on all strains encountered during infection and therefore provide universal protection. This probably holds true for some pathogenic species. In the case of *S. agalactiae*, however, no individual core protein or combination thereof provides high levels of immunity against a panel of *S. agalactiae* isolates. This could be due to the fact that these core genes are absent in strains that have not been sequenced. Another factor is that not all potential candidates are accessible to antibodies *in vivo*, for instance because of the presence of capsular polysaccharides. The best four candidates identified through the screening process described above include only one protein from the core genome while the three other candidates belong to the dispensable genome. Each candidate was used individually in protection assays. They conferred 0 to 88 percent protection individually when tested against a panel of 6 challenge *S. agalactiae* strains belonging to the 5 major disease-causing serotypes. The best results in protection were only achieved when the four best candidates were combined together. The four-protein cocktail conferred 59 to 100 percent protection against a panel of 12 *S. agalactiae* isolates that included the major serotypes as well as two strains from the less common serotype VIII (81 percent and 94 percent protection). Finally, bacterial killing was assayed *in vitro* in the presence of polymorphonuclear leukocytes and rabbit complement, a strong indicator of a promising vaccine candidate. Again, the most efficient killing was achieved with sera from mice vaccinated with the four-protein cocktail, indicating that the protection each antigen confers complements that of the other antigens [14].

Conclusion

Reverse vaccinology alleviates a major problem inherent to classical approaches to vaccine development that identify only a limited number of highly accessible and/or highly expressed antigens. All potential antigens are encoded by the pathogen's genome, and a systematic prediction and characterization of all possible candidates results in a large number of antigens available for vaccine development. The approach has been applied to a growing number of eukaryotic and prokaryotic species, including the following bacteria: *N. meningitidis* [1-3], *S. agalactiae* [9, 11, 14], *S. pneumoniae* [15], *S. pyogenes* [16], *Porphyromonas gingivalis* [17], *Chlamydia* [18], *S. aureus* [19], and the SARS coronavirus [20, 21].

While a single genome sequence proved sufficient for the design of a vaccine against a specific serogroup of *N. meningitidis*, species with an open pan-genome encode too much diversity to enable identification of reliable candidates from a single genome sequence. The availability of a single genome is further limited by the fact that the first genome sequence of a given species is typically the type of strain that is well characterized and genetically tractable but often poorly represents the gene repertoire and diversity encountered in the wild [22]. Fux et al. further indicate that "this limitation might be overcome by the summation of individual genomes

to produce a species-specific virtual "supragenome" that corresponds to our definition of the pan-genome [22]. Shen et al. surveyed the supragenome (pan-genome) of non-typeable strains of *Haemophilus influenzae* through low pass whole genome sequencing [23]. They concluded that each strain contained about 10 percent of new genes and that the supragenome of *H. influenzae* is significantly larger than the genome of any individual strain.

Most of the recent genome sequencing efforts were based on the classical Sanger dideoxy-sequencing or chain termination method, which has been greatly automated by the use of capillary sequencing machines. More recently, however, novel sequencing technologies that allow for higher throughput and greater levels of automation have emerged as viable complements to the Sanger methods [24]. A very promising novel technology is based on the automation of the pyrosequencing technique that was applied to the resequencing of a number of species, including some pathogens with an open pan-genome [25]. While this technology is currently limited by short sequence read length (100-150 nucleotides) and the inability to accurately determine the number of bases in homopolymeric nucleotide tracts (*e.g.,* eight As in a row), it holds great promise for sequencing many additional genomes of species of interest cheaply and rapidly when a complete reference genome is already available. It is predicted that within the next few years this and other new technologies will revolutionize the ability to sequence hundreds of genomes of species of interest and provide the amount of data that will enable proper determination of any species' pan-genome.

The combination of reverse vaccinology with large-scale comparative genomics provides for a more informed selection of vaccine candidates and increases the chances of obtaining a successful vaccine product. These approaches should also be combined with functional genomics studies such as microarray transcriptional profiling and proteomics to characterize the level, localization, and timing of protein expression. Indeed, such data allow refining of the candidate selection such that the antigens best suited to tackle the pathogen and its lifestyle, including its mode of interaction with the host, are pursued.

Ultimately, the entire process—whole genome sequencing of multiple isolates of a pathogen followed by *in silico* analyses and experimental characterization of the promising candidates—needs to be streamlined and automated in order to get to the human clinical trial stage faster and with better candidates. Efforts funded by the National Institute of Allergy and Infectious Diseases are currently under way to (1) generate the sequence of multiple strains of many important pathogens under the Microbial Sequencing Centers (www.niaid.nih.gov/dmid/genomes/mscs), and (2) produce databases and interfaces to enable large-scale genome comparisons and data mining of these pathogen genome sequences under the Bioinformatics Resource Centers (www.niaid.nih.gov/dmid/genomes/brc). These efforts, combined with microarrays, proteomics, and the exploration of medium to high throughput platforms for the immunological characterization of candidates such as the VaxDesign system (www.vaxdesign.com) hold promise to remarkably accelerate vaccine development in the very near future.

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During his Ph.D. thesis, Dr. Tettelin coordinated the work of 25 laboratories for sequencing yeast chromosome VII. Upon joining TIGR, he was the primary person responsible for the bioinformatic analysis of chromosome 2 of the human malarial parasite *P. falciparum*. Since then, Dr. Tettelin has led several published whole genome projects on human pathogens including *N. meningitidis, S. pneumoniae*, multiple strains of *S. agalactiae, E. chaffeensis, A. phagocytophilum*, and *N. sennetsu*. Dr. Tettelin is also the principal investigator for microarray-based functional genomics projects on these and other pathogens, conducting studies both at the DNA level (comparative genome hybridizations), and the RNA level (transcriptional profiling).

Dr. Tettelin is the author of more than 50 papers in the field of genomics. His most prominent scientific contributions relate to pioneering work on the reverse vaccinology approach, in collaboration with Novartis Vaccines and Diagnostics (formerly Chiron Vaccines). This work resulted in new vaccines currently being tested in clinical trials and also led to the new concept of the bacterial pangenome (both described in this article).

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He is the co-founder of the field of cellular microbiology, a discipline that has merged cell biology and microbiology. He is a member of the U.S. National Academy of Sciences and of the European Molecular Biology Organization. In 2005, he was awarded the Gold Medal by the President of the Italian Republic for his contributions to public health care.

Dr. Rappuoli has developed the first recombinant bacterial vaccine (against pertussis) and a conjugate vaccine against meningococcus C. Both products have been approved for human use. Currently, he is involved in the development of a vaccine against group B meningococcus using reverse vaccinology (described in this article), the development of influenza vaccines produced in cell culture, and the development of vaccines against avian influenza.

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Dr. Fraser-Liggett's academic and professional honors have included the 2005 Promega Bio-technology Research Award from the American Society of Microbiology, the 2005 Charles Thom Award from the Society for Industrial Microbiology, the 2002 E. O. Lawrence Award from the Department of Energy, election as a Fellow in the American Academy of Microbiology and AAAS, and recognition as one of Maryland's Top 100 Women. She holds professorships in Microbiology and Tropical Medicine as well as in Pharmacology at The George Washington University School of Medicine.

Adolescent Immunizations
Will the Shots Hit Their Targets?

Amy B. Middleman, M.D., M.P.H., M.S.Ed.

New Immunizations for Adolescents

Adolescent immunizations are currently being recommended at a rapid pace. Prior to May 2005, the only *routine* adolescent vaccination recommendation was for the tetanus/diphtheria booster (Td). Hepatitis B vaccination has been recommended universally as a catch-up for all adolescents who have not previously received the vaccine, and adolescents who have not yet received the varicella vaccine or second measles, mumps, rubella (MMR) vaccine should also receive these vaccinations as soon as possible. Other vaccines, including the hepatitis A vaccine, pneumococcal vaccine, and the influenza vaccine, have been recommended for adolescents in certain high-risk groups. In May 2005, the Advisory Committee on Immunization Practices (ACIP) recommended routine vaccination of adolescents with conjugate meningococcal vaccine (MCV4). The vaccination was recommended for the 11- to 12-year age group with catch-up recommended for those approximately 15 years of age and those entering college and expecting to live in dormitories [1]. In June 2006, the ACIP also recommended that the human papillomavirus (HPV) vaccine be administered routinely to females age 11 to 12 years, with catch-up among females age 13 to 26 years (9- to 10-year-olds may also be immunized if desired) [2]. These new vaccine recommendations and new vaccines on the horizon for adolescents have focused a spotlight on this age group's likelihood of achieving high vaccination completion rates. The question is: Will these new shots hit their marks? What are the factors influencing the likelihood of adolescent immunization, and what are some possible strategies to address these factors?

Adolescent Immunization Rates

When tracking adolescent vaccination completion rates for vaccines such as hepatitis B, which has been recommended universally for neonates since the early 1990s as well as for catch-up among adolescents, it is clear that vaccination rates of adolescents have risen steeply in recent years. National data from commercial health maintenance organizations indicate that in 2004, for example, 67 percent of adolescents had received three doses of hepatitis B vaccine by the age of 13 years, compared to 18 percent in 1997 [3, 4]. Interpreting these data is difficult as the data sets do not reveal the year in which the shots were given. Thus, one can not discern if rates are going up among adolescents due to more shots being given to adolescents themselves, due to the aging of immunized infants who are now adolescents and are up to date from childhood immunization, or some combination of these two possibilities. Unfortunately, although the rates are rising, they are still below the Healthy People 2010 goal of 90 percent coverage among adolescents 13 to 15 years of age for all universally recommended vaccinations, especially for those vaccinations recommended within the past 10 years, such as hepatitis B and varicella. Although the data include shots that are completed per parent recall and not necessarily confirmed by provider record, the National Health Interview Survey, the Centers for Disease Control and Prevention (CDC), and the National Center for Health Statistics (NCHS) indicate that rates of immunization for hepatitis B and varicella vaccines remain significantly higher among younger children age 19 to 35 months than for adolescents [5]. For some experts, these data indicate that adolescents are "traditionally" non-compliant with vaccination regimens, and that the only way to achieve successful immunization rates is to immunize when children are young.

The Provider and Patient Factors Affecting Adolescent Immunization

There are some challenges inherent in reaching adolescents, and multiple factors contribute to the difficulty in immunizing the adolescent age group. Some of them are provider/systems-based factors and some are patient/parent-based factors; however, interestingly, these two factions have many concerns in common (see Figure 1). The oft-cited barriers for providers include not seeing enough adolescents, lack of time during the visit, reimbursement concerns, difficulty accessing and verifying past immunizations, and lack of confidence/self-efficacy in addressing adolescent issues—especially those related to reproductive health. Patient/parent issues include lack of

knowledge regarding vaccine-preventable diseases and the associated vaccines, lack of time, reimbursement concerns, transportation issues, and lack of self-efficacy in getting to provider visits. All of these factors potentially influence each other to determine the likelihood of adolescent immunization.

Are Providers Seeing Adolescents?
Although the impression is that providers are not seeing adolescents, the data indicate otherwise. In fact, although data from the Health Plan Employer Data and Information Set indicate that only 34 percent of adolescents access annual preventive visits within the health plan population [6], 2003 data from NCHS indicate that 86 percent of children 6 to 17 years of age and 76 percent of adolescents and young adults age 18 to 24 years reported at least one visit to a doctor's office or emergency department or a home visit within the past 12 months [7]. Studies support that the majority (88 to 92 percent) of adolescents have an identified source of primary care [8, 9]. Data indicate that it is more likely that a younger adolescent will present for preventive care; in addition, research supports that physicians are more likely to screen for and provide vaccination to younger adolescents [10]. Although parents and patients cite time and transportation as barriers to immunization, adolescents are accessing the health system at a rate that could be exploited for increased immunization compliance. The opportunity is there to capitalize on these visits and encourage patients and providers alike to avoid missed opportunities for vaccination.

Reimbursement
Reimbursement for immunization is an important concern affecting provider and patient behavior [6, 11]. With the advent of the Vaccines for Children Program (VFC) in 1994 as well as state children's health insurance programs, public funding has increased to provide vaccinations to children from birth to the nineteenth birthday for those who are eligible. Improved funding, which is a response to Healthy People 2000 goals requiring increased immunization rates among children, has had a significant impact on immunization completion rates. In 2004, for example, VFC purchased an estimated 40 percent of all of the doses of childhood vaccines distributed in the United States [12]. Many insurance companies follow VFC resolutions to determine coverage for specific vaccines. Funding for vaccination of young adults over the age of 19 years is more complex; there are some funding streams that can be utilized, including federal dollars via Vaccination Assistance Act, Section 317 funds. Overall, improved reimbursement for vaccinations, especially for children and adolescents, has impacted immunization rates. Financial policy discussions are ongoing [13, 14], and, with increasing numbers of vaccinations available, continued change in policy is required to improve immunization rates for all.

Parent/Patient/Provider Education
There is a significant body of literature emphasizing positive adolescent and parent perceptions regarding the use of adolescent immunizations, including those pertaining to reproductive health [15-20]. In a concrete sense, parents are clearly important for transportation, insurance coverage, and authorization for vaccination, but studies also indicate that parental influence regarding vaccination is an important factor affecting adolescents' vaccination decisions [21,22]. Provider acceptability is an important determinant of parent and adolescent acceptability [22]. Data also support the role of education in determining parental support of vaccination [19]; parents who received education regarding HPV were more likely to indicate acceptance of vaccination than those not receiving an educational intervention. Providers also indicate support for vaccination in general; a national survey of U.S. physicians in 1997 revealed that even then, 82 percent recommended hepatitis B vaccination for all eligible adolescents, and 84 percent preferred that immunizations be administered at their own practices [23]. However, it may be an overstatement to assert that the key factor in immunization is a parent's or patient's complete understanding of the disease and the vaccine being considered. Anecdotally, most parents (and some providers) are not aware of the specifics of diphtheria and yet immunization rates against this disease remain high. There are clearly other factors influencing immunization. The general recognition and parental and provider expectation of compliance with established immunization visits during the adolescent years is a critical component to vaccination compliance.

How to Address These Factors

The Society for Adolescent Medicine (SAM) has developed position statements to address many of the barriers to successful immunization of adolescents [24]. They include

(1) The use of all ACIP-recommended vaccines and vaccination schedules in the adolescent age group, without prejudice against the type of infection or mode of transmission targeted by the vaccine.
(2) The development of three distinct adolescent immunization visits/plat-

forms for adolescents (11- to 12-year visit, 14- to 15-year visit, and 17- to 18-year visit) to integrate and emphasize the role of vaccination in already recommended comprehensive health care screening and provision visits. The 11- to 12-year platform is the primary immunization platform promulgated by ACIP. SAM endorses emphasizing a 14- to 15-year visit/platform as a time to catch-up on missed vaccines or complete multi-dose regimens, and a 17- to 18-year visit/platform as an opportunity to update all vaccinations that may have been missed or are newly recommended while the patient is still covered by third-party payers, including the VFC program.

(3) The use of standing immunization orders, immunization screening tools, immunization registries, immunization reminder systems (for both provider and patient), and recall systems, whenever available, to increase rates of vaccination among this age group.

(4) The simultaneous administration of multiple vaccines to increase vaccination rates and utilize/capitalize on currently required and mandated vaccination regimens.

(5) The use of "non-comprehensive" visits (e.g., minor illness visits, camp/sports physicals, pre-college visits) and qualified "alternative" vaccination sites (e.g., pharmacies, schools) for adolescents unable to access comprehensive preventive care. SAM urges the alternative vaccination sites to provide adolescent clients with referral lists of adolescent care providers in their area as well as appropriate adolescent health education materials.

(6) The continued and increased education of health care providers, parents, and teens regarding the health promotion benefits of immunization against vaccine-preventable disease.

These positions promote a relatively complex assortment of approaches that include strategies to improve adolescent immunization rates, some of which must be implemented at a public health level and some at a more individual provider/client level. Individualized practice strategies that have a history of improving compliance among other target groups, such as education, the use of standing orders, the implementation of recall systems, the simultaneous administration of vaccines, and the use of non-comprehensive visits for vaccinations, will only be implemented after a priority on adolescent preventive care in general, and adolescent immunization in particular, has evolved. Study has revealed that one of the most important factors in provider acceptability regarding immunizations is the recommendation and standard of care developed by national standards promulgated by their own professional organizations [25].

It will most likely be the changes that occur at a public health level as a result of evidence-based deliberation that will stimulate the most significant changes in the ultimate vaccination behavior of both providers and patients. The key public health constructs that could potentially change the culture of adolescent immunization include: the development of distinct and expected adolescent immunization platforms/visits; continued mandates requiring vaccination for school entry (a state-level issue at this time); resources and emphasis placed on the use of immunization registries that will prevent over- and under-immunization of adolescents who often visit multiple sites for care; and the potential use of alternative immunization sites to augment the options for immunizations and multi-dose series completion for busy families. The issue of reimbursement for vaccinations, already discussed above, will also require continued advocacy from multiple constituencies (see Figure 2).

Public Health Solutions to Help "Hit the Targets"

The Need for Established Adolescent Immunization Platforms

Historically, there has been no immunization platform for adolescents. Immunizations have been emphasized for infants and young children and for very good reason: the majority of deaths from vaccine-preventable diseases occur in these age groups. The structure for effective and efficient immunization of infants and children has been in place for many years. The first "official" immunization schedule published in 1983 by the CDC depicts the immunization visit structure for infants and children that had been developing in the United States since the introduction of the diphtheria, pertussis, tetanus (DPT) vaccine in the 1940s. The only adolescent immunization recommendation at that time was for the Td booster at age 14 to 16 years. Adolescents' importance in the overall scope of the early immunization schedules is perhaps most clearly represented by their complete omission from the 1994 schedule [26, 41]. Now, as the idea of using immunization to protect against disease across the life span burgeons, the establishment of immunization platforms that encompass older children in the adolescent age range and provide an expectation and standard of care to be followed would address a significant barrier to adolescent immunization completion.

The same parents who brought their children in for infant and child immunizations are, for the most part, the same parents who will bring in their children as adolescents. A large part of the current difficulty in having parents know to bring their children in to the provider for immunizations is the lack of an expected and standard immunization or preventive visit. Annual preventive health care visits for adolescents have been recommended by multiple agencies for many years; the most notable recommendations include Guidelines for Adolescent Preventive Services (promulgated by the American Medical Association in 1992), Bright Futures (a Maternal Child Health Bureau product), U.S. Preventive Health Task Force recommendations, and recommendations by the American Academy of Pediatrics and the American Association of Family Physicians. However, the preventive strategies implemented during annual visits rarely include the type of tangible service such as immunization that draws patients in for care. Although most adolescents eventually receive their Td booster and compliance rates for this vaccination are relatively high, this is most likely due to state mandates and national recommendations that, although variable, have been in place for many years. However, there is no consistent national standard that guides the implementation of a distinct immunization platform similar to the one that exists for infants and young children. One of the most significant barriers to effective adolescent immunization delivery is the lack of structure provided by well-established immunization platforms that so efficiently guide the parents of younger children. It is also important to establish further immunization platforms within the adolescent age range as suggested by SAM. Multiple encounters for immunizations and immunization catch-up will increase the likelihood of delivery of other preventive health services.

ACIP has been methodical in its recommendations for adolescents thus far. Most recommendations are geared, when epidemiologically appropriate, to the 11- to 12-year age group to build a strong platform that will, with continued dissemination, become a national standard for the immunization of adolescents. Data indicate that younger adolescents have higher rates of accessing preventive health care than older adolescents [27]. One of the greatest challenges will be remaining patient as time, education, and resources filter through to help hold the platform together. Vaccine utilization data compiled after the initial recommendation of MCV4 reveal a pattern of immunization that could have been predicted given the lack of immunization platforms among the adolescent age group. Physician claims data through March 2006 revealed a routine immunization across all adolescents regardless of age, despite the recommendations for immunization of 11- to 12-year-olds, 15-year-olds, and 18-year-olds entering college to live in dormitories [28]. These data indicate that without standard and established visit patterns for adolescents, recommendations that target distinct, intermittent age groupings within the adolescent years are unlikely to be followed. Currently, providers cannot be sure when they will see adolescents again and are understandably anxious to vaccinate while they have the opportunity. There have been subtle changes noted, however, as adolescent immunization has increased over the past several years. In a personal communication with sanofi-pasteur, it was noted that as providers have developed familiarity with the meningococcal vaccine recommendation for freshmen entering college, vaccine supply requirements to colleges have been decreasing as more providers have started immunizing in the medical home [personal communication, July 31, 2006]. Change will take time—and patience—but the long-term effects are potentially greater than those associated only with immunizations.

Platform development for adolescents will be as important for general preventive health care of adolescents as it is for infants. The primary sources of morbidity and mortality among adolescents as they begin to change and develop physically, emotionally, and cognitively are the result of risk behaviors. With the advent of immunizations, the possibility exists that adolescents coming in for the effective prevention of vaccine-preventable disease will also be able to receive further services that address multiple health care concerns that would otherwise have been missed. As new recommendations are developed for adolescents, the importance of developing a similarly strong structure, upon which the preventive health care of adolescents can be built, represents an opportunity to affect adolescent health on a scale much larger than vaccine-preventable disease alone.

School Mandates

Experience with immunizing adolescents against hepatitis B has provided data that make it quite clear that state mandates significantly affect immunization rates among adolescents in the United States. In a recent study of the effect of school mandates, adolescents were significantly more likely to have completed the hepatitis B vaccination series in states with mandates (75 percent) versus in states without mandates (39 percent, $p<0.001$) [29]. A study was conducted during the year before and year after a new state

law was passed in California requiring that students entering the seventh grade have received three doses of hepatitis B vaccine and two doses of MMR. The researchers found that vaccination coverage rates were greatly increased among seventh graders in the year after the law was put into effect (60 percent) than among fifth and sixth graders the year before (13 percent), or eighth through twelfth graders not affected by the legislation (27 percent) [30]. Although not affected by the law, there was an encouraging increase in the vaccination coverage achieved among the eighth to twelfth grade group, perhaps indicating that the educational effects of perceived benefit from the law affected immunization rates among other adolescents and their parents. The power of mandates may not only be the direct effect of the actual law on coverage rates in the schools, but the message that is tied to the mandate—that immunizations are deemed an important preventive health strategy for everyone.

As of July 2006, according to the Immunization Action Coalition Web site, only 14 of the 50 states plus the District of Columbia do not have a middle school mandate for hepatitis B vaccine [31]. Tetanus, diphtheria, and pertussis booster mandates change relatively frequently, but states clearly differ in their mandates and the ages at which they require immunization, specifically the booster dose for adolescents. There are still many states that do not have mandates for the booster dose of Td or Tetanus, Diphtheria, Pertussis vaccine (Tdap) [32, 33].

Despite the success of school mandates in increasing adolescent immunization rates, it is unlikely that all adolescent vaccinations will be mandated by each state. By educating providers on the importance of simultaneous administration of vaccinations (same day, different anatomic sites), and by taking advantage of immunization mandates that are already in place, immunization rates for all vaccines can be increased. In general, more coordinated state efforts that reflect national policy recommendations would help increase immunization rates for all children and adolescents.

The Use of Registries

By 3 years of age, 25 percent of children in the United States receive immunizations from more than one provider [14]. It is also estimated that 27 percent of adolescents use multiple sources of care depending upon the health issues for which they seek guidance [8]. Providers often have incomplete or inaccurate immunization records, leading to over- or under-immunization. The National Vaccine Advisory Committee and the National Immunization Program of the CDC advocate the use of computerized immunization information systems (IIS) as a solution to these problems. These systems have been shown to increase documented up-to-date rates and provide insights into patterns of immunization delivery [34]. IIS can consolidate fragmented records, provide immunization needs assessments for each patient, keep track of patients needing recommended or catch-up vaccination, provide automated reminder, assist in the management of vaccine supply, and generate vaccination records for parents, schools, and others [35, 36]. Data as of 2004 indicate that 48 percent of U.S. children are involved in an IIS, and 76 percent of public and 39 percent of private providers participate, an increase over 2003 numbers [1]. Given the fragmented health utilization patterns in this country as insurance plans change and people change jobs and move around the country, and given the strong possibility that alternative sites will be used for immunization among the adolescent age group as well as adults, federal and state support of registries will be a key component to the success of immunization programs across the life span.

The Use of Alternative Sites

Alternative immunization sites have been used successfully among adults. Pharmacies, in particular, have played an ever-increasing role in immunizations since the 1980s when pharmacies hosted nurses to administer vaccines [37]. As of December 2004, 43 states allow pharmacists to immunize [38]. Recent research indicates a high level of safety associated with mass influenza vaccination clinics in non-traditional settings [39]. Pharmacies are becoming increasingly popular sites for adult immunization.

Sites other than the medical home for adolescent immunization might include schools, city clinics, family planning clinics, gynecology offices, emergency departments, and pharmacies. There is evidence that adolescents seek care at these sites: in 1994, 14.8 million adolescent health visits were to emergency departments, 7.3 million were to outpatient departments, and 5.2 million female visits were to family planning clinics [40]. Approximately 4 percent of teens and 11 percent of impoverished adolescents use community clinics for routine health care [41]. School clinics have already been enlisted in several immunization programs with great success [42-47], and city clinics are already often used as immunization sites for adolescents. With the advent of the HPV vaccine, it is expected that gynecology offices will also become more involved in the administration of at least some vaccines.

An important issue that will need to

be addressed, especially with the use of alternative sites for adolescent immunization, is that of "consent" for adolescent vaccinations. The CDC notes in its General Recommendations on Immunization that, "the National Childhood Vaccine Injury Act of 1986 (42 U.S.C. § 300aa-26) requires that all health care providers in the United States who administer any vaccine covered by the act must provide a copy of the relevant, current edition of the vaccine information materials that have been produced by CDC before administering each dose of the vaccine. The vaccine information material must be provided to the parent or legal representative of any child or to any adult to whom the physician or other health care provider intends to administer the vaccine. The Act does not require that a signature be obtained, but documentation of consent is recommended or required by certain state or local authorities [48]." It will be important for states to develop and support consent procedures that do not hinder the immunization of adolescents yet also allow for parents to be involved with the decision to immunize when appropriate. Studies have shown that school-based immunization initiatives have experienced parental consent for immunization as the single most difficult barrier to immunizing students [49, 50]. In a hepatitis B school-based immunization initiative in Texas, the state required parental consent for each of the three doses of vaccine, representing a significant barrier to the efficient delivery of the full vaccination series [49]. However, this vigilance is often needed when immunizations are administered in schools, in particular. Each school district has the ability to determine what will occur within its schools. Each state determines the level of consent required for vaccines, and the conditions of this consent may vary based on whether the vaccine addresses reproductive health diseases for which adolescents have the ability to consent for confidential care. The details of consent for immunizations targeting adolescents, especially at alternative sites, will be an interesting challenge for public health officials in each state.

The primary morbidity and mortality among adolescents, however, is not due to vaccine-preventable disease, but, rather, to preventable behaviors [51]. Primary care providers are an important component in the care of adolescents; the advent of new vaccine recommendations could serve as an important impetus pushing adolescents to receive other needed preventive health services. If alternative sites of immunization are made readily available, adolescents may bypass this opportunity to receive comprehensive preventive care and opt for the quicker use of an alternative immunization site. This creates an obvious tension between the opportunity to provide effective immunizations to as many adolescents as possible and the desire to deliver more comprehensive preventive health care to adolescents. Other potential issues with alternative immunization sites include poor follow-up and lack of access to IIS. However, alternative sites for immunization seem like a practical option for those without a medical home, or those who have already received other preventive care services and only require a vaccination to complete a series or quickly comply with school mandates. Studies are being initiated to investigate the feasibility and acceptability of the use of alternative immunization sites for adolescents among providers and patients. The results are awaited with interest.

Summary

In general, the individual adolescent is not keen on receiving shots. Adolescents and young adults report fear of pain and needles [52, 53]. Until some of the new, less painful immunization delivery systems are marketed, however, adolescents need to access vaccinations in their current form to be protected against vaccine-preventable disease. The public health focus to this point has understandably been on immunizing infants and younger children. With the advent of newer vaccines targeting the adolescent age group, the opportunity exists to greatly affect adolescent health in general in this country. Vaccination is an effective preventive measure; the tangible delivery of such a service will also function as an important "carrot" to draw adolescents in to providers to receive other critical preventive health care. The key to the successful implementation of adolescent immunization will be patience; each factor associated with increasing the likelihood of adolescent immunization has many complexities associated with it. The strong structure of infant and childhood immunization was not built particularly expeditiously and required significant time and resources. The same will be true for an adolescent immunization structure. The long-term advantages to building the structure will be multifold: disease prevention among adolescents, increased comprehensive care applying to other important health issues, and the establishment of immunization patterns that, if successful, could help set the stage for the public to view immunizations as a lifelong preventive strategy that requires maintenance through adulthood. The goal is to have those shots hit their targets, and the targets will benefit in many ways for a long time to come.

Figure 1: **Patient and Provider Factors Associated With the Likelihood of Adolescent Immunization**

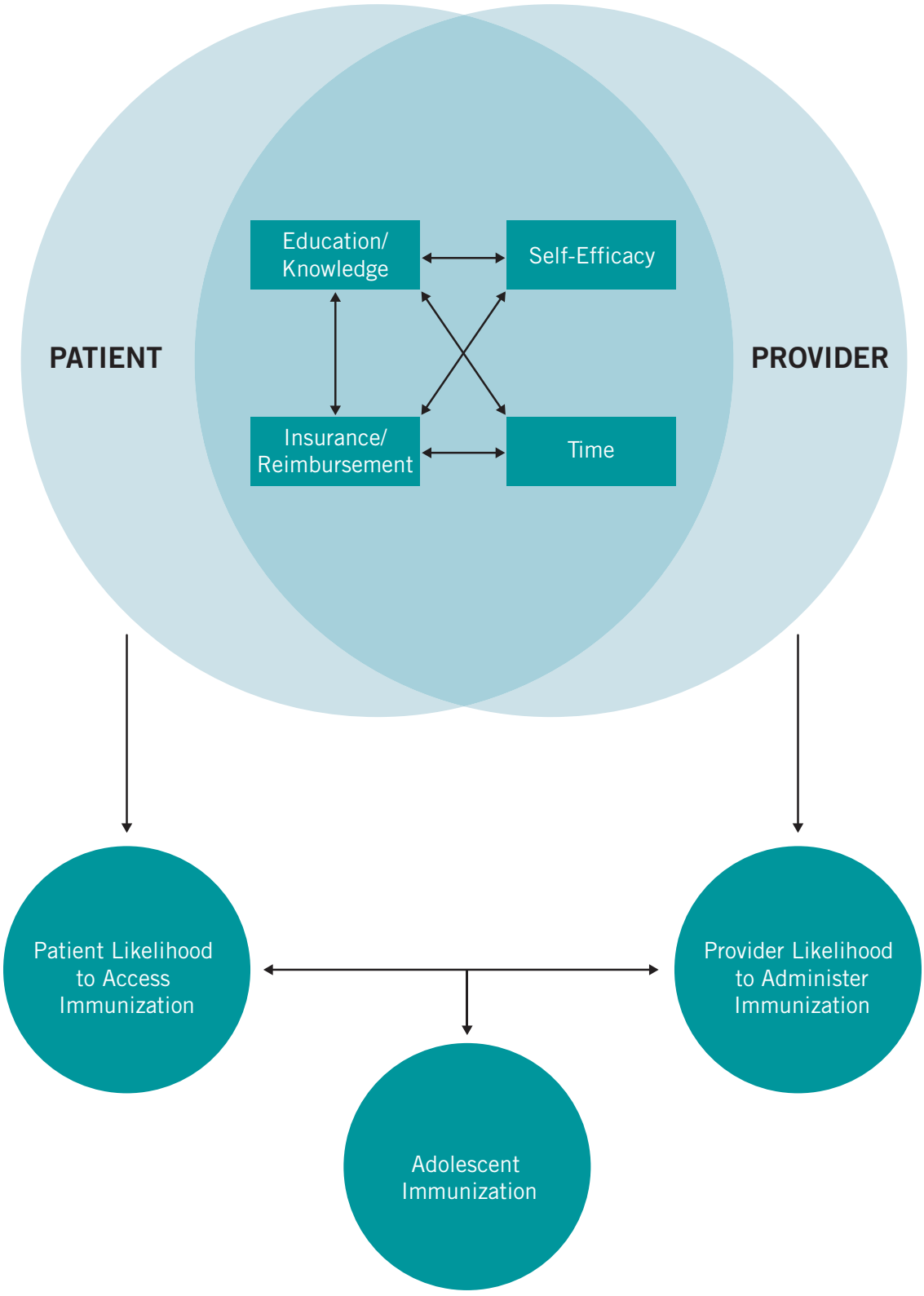
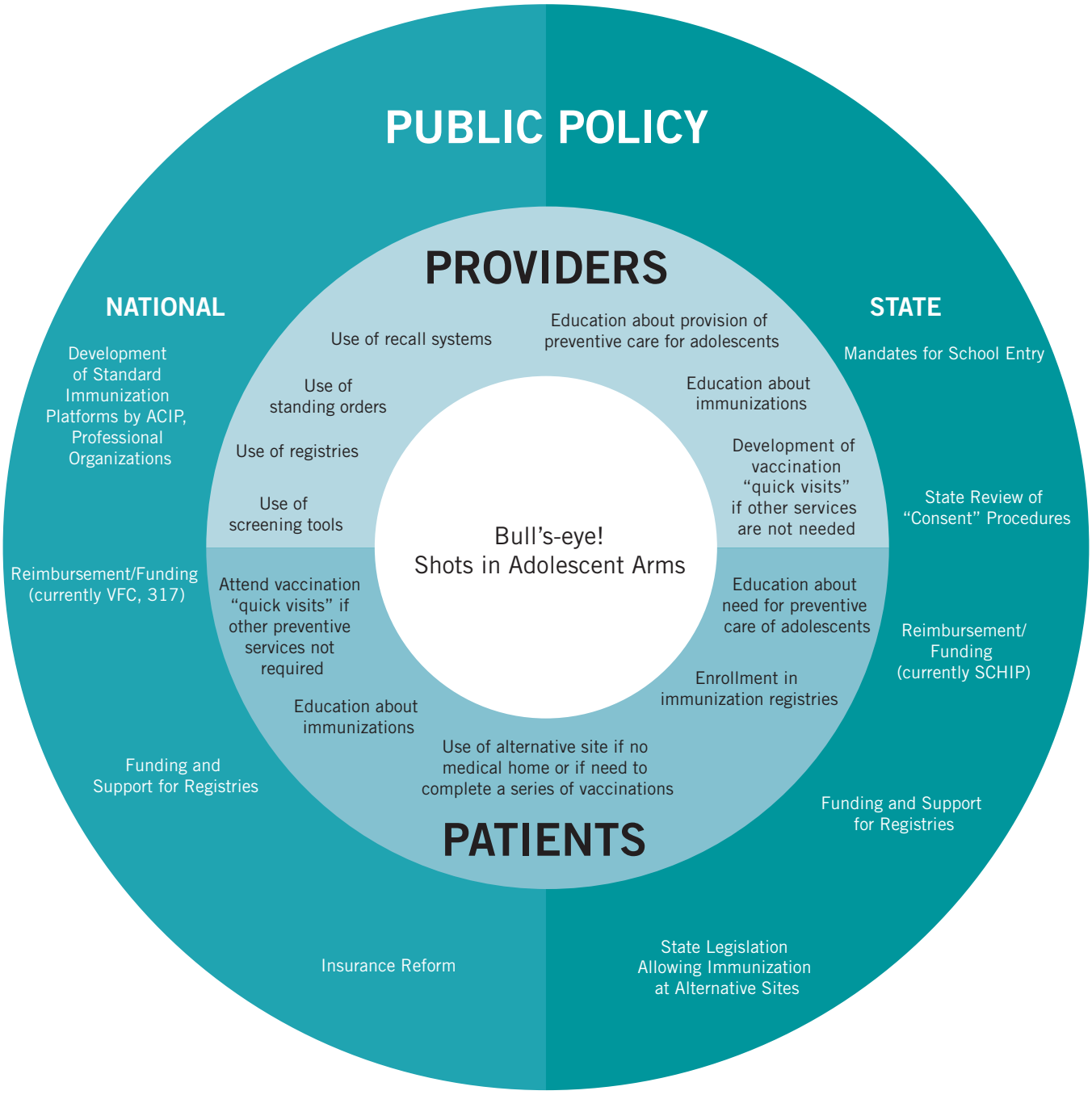


Figure 2: **Constituencies and Issues Related to Vaccine Reimbursement**



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Phase IIB/Test of Concept Trials for HIV Vaccine Efficacy Evaluation

Susan P. Buchbinder, M.D.

Among the many challenges in human immunodeficiency virus (HIV) vaccine development, one of the most critical is the lack of clearly defined immune correlates of protection from HIV acquisition or development of disease. Although protection has been demonstrated in animal models of closely related retroviral infection, the relevance of these models to human protection is not yet known, and no clear immune correlates have been identified in human or animal studies. Without a clear bar against which to compare immune responses from vaccine candidates in early clinical trials, it is difficult to define the appropriate time to move these vaccines into efficacy evaluation. Efficacy evaluation is further complicated by the lack of clear consensus on appropriate endpoints for HIV vaccine trials. Rather than being able to rely on classic vaccine trial endpoints such as protection from acquisition of infection or development of clinical disease, HIV vaccine trials may only be able to measure protection against surrogate measures of clinical progression, such as HIV viral load, or time to initiation of antiretroviral therapy.

These challenges may be most appropriately addressed by the selective use of Phase IIB or "test of concept" (TOC) trial designs. By definition, Phase IIB/TOC trials are designed to accrue fewer endpoints than Phase III trials, and can generally be completed more quickly or with fewer resources than a pivotal licensure trial. They are not "underpowered Phase III trials." Instead, Phase IIB/TOC trials are designed to address a different set of questions than would a Phase III trial, and, in doing so, help in the design and execution of later Phase III trials.

Questions Addressed in Phase IIB Trials

Phase III trials are designed to evaluate the efficacy of promising products, in preparation for licensure. However, when considerable uncertainty exists about the likelihood of success of a particular vaccine product or class of products, or when additional information is needed to further define the appropriate endpoints, target populations, or immune correlates of protection, Phase IIB/TOC trials may provide strategic advantage as an intermediary step between immunogenicity studies and Phase III pivotal trials. In the case of HIV vaccines, uncertainty currently exists for all four questions: (1) likelihood of success; (2) appropriate trial endpoints; (3) appropriate populations; and (4) immune correlates of protection.

Likelihood of Success

Phase IIB/TOC trials can be used to eliminate unsuccessful vaccine candidates from further evaluation relatively early in the development process, or to build upon successful vaccine prototypes. For example, Merck evaluated a monovalent human papillomavirus (HPV) vaccine prototype with a Phase IIB/TOC trial prior to evaluating a quadravalent vaccine candidate [1]. Both Merck and GlaxoSmithKline evaluated HPV vaccine candidates initially with Phase IIB/TOC trials [2, 3] prior to moving to pivotal Phase III trials [4, 5].

Only two efficacy trials of HIV vaccines have been completed to date and neither demonstrated efficacy. One trial evaluated the AIDSVAX B/B' vaccine in North America for protection against sexually acquired HIV acquisition; the other evaluated the AIDSVAX B/E' vaccine in injection drug users. Both vaccines included two recombinant glycoprotein (rgp) 120 subunit proteins: AIDSVAX B/B' used the laboratory-adapted isolate (MN) with a primary clade B isolate (GNE8), while the AIDSVAX B/E' also included the MN component mixed with a primary clade E isolate (A244). To demonstrate the public health utility of these vaccines, both were also powered to definitively identify at least 30 percent reduction in HIV infection rates in the vaccine recipients. Neither trial demonstrated any overall efficacy despite achieving the anticipated humoral immune response to vaccine [6-9]. The combined trials enrolled nearly 8,000 volunteers, lasted five years, and are estimated to have cost $130 million.

The next product to enter efficacy testing was a prime-boost strategy using a canarypox vector (vCP 1521), followed by an rgp120 boost (AIDSVAX B/E'). The canarypox product generates a CD8+ cytotoxic T-lymphocyte (CTL) response in approximately 25 percent of recipients [10], and trial investigators hope that, in combination with the

CD4+ T-lymphocyte and antibody responses generated by the AIDSVAX B/E' vaccine, this prime-boost approach will protect against HIV acquisition. Scientific opinion remains divided about the likelihood of success of this vaccine approach [11, 12]. The trial has fully enrolled 16,000 volunteers, and trial duration is anticipated to be more than five years.

A newer vaccine candidate has proven substantially more immunogenic than the canarypox vaccines in Phase I and II testing, and has also moved into efficacy evaluation. Merck has developed several generations of replication-incompetent adenoviral vector HIV vaccines. The trivalent adenovirus type 5 (Ad5) vaccine (MRKAd5 HIV-1 gag/pol/nef) was well tolerated at the 1.5×10^{10} viral particle dose, and generated cellular immune responses in more than three-fourths of individuals with low pre-existing Ad5 neutralizing antibody (Nab) titers, and in more than half of persons with high pre-existing Ad5 Nab titers [13]. Rather than move this product directly into a Phase III trial, investigators from Merck and the National Institute of Allergy and Infectious Diseases (NIAID)-sponsored HIV Vaccine Trials Network (HVTN) opted to develop a collaborative Phase IIB/TOC trial. The rationale for getting data from Phase IIB/TOC trials first, before moving to Phase III trials, was to obtain preliminary data on the plausibility that this approach will be successful, both for this specific vaccine candidate, and for other adenoviral vector prototypes that have also entered the clinical trial pipeline [14]. Unlike Phase III trials that are designed to show public health utility (e.g., minimum 30 percent protection from acquisition), Phase IIB/TOC trials are designed to identify whether a product shows any promise (e.g., lower bound of the 95 percent confidence interval (CI) greater than 0 percent) and can thus be smaller in sample size. Phase IIB/TOC trials can also define appropriate trial endpoints and populations for Phase III trials, and identify potential immune correlates of protection. Details of the collaborative Merck/HVTN trial Phase IIB/TOC trial, named the STEP study, will be provided throughout this article to illustrate the potential utility of the Phase IIB/TOC approach.

Appropriate Trial Endpoints

If a vaccine candidate is designed to prevent HIV acquisition, measurement of that trial endpoint is relatively straightforward. Serologic assays licensed by the U.S. Food and Drug Administration (FDA) can generally be used to differentiate vaccine-induced antibody from true HIV infection, and these tests can be supplemented by assays to detect viral nucleic acid sequences. Experienced, independent investigators can be assembled to review assay results and adjudicate endpoint determinations while remaining blinded to study assignment.

However, the current generation of viral vector vaccines is designed to generate a robust CD8+ CTL response, with no substantial ability to generate Nab against HIV primary isolates. Because CTLs are active only after productive HIV infection has occurred, investigators believe that these vaccines are more likely to control viral replication than provide sterilizing immunity, although both outcomes will be important to measure. The challenge in measuring clinical endpoints is the long latency period between HIV acquisition and development of clinical disease. The median time from HIV acquisition to AIDS in the developed world was 8 to 10 years prior to the introduction of highly effective antiretroviral therapy [15, 16], and that duration has been extended considerably through early access to antiretroviral therapy [17, 18]. Antiretroviral therapy is also making a difference in the developing world, where untreated HIV infection may progress to AIDS more rapidly [19, 20]. As global access to antiretroviral therapy becomes an increasingly achievable (and important) public health goal, the relevant research question for CTL-based HIV vaccines becomes whether such vaccines can delay the onset of need for antiretroviral therapy, rather than the more traditional endpoint of clinical AIDS diagnosis or death. Surrogate measures of clinical progression are needed to ensure more rapid assessment of vaccine effects [6].

Early measures of plasma viral load and CD4+ T-cell counts have prognostic significance for later HIV disease progression and survival, and both factor into recommendations for antiretroviral treatment [21]. Viral load has been useful as an early surrogate marker of clinical disease progression in antiretroviral treatment trials, and has served as the basis for licensure decisions. If viral load measures are taken early after peak viremia but before antiretroviral therapy is likely to be initiated—for example, within three months of detection of HIV infection—such measures are also unlikely to be confounded by early initiation of antiretroviral therapy. In addition, such a measure could provide important surrogate information on the potential impact of vaccine on secondary HIV transmission rates, as pre-treatment viral load has been shown to be strongly associated with the risk of HIV transmission in serodiscordant partner studies [22, 23]. Direct measurement of the impact of vaccine on secondary transmission,

on the other hand, will likely be challenging and require cluster randomized controlled trials [24] or recruitment of HIV-negative partners of infected volunteers [25]. Such studies would only be undertaken if earlier trials demonstrate the plausibility of vaccine effects on transmission through persistent reduction in HIV viral load among infected vaccinees.

To measure the durability of vaccine responses, later time points post-infection should also be measured and compared, in a blinded fashion, between vaccine and placebo recipients. Because of the potential for confounding by early initiation of antiretroviral therapy, a composite endpoint might be chosen to include initiation of antiretroviral therapy or reaching a CD4+ T-cell count or viral load for which antiretroviral therapy is recommended [26, 27]. Other strategies may be useful in the analysis of post-infection endpoints, to minimize the risk of introducing bias from evaluating only the subset of volunteers who become HIV-infected. For example, if the vaccine were to protect against less virulent quasi-species but not against those that cause rapid disease progression, analyses comparing only the subset of infected trial volunteers (excluding those protected from less virulent quasispecies) to the entire group of infected placebo recipients would lead to the erroneous conclusion that the vaccine enhanced disease progression. Some analyses might include the entire population of vaccine and placebo recipients to ensure that overall disease (or surrogate markers of disease) incidence is compared between the entire randomized population [6].

All three of the Phase III HIV vaccine trials launched to date have used HIV acquisition as the only primary efficacy outcome variable. The STEP study has two co-primary endpoints: HIV acquisition and post-infection viral load. The sample size for STEP is driven largely by acquisition endpoint, rather than the viral load endpoint. For example, the trial is powered to identify only substantial (i.e., greater than or equal to 50 percent) reduction in HIV acquisition rates. This sample size also provides power to identify relatively modest (i.e., greater than or equal to 0.7 $\log_{10}$) reduction in early HIV viral load. It is not yet clear how the FDA and other national regulatory bodies will view surrogate measures of disease progression in licensure decisions. To provide compelling evidence of public health benefit, how large a reduction in viral load would be required for how long a period of time? Will benefits also be required to be seen in CD4+ T-cell counts and time to initiation of antiretroviral therapy? Data generated from this trial should be quite helpful in making decisions about whether to take this product and other products with similar immunologic profiles forward into Phase III testing. The results will also provide useful concrete data for discussions with regulatory agencies about specific Phase III trial endpoints and goals for ultimate licensure. If the HIV acquisition endpoint is not met, future studies of this particular vaccine candidate may focus more appropriately on surrogate markers of disease progression. On extended follow-up of infected trial participants, this study will also provide data on the rate of development of clinical endpoints, composite endpoints, and the relationship of surrogate markers to clinical disease (e.g., AIDS and survival) that will be useful ultimately for validation of surrogate markers.

Appropriate Populations for Testing Vaccines

Vaccine efficacy may differ in different populations, based on the mix in the trial population of age, gender, route of HIV exposure, host genetics, circulating viral subtype, pre-existing immunity to the viral vector used in the vaccine, or other environmental factors (e.g., nutrition, co-infections). To limit the potential for confounding as a result of any of these factors, early efficacy trials may best be conducted in relatively homogeneous populations. For example, the STEP study is being conducted in populations at highest risk for clade B infection in the Americas and Australia: men who have sex with men [28], with a smaller population of heterosexually exposed women and men. Because preclinical and clinical data suggested that pre-existing neutralizing antibody to Ad5 could attenuate vaccine-induced immune responses, the initial trial was limited to persons with low pre-existing Ad5 titers. When Phase I data became available that demonstrated substantial immune responses in the subgroup of participants with high pre-existing Ad5 titers, the STEP study was expanded to include a stratum of high-risk volunteers with high pre-existing Ad5 titers. A follow-up Phase IIB/TOC trial (HVTN 503) is being planned with the same product in South Africa, an area with a substantial clade C epidemic. Although this candidate vaccine was created using clade B sequence isolates, the antigens included in the product are relatively conserved across clades, and induce immune responses with cross-clade reactivity among HIV-infected individuals [29, 30] and vaccinees [31]. The HVTN 503 Phase IIB/TOC will evaluate the impact of clade on efficacy and provide guidance on the degree to which vaccine antigens

need to match circulating strains. HVTN 503 will also evaluate vaccine efficacy in a population of heterosexually exposed volunteers, including a large population of women, while the STEP study will largely comprise men who have sex with men. Data combined from the two Phase IIB/TOC trials will allow a measure of the robustness of protection provided by the vaccine, and will direct future trials to appropriate populations based on pre-existing Ad5 titer, gender, route of exposure, and viral subtype.

There is also considerable uncertainty in projecting HIV seroincidence in trial populations. Risk generally declines in HIV vaccine trials [32], as participants receive regular risk reduction counseling, and through other cohort effects such as early infection of those who are most susceptible or most highly exposed. Other population-level factors may affect seroincidence during trials, such as widespread use of antiretroviral therapy among HIV-infected persons, or rollout of other effective prevention strategies. Because the power of efficacy trials is driven by the number of anticipated endpoints, accurate projection of seroincidence is critical to ensure adequate power and efficient use of resources. One way to deal with this uncertainty is to create endpoint-driven studies, in which the total number of endpoints drives the timeline for interim and final evaluation of trial results, rather than setting a pre-defined duration of study follow-up. Both the STEP study and HVTN 503 trials are endpoint-driven trials. In addition, both will provide important data on current HIV seroincidence rates in trial populations that will be helpful in planning future Phase III trials.

Immune Correlates of Protection

Identification of vaccine-induced immune correlates of protection could help move HIV vaccine science forward immeasurably, by providing qualitative and quantitative targets for vaccine design and development. Phase IIB/TOC trials can provide substantial power to measure potential immune correlates of protection, particularly for continuous outcomes, such as plasma viral load. For example, in the low Ad5 titer stratum alone, the STEP study is powered to detect 0.25 $\log_{10}$ difference or greater in plasma viral load between those with high versus low immune response to the vaccine, if at least 30 percent of participants develop that immune response (e.g., positive ELISpot response). Considerably more power is available if the low and high Ad5 titer strata can be pooled, or if data can be pooled across the STEP study and HVTN 503. However, there is very limited power to evaluate any but the strongest correlates of protection from HIV acquisition, as this would require a substantially greater trial sample size.

Sequencing Efficacy Trials

Phase IIB/TOC trials can provide important preliminary information on the promise of a given vaccine candidate and provide additional data in support of specific surrogate markers and use of appropriate populations. But a single Phase IIB/TOC trial is not of sufficient size or duration to qualify a product for licensure. How does use of Phase IIB/TOC trials affect overall product development timelines?

If a Phase IIB/TOC trial can show that a vaccine product does not meet a minimum threshold of efficacy, the trial will have been "successful" in determining that the product should be reformulated or discarded before proceeding to Phase III testing. In that situation, Phase IIB/TOC trials are both time- and cost-effective compared with having moved directly from Phase II to Phase III testing of an unsuccessful vaccine candidate.

If a Phase IIB/TOC trial demonstrates sufficient efficacy to warrant that the product proceeds to Phase III testing, decisions will need to be made about the appropriate next steps for licensure of the product. In this situation, the Phase IIB/TOC trial could either have accelerated or slowed down the timeline for product development, depending on what is learned from the Phase IIB/TOC trial. For example, if the Phase IIB/TOC trials are able to rule out (or in) a particular population, make large corrections in anticipated seroincidence rates, provide critical data for use of surrogate markers in trials, or identify potential immune correlates of protection earlier than a Phase III trial, then such trials can still be cost- and/or time-saving for that vaccine candidate or for others following in the clinical trials pipeline. However, it is also possible for Phase IIB/TOC trials to slow down the development timeline of successful vaccines, by inserting this intermediate step between immunogenicity and efficacy studies. To minimize such potential delays, it is important to create a development pathway through licensure that weighs the appropriate timing of each step in the development process.

Plans for evaluating the Merck MRKAd5 gag/pol/nef product can serve as a useful example of how such sequencing can occur to minimize potential delays in the development timeline. The STEP study expanded to include a high-Ad5 stratum before data were available on the efficacy in the low Ad5 stratum for two reasons: (1) clinical

data were available from Phase I trials suggesting similar immunogenicity in the two strata between these products, and (2) the majority of individuals in developing countries have high Ad5 titers, making testing the vaccine in this population of critical importance.

Plans were then initiated for a parallel Phase IIB/TOC trial of this vaccine candidate in South Africa (HVTN 503) before data were available from STEP, utilizing the same data and specimen collection time points and methods for the two trials. The rationale for expanding geographically again relies, in part, on early clinical data suggesting similar immunogenicity across populations, and on the cross-clade immune responses generated by this vaccine. It is also plausible that vaccines may more readily protect against heterosexual HIV transmission than homosexual transmission, and/or that protection will be greater in women than men [33]. Thus, whether or not the STEP study shows some protective efficacy, it will be useful to also have data on vaccine efficacy in women and heterosexual populations in other regions of the world. Taken together, the STEP study and HVTN 503 trial have power similar to a single, small Phase III trial. However, conducting this series of Phase IIB/TOC trials provides important data on appropriate trial endpoints, populations, and correlates of protection that will be important in moving this and other vaccine candidates into pivotal Phase III trials.

HIV presents a unique set of challenges for vaccine development. The selective use of Phase IIB/TOC trials, appropriately timed to avoid delays in product development, may ultimately speed development of a safe and effective HIV vaccine.

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The Animal Rule from a Vaccine Development Perspective

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The threat of biological weapons has stimulated the development of a number of new vaccines to prevent diseases such as smallpox, anthrax, Ebola, and plague—diseases which heretofore either have been eradicated or occur at a very low incidence in the human population. As for any vaccine, these products must be demonstrated to be both safe and effective before they can be approved for use by the U.S. Food and Drug Administration (FDA). Safety of such vaccines can be evaluated in Phase I, II, and III clinical trials. However, the efficacy of many of these vaccines cannot be assessed in clinical field trials due to the epidemiology of the respective diseases, and human challenge studies cannot be conducted because it would be unethical to deliberately expose volunteers to life-threatening infectious agents. In response to this problem, FDA issued a new rule in May 2002 entitled "*New Drug and Biological Drug Products; Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible.*" [1].

This rule, commonly referred to as the "Animal Rule," allows appropriate studies in animals to provide evidence of efficacy for new drug and biological products, such as vaccines, in certain specific cases. The rule can apply only when human efficacy studies are not feasible or ethical. Moreover, the rule is applied only when appropriate animal model and study design criteria can be met that provide a solid scientific basis for extrapolation of efficacy from animals to humans.

Despite its commonly referred to name, a search for the Animal Rule in the *Code of Federal Regulations* [CFR] will yield negative results. The relevant regulations are published in two locations in the CFR, namely 21 CFR§601.90-95 [Biologicals], "*Approval of Biological Products When Human Efficacy Studies are Not Ethical or Feasible,*" and 21 CFR§314.600-650 [Drugs], "*Approval of Drug Products When Human Efficacy Studies are Not Ethical or Feasible.*"

To date, the Center for Biologics Evaluation and Research (CBER) has not approved any products under this rule. The sole product currently approved by FDA using the rule is pyridostigmine bromide, a drug regulated by the Center for Drug Evaluation and Research (CDER) that increases survival after exposure to the nerve agent Soman. The scope of the Animal Rule is broad and may be applied to any drug or vaccine that can ameliorate or prevent serious or life-threatening conditions caused by exposure to lethal or permanently disabling chemical, biological, radiological, and nuclear substances. Thus, the scope is not limited to products related to counterterrorism. Importantly, the rule does not apply if the drug or vaccine can be licensed using standards described elsewhere in the FDA regulations. The Animal Rule does not represent a shortcut to licensure. Moreover, human safety and immunogenicity data must still be generated in traditional clinical studies [2]. For vaccines, these clinical studies will also serve as the means to collect immune response data from vaccinated humans that may allow for making comparison between animal and human studies, and ultimately bridging of immune response data between the species.

Requirements for Animal Vaccine Studies

Under the Animal Rule, animal data can be used to provide evidence of efficacy of a vaccine only if all of the following four criteria are met:

(1) There is a reasonably well understood pathophysiological mechanism of toxicity of the substance and its prevention or substantial reduction by the product;

(2) The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans;

(3) The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity; and

(4) The data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans.

These conditions can only be met when well-designed and well-controlled animal studies are conducted. A number of factors should be taken into account when designing these studies, including the expected route of exposure to the biological agent, expected exposure dose, and the vaccine indication (e.g., pre-exposure prophylaxis, post-exposure prophylaxis). Importantly, the animal species used in the studies should be those in which the animal disease mimics that of humans as closely as possible.

In addition to the requirements that have been discussed, a number of post-marketing and post-event requirements exist for approval of a product using the Animal Rule [1].

Animal Study Designs to Support Vaccine Licensure

Each different type of vaccine (e.g., anthrax, smallpox, plague, Ebola) and vaccine indication will require specialized animal model development and study design. The rule does not state which animal species or strain(s) should be used for evaluation of any given vaccine. Thus a vaccine manufacturer can develop an animal model of its choosing that meets the aforementioned four criteria. The choice of each model will need to be justified to CBER's Office of Vaccines Research and Review (OVRR). Data in the existing scientific literature can help serve as the basis for animal study design. (For example, see references [3, 4].) In addition, workshops such as those held on this topic for anthrax and plague vaccines [5, 6] have provided valuable scientific consensus on the appropriate design of studies. The design of any animal study conducted to support efficacy of a vaccine in humans must include a scientifically sound basis for extrapolating data from the animal study to humans. The scientific validity of the extrapolation will be considered by FDA scientists, expert panels such as FDA Advisory Committees, and the general scientific and medical communities.

Because study design will depend on vaccine type and indication, it is not possible here to provide specifics for a study design that can be applied generally. However, we would like to present examples of two vaccines that illustrate the types of thought processes used to design studies that are expected to fulfill the four criteria of the Animal Rule in a scientifically rigorous manner.

New Generation Anthrax Vaccines

New generation anthrax vaccines are currently being developed, e.g., vaccines composed of a recombinant form of a *Bacillus anthracis* protein known as protective antigen (rPA). The choice of this protein was based on knowledge of *B. anthracis* pathogenesis, which has been studied extensively. Studies have shown that upon inhalation of *B. anthracis* into the lungs, the spores are engulfed by alveolar macrophages. During transit of the macrophages to the lymph nodes, the spores germinate. Vegetative bacteria are eventually released into the bloodstream where they produce copious quantities of anthrax toxin, a tri-partite toxin composed of a binding component, protective antigen, and two enzymatically active components, lethal factor and edema factor. The resulting toxemia is thought to result in the clinical manifestations of the disease. (For reviews of *B. anthracis* pathogenesis and anthrax toxin, see [7, 8].) Thus, neutralization of the toxin by antibodies induced by immunization with rPA vaccines would be expected to prevent disease. The significant body of knowledge available concerning pathogenicity of *B. anthracis*, the mechanism of action of anthrax toxin, and the role of toxin-neutralizing antibodies (TNAs) in protection against disease satisfies the first criterion of the Animal Rule. Moreover, the role of TNAs in protection against disease provides a basis for use of TNA levels as a measure of protective response [9, 10].

When designing the anthrax vaccine animal studies, one of the first questions that had to be addressed was which animal species should be used. The two animal species currently regarded as the most appropriate for use are the rhesus macaque (*Macaca mulatta*) and the New Zealand White rabbit based upon the resemblance of the pathology of experimental anthrax in these animals to that of human disease [5, 3, 11]. A second consideration was animal study endpoint. Because anthrax is generally a lethal disease, survival after challenge with *B. anthracis* is considered to be an appropriate endpoint. Additionally, the vaccine indication must be considered. Since anthrax vaccines might be used for either pre- or post-exposure prophylaxis, the studies should be designed to reflect the desired clinical indication, i.e., challenge of the animals with *B. anthracis* should occur post-vaccination if a pre-exposure indication is being sought and pre-vaccination for a post-exposure indication. The challenge strain and the challenge dose must be carefully considered, should be relevant, and the choice should be discussed with CBER before initiation of an animal efficacy study. These considerations addressed the second and third animal study criteria of the rule.

Perhaps the most complex aspect of the Animal Rule to fulfill is the fourth criterion that efficacy data should be extrapolated from animals to humans in a scientifically valid and rigorous manner.

In the case of rPA anthrax vaccines, TNA titers induced by the vaccine will likely be the "common language" that can be used to translate a protective immune response in animals to that of humans, although additional data are needed to confirm this. If this is found to be the case, then extrapolation of protection data from animals to efficacy in humans may be possible. First, TNA titers in vaccinated animals protected from challenge with *B. anthracis* could be used to estimate the level of TNA required for protection in the animal. This level could be compared to that achieved upon vaccination of humans in clinical immunogenicity trials. From this information, efficacy of the vaccine at the dose and schedule administered in humans might be inferred.

New Generation Smallpox Vaccines

Certain new generation smallpox vaccines may need to use the Animal Rule as a regulatory pathway to approval [12]. The Animal Rule criteria for a new generation (attenuated) smallpox vaccine, such as a modified vaccinia Ankara (MVA) vaccine, are more challenging than those described for the new generation anthrax vaccines. Since variola virus has no identified animal reservoir and smallpox is not considered a zoonotic disease, there are no natural animal models for variola. Secondly, since the disease was eradicated from the human population almost 30 years ago, not enough is known about the pathophysiology and pathogenesis of smallpox infection in people. The correlates of immunity and the mechanism of immune protection are not fully understood, and animal models mimicking the natural respiratory exposure route for human infection, and in particular a non-human primate model, have not been developed. Clinically, smallpox spreads from person to person via the oropharyngeal route. Following a 7- to 17-day incubation period, the disease manifests as a disseminated viral infection with high fever and a pustular rash that spreads over the body [13]. However, there is still no consensus as to the actual cause of death from smallpox in humans, thus complicating the ability to meet the first animal study criterion of the rule.

Each of the current animal models used for evaluation of smallpox vaccines presents specific strengths and weaknesses. The lack of well-characterized animal models that fully mimic a smallpox infection in humans means that the evaluation of a new generation smallpox vaccine under the auspices of the Animal Rule will require a combination of animal models to provide a measure of confidence in the efficacy of the vaccine to protect against severe disease or to prevent virus entry and/or spread. OVRR has indicated that, if feasible, the animal challenge studies should use the route of exposure that best mimics the anticipated natural route of exposure.

The primary endpoint of the animal efficacy studies should be protection by the candidate vaccine against lethal challenge with two relevant pathogenic orthopoxviruses in at least two mammalian species. Presently, most research has been focused on the development of suitable animal models using mice, rabbits, and cynomolgus macaques (*Macaca fascicularis*). Each of these models comes with its own set of challenges, including small sample size, perfecting the respiratory challenge, working in biosafety level (BSL)-3 and -4 laboratories, and cost.

The BALB/c strain of mice is the most commonly used mouse model in orthopoxvirus research and is best understood with regard to disease pathogenesis and the immune response. Mice are susceptible to only a few plaque-forming units of ectromelia virus, or mousepox, and moderate doses of cowpox virus or vaccinia virus strain Western Reserve (WR). Additionally, most of the challenge experiments using cowpox and vaccinia have used respiratory routes of virus challenge. Many of the components of the humoral and cellular immune response to vaccination and the basis for resistance to orthopox virus infection have been studied in mice. More recently, the disease caused by rabbitpox virus in New Zealand White rabbits has been studied as a model for smallpox in humans. Rabbitpox infection produces a relatively large amount of extracellular enveloped virus (EEV) when compared to other orthopoxviruses, making it a more stringent model for virus spread within an infected host. Most recently, a number of laboratories have pursued the development of a non-human primate model in cynomolgus macaques [14, 15]. In this model, monkeys are challenged with a lethal dose of the Zaire strain of monkeypox virus that is known to cause fatalities in humans. While other monkey species have been used in orthopoxvirus research, the demand and supply dictate that cynomologus monkeys be used most often today. In the future, other alternatives may exist, as research is continuing in the development of animal models for vaccine evaluation.

The evaluation of vaccines using smallpox (Variola major) is complicated by the restriction of research using live variola virus to two BSL-4, World Health Organization-sanctioned facilities, namely at the Centers for Disease Control and Prevention in Atlanta and Russia's State Research Center of Virology and Biotechnology (VECTOR).

Although smallpox infections in cynomolgus monkeys were recently reported [16], this model used an extremely high challenge dose, had an atypical disease course, is exploratory, and is not available to the wider orthopoxvirus research community. At present, OVRR does not require a demonstration of efficacy in a variola virus animal model for evaluation of a smallpox vaccine. However, companies pursuing the approval of new smallpox vaccines can use Wyeth Dryvax, an effective, approved smallpox vaccine, as a comparator. The ability of the candidate vaccine to protect against lethal orthopox virus challenge and to elicit an immune response in animal models and humans can be compared to Dryvax. This will be important in meeting the fourth animal study criterion of the rule.

The animal efficacy studies should use appropriate orthopoxviruses—those closely related to smallpox virus—and challenge doses that induce clinical disease that best mimics human smallpox disease, including the normal route of exposure. These viruses could include ectromelia virus, cowpox virus (Brighton strain), vaccinia virus (WR strain), rabbitpox virus, and monkeypox virus, depending on the susceptible animal species. The appropriate challenge dose will be dependent on the individual circumstance, since the species of animal to be challenged, route of challenge, and type of virus are all variables.

The final animal study criterion of the rule is to allow for the selection of an effective human dose and vaccine schedule. This will require bridging between the animal and human immune response data. Although we do not currently know the critical correlates of the protective immune response, vaccination with Dryvax is associated with a broad humoral and cellular immune response. The immunological investigation of new smallpox vaccines will use a combination of immunological assays, some still undergoing validation. These assays will likely include Plaque Reduction Neutralizing Titers (PRNT), ELISA anti-vaccinia antibody levels, and ELISPOT assays for the cell-mediated response. One reason for fully characterizing the immune response to vaccination will be to compare the response elicited by new vaccines to the immune response induced by Dryvax used in the same animal model, thus enabling comparison to a vaccine with known efficacy against smallpox.

Summary and Conclusions

The development of vaccines for most biothreat agents and some emerging diseases presents unique challenges for clinical development and licensure. The worldwide incidence of natural disease caused by many of these agents is usually low, so classical field trials are not feasible. This, along with the fact that it is unethical to conduct human challenge studies for most of these agents, makes direct demonstration of efficacy in humans virtually impossible. That problem precluded the licensure of vaccines for such infectious agents prior to the Animal Rule going into effect. The Animal Rule can address this problem and provides an avenue to vaccine licensure, provided that the four-animal study criteria of the Animal Rule can be fulfilled in a scientifically sound and rigorous manner. However, it is important to note that the Animal Rule does not specify which species should be used for a specific situation, and thus any animal model may be used as long as it rigorously meets the four critical requirements previously discussed. Finally, the rule pertains only to efficacy data. Vaccine immunogenicity and safety data will need to be generated in human clinical trials.

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VACCINE UPDATES

Supporting the Nation's Biodefense

Introduction

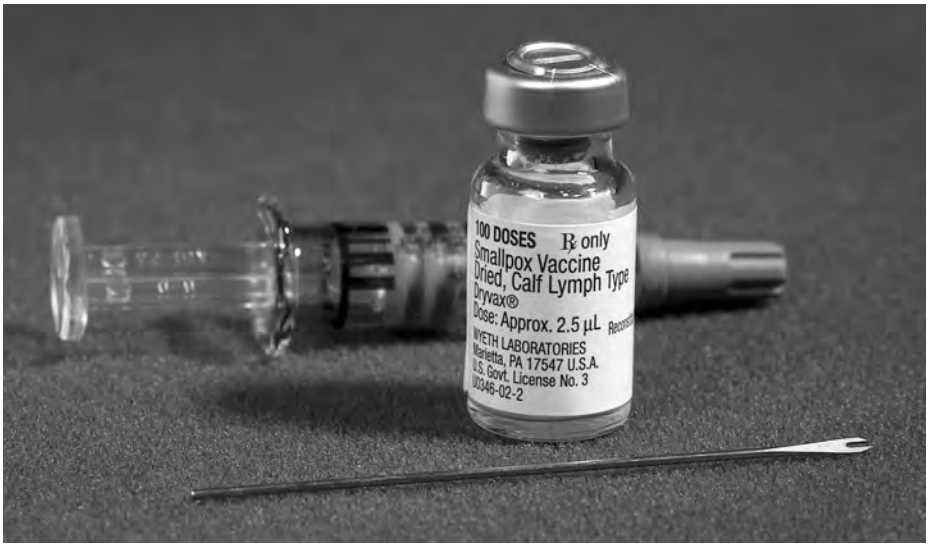
Biological weapons present a unique threat because contagious diseases can spread rapidly beyond the area of initial outbreak with potential to cause massive casualties and economic disruption. In 2001, the United States encountered bioterrorism in the deliberate exposure of the civilian population to anthrax spores hidden in several pieces of mail delivered via the U.S. Postal Service. The anthrax attacks revealed a gap in the nation's overall preparedness against bioterrorism.

Other events, such as the first documented instance of avian influenza virus (H5N1) transmission from birds to humans in 1997, the arrival of West Nile virus in North America in 1999, and the emergence of severe acute respiratory syndrome (SARS) in China in 2002, highlight the continuing threat that emerging and re-emerging infectious diseases pose to public health. They illustrate the need for the federal government to be prepared to respond to potential infectious disease outbreaks caused either naturally or by deliberate release of pathogens.

The National Institute of Allergy and Infectious Diseases (NIAID) is the lead agency within the U.S. Department of Health and Human Services (HHS) for conducting research concerning potential agents of bioterrorism that directly affect human health. NIAID's long institutional experience with infectious disease research provided a foundation for the seamless adoption of a biodefense role that has expanded greatly over the past five years.

In the wake of the 2001 terrorist attacks, the National Institutes of Health (NIH) embarked on a systematic strategic biodefense planning process by convening the Blue Ribbon Panel on Bioterrorism and Its Implications for Biomedical Research, comprised of distinguished researchers representing academia, private industry, civilian government agencies, and the military. Based on the panel's recommendations and extensive discussions with other federal agencies, NIH developed three key documents to guide its biodefense research program: the NIAID Strategic Plan for Biodefense Research, the NIAID Research Agenda for CDC Category A Agents (covering agents that pose the gravest threat to human health, such as those that cause smallpox, anthrax, botulism, and plague), and the NIAID Research Agenda for Category B and C Priority Pathogens (for agents whose biological properties make them more difficult to deploy or less likely to cause widespread harm than Category A agents).

The Strategic Plan provides a blueprint for constructing three essential pillars of the biodefense research program: (1) **basic research** on microbes and host immune defenses, which serves as the foundation for applied research; (2) targeted, milestone-driven **medical countermeasure development** to create the vaccines, therapeutics, and diagnostics that will be crucial in the event of a bioterror attack; and (3) **infrastructure** needed to safely conduct research on dangerous pathogens. The Biodefense Research Agendas present detailed descriptions of short-term, intermediate, and long-term goals for research on the wide variety of potential bioterrorism agents.

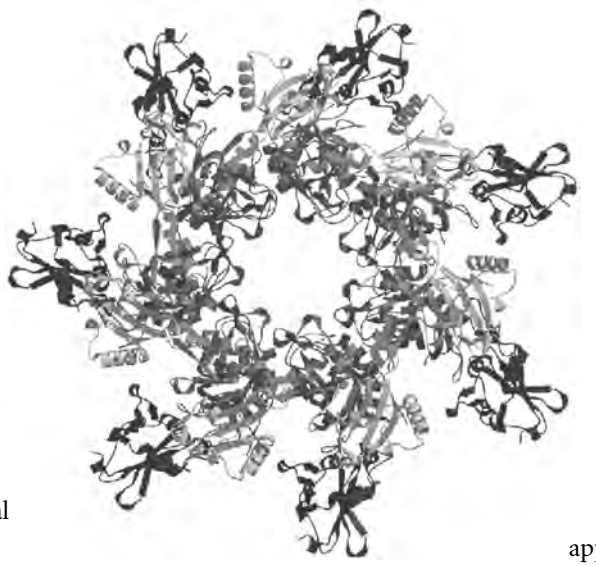


The components of a smallpox vaccination kit including the diluent, vial of Dryvax smallpox vaccine, and a bifurcated needle. Courtesy of CDC / James Gathany

NIAID Category A agents represent the most dangerous threats. These pathogens, including smallpox and anthrax, are given the Institute's highest priority because they (1) are easily dis-

seminated or transmitted from person to person, (2) result in high mortality rates with the potential for major public health impact, (3) would likely cause significant social disruption, and (4) require special action for public health preparedness.

One of the key elements of the Institute's research agenda for Category A agents is developing medical countermeasures, including vaccines, therapeutics, and diagnostics, that can protect the public in the event of a public health emergency. Vaccines play a crucial role in preparedness in that they prevent infection, and therefore have the potential to maintain the vaccinated population's health in the midst of an outbreak. Below are some examples of progress being made in developing vaccines and vaccine strategies for Category A priority agents.



Anthrax heptamer structure. Courtesy of John R. Collier, Harvard Medical School 2002

Current State of Science

Researchers Make Rapid Advances in Vaccine Development

The vaccine resources of the United States are far stronger than they were just five years ago. For example, in September 2001, the United States had only 15.4 million doses of smallpox vaccine available; today there are more than 300 million doses, due in part to NIAID-supported clinical research on dose requirements of older smallpox vaccines.

However, the vaccines currently available are not without their faults. The smallpox vaccine currently used, Dryvax, is highly efficacious, but is associated with significant local and/or systemic reaction in more than 90 percent of vaccinees. In addition, Dryvax should not be given to individuals who are immunocompromised, such as those with human immunodeficiency virus (HIV), leaving a significant portion of the U.S. population without access to immunization.

The anthrax vaccine currently licensed in the United States, BioThrax, is a crude mixture that consists of filtered *Bacillus anthracis* culture supernatant treated with formaldehyde and mixed with an aluminum adjuvant. The vaccine includes a subunit of anthrax toxins called Protective Antigen (PA), which is known to generate an antibody response. A 2002 Institute of Medicine report entitled "The Anthrax Vaccine: Is It Safe? Does It Work?" recommended that research be pursued and encouraged to develop other possible anthrax vaccine products that are less reactogenic than BioThrax and can be produced more consistently.

NIAID is supporting research on next-generation vaccines against smallpox and anthrax that are not only safer and more reliable, but may also be used by the entire U.S. population, including those with compromised immune systems. For anthrax, NIAID is supporting the development of vaccines comprised of only PA produced by modern recombinant technology (rPA) and combined with aluminum adjuvant. The rPA vaccines have been tested for safety and efficacy in rabbits and monkeys, and subsequently underwent clinical safety testing in people. To date, large-scale manufacturing capability of rPA vaccines has been demonstrated and the vaccines appear to be safe and immunogenic in people.

One of the most promising approaches against smallpox is the modified vaccinia Ankara (MVA) vaccine. This vaccine is unable to grow in human cells and cannot form a lesion at the site of vaccination, making it likely to be safe for use in individuals who should not receive Dryvax. NIAID-supported researchers successfully performed small-scale manufacturing of the MVA vaccines and conducted small Phase I clinical trials in healthy volunteers. These early development studies showed that MVA could be manufactured in compliance with current laws and regulations, and that it appeared to be safe and immunogenic in healthy volunteers.

Following these successes, large-scale manufacturing of MVA was performed and Phase II clinical studies have been planned in both healthy individuals and those who are immunocompromised. The purpose of these studies will be to further assess the safety of these vaccines, and begin to assess effectiveness based on the immune response. Several of these clinical trials have started, including trials in volunteers with HIV.

Additional progress in developing new vaccines against Category A agents includes the first human clinical trial of a DNA vaccine designed to prevent Ebola infection. This recently completed trial was conducted by researchers at the Institute's Dale and Betty Bumpers Vaccine

Research Center (VRC). The vaccine, composed of three DNA plasmids, was well-tolerated and elicited both humoral and cellular immune responses at all doses. In parallel, non-human primate clinical studies have refined the design of the Ebola vaccine products. The DNA plasmid product is currently being manufactured for clinical testing.

Novel Strategies Tackle Challenging Pathogens

NIAID researchers are also designing new strategies to tackle the complexities of Category A agents. For example, developing a vaccine against dengue has been extremely complicated due to the virus' four subtypes. While infection with one dengue serotype results in lifelong immunity against that serotype, at best it provides only temporary cross-protection against the others. Worse yet, subsequent infection with a different dengue serotype can be much more severe, a phenomenon called antibody-dependent disease enhancement (ADDE). Therefore, a dengue vaccine must be tetravalent and provide protection against all four dengue serotypes.

A second major impediment to dengue vaccine development is the lack of an animal model of dengue that mimics human disease. The effectiveness of a vaccine candidate is inferred by the levels of antibodies it induces and by determining the level of dengue wild-type virus detected in the blood of vaccinated animals (vs. unvaccinated controls) following challenge with wild-type dengue virus.

Researchers in the United States and abroad have worked to develop a tetravalent dengue vaccine for years. Most have used the live attenuated vaccine approach, which was used in the successful yellow fever vaccine and is also the most economical method of vaccine development. However, this approach is especially slow and difficult for dengue because of the need to develop four different vaccine viruses that must be assessed separately and in combination both *in vitro* and in animals before advancing to clinical trials. Classic *in vitro* tests such as plaque reduction assays do not reliably predict the behavior of dengue viruses in animals, which in turn, are imperfect models of dengue in humans. And often a single serotype vaccine shows promise in clinical trials but fails when included in a tetravalent vaccine as it competes with the other three serotypes to stimulate immunity.

As with other live attenuated vaccines, success lies in making the viruses sufficiently weak to be safe to administer yet still able to induce a protective immune response. Two other qualities are important in a dengue vaccine as well: the vaccine viruses should not be transmissible to mosquitoes that bite a vaccinee, and the viruses should be cultivable to high titer in a cell line that permits cost-effective manufacturing.

Dengue researchers have used several methods to weaken the vaccine viruses, including serial infection in cell cultures or animals and reverse genetics to introduce attenuating nucleotide deletions and point mutations. Chimerization, a method developed by NIAID researchers in the early 1990s, also has been found to attenuate the viral hemorrhagic fever flaviviruses, such as dengue. Chimerization involves replacing the genes of an attenuated virus (the backbone or recipient virus) with those of another (the donor virus) to develop a third virus—a chimera—that is attenuated but induces immunity to the donor virus.

NIAID researchers have used both recombinant DNA techniques and chimerization to develop a tetravalent dengue vaccine slated for clinical trial in the near future. In addition, they continue to create dengue viruses with novel attenuating mutations for use in the event that ongoing clinical trials suggest that additional changes in the tetravalent formulation are needed. Each of the four components of NIAID's dengue tetravalent vaccine has a large, attenuating, and genetically stable 30-nucleotide deletion in its genome. Because chimerization is also attenuating, the two chimeric viruses in this tetravalent vaccine are even more stable and less likely to revert back to non-attenuated forms, as well as less transmissible to mosquitoes.

NIAID's chimerization methodology has been licensed to Acambis, whose tetravalent vaccine appears promising in early clinical trials. NIAID is working with scientists in India and Brazil to further dengue vaccine efforts in those countries as well. Using different attenuation techniques, investigators from Mahidol University in Thailand, Walter Reed scientists, and others continue their long-term work toward development of a successful dengue vaccine.

Basic Research Holds the Key for New Countermeasures

A key component of the NIAID biodefense research program is basic research to understand how pathogens interact with human hosts. Recognizing the significance of genomic sequencing to biodefense, NIAID-supported researchers and their international colleagues sequenced complete genomes of at least one strain of each Category A agent. This includes multiple strains of the anthrax bacterium. The Institute has also expanded its functional genomics program to provide comprehensive genomic, bioinformatic, and proteomic resources for basic and applied research

to rapidly address the nation's biodefense needs. These resources include the Pathogen Functional Genomics Resource Center, Microbial Sequencing Centers, Bioinformatics Resource Centers, and Biodefense Proteomics Research Centers.

The human innate immune system is comprised of broadly active "first responder" cells and other non-specific mechanisms that are the first line of defense against infection. NIAID-supported research on methods to boost innate immune responses could lead to fast-acting countermeasures to mitigate the effects of a wide variety of bioterror pathogens or toxins. In addition, manipulation of the innate immune system could pave the way toward powerful adjuvants that can be used to increase the potency and effectiveness of vaccines.

Qualitative and quantitative assessments of host response also are crucial for creating vaccine candidates. For example, *Francisella tularensis*, the bacterium that causes tularemia, is a highly infective, Gram-negative bacterium that is a potential bioterrorist threat. As few as 10 organisms may cause disease and the bacterium can survive at low temperatures for weeks. Studies of the immune response to a live attenuated vaccine derived from the avirulent Live Vaccine Strain (LVS) suggest that it induces an incomplete immune response, activating some pathways but not others. In addition, the effectiveness of LVS depends on a number of factors, including the route of tularemia infection. While LVS protected mice against systemic infection, it did not protect against aerosol infection. This suggests either that immune responses elicited by vaccination with LVS are not as well expressed in the lungs, or that immune responses raised by LVS are less able to combat pulmonary tularemia than systemic tularemia. Understanding the host response to this highly virulent pathogen is a vital step toward developing effective vaccines for systemic and pulmonary manifestations of the disease.

Challenges and Opportunities

The new emphasis placed on biodefense as a national priority has led the National Institutes of Health (NIH) to develop an expanded paradigm with respect to biodefense product development. NIH has always supported research that generates new knowledge about disease and has worked to translate these findings into vaccines, therapeutics, and diagnostics that protect public health. But to develop safe and effective products for biodefense as quickly as possible, NIH needed to intensify and accelerate this process. Working in close collaboration with industry and academia, NIH is taking an active role in moving promising concepts into advanced product development.

The enhancements of NIH's traditional process of research and development have strengthened public health preparedness not just against Category A agents, but against emerging and re-emerging infectious diseases in general. The viruses, bacteria, and parasites that cause infectious diseases do not remain static, but continually and dramatically change over time as new pathogens emerge and as familiar ones re-emerge with new properties or in unfamiliar settings. Emerging infections such as HIV, Ebola, and SARS and re-emerging infections such as influenza have shaped the course of human history while causing incalculable misery and death. Fortunately, the knowledge and products that will flow from the NIH biodefense research program, including research results, intellectual capital, laboratory resources, and countermeasures in the form of diagnostics, therapeutics, and vaccines, will help us cope with naturally emerging, re-emerging, and deliberately released microbes alike.

For more information about NIAID biodefense research, including research agendas and progress reports, visit www.niaid.nih.gov/biodefense.

ANIMAL MODELS FOR EMERGING INFECTIOUS DISEASES

Animal models are critical research tools in developing effective countermeasures against emerging and re-emerging infectious diseases, including potential agents of bioterror. Scientists utilize animal models to evaluate novel therapeutics, diagnostics, drug treatments and vaccines, and to learn more about disease pathogenesis and host response. Such research has resulted in important public health advances, ranging from the development of vaccines for diseases such as Hepatitis B, to drugs for chronic disorders such as diabetes.

The NIAID *In Vitro* and Animal Models Program has made many significant contributions to the development of new therapies and continues to provide NIAID researchers with a range of resources to bring new therapies and preventive measures from the laboratory to initial clinical testing in humans.

Examples of research advances made include those for anthrax and smallpox animal models. One of the challenges in anthrax models is that multiple interventions are often given. As each intervention is highly effective when used alone, it can be difficult to show the added value of multiple interventions. NIAID researchers have used rabbit animal models to demonstrate that antibiotics and vaccines can be additive, and have demonstrated in mice and guinea pig models that antibiotics and immunotherapeutics can be additive as well. Tests were conducted in post-exposure models, where animals have been exposed but are not yet showing clinical signs of disease. NIAID is currently exploring the rabbit and non-human primate as potential models for testing products.

CDC has the only laboratory in the United States permitted to conduct research using the smallpox virus. Therefore, smallpox animal model research is dependent on related, less lethal poxviruses, such as monkeypox. Less is known about these related viruses regarding the amount of virus needed to cause disease by various routes of transmission and the way in which the disease develops. Although scientists have collected promising proof-of-concept data for several drugs and vaccines against intravenously-acquired infection, it will be important to demonstrate efficacy against respiratory-acquired disease as well. To this end, NIAID is exploring intranasal, intratracheal and aerosol infection models and assessing the impact of route of infection on disease progression.

Hepatitis C

Approximately 85 percent of individuals infected with hepatitis C virus (HCV) become chronic carriers of the virus. Chronic HCV infection greatly increases the risk of developing liver cirrhosis and/or liver cancer. The World Health Organization estimates that there are 170 million chronic carriers of HCV, equaling about 3 percent of the world's population. In the United States, an estimated 3.9 million people (1.8 percent of the population), are infected. The rate of infection is substantially higher in African Americans (8 to 10 percent), who are also more refractory to current therapies. There are about 25,000 new infections annually, mostly among young adults who are intravenous drug users (IDU); sexual transmission may also account for a proportion of cases. About 40 percent of liver transplants are due to HCV-related liver failure and 8,000 to 10,000 deaths per year result from complications of chronic liver disease. Even though HCV infection rates have fallen dramatically (declining from 180,000 over the past decade), the economic toll exacted by HCV infection in the United States is enormous, estimated at $1 billion per year.

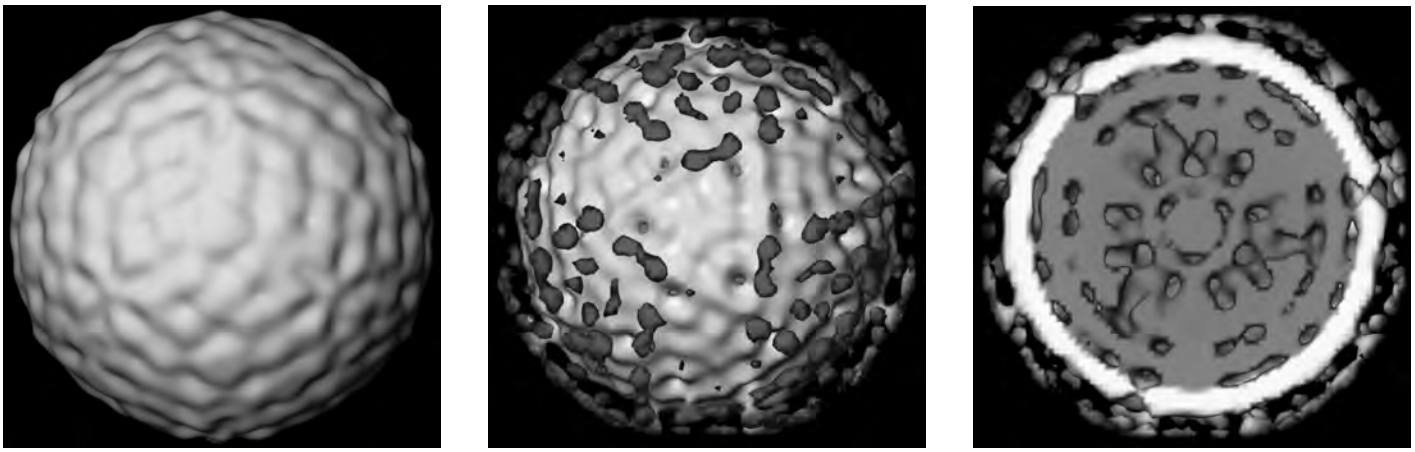
Several investigators have reported a relatively high efficiency vertical transmission of HCV from mothers co-infected with human immunodeficiency virus (HIV). Other major studies in the United States and Europe have failed to demonstrate transmission from HCV-positive mothers. Risk factors for transmission, which is assumed to occur *in utero*, include a high HCV RNA level in the mother and the presence of specific HCV variants. Results of a study of infants born to HCV-infected mothers demonstrated biochemical features of liver damage (alanine aminotransferase (ALT) abnormalities) during the first 12 months of life, although HCV-associated liver disease is likely to be mild throughout infancy and childhood. Multivariate analyses of risk factors for cirrhosis and/or liver cancer in HCV-infected people demonstrated that increased age, male gender, and excessive alcohol consumption are all important factors. Additional risk factors for liver cancer are the presence of hepatitis B antibodies and HCV genotype 1 and 2.

Although HCV is the leading cause of chronic viral hepatitis in the United States, a vaccine has been difficult to develop because of extensive genetic and possibly antigenic diversity among the different strains. New variants known as quasi-species arise quickly and frequently, thus allowing escape from neutralizing antibodies and cytotoxic T lymphocytes. Amino acid changes in many epitopes, and particularly in the hypervariable region 1 (HVR1) of the HCV envelope glycoprotein E2, may play a role in escape from neutralizing antibodies.

Recently, several new advances, including the development of a fully permissive cell culture system to produce infectious HCV, have become available, opening up more opportunities to study virus infection, to analyze the immune responses associated with virus clearance, and to test new anti-HCV drugs. However, the chimpanzee remains the only HCV infection animal model available for immunological studies to define the correlates of immunity and protection. Chimpanzees are an endangered species and very expensive. Their continued use is expected to be very limited.

The current perception is that successful vaccines will need to induce both strong antibody responses and robust, long-lived, cytotoxic T-lymphocyte responses to overcome immune evasion by continual evolution of variant HCV species. Even though infection by HCV generates antibodies, these antibodies cannot resolve or neutralize the infection. Vaccine studies in chimpanzees using recombinant envelope glycoproteins showed limited protection upon challenge with virus, but both chimpanzee experiments and epidemiological studies in humans suggest that vaccines may be developed to prevent chronic infection. DNA vaccines are now being tested in chimpanzees, using envelope as well as core protein constructs. Virus-like particles (VLPs) made up of structural HCV proteins have been produced successfully in insect cells and may serve as a potential vaccine model. Ribozymes, catalytic RNA molecules that bind specifically to target RNA by an antisense mechanism, are also being tested as a possible strategy for the treatment of HCV infection.

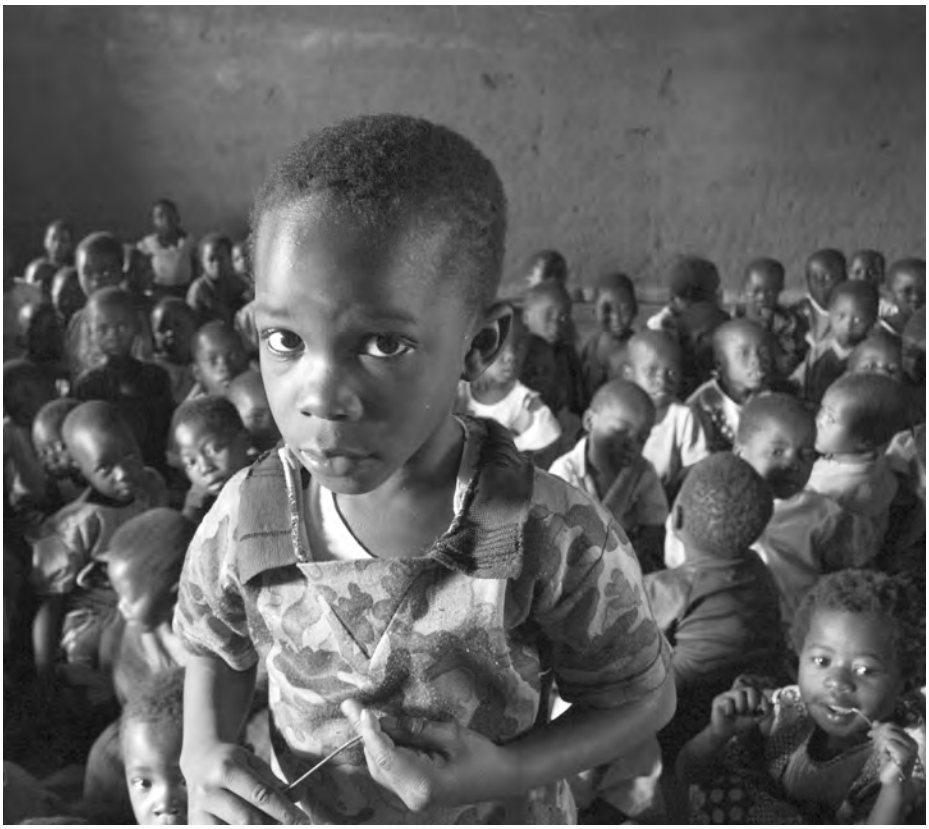
A prophylactic vaccine candidate, based on the envelope glycoproteins E1 and E2 of HCV, is currently being tested by NIAID in the second of two Phase I trials, using different adjuvants. Initial results from the first completed trial indicate that the vaccine is safe and capable of eliciting immune responses, which are still being characterized. A second vaccine, for use primarily in chronically infected individuals as an immunotherapeutic vaccine, is expected to enter clinical trials in 2007.



The reconstructed images of a recombinant hepatitis C virus-like particle produced in cell culture. These images are three-dimensional representations of the HCV-like particle at high resolution. Visualizing the particles in this way enables researchers to understand how they interact at the molecular level with compounds important in fighting against HCV infection, and how to develop a vaccine to prevent HCV infection. Dr. Liang and his colleagues have shown that these HCV-like particles are promising as a vaccine candidate in animal models. They plan to conduct clinical studies in the near future. Courtesy of Dr. T. Jake Liang, Chief, Liver Diseases Branch, NIDDK Division of Intramural Research, NIH.

HIV/AIDS

Overview



A school for orphans in Mlomba, Malawi houses 100 children, most of whom lost their parents to AIDS. Courtesy of Yoshi Shimizu / International Federation of Red Cross and Red Crescent Societies

Although there have been ambitious human immunodeficiency virus (HIV) prevention campaigns over the years, HIV/AIDS continues to ravage many parts of the world, with approximately 40 million people living with HIV/AIDS globally. First reported in the United States in 1981, HIV/AIDS has become a worldwide pandemic. According to the United Nations, in 2005 there were an estimated 5 million new infections, more than 95 percent of which occurred in developing countries, and approximately 3.1 million deaths due to AIDS. Sub-Saharan Africa is the hardest hit, with more than 25.4 million people infected. South and Southeast Asia together account for more than 7.1 million infected people, with 1.4 million more in Eastern Europe and Central Asia, 2.1 million in Latin America and the Caribbean, 1.1 million in East Asia, 1 million in North America, 610,000 in Western and Central Europe, and 35,000 in Oceania. Approximately 14,000 people worldwide are newly infected with HIV every day. In the United States alone, despite intensive HIV prevention efforts and strong care and treatment programs, the number of annual new infections has not decreased over the past 10 years. More than 40,000 new HIV infections are estimated to occur in the United States every year with 50 percent of those in persons under the age of 25 and the majority in racial and ethnic minorities, women, and men who have sex with men.

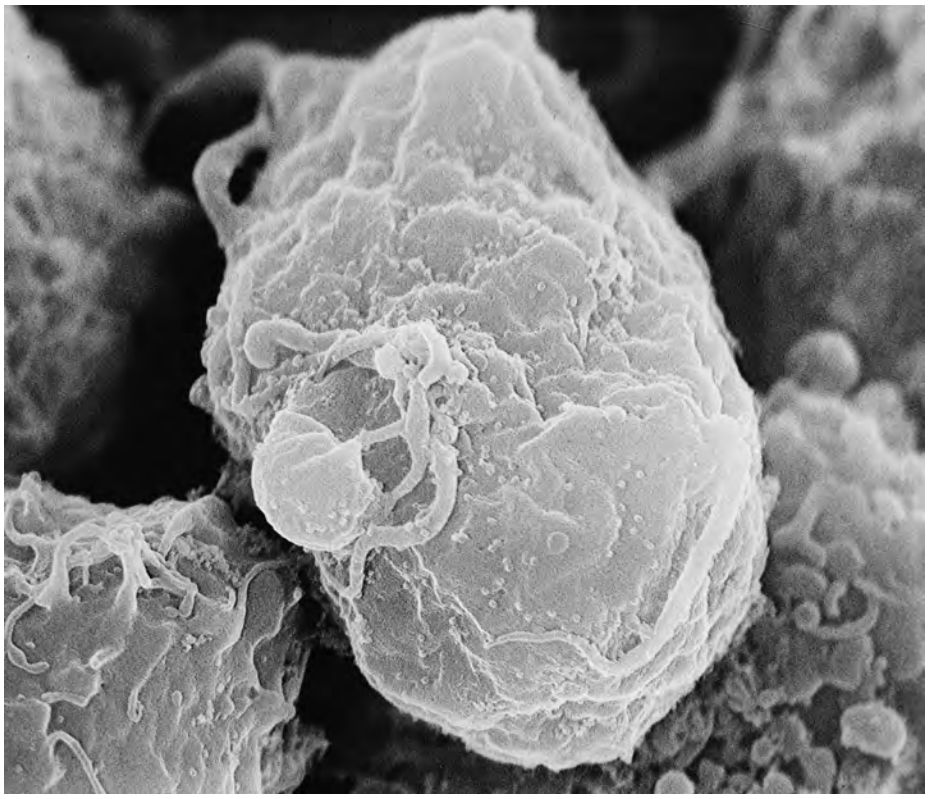
Scientists and public health officials agree that a preventive HIV vaccine remains the best hope for ending the AIDS pandemic. Although progress has been made, a safe and effective vaccine has not yet been identified and much needs to be done. The National Institute of Allergy and Infectious Diseases (NIAID) is committed to developing a preventive HIV vaccine and, toward this end, supports basic biomedical research to better understand the relationship between HIV and the immune system, preclinical development of new vaccines, and clinical research and evaluation of novel vaccines in all phases of clinical trials.

The most rational way to design an effective vaccine is to identify which immune responses protect against the specific infection and to construct a vaccine that stimulates those responses. Since HIV can be transmitted through systemic and mucosal routes of exposure, by cell-associated and cell-free virus, researchers are working to identify the components of the immune system that are essential to inducing immunity and preventing or controlling infection. The two main types of immune responses are humoral immunity, which uses antibodies to defend against free virus, and cell-mediated immunity, which uses cytotoxic T lymphocytes (CTLs) to directly kill or control infected cells. While earlier vaccine research focused primarily on vaccines that

elicited antibodies, it is now generally believed that both arms of the immune response are needed to prevent or control HIV infection. As a result, vaccine concepts that induce a strong cellular response by eliciting CTLs are now being tested. More recently, vaccine concepts involving a "prime-boost" strategy—a combination of vaccines—has been tested. These vaccines stimulate a cellular immune response via CTLs (prime) as well as antibodies that bind to the virus (boost). Furthermore, in addition to systemic immunity, mucosal immunity, which includes antibodies in mucosal secretions and cells in the lining of the reproductive tract and nearby lymph nodes, may also be required.

Currently, NIAID is designing and testing new vaccine candidates based on research findings on the structural components of HIV and on studies of immune response in small animals and non-human primates. Vaccine candidates are also being constructed based on isolates from many regions of the world, and several research groups are exploring mixtures of viral components from different isolates and clades. NIAID is also testing new vaccine strategies using different adjuvants, immune modulators, and delivery components to optimize the immune response.

When a vaccine is developed, the effectiveness of the vaccine will be critical. While the goal is to find a vaccine that is 100 percent effective in preventing infection, the initial HIV vaccines may not protect all vaccinated people from infection or may work to delay or prevent disease rather than prevent infection. Nonetheless, researchers recognize that even a partially effective vaccine could have a significant impact on the worldwide spread of new infections. By decreasing the number of people susceptible to HIV infection and/or able to infect others, fewer people would be passing the virus to others. If this occurs in a high percentage of people within a given population, new infections could be reduced dramatically or even eliminated. The potential drawback is that the benefits of a partially effective vaccine could be offset by relaxation in the practice of safe behaviors, education, and other prevention efforts because of perceived protection from infection. Thus, partially effective vaccines will have to be delivered in the context of a broad prevention program.



Scanning electron micrograph of human immunodeficiency virus (HIV), grown in cultured lymphocytes. Virions are seen as small spheres on the surface of the cells. Courtesy of CDC / C. Goldsmith, P. Feorino, E. L. Palmer, W. R. McManus

Vaccine Discovery

With every new scientific advance, scientists move one step closer to the discovery and development of a safe, efficacious, cost-effective vaccine to prevent HIV infection around the world. The road to a successful vaccine begins with the identification of new vaccine concepts. Basic research in the fields of HIV pathogenesis, microbiology, immunology, virology, and animal model development leads to discoveries about the biology of HIV such as the virus' life cycle, virus-host interactions, and mechanisms of disease progression and transmission. These discoveries, coupled with knowledge gained through learning how the immune system responds to the virus, enhance scientists' ability to create and design new vaccines to combat HIV infection.

One example of an important scientific advance is the elucidation of the three-dimensional structure of the HIV envelope and of broadly neutralizing antibodies, which has helped reveal specific targets for HIV vaccines and highlight several defenses that the virus uses to evade attack. Research has also

provided information on how the HIV envelope changes structure as HIV enters target cells, improved understanding of the specificity and role of antibodies and CTLs in HIV/simian immunodeficiency virus (SIV) infection, classified important subsets of T cells that affect HIV replication, and identified new potential targets for HIV vaccines, such as the HIV regulatory proteins rev and tat.

There also have been important advances in vaccine technology, such as improved systems for vaccine delivery (e.g., codon-optimized DNA, novel viral and bacterial vectors, and cytokine adjuvants); vaccination method or strategy (intramuscular, subcutaneous); and laboratory techniques. These include the development of the enzyme-linked immunospot (ELISPOT) assay, which allows researchers to detect and count cells producing cytokines in response to specific HIV peptides; tetramer binding assays that detect T cells that recognize specific HIV peptides bound to major histocompatibility complex (MHC) class I molecules; multi-color flow cytometry, which enables enumeration of different subsets of T cells from a single specimen; and easier assays to measure neutralization of primary HIV isolates. All of these new discoveries serve to further the development of HIV vaccines.

Preclinical Development

All promising vaccine candidates begin in the lab as a concept or design. However, there are many obstacles and scientific challenges along the way. Overcoming these challenges requires a better understanding of the interaction between HIV and the immune system.

One critical area of investigation seeks to address the difficulty of inducing broadly reactive neutralizing antibodies against HIV. Despite significant advances in the understanding of the HIV/SIV envelope structure and function, no HIV envelope vaccine has thus far succeeded in eliciting broadly reactive antibodies similar to those sometimes observed in individuals who have been infected for a long period of time. One theory is that a few of the human broadly neutralizing antibodies are actually derived from a pool of B cells that is usually deleted or anergic in most individuals. Such rare, broadly neutralizing antibodies may have been isolated from individuals with immune dysfunctions that cause the body to make antibodies that attack an individual's own tissues and cells. In one particular study, NIAID-supported researchers discovered human antibodies that not only bind and neutralize HIV, but also recognize cardiolipin, a lipid molecule present on membranes of heart muscle, as well as other lipid moieties normally present on cell membranes.

Another NIAID-supported investigator deciphered the crystal structure of SIV gp120 envelope glycoprotein. High resolution X-ray diffraction patterns of crystallized protein can reveal the molecular interactions and structure of proteins. Such studies have information that significantly enhances the understanding of the structure of the HIV outer envelope and how neutralizing antibodies bind to it. Ultimately, discoveries resulting from crystallographic studies will permit the design and engineering of HIV envelope vaccines that may elicit broadly neutralizing antibody responses. Preclinical studies of these constructs are essential for developing more promising vaccine candidates.

There are now more promising vaccine candidates in the preclinical pipeline than ever before. In light of this fact, HIV vaccine testing in animal models is becoming an important step in determining whether a vaccine candidate should proceed into human clinical trials. While these studies provide critical information regarding safety and potential efficacy, they also help scientists understand how the body responds to infection. Since HIV does not infect monkeys naturally, researchers instead carry out experiments with the closely related SIV. Combining parts of the HIV envelope and the inner core of SIV, researchers have engineered simian-human immunodeficiency viruses (SHIVs), which mimic HIV infection and can cause AIDS-like illness in macaque monkeys. These chimeric SHIVs allow researchers to study the immune responses to the envelope-based HIV vaccines in a live model, and the ability of these responses to stop or control the virus.

In addition to NIAID-supported investigator-initiated research, NIAID carries out AIDS vaccine-related studies in the non-human primate model through the Simian Vaccine Evaluation Units (SVEUs). The SVEUs provide non-human primates for immunization with candidate SIV or HIV vaccines selected by NIAID, conduct initial assessment of the resulting immune responses, challenge the animals with infectious virus, determine parameters of infection, and collect samples for evaluation of immune responses and protection. There are currently about 20 active protocols involving more than 350 macaques. These studies help identify optimal vaccine vectors and are revealing whether vaginal viral loads can be detected in animals chronically infected with SHIV and SIV. Some initial reports have been encouraging. The eventual aim is to determine whether vaginal "shedding" of virus is still detectable when blood viral loads become undetectable, either as a result of vaccination or from "natural" control, as has been reported in some

human studies among women on highly active antiretroviral therapy (HAART). These studies are important components of NIAID's preclinical vaccine development process.

A NIAID-funded scientist designed a study to test whether vaccines containing the HIV gene env bolster protection mediated by cellular responses to two other HIV genes, gag and pol. The study compared protection of macaques against a mucosal rectal challenge following immunization with DNA/modified vaccinia Ankara (MVA) vaccines expressing either gag-pol-env or gag-pol. The study showed that all animals immunized with vaccines expressing gag-pol-env rapidly controlled the challenge infection, whereas some of the animals immunized with only gag-pol failed to control the challenge. The gag-pol-env immunizations helped in conserving CD4+ cell numbers. These results demonstrate that while cellular immune responses to gag and pol can control an immunodeficiency virus challenge, immune responses to env synergize with those of gag and pol in achieving efficient and consistent control of the challenge. These results also suggest that in the presence of a strong T-cell response, inclusion of env may help protect against early programmed cell death of uninfected CD4+ T cells, a hallmark of the acute phase of HIV infection.

In other recent preclinical studies, researchers have identified the critical parts of HIV gp120 responsible for inducing protective neutralizing antibodies. Human monoclonal antibodies (MAbs) are capable of neutralizing a broad spectrum of HIV primary isolates. Specific MAbs can bind to an important structure called the V3 loop of the HIV-1 envelope gp120, which helps the virus gain entry to and infect target cells. Scientists have found that MAbs directed to non-linear V3 conformations (molecular shapes) are able to neutralize more primary HIV isolates compared to MAbs directed at other V3 regions. Experiments in macaques have shown that low amounts of antibodies specific for the V3 region were able to provide partial protection against viral challenge.

To circumvent the potential dampening of immune responses to candidate adenovirus vector vaccines caused by preexisting immunity to common human adenoviruses, NIAID-supported investigators developed a recombinant chimpanzee adenovirus that is not neutralized by antibodies against that common human adenovirus. Other NIAID supported approaches include the use of "novel" human adenovirus serotypes as vectors to reduce neutralization by human antibodies.

Clinical Research

Vaccine development is a lengthy process, as each stage of a clinical study can take several years. Vaccine candidates currently in Phase I, Phase II, and Phase IIB clinical trials are at least a few years away from being tested in large Phase III efficacy trials, which assess protective effects of the product and typically continue for several years after enrollment of the last patient.

To accelerate the development and conduct of these clinical trials, NIAID currently supports a variety of collaborations, programs, and networks. The majority of the NIAID-supported HIV vaccine clinical trials are conducted through the HIV Vaccine Trials Network (HVTN). Established in 1999 by NIAID, the HVTN is a comprehensive global network of international scientists and researchers whose mission is to develop and test preventive vaccines against HIV/AIDS. The HVTN's conduct of clinical trials is to include all phases, from evaluating experimental vaccines for safety and ability to stimulate immune responses, to testing vaccine efficacy. Spanning four continents, the network includes more than 25 clinical sites in the United States, Africa, Asia, South America, and the Caribbean; an operations management center; a statistical and data management center; and a central laboratory.

Within the National Institutes of Health (NIH), the NIAID Dale and Betty Bumpers Vaccine Research Center (VRC) conducts research that facilitates the development of effective vaccines for human disease, with a primary focus on the development of vaccines for HIV/AIDS. The VRC's activities include basic research to establish mechanisms of inducing long-lasting protective immunity against HIV and other pathogens that present special challenges to vaccine development; the conception, design, and preparation of vaccine candidates for HIV and related viruses; laboratory analysis and animal testing of vaccine candidates; and clinical trials of vaccine candidates.

To ensure the effective integration and coordination of HIV vaccine research efforts, in 2002 NIAID and the United States Army Medical Research and Materiel Command (USAMRMC) of the Department of Defense (DoD) forged a collaboration through an interagency agreement. A team consisting of U.S. Military HIV Research Program (USMHRP) and NIAID staff was formed to coordinate preclinical and clinical vaccine research and development efforts. As part of this collaboration, a vaccine preclinical testing laboratory utilizing standardized *in vitro* assays and animal models has been established to test vac-

cines supported by NIAID. This laboratory will help identify assays and animal models that predict human immunogenicity and thereby help NIAID prioritize vaccine candidates for further development. In addition, NIAID also supports the HIV Vaccine Design and Development Team (HVDDT) contract program which is exploring development of more than 15 candidates. This program brings together the skills and expertise of private industry and academic research centers that have identified promising vaccine concepts and have plans for targeted testing in humans. All of the original four contactors—Wyeth-Lederle, Advanced BioSciences Laboratories, Chiron Corporation, and a consortium headed by the University of New South Wales in Australia—have made experimental AIDS vaccines that have entered human clinical trials. Two more HVDDT contracts were made in fiscal year 2005 to the Children's Research Institute and Chiron Corporation, making a total of 11 awards since the program's inception.

Vaccine Strategies

Currently, there are a number of vaccine strategies under investigation, including component or subunit vaccines (a structural piece of HIV such as an envelope or a core protein); live vector vaccines (a live bacterium or virus modified to carry genes that encode HIV proteins); peptide (small pieces of HIV proteins) or fusion protein vaccines (two proteins merged together); DNA vaccines (direct injection of HIV DNA sequences) and vaccine combinations such as the prime-boost strategy.

Early in the AIDS epidemic, most of the initial HIV vaccine research focused on component or subunit vaccines directed against the HIV envelope proteins gp160 and gp120, as they represent the primary targets for neutralizing antibodies in HIV-infected individuals. The first HIV vaccine clinical trial opened in 1987 at the NIH Clinical Center. Tested in healthy, uninfected volunteers at low risk of HIV infection, the gp160 subunit candidate vaccine caused no serious adverse effects. In 1992, NIAID launched the first Phase II HIV vaccine clinical trial, testing a recombinant subunit gp120 vaccine in uninfected volunteers with a history of high-risk behavior such as injection drug use, multiple sex partners, or sexually transmitted infections. More recently, the HVTN conducted a Phase I trial using a nef-tat fusion protein (HVTN 041), followed by varying doses of gp120. Although these early candidates, as well as many others that target HIV envelope proteins, stimulated production of antibodies, antibody levels decreased within a relatively short period of time. The formulations and dosages used in the vaccine clinical trials induced low levels of neutralizing antibodies and rarely elicited cytotoxic T cells, which kill HIV-infected cells.

A major challenge in HIV vaccine development arises from the vast genetic diversity of the virus and its structural proteins. As the virus replicates within infected individuals, and after transmission to others, it continues to mutate genetically, evolving into different subtypes around the world. Thus, for a vaccine to be effective on a global scale, it will need to induce immune responses that are broadly reactive to the many different subtypes of HIV. The initial envelope vaccines induced antibodies that were largely specific for clade B isolates, the subtype of HIV that is predominantly found only in the United States and Europe. As research unlocks additional information about the HIV envelope protein and its genetic diversity, scientists learn more about its complex three-dimensional structure, conformation, and interaction with the HIV receptor. These advances may allow researchers to create vaccines that more closely resemble the natural conformation of the HIV envelope on the virion surface.

Studies have demonstrated that protection against HIV may also require cell-mediated immune responses that eliminate HIV-infected cells. The cell-mediated immune response involves the activation of specific CD8+ CTLs that target HIV-infected cells. To elicit CD8+ CTL responses, scientists employ viral or bacterial vectors to mimic infection by safely delivering specific HIV genes and inducing production of HIV proteins within cells. Because scientists design vectors to carry only a small part of the total HIV genetic material, these vectors cannot cause HIV infection. There are different types of viral vector vaccines currently being used, including poxviruses such as canarypox, fowlpox, and MVA, which is a weakened vaccinia virus; alphavirus; and adenovirus type 5 (Ad5), which is related to the virus that causes some forms of the common cold. The canarypox vaccine was the first candidate HIV vaccine shown to induce a CTL response against diverse HIV genetic subtypes. As a result, NIAID conducted a subsequent study in Uganda using a recombinant canarypox based on clade B. This study demonstrated that a vaccine could induce cross-clade cellular reactivity against various subtypes of HIV. In addition, by successfully completing this trial, researchers showed that a vaccine trial could be conducted in Africa with high scientific and ethical standards, thus

paving the way for additional international HIV vaccine trials in Africa.

Since then, there has been an expanding number of Phase I and II vaccine trials, with increasing participation in the developing world, using strategies that elicit cell-mediated immune responses. In 2005, NIAID's HVTN, in collaboration with Merck, initiated a Phase IIB "test-of-concept" trial (HVTN 502) of a recombinant adenovirus vaccine at sites in the United States, the Caribbean, and South America. Designed by Merck, the investigational vaccine contains three replication-defective adenoviruses, each expressing one of three HIV genes: gag, pol, and nef.

Researchers also have been exploring other possible vaccines, including DNA vaccines (containing one or more HIV genes). Injection of a DNA vaccine, usually intramuscularly, causes cells to take up the DNA and produce HIV proteins by normal cellular mechanisms, stimulating cell-mediated immune responses. Early studies demonstrated that the first DNA candidates were safe but did not induce strong immune responses. However, new technologies are being developed for DNA vaccines, such as codon-optimized and particle-formulated DNA vaccine candidates, that are expected to enhance their performance.

In 1992 researchers turned their attention to a prime-boost approach to improve the immunogenicity of HIV vaccines. In this strategy, the first vaccine primes the initial immune response, followed by another vaccine to boost the response. Prime-boost approaches have utilized combinations of DNA vaccines, viral vector vaccines, and subunit or peptide vaccines. The combination vaccine approach has been shown to be safe and immunogenic in volunteers at low and high risk of HIV infection. Studies have shown that this approach can stimulate cellular immunity, resulting in CTLs that can kill infected cells, as well as the production of HIV-neutralizing antibodies, which can stop HIV from infecting cells. Thus, the combination approach continues to hold promise because it stimulates production of HIV-neutralizing antibodies and cellular immunity.

At present, the HVTN has eight clinical trials under way to further evaluate the combination vaccine approach. In addition, in 2003, NIAID began working with the DoD and the Royal Thai Government to initiate a Phase III efficacy trial (RV144) using a prime-boost combination comprised of Aventis Pasteur's recombinant canarypox vector vaccine candidate (ALVAC-HIV vCP1521) and VaxGen's recombinant gp120 (AIDSVAX B/E) designed for Southeast Asia. The goal of this trial is to determine if these vaccines can either prevent infection or control HIV, if infection occurs. By November 2005, this trial fully enrolled more than 16,000 uninfected volunteers.

In 2002, VRC initiated the first multiclade vaccine clinical study, a Phase I trial using a multiclade, multigene DNA plasmid vector, which was later combined with an adenoviral vector (ADV) boost. The VRC subsequently conducted other Phase I vaccine trials using the prime-boost strategy in 2005. These vaccines were shown to be well-tolerated and elicited cellular and humoral responses. The vaccines incorporate HIV genetic material from clades A, B and C, which cause about 90 percent of all HIV infections around the world. Recently, the VRC candidates have progressed into Phase II testing of VRC's multiclade HIV-1 six-plasmid DNA vaccine in combination with a recombinant ADV boost in HIV-l uninfected adults. These are the first multigene, multiclade HIV vaccines to reach clinical Phase II, marking an important milestone in the search for a single vaccine strategy that targets U.S. subtypes of HIV as well as clades causing the global pandemic. The partners in the coordinated trials include the HVTN, the USMHRP, and the International AIDS Vaccine Initiative (IAVI). The HVTN is conducting a trial in the United States, Caribbean, South America and South Africa; the USMHRP is conducting a trial in Kenya, Tanzania, and Uganda; and IAVI is conducting a trial in Kenya and Rwanda.

Challenges and Future Directions

The scientific challenges that must be solved to develop an effective preventive HIV vaccine have proven more daunting than those faced by researchers developing vaccines against other diseases. For virtually all infections, including most viral infections, if the patient does not die the immune system ultimately clears the infection and the person becomes immune to subsequent exposure to the infectious agent, sometimes for life. This is not the case for HIV. The biggest obstacle is that immune-mediated eradication of HIV from the body, with subsequent naturally induced immunity, simply does not occur. Even after more than 60 million cumulative HIV infections since the beginning of the pandemic, there has not been a single documented case in which a person with established HIV infection has completely eliminated the virus from the body. This fact alone makes it difficult for scientists to develop a vaccine that can induce a protective immune response. This means a vaccine that only mimics natural infection will likely not be sufficient.

DNA vaccination using the needle-free Biojector device. Credit: NIAID

Scientific Challenges

Years of research have helped provide a solid understanding of how HIV evades and ultimately defeats the immune response. First, because the primary target of its devastation is the immune system itself, HIV disables the very cells that are responsible for fighting it. Second, HIV is a retrovirus, which means that it can integrate its viral sequence into the chromosomes of infected cells. Thus, the virus can shield itself from immune attack for many years, only to emerge when the infected cell is activated by the immune system to fight another infection. Third, HIV conceals the protein components that can induce a protective immune response, and therefore presents itself to the body in a way that makes it difficult for the immune system to respond effectively. Fourth, HIV is genetically diverse and rapidly changing, especially in its outer coat proteins; its mutability allows HIV to evade the modest protective responses the immune system is naturally able to make. Initially, a person is infected with only one or a limited number of HIV variants. Once HIV infection becomes established, the virus continually undergoes genetic changes, and many variants may arise within an infected person. Thus, researchers will need to determine the significance of strain variation within individuals and among populations when developing HIV vaccines. Given these scientific challenges, the need to better understand the intricacies and complexities of the immune response against HIV has never been greater.

Researchers are seeking to improve upon current vaccine designs so that the vaccines will induce broadly reactive, long-lasting neutralizing antibodies and CTL responses. Although scientists have found clues about the correlates of immunity for HIV, efficacy trials will be required to confirm immune correlates. It is hoped that once a vaccine is shown to induce at least partial protection in humans, researchers will be able to decipher the type, magnitude, breadth, and/or location of the immune responses associated with that protection. Additionally, the issue of clade or subtype diversity around the world must be addressed for an HIV vaccine to be effective on a global scale.

Researchers also need to be prepared for the various possible clinical trial outcomes, so as to assess the value from an individual and a public health perspective. Outcomes may range from preventing the establishment of infection (sterilizing immunity), to preventing or delaying disease of a volunteer who becomes infected after vaccination (controlled infection). Even if a vaccine were not able to prevent infection, it is hoped that it may keep the level of virus in the blood low enough in the vaccine recipient so that the recipient remains healthy and is not able to infect others. The greatest public health value of a vaccine will be in its ability to prevent transmission.

Animal studies can help answer critical questions that cannot be answered either in humans, because of undue risk, or by using computer modeling or laboratory tests. Although non-human primates are not the ideal animal model, they represent the best available surrogate model for research on AIDS pathogenesis and vaccine development.

Expanding Global Vaccine Research

In response to the changing HIV pandemic and the relatively low incidence of HIV infection in industrialized countries, even among higher risk groups, HIV vaccine testing must in large part be carried out in countries where the rate of new infections is highest and where different subtypes of HIV are being transmitted. NIAID established the HVTN to build global capacity and infrastructure with a special focus on pursuing an international vaccine research agenda. To expand upon and better coordinate global vaccine research activities, in addition to increasing collaboration, efficiency, and flexibility, NIAID is

restructuring all of its HIV clinical trials research networks. The new structure is designed to encourage greater integration of vaccine, prevention, and treatment research and address high-priority research questions, particularly in resource-limited settings where AIDS is most devastating.

Recruitment and Community Support

Another major challenge is to recruit enough volunteers willing to participate in clinical trials. More vaccines will be studied in the next two years than in the past five years combined. The 97 Phase I, II, and III HIV preventive vaccine clinical trials currently under way or planned will require thousands of volunteers, both in the United States and internationally. Within the United States, one of the biggest challenges is the low participation of women, minorities, and high-risk populations in clinical trials. These groups are the most in need of an HIV vaccine because they are disproportionately affected by HIV/AIDS, and their participation is needed to ensure that a potential vaccine is safe and effective in all groups of people. In developing countries, researchers are taking care to maintain equal partnerships with local researchers and help ensure infrastructure (clinical laboratories, supplies, and equipment) and increase training. Some populations that are at higher risk of HIV infection (such as high-risk women and injection drug users) are often harder to recruit and retain in a clinical trial. In some populations, there may be a general mistrust and misunderstanding of vaccine research that creates barriers to HIV vaccine trial recruitment, which researchers are working to address.

To help raise awareness and create and sustain a supportive environment suitable for clinical HIV vaccine research, NIAID has invested in building a working partnership with community representatives around the world. Among these efforts, community advisory boards are essential components at all NIAID-sponsored vaccine trial sites and within the research network. Community advisory boards provide advice and perspective on whether trials are ethical and reasonable based on the concerns and needs of the community. In 2001, NIAID launched the National HIV Vaccine Communications Campaign to stimulate and enhance the national dialogue concerning HIV preventive vaccines and to create a supportive environment for future vaccine studies. The campaign's activities include partnerships with national and local community groups, the development and provision of resources and materials, and promotion of HIV Vaccine Awareness Day on May 18th of every year.

Collaborations and Partnerships

The Partnership for AIDS Vaccine Evaluation (PAVE) was created in 2003 as a voluntary consortium of U.S. government agencies and key U.S. government-funded organizations involved in the development and evaluation of HIV/AIDS preventive vaccines and the conduct of HIV vaccine clinical trials. It is a collaborative effort to achieve better harmony and increased operational and cost efficiencies in HIV vaccine development and in the conduct of HIV vaccine clinical trials, especially Phase III trials, through serving as a forum and clearinghouse for information sharing and planning. PAVE is currently assessing international trial site capacity and standardizing laboratory procedures among partner organizations.

Although substantial resources have been devoted to HIV vaccine research around the world, until recently there was minimal international coordination and support for HIV vaccine development efforts. Following a proposal by a group of scientists, the Global HIV Vaccine Enterprise was created to foster collaboration, cooperation, and transparency in the conduct of HIV vaccine clinical trials on a global scale. In June 2004, the "Group of Eight" (G8) countries and President George W. Bush endorsed the Enterprise at the 30th G8 Summit at Sea Island, Georgia. The Enterprise is a consortium of international scientists and organizations committed to accelerating the development of preventive vaccines for HIV/AIDS. The overarching purpose is to efficiently bring resources to bear on gaps in HIV vaccine research, while allowing flexibility in how research is carried out. A strategic plan was published online in January 2005 in the journal *Public Library of Science Medicine*. Importantly, the plan emphasizes that the major difficulties encountered in the development of an HIV vaccine are scientific. The plan proposes five major activities to address the scientific priorities: (1) creation of HIV vaccine development centers or consortia to address the key scientific obstacles; (2) creation of a network of individuals and companies with vaccine manufacturing expertise to facilitate advancement of improved candidates; (3) development of a global system of laboratories that will standardize laboratory evaluation parameters; (4) sharing of common reagents; and (5) development of a network of clinical research training centers, all with the full engagement of scientists from developing countries.

In response to recommendations by the Global HIV Vaccine Enterprise, in 2005, NIAID created a Center for HIV/AIDS Vaccine Immunology (CHAVI), a virtual center that links a large group

of domestic and international scientists working to elucidate the correlates of immune protection against HIV, and to use that knowledge to design a vaccine that elicits protective immune responses. CHAVI's mission includes addressing other key immunological roadblocks to HIV vaccine development and designing, developing, and testing novel HIV vaccine candidates, as defined by NIH and as identified by the strategic plan of the Global HIV Vaccine Enterprise.

Conclusion

Although many difficult challenges lie ahead, both scientific and technological, scientists are moving closer to finding an HIV vaccine. They continue to expand their knowledge of the science, overcome obstacles in development, and advance candidate HIV vaccines through clinical trials, all in collaboration with scientists and organizations from around the world. The combined efforts of NIAID's HVTN, the Dale and Betty Bumpers VRC, industrialized partners on HIV Vaccine Design and Development Teams, collaborators in the U.S. military, and other partnerships are moving HIV vaccine research forward and providing hope and optimism for realizing the goal of identifying a safe and effective HIV vaccine.

Sources

HIV vaccine research has long been an integral part of NIAID's research portfolio, with the goal of identifying a safe and efficacious vaccine to prevent HIV infection and/or disease. Over the past 10 years, the HIV/AIDS program has received an influx of funds that has enabled it to grow exponentially. From 1996 to 2005, funding for HIV vaccine research at the NIH increased from slightly more than $100 million to more than $600 million. These funds have enabled NIAID to establish a comprehensive and vibrant set of programs that support all stages of the vaccine development pipeline, including

Dale and Betty Bumpers Vaccine Research Center (VRC)—Intramural vaccine research with a primary focus on the development of HIV vaccines

Phased Innovation Grant Program—Investigator-initiated HIV vaccine research involving high-risk/high-impact studies at the earliest stages of concept genesis and evaluation

HIV Research and Design Program—Grants to support concept testing in animal models, development of potential vaccine candidates, studies of immune correlates, and animal model development

Integrated Preclinical/Clinical AIDS Vaccine Development Program—Grants that target research at the preclinical/clinical interface *HIV Vaccine Design and*

DALE AND BETTY BUMPERS VACCINE RESEARCH CENTER

The primary focus of activities at the Dale and Betty Bumpers Vaccine Research Center (VRC) remains the development of an effective HIV/AIDS vaccine. However, over the last several years, the VRC research program has also expanded its activities to include vaccine development for Ebola, severe acute respiratory syndrome (SARS), West Nile virus, and influenza. Since being established in 1999, the VRC has made 22 clinical grade vaccine products, including 8 HIV vaccine products. Twenty-four clinical trials have been completed or are ongoing, and 7 more are planned in the next year. More than 70 percent of HIV research funds at the VRC are allocated to translational product development, as compared to basic research. In the process of addressing the need for an HIV/AIDS vaccine, novel technologies—such as DNA vaccines, viral vectors, and recombinant proteins—have been developed. When appropriate, these technologies have been applied to other emerging diseases as noted above.

Activities at the VRC stem initially from basic science discovery programs in immunology and virology. The strategy has been to identify the most relevant vaccine candidates, move these candidates quickly into production, and then into clinical trials. This effort requires robust and reliable production and immune assay technologies. Major advances in the past year include completion of the Vaccine Production Plant and initiation of its first cGMP manufacturing effort, Ebola cell banking, as well as completion of the National Vaccine, Immune, T-Cell, and Antibody Laboratory.

Development Teams—Consortia of scientists from industry and/or academia who have identified promising vaccine concepts and work under milestone-driven contracts

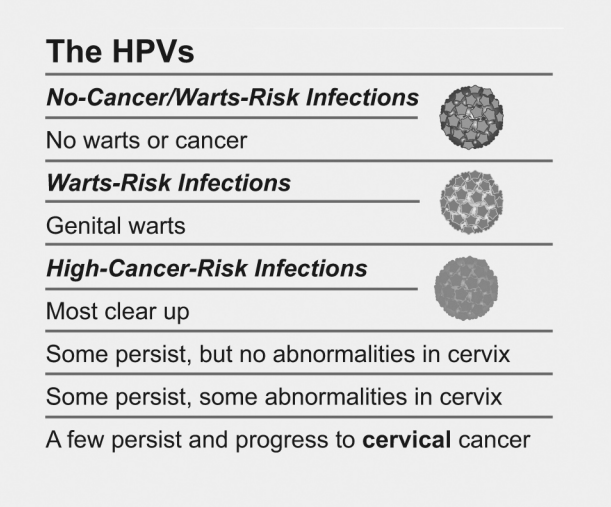
Vaccine Development Resources—Contracts for the manufacture and testing of vaccine candidates

Simian Vaccine Evaluation Units—Testing of promising SIV and HIV candidates in non-human primates

HVTN—Global research network with the capacity to conduct all phases of clinical trials, from evaluating candidate vaccines for safety and the ability to stimulate immune responses, to testing vaccine efficacy

Human Papillomavirus (HPV)

Human papillomavirus (HPV) is the name given to a group of viruses that includes more than 100 different strains. Almost every cervical cancer in the United States and abroad is caused by sexually transmitted infection with HPV. Two dominant strains of HPV, types 16 and 18, together cause 70 percent of new cases of cervical cancer. In 2006, a vaccine to prevent infection by these two HPV types was approved by the U.S. Food and Drug Administration (FDA). This vaccine, based primarily on technology developed by scientists from the National Cancer Institute's (NCI) Center for Cancer Research, offers great hope for reducing the global burden of cervical cancer.



Low-Risk and High-Risk HPVs. This chart shows the three groups of genital HPV strains. While the majority of infections with high-risk HPVs clear up on their own, a few can trigger cervical cancer over time. Courtesy of NCI

Using molecular biology techniques, NCI scientists engineered a vaccine to prevent HPV infection. They demonstrated that the major HPV capsid protein by itself can self-assemble into non-infectious virus-like particles (VLPs) that are highly immunogenic. Immunization with these VLPs, which lack any infectious or oncogenic genetic material, stimulates production of large quantities of antibodies that prevent HPV virus infections in humans.

NCI licensed the technology to two pharmaceutical companies, Merck and GlaxoSmithKline (GSK), to develop HPV vaccines commercially. Both companies are running large-scale Phase III trials of their versions of an HPV vaccine. GSK's targets the two most oncogenic HPV strains, 16 and 18. Merck's vaccine also targets strains 16 and 18, as well as strains 6 and 11, which cause about 90 percent of genital warts. Phase II trials by both companies produced encouraging results. The VLP vaccines were more than 90 percent effective at preventing infection with the virus that can lead to the development of premalignant cervical abnormalities. Studies to date have demonstrated that the vaccine prevents infection for up to four years after vaccination. Studies are under way to determine if a booster, in addition to the three initial intramuscular injections, will be necessary for long-term protection. Merck submitted an application for FDA approval and on June 8, 2006, Gardasil was approved as the first vaccine developed to prevent cervical cancer, precancerous genital lesions, and genital warts due to HPV types 6, 11, 16, and 18.

NCI's involvement in optimizing HPV vaccine development continues. NCI scientists have developed the first high-throughput assay to enable HPV vaccine developers to monitor protective antibody responses long term, and test whether their new vaccines can induce potentially protective antibody responses against other strains of HPV.

In anticipation of FDA approval of HPV vaccines, the Gates Foundation announced in June 2005 that it would grant $12.9 million to the World Health Organization, the International Agency for Research on Cancer, Harvard University, and the Program for Appropriate Technology in Health to establish systems to ensure quality control in vaccine distribution, monitor the impact of different HPV vaccination strategies, and facilitate introduction of the vaccines worldwide.

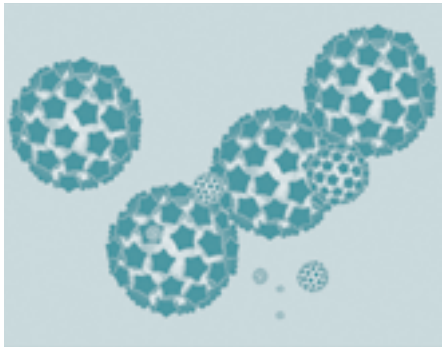


Illustration of the virus-like particles in the HPV vaccine. Like the real human papillomavirus, these vaccine participles have the same outer L1 protein coat, but they have no genetic material inside. This structure enables the vaccine to induce a strong protective immune response. Courtesy of NCI

CANCER VACCINE DEVELOPMENT AT NCI

The National Cancer Institute's (NCI's) Center for Cancer Research (CCR) is home to one of the strongest immunology and virology communities in the world. CCR scientists perform basic, translational, and clinical research as a collaborative unit in the Center of Excellence for Immunology (CEI). Their goal is to further the discovery, development, and delivery of novel immunologic approaches to the prevention, diagnosis, and treatment of cancer and cancer-associated viral diseases.

Therapeutic Cancer Vaccines

CEI researchers have made significant contributions to the ever-expanding field of therapeutic cancer vaccines. They have identified numerous novel cancer antigens, devised novel approaches to vaccine design, improved vaccine delivery, and discovered ways to optimize vaccine-induced immune responses with cytokines and co-stimulatory molecules. CEI clinicians recently initiated a pilot study that was the first clinical trial to combine radiation with a cancer vaccine for treating prostate cancer. By showing that such combination therapy is safe and well-tolerated, CCR is leading the way toward finding alternative treatments for patients with localized disease who receive radiation or surgery and then relapse. In several clinical trials, there is evidence that immune responses to vaccines are associated with prolonged survival. Several vaccines and combination protocols are being developed at NCI and are being evaluated at more than 60 cancer centers around the country for testing in clinical trials. At least two of these vaccines are progressing successfully from Phase II to Phase III studies.

One promising immunotherapy under development at NCI is interleukin-15 (IL-15), which is being investigated for inclusion in cancer vaccines. IL-15 seems to improve the body's natural response to infection and disease. In addition, IL-15 helps generate cytotoxic T lymphocytes and natural killer cells; inhibits activation-induced T-cell death and the generation of suppressor T cells that can suppress immune response against a tumor; and facilitates the survival of memory T cells that identify antigens previously encountered by the immune system.

Influenza

Introduction

Influenza virus infection remains among the leading causes of preventable morbidity and mortality. Annual epidemics and infrequent pandemics occur in all age groups worldwide. In the United States, pneumonia and influenza together are among the top 10 causes of mortality and influenza viruses are estimated to be associated with 36,000 deaths annually.

Despite prior vaccination or infection, the population's susceptibility to influenza infection continues because point mutations accumulate in the two major surface glycoproteins of the virus, hemagglutinin (HA) and neuraminidase (NA). Over time, often within a single year, accumulation of mutations ("antigenic drift") results in sufficiently different antigenic characteristics of the HA and NA proteins, making previous influenza vaccines less effective. Vaccines therefore must be reformulated annually to combat the coming year's prevalent strains.

While antigenic drift is the basis for the yearly review and frequent update of seasonal influenza vaccines, the form of antigenic variation that results in pandemics ("antigenic shift") occurs when a completely new subtype of type A influenza is introduced into the human population and begins to transmit efficiently. Because of the segmented nature of the influenza virus genome, co-infection of animals or humans with two influenza viruses can result in the exchange of genetic material between the viruses. The resulting viruses, which have exchanged genetic material, may have acquired a new HA or NA. If a reassorted influenza A virus acquires the ability to spread efficiently from person to person, a pandemic may result. This is thought to be the mechanism by which H2 and H3 avian influenza virus subtypes were introduced into human influenza viruses to spark global influenza pandemics that began in 1957 and 1968, respectively.

Vaccine History

Influenza vaccines are the primary means of preventing influenza disease and related complications in all age groups. Influenza viruses were first isolated in the early 1930s. The earliest influenza vaccines were whole virus vaccines that were produced by growing the virus in embryonated chicken eggs and inactivating it with formalin. Clinical trials sponsored by the U.S. military were conducted in healthy adults in the 1940s and demonstrated that the vaccine was highly effective in preventing influenza illness and its complications, provided there was a good match between the viruses in the vaccine and those causing the epidemic. As a result of those clinical studies, licenses were issued in 1945 to several companies in the United States for production of the first influenza vaccines for commercial use. While contemporary inactivated influenza vaccines are still produced in embryonated eggs, a series of improvements in manufacturing over the years has resulted in purified vaccines that maintain their immunogenicity but are less reactogenic. Additional progress and advances in vaccine technology are continuing.

Current State of Science

At present, licensed trivalent vaccines of two types of influenza vaccine are available to prevent seasonal influenza: the inactivated vaccine (TIV) and the live attenuated influenza vaccine (LAIV). TIV and LAIV are similar in that they incorporate three strains of influenza and are updated each year to reflect the circulating strains identified during global surveillance and recommended for use in vaccine preparation.

MedImmune, Inc., received the first license in the United States for LAIV in 2003 for its product, FluMist, derived from cold-adapted live attenuated donor strains of influenza A and B. More than 25 years of research and development were required to bring this product to licensure. Much of this research was conducted in National Institute of Allergy and Infectious Diseases (NIAID) labs and in clinical trials supported by NIAID and the private sector. The unique characteristic of these cold-adapted influenza viruses is their ability to grow at 25°C and inability to grow at temperatures greater than 38°C (temperature sensitivity). This characteristic allows the virus to undergo limited replication in the cooler nasopharynx, but not the warmer lower respiratory tract. FluMist is approved to prevent influenza illness in healthy children and adolescents, ages 5 to 17 years, and healthy adults, ages 18 to 49 years. In July 2006, MedImmune began the process of obtaining approval for a refrigerator-stable formulation of FluMist, and for expansion of the age indications to include children 6 to 59 months of age.

A CDC researcher in a BSL-3 laboratory conducts an experiment to investigate the pathogenicity and transmissibility of emerging H5N1 viruses.
Courtesy of CDC / Greg Knobloch

There are continuing studies to look at the immune response to live attenuated virus vaccines in persons less than 5 years of age and greater than 50 years. Additional investigations are needed to determine whether it is safe to use LAIV in persons with asthma, reactive airway diseases, or other chronic disorders of the pulmonary or cardiovascular systems or other current contraindications, including children or adolescents receiving aspirin or other salicylates, persons with a history of Guillain-Barré Syndrome, pregnant women, and persons with a history of hypersensitivity to any component of the vaccine or to eggs.

In 2003 there were three manufacturers licensed to provide influenza vaccine in the United States: Aventis Pasteur (producer of the TIV called Fluzone and now merged to become sanofi pasteur); Chiron Corporation (producer of the TIV Fluvirin and now merged with Novartis), and MedImmune, Inc., producer of the LAIV marketed as FluMist. In October 2004, Chiron Corporation in Liverpool, United Kingdom, was notified by the Medicines and Healthcare products Regulatory Agency (MHRA) that the company's license was being suspended for three months. As a result, Chiron notified the U.S. Food and Drug Administration (FDA) that none of its Fluvirin influenza vaccine would be available for distribution in the United States during the 2004-2005 influenza season [1].

To bring additional manufacturers to the United States and lessen the impact of future delays or shortages of influenza vaccines, NIAID rapidly initiated a Phase III trial to evaluate safety and immunogenicity of GlaxoSmithKline's (GSK) TIV, called Fluarix, for use in healthy adults. This multi-center clinical trial was conducted by the NIAID-supported Vaccine and Treatment Evaluation Units (VTEUs) [2]. The trial began in December 2004 and provided the clinical information needed by GSK to support approval by the U.S. FDA in August 2005 of the Biologics License Application for Fluarix. Fluarix became the first vaccine approved through the FDA's new accelerated approval process. In March 2006, the FDA issued two draft guidance documents to further

delineate how accelerated approval might be implemented for interpandemic and pandemic influenza vaccines.

Expanding Population Protection

Populations at special risk for complications of influenza virus infection have been expanding, both because of the aging of the population and because additional risk factors for significant morbidity and mortality related to influenza have been recognized. Recommendations for the use of inactivated influenza vaccines in the United States include individuals 50 years of age or older and individuals 6 months of age and older with chronic underlying diseases that place them at increased risk for complications from influenza infection. In 2006, the Advisory Committee for Immunization Practices (ACIP) extended the recommended age for influenza vaccination to include all children age 6 months to 5 years, because of their high risk for increased clinic and emergency room visits for complications from influenza. The expanded recommendation is a step toward increasing rates of influenza vaccination for the population as a whole [3].

Over the last 10 years, annual influenza vaccination rates in persons 65 years of age or older have steadily risen; however, the effectiveness of the current vaccine in preventing influenza illness in some elderly populations can be as low as 30 to 40 percent. The NIAID Influenza Research Program supports research to improve the effectiveness of influenza vaccines in naïve populations and those at high risk, especially the elderly. NIAID-supported VTEUs conducted a study to assess the immunogenicity and reactogenicity of a current U.S. vaccine formulation given at increasing doses in the elderly population [4]. The study enrolled approximately 200 people over the age of 65 and administered one of three different doses of the influenza vaccine: the standard dose (15 mcg), two times the standard dose (30 mcg), and four times the standard dose (60 mcg). Study results showed that participants in the highest-dose group (60 mcg) had 44 to 79 percent higher levels of antibody than did those who received the standard dose of vaccine. The higher doses also increased the number of elderly volunteers achieving levels of antibody that have been associated with protection against influenza. Increasing the antigen content of inactivated vaccines may provide a straightforward approach to improving protection in the elderly.

New Vaccine Strategies

Influenza vaccines have been prepared in eggs for many years, but new vaccine technologies are facilitating the development of innovative types of vaccine production platforms. For example, the Department of Health and Human Services (HHS), including NIAID, has encouraged and supported multiple manufacturing efforts to develop cell-based influenza vaccines.

Cell- and egg-based production of influenza vaccines has been aided by advances made in reverse genetics techniques, which allow a vaccine strain to be designed rather than selected. Some influenza viruses do not grow well in eggs and therefore cannot be used in the production of a vaccine. Reverse genetics improves the chances that a new virus can be generated that does grow well in eggs or tissue culture.

Innovative vaccine strategies are being developed that do not require replication of the whole influenza virus, including purified protein vaccines produced by recombinant DNA technology. These are comprised of individual viral proteins produced in cells and purified to a level not possible with vaccines started from a whole virus. These purified protein vaccines include vaccines using only the HA protein, or the HA protein in combination with NA or internal proteins. Additionally, a variety of DNA vaccines are being developed. In these vaccines, viral DNA sequences are included in a plasmid or viral vector, which, once injected into a person, enter the cells of the host where they cause production of limited amounts of the viral proteins that in turn elicit an immune response.

The ideal vaccine, one providing protection against any strain of influenza and not needing to be updated or administered every year to protect against newly emerging strains, is a goal not yet realized. However, research to develop such a universal vaccine is currently being supported by NIAID and others. One strategy being pursued is a "common epitope" vaccine, which utilizes highly conserved influenza proteins as targets. Although the HA and NA surface glycoproteins of influenza change frequently, many of the internal proteins are less variable. In particular, the M2 protein is being explored as an option. The M2 protein acts as an ion channel between the outside and inside of the virus membrane. A small portion of the M2 protein, its ectodomain or M2e, is exposed on the surface of the influenza virus. While it is still in early stages of investigation, M2e may act as an additional immune stimulus to augment the immune response and increase protection.

Innovative vaccine strategies also provide new options to develop vaccines rapidly in response to a newly emerging strain. If successful, these strategies could further increase vaccine production capacity and enhance preparedness

against seasonal influenza and potential pandemic strains of influenza [5].

Pandemic Influenza

As the understanding of influenza viruses has increased, it has become clear that wild aquatic birds, such as ducks and shore birds, are the most important reservoir of influenza A viruses. Strains containing all 16 HA and all 9 NA subtypes have been isolated from these birds. The majority of wild birds infected with influenza A viruses have no symptoms, although rarely strains emerge that can cause severe disease or death. Those strains that cause severe disease and death in the domestic chicken are termed highly pathogenic avian influenza (HPAI) viruses. HPAI viruses share a characteristic motif in the HA that permits efficient virus replication in organs not normally affected by influenza viruses including the brain, heart, liver, and kidneys. The subtypes most likely to be or develop into HPAI include H5 and H7 viruses.

Influenza A viruses can readily jump genus and species barriers. Wild birds may infect domestic poultry such as ducks and chickens. From there, the next step in the infection chain can be other farm animals such as pigs and horses. An intermediate host is not needed for humans to be infected, as has been amply demonstrated by H5N1 influenza A viruses in Asia and Europe and H7N7 viruses in the Netherlands since 2003. Typically, direct infection of humans with an avian influenza virus requires intense, close contact with sick or dead birds or feces from sick birds.

Human and avian influenza viruses can reassort to form a chimeric virus with features from both human and avian viruses. The human features of the virus may allow it to readily infect and transmit between humans, but the avian features may result in a virus that is new to humans and is therefore pathogenic. The human population has little or no immunity to a novel influenza subtype that emerges in this way, and these strains can cause very severe flu epidemics or pandemics. This is thought to have occurred in 1957-58 (Asian Flu) and 1968-69 (Hong Kong Flu). However, sequence and phylogenetic analyses of the complete genome of the 1918 influenza virus suggest that the 1918 pandemic strain was an avian strain that adapted to a human host and did not reassort in a secondary host [6].

In 1997, the H5N1 strain of avian influenza infected humans in Hong Kong directly from infected poultry. During this outbreak, 18 people were confirmed as having H5N1 infection, and 6 of these people died. The outbreak was successfully controlled by culling approximately 1.5 million chickens during a 3-day period.

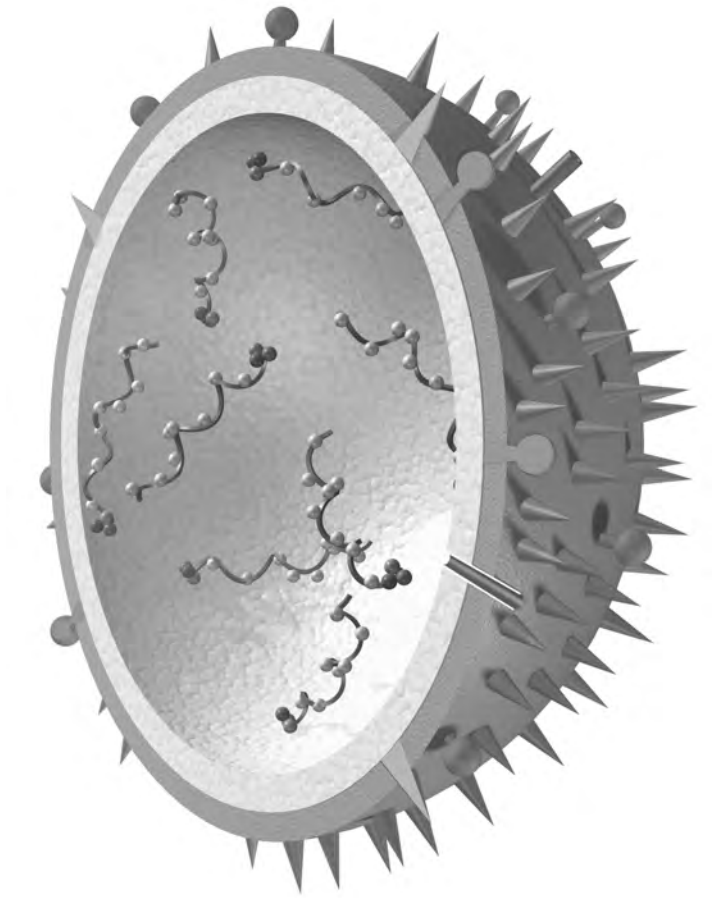
In 2003, H5N1 reappeared with two cases (one fatal) in family members from Hong Kong who had recently traveled to China; a third member of the family died in mainland China from an unconfirmed respiratory illness. As of early 2007, H5N1 influenza cases in humans have been confirmed in Azerbaijan, Cambodia, China, Djibouti, Egypt, Indonesia, Iraq, Thailand, Turkey, and Vietnam. Worldwide, as of early 2007, 270 human cases had been confirmed, with more than 160 deaths.

Extensive H5N1 outbreaks in poultry have occurred in Asia and Europe, raising concerns that transmission to humans may become more widespread. Clustering of H5N1 cases suggests that limited human-to-human transmission has occurred among persons with intense, close contact. However, it is not clear whether genetic changes other than reassortment could permit H5N1 influenza A viruses to acquire the ability to transmit efficiently from one person to another, precipitating a pandemic.

Limited human transmission associated with poultry outbreaks of other avian influenza subtypes has also been observed. H9N2 virus infections occurred in two children in China in 1999 and one child in Hong Kong in 2003, all of whom recovered. In 2003, 89 cases of H7N7 were reported in the Netherlands, with most victims reporting only mild symptoms, including conjunctivitis, although 1 death occurred in an infected person. Serological evidence was found for a human H7N2 infection in Virginia in 2002 and a patient with an underlying medical condition was hospitalized for influenza that was later determined to be an H7N2 subtype. In 2004, Canadian poultry workers contracted H7N3, resulting in mild illness in each patient.

Historically, emergence of a pandemic influenza strain is both infrequent and unpredictable; however, because of the number of human infections that have occurred, the public health community is concerned that H5N1 may emerge as the next pandemic strain. Recent NIAID vaccine development efforts have focused on vaccines against H5N1 influenza strains that have infected people in Asia. World Health Organization reference laboratories have produced several reference virus strains for use in manufacturing vaccines against H5N1, using representative H5N1 strains including A/HongKong/213/2003, A/Vietnam/1194/2004, A/Vietnam/1203/2004, A/Indonesia/5/2005, A/whooper swan/ Mongolia/244/2005, A/barheaded goose/Qinghai Lake/1A/2005, and A/turkey/Turkey/1/2005.

In 2004, NIAID awarded contracts to support the production and clinical testing of H5N1 vaccines to sanofi pasteur

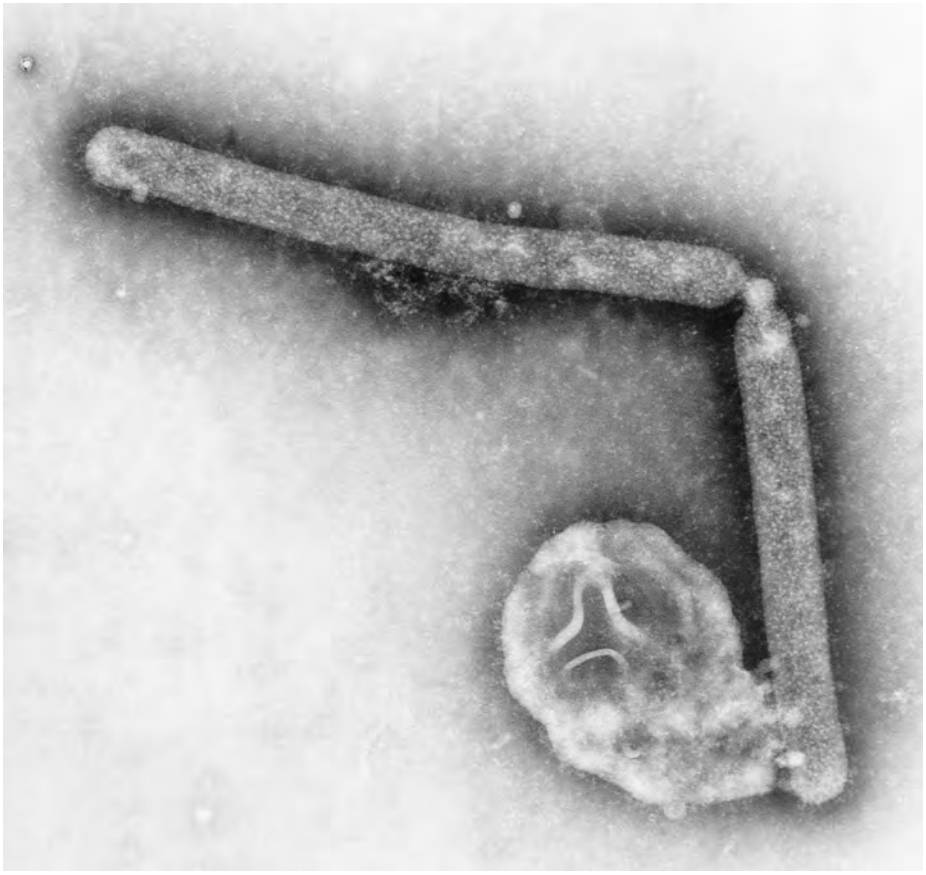


An illustration of the flu virus depicting the layer of HA and NA protein spikes projecting from its surface, as well as the eight segments of single-strand RNA inside the virus. Credit: NIAID

and Chiron Corporation. The reference virus for production of these vaccines was generated at St. Jude Children's Research Hospital using reverse genetics to provide the HA and NA genes from A/Vietnam/1203/2004 (isolated from a Vietnamese patient who died from H5N1 infection) and the remaining genes from A/PR/8/34, a non-virulent strain. The HA protein from A/Vietnam/1203/2004 was modified by replacing the HA cleavage sequence, which has been shown to be directly linked to virulence in H5 viruses, with a sequence from an avirulent strain, making the virus safe for use in vaccine production.

NIAID has recently supported the production of several lots of inactivated H5N1 vaccine and is conducting a series of safety and dose-ranging immunogenicity studies through its VTEU network. In a multi-center, double-blind two-stage Phase I/II study, the vaccine produced under NIAID contract by sanofi pasteur was evaluated in 451 healthy adults aged 18 to 64 years. Two intramuscular doses of the subunit, inactivated H5N1 influenza vaccine were administered at 90 mcg, 45 mcg, 15 mcg, or 7.5 mcg of HA per dose, or placebo [7], and subjects were followed for safety analysis throughout their participation. Sera were obtained before each vaccination and 28 days after the second vaccination, and tested for H5 antibody by neutralization and hemagglutination-inhibition. All doses of vaccine were well tolerated. The antibody response to the vaccine correlated with dosage, with the highest response occurring in subjects receiving the 90 mcg dose. To evaluate the ability to boost individuals previously immunized with an H5N1 vaccine, subjects in the above study who consented to be in a follow-on study received a third identical dose of the H5N1 vaccine. Immunogenicity results are expected shortly. Intradermal administration of 3 mcg or 9 mcg of the H5N1 vaccine produced by sanofi pasteur was also evaluated in healthy adults and found to be less immunogenic than two 45 mcg doses given via intramuscular injection. Research to evaluate higher intradermal doses of the H5N1 vaccine is underway. Testing the sanofi pasteur H5N1 vaccine in the elderly and children began in early 2006 and preliminary results suggest that the vaccine is well tolerated and immunogenic, similar to the results in adults. In addition, evaluation of adjuvanted H5N1 vaccines, including those formulated with aluminum hydroxide (sanofi pasteur and Novartis) or MF59 (Novartis) is currently under way through the NIAID VTEUs.

A 96-patient study explored the safety and immunogenicity of 4 different doses of an investigational vaccine for H9N2 influenza with and without Chiron's adjuvant MF59 [8]. The vaccine was well tolerated and all vaccine formulations containing the adjuvant MF59 proved highly immunogenic. The lowest dose contained 3.75 mcg of antigen per dose, a quarter of the dose used in seasonal influenza vaccines. In marked contrast, the unadjuvanted vaccine induced significantly lower antibody titers and did not reach levels achieved by the adjuvanted vaccine following any of the antigen doses tested, which ranged from 3.75 to



A transmission electron micrograph (TEM) of two avian influenza A (H5N1) virions. Courtesy of CDC/ Cynthia Goldsmith/ Jackie Katz

30 mcg.

In 2005, NIAID announced a cooperative research and development agreement with MedImmune to produce and test at least one LAIV for each of the 16 variations of HA, beginning with vaccines for the highest priority HA subtypes, including H5. These vaccines will be based on the same cold-adapted virus currently used for the licensed live attenuated FluMist vaccine. However, like the inactivated vaccine used to manufacture vaccines for clinical trials, the HA gene will be modified as necessary to alter virulence determinants.

Both NIAID and MedImmune will conduct laboratory studies to assess the safety of the vaccines before they are used for clinical trials. MedImmune is manufacturing the vaccines, and NIAID is testing the vaccines in an isolation unit at Johns Hopkins Bloomberg School of Public Health Center for Immunization Research in Baltimore, Maryland, to assess vaccine safety, infectivity, and immunogenicity. Results of preclinical testing of the team's H5N1 vaccine candidates were reported in September, 2006 [9]. The candidate vaccines were prepared using H5N1 viruses isolated from human cases in Hong Kong in 1997 and 2003, and Vietnam in 2004. The resulting vaccines protected mice and ferrets from infection with homologous and antigenically distinct heterologous wild-type H5N1 viruses that were isolated in Asia between 1997 and 2005. Results from a clinical trial using an H5N1 LAIV and from an earlier clinical trial of a similar H9N2 LAIV are not yet available.

In December 2006, the first human trial of DNA vaccine to prevent H5N1 avian influenza began at the National Institutes of Health Clinical Center in Bethesda, MD. The vaccine was designed by scientists at NIAID's Dale and Betty Bumpers Vaccine Research Center, and targets newer strains of the H5N1 virus currently circulating in Indonesia. The trial will enroll 45 volunteers between the ages of 18 and 60. Fifteen will receive placebo injections and 30 will receive three injections of the investigational vaccine over two months and will be followed for one year.

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Malaria

While significant advances have been made in malaria research, the disease remains a major health problem in the world's tropical areas. More than 40 percent of the world's population lives in areas at risk for malaria transmission. According to the World Health Organization (WHO), approximately 300 million to 500 million cases of malaria occur worldwide each year, resulting in more than 1 million deaths, primarily among young children in Africa. Given the disease's devastating toll on public health, and the fact that disease control has been further inhibited by the spread of drug-resistant parasites and insecticide-resistant mosquito vectors, the development of a malaria vaccine has been given high priority throughout the research community.

In 1997, the National Institute of Allergy and Infectious Diseases (NIAID) launched its Research Plan to Accelerate Development of Malaria Vaccines. Four elements were identified as being critical: (1) improved access to well-characterized research materials; (2) discovery and preclinical testing of new vaccine candidates; (3) production and evaluation of candidate malaria vaccines; and (4) clinical research and trial preparation sites in endemic areas. Through the systematic implementation of this plan, NIAID has sought to define an accessible development pathway for promising malaria vaccines.

As a result of the increasing attention that malaria has received in recent years, coupled with the improved infrastructure to produce and evaluate candidate malaria vaccines, an increasing number of malaria vaccines have been tested in clinical trials. As of December 2006, 25 vaccine candidates were being tested in clinical trials (see Table 1). Based on the results of the clinical trials, further clinical evaluation is planned for 20 candidates.

Table 1: Candidate Malaria Vaccines in Clinical Development

(Source: Adapted from Initiative for Vaccine Research, World Health Organization: www.who.int/vaccine_research/documents/RainbowTable_ClinicalTrials_December2006.pdf; accessed February 22, 2007)

Targeted Stage	Recent Clinical Trials of Candidate Vaccines	
	Phase I	Phase II
Pre-erythrocytic Stages	3	6
Asexual Erythrocytic Stages	11	2
Sexual Stages	1	0
Multistage Combination	0	2
Total	15	10

Vaccines Against Pre-Erythrocytic Stages of Malaria Parasites

During the 1990s, many studies were carried out with various candidate malaria vaccine formulations that included different adjuvants. These studies either failed to demonstrate adequate immunogenicity or failed to demonstrate adequate protection against challenge infection. In early 1997, however, investigators working at the Walter Reed Army Institute of Research (WRAIR) reported that a candidate vaccine (RTS,S), based on recombinant fusion proteins of the malaria CS protein and the hepatitis B surface antigen, could provide protection against challenge infection with a homologous parasite when the vaccine was formulated with an appropriate novel adjuvant. These results were encouraging and validated the importance of incorporating into vaccine formulations strong adjuvants that elicit appropriate immune responses. Subsequent studies, however, indicated that the protection conferred against experimental challenge by this vaccine alone was not long lived. An additional study carried out in The Gambia demonstrated that under conditions of natural exposure to malaria, the candidate vaccine could elicit protection, defined as a delay in time to first infection, in semi-immune adult men. Such protective immunity did not appear to be restricted to homologous parasites, but again was short lived. Overall vaccine efficacy was 34 percent, but was higher (71 percent) in the first 9 weeks of follow-up than in the last 6 weeks. Volunteers who received a fourth dose the next year, prior to the onset of the malaria season, again exhibited statistically significant protection (47 percent) over a 9-week follow-up period. In subsequent studies carried out in children in Mozambique, the vaccine also elicited 30 percent protection against clinical malaria; in contrast to prior studies, however, the duration of protection appeared to last at least 18 months. Furthermore, somewhat unexpectedly, the vaccine in this study also appeared to confer 58 percent protection against severe malaria. Based on these findings, plans are now under way to carry out additional clinical studies of this vaccine in infants and in different epidemiologic settings. GlaxoSmithKline Biologicals, the corporate sponsor for these studies, anticipates licensure as early as 2011 and has recently committed funds for the construction of a manufacturing facility.

In Niger, a quarter of all children do not reach their fifth birthday. Half the deaths among children under five are from malaria. Courtesy of John Haskew / International Federation of Red Cross and Red Crescent Societies

Studies are already under way to improve the formulation and investigate other means by which the immunity provided might be enhanced. Recently, vaccine- or antigen-specific production of interferon (IFN) has been identified as an important correlate of protection. Although further studies are needed to confirm these preliminary findings, they suggest specific strategies and priorities for future development. Recent studies also suggest that an alternative adjuvant may elicit greater protection against clinical malaria, but further studies with larger numbers of participants will be required to verify this. An initial study to assess the combination of RTS,S and another recombinant protein corresponding to the pre-erythrocytic antigen thrombospondin-related adhesion protein/sporozoite surface protein 2 (TRAP/SSP2) resulted in an apparent loss of protective efficacy compared to RTS,S alone. These results suggest that interactions among constituent antigens in vaccines may actually be detrimental rather than beneficial, and thus serve as a cautionary note for combination of RTS,S with other antigens.

Building on the increased awareness of the importance of strong adjuvants, some investigators have returned to the

concept of immunizing with long synthetic peptides (LSPs) formulated with stronger adjuvants. Investigators at the University of Lausanne in Switzerland carried out a Phase I clinical trial of an approximately 100-amino-acid synthetic peptide corresponding to the C-terminal portion of the CS protein, formulated with a strong adjuvant (Montanide ISA 720). Subsequent analyses showed that the vaccine was safe and well tolerated and elicited antibody and cellular immune responses, including antigen-specific production of IFN.

An alternative approach that appears promising is to identify specific regions of the CS protein that stimulate immune responses and then incorporate several copies of those regions into a synthetic structure called a multiple antigenic peptide (MAP). MAPs based on CS protein structures have been shown to elicit high antibody titers in animal models and are capable of boosting preexisting malaria-specific immune responses. One potential problem associated with evaluation of synthetic peptide-based vaccines such as MAPs is that human genetic variations may limit immune responses to the vaccine. This is particularly important because the candidate vaccine might be rejected as non-immunogenic if the responsive individuals are not adequately represented in the initial immunogenicity study. Two approaches have been studied to determine how genetic factors impact immune responses to CS-based MAP vaccines, including a recent innovative Phase I clinical trial designed to ensure that an adequate number of pre-identified responder individuals was included. Though these individuals mounted significant immune responses, hypersensitivity reactions were seen in a significant number of trial participants. In another approach to overcome the genetic restriction, investigators created a construct that also incorporated a "universal" T-cell epitope (i.e., one that was not subject to narrow genetic restriction) and was subsequently shown to elicit robust immune responses in mice and in humans with diverse genetic backgrounds. In contrast to the preceding MAP vaccine, this vaccine was not associated with significant hypersensitivity reactions in trial participants.

Other investigators have made LSPs based on the sequence of various malaria antigens, including the CS antigens of *Plasmodium falciparum* and *Plasmodium vivax*, a number of which have completed Phase I trials. In general, these studies have demonstrated that LSP-based vaccines are safe and immunogenic. A Phase II experimental challenge study has also been completed for a *P. falciparum* construct, but the results have not yet been reported.

Two critical issues for synthetic peptide vaccines have been the difficulty of manufacturing such candidates reproducibly, and the likelihood that linear peptides may not adopt the conformation of the native parasite protein. Alternative strategies, therefore, have been pursued to develop new platforms for delivery of epitopes. Recently the B- and T-cell epitopes studied in the MAP trials have been incorporated into a recombinant virus-like particle based on a molecularly engineered version of the hepatitis B core antigen (HBc). This particle appeared to function as a particularly immunogenic platform, and the engineered CS-HBc particle elicited robust immune responses to *P. falciparum* sporozoite antigens in animal studies. In Phase I clinical trials, however, the immunogenicity of the construct in humans was shown to be much lower, and further work will be needed to improve the characteristics of this candidate.

An alternative approach is to couple synthetic peptides to virus-like particles. One advantage of this approach is that the peptides can sometimes be constructed so as to mimic the structure that occurs in the native parasite protein (these are often called "mimotopes"). Both the CS repeat B-cell epitope and the apical merozoite antigen 1 (AMA1) ectodomain have been constructed as mimotopes and coupled to viral-like particles based on influenza. In a Phase I trial these constructs were reported to be well tolerated and immunogenic. In addition, the antibodies elicited inhibited parasite invasion of liver cells in an *in vitro* assay.

Attention also has been directed to the non-repeat domains of the CS polypeptide. A genetically conserved region within these domains has been implicated in parasite attachment to liver cells. Although shown to be safe and immunogenic in a clinical trial, a vaccine based on a genetically engineered CS-derived polypeptide from which the central repeat region was excised failed to confer protection against experimental challenge in the immunized volunteers.

While malaria vaccine efforts in the past have focused primarily on the humoral aspects of immunity, increasing attention is being directed to the important role played by T cells. In addition to enhancing antibody responses and conferring immunological memory, T cells also mediate cytotoxic immunity and induce the production of cytokines, such as IFN. CS-responsive T-cell clones have been established from cells of vaccinees immunized with attenuated parasites, and may prove to be useful in future studies on the development of immune

responsiveness. Epitopes of CS polypeptides recognized by helper T cells, as well as by cytotoxic T cells, have been identified and are being incorporated into recombinant vaccine candidates for further testing.

To identify new candidate vaccine components, investigators have used a variety of techniques. For example, reverse immunogenetics allowed investigators to identify a peptide component of a liver-stage parasite protein (LSA-1) from individuals who are resistant to severe malaria that is efficiently recognized by cytotoxic T cells. This result, in combination with epidemiologic data, supported the case for evaluating an LSA-1 based vaccine and new recombinant viral vectors expressing LSA-1. Recombinant proteins based on LSA-1 sequences have now been made and are beginning to enter preclinical and clinical development. Other liver-stage antigens (e.g., LSA-3) are also being evaluated in preclinical and clinical studies for their potential as candidate malaria vaccines.

The availability of the genomes for *P. falciparum* as well as *Plasmodium yoelli* has also allowed investigators to identify novel antigens that might be developed as potential vaccines. One approach has been to identify genes that are essential to parasite development and might be susceptible to genetic or immunologic manipulation. In an alternate approach using a combination of proteomic and genomic data, researchers were able to identify 27 potential sporozoite antigens. Sixteen of these antigens were recognized by blood cells from individuals immunized with irradiated sporozoites, suggesting they could be targets of protective immunity. Indeed, one antigen, PFL0800C, appeared particularly promising in that it was recognized by cells from all the volunteers, and further analysis indicated that it is highly expressed in sporozoites.

Pre-erythrocytic antigens also have been incorporated into multicomponent vaccines. In the case of DNA vaccines, a construct incorporating the gene for the CS antigen was evaluated as a "proof of concept" in a clinical study carried out by the U.S. Navy Malaria Program and its collaborators. The construct elicited cell-mediated immune responses in study volunteers, but did not elicit antibody responses and did not confer protection against experimental challenge. The possibility has also been raised that incorporating additional pre-erythrocytic antigens or epitopes into a DNA vaccine might improve efficacy. In a subsequent Phase I/IIa clinical trial, DNA plasmids for five different antigens (CSP, TRAP/SSP2, Exp1, LSA-1, LSA-3) plus a plasmid for human GM-CSF were evaluated. The five different antigens all elicited T-cell responses and did not appear to interfere immunologically with each other; however, they failed to elicit an antibody response, and none of the vaccinees were protected against experimental challenge.

Investigators are expressing pre-erythrocytic stage antigens in a variety of viral and bacterial vectors and evaluating their potential either as vaccines by themselves or as part of a heterologous prime-boost strategy in which one type of vaccine is used to prime and a second is used to boost the immune response. Heterologous prime-boost was first demonstrated as a strategy in malaria vaccination more than 10 years ago when NIAID-supported investigators showed that immunizing mice against rodent malaria could be done most effectively when using different viral vectors encoding the gene for the rodent CS protein. Since then various investigators have initiated studies of different prime-boost combinations for a number of different antigens. Although none of the regimens studied to date have demonstrated clinically relevant protection, results have been encouraging, and these regimens are still under active investigation. Under a recent contract, NIAID-supported investigators produced a novel candidate malaria vaccine based on a replication-deficient adenovirus serotype 35 vector encoding the CS antigen; clinical trials of this candidate began in December, 2006. This construct is especially interesting because it was previously shown that a single dose of an adenovirus encoding the CS gene could confer protection against rodent malaria, and also could serve as an effective prime in a prime-boost regimen with RTS,S in a study of immune responses in rhesus macaques.

Although these advances are promising, some investigators have returned to the concept of an attenuated sporozoite vaccine for malaria. In the past, concerns have been raised about the safety, reproducibility, production scaleability, and distribution of such a vaccine. Considerable progress has been made, however, toward the various steps necessary for commercial development and distribution of an irradiated sporozoite vaccine. A related approach, which builds on the recent availability of the *Plasmodium* genomes, has been to identify genes that are critical for parasite development (e.g., in the liver stage), and then to use molecular genetic techniques to knock out expression of those genes. Using this approach, NIAID-supported investigators demonstrated the feasibility of a genetically attenuated sporozoite vaccine in a murine model.

The *Anopheles gambiae* mosquito, a malaria vector. Courtesy of CDC / Jim Gathany

That result was independently confirmed for a different gene by other investigators. Efforts are now under way to translate these findings into a comparable vaccine for *P. falciparum*.

Vaccines Against Asexual Blood Stages of Malaria Parasites

In collaboration with GlaxoSmithKline and the U.S. Agency for International Development, investigators at WRAIR recently expressed the 42-kD C-terminal fragment of the major MSP1 in *Escherichia coli*. Based on reactivity with a panel of monoclonal antibodies, the antigen appears to be conformationally correct. The antigen was subsequently formulated with the same adjuvant used in the RTS,S studies. In clinical trials carried out in the United States, this vaccine appeared to be safe and immunogenic, although the addition of the MSP1 42-kD antigen to RTS,S did not appear to enhance protective efficacy against experimental challenge. A clinical trial of the recombinant MSP1 42-kD fragment for assessment of safety and immunogenicity in malaria-endemic populations has been carried out in adults in Kenya and in Mali, and plans are under way for pediatric trials.

Investigators at the NIAID Malaria Vaccine Development Branch (MVDB) have also made recombinant proteins based on two allelic variants of the *P. falciparum* MSP1 42-kD protein. The recombinant proteins were expressed in *E. coli* and then allowed to refold to obtain their native conformations. In *Aotus* monkeys, these proteins were shown to be immunogenic, and protection against experimental challenge with infected red blood cells was associated with antibody titers to the immunogen. Phase I clinical trials are currently under way.

Under a cooperative research and development agreement, NIAID and Genzyme Transgenics Corporation evaluated the feasibility of producing genetically engineered animals capable of secreting a recombinant version of the MSP1 42-kD C-terminal fragment in the animals' milk. Because *Plasmodium* species do not carry out substantial N- or O-linked glycosylation, site-specific mutations were introduced into the native sequence to prevent glycosylation in the transgenic animals. When glycosylated and non-glycosylated versions of these recombinant proteins were compared in a head-to-head study in *Aotus* monkeys, only the non-glycosylated version elicited protective immunity. Together with studies of the same recombinant protein expressed from a baculovirus construct in insect cells, these results suggest that the extent of glycosylation in some expression systems may alter or obscure the immunogenicity of protective epitopes. A recombinant version of the ectodomain of AMA1 in which the glycosylation sites were also mutagenized has been shown to elicit protective immunity in *Aotus* monkey studies as well.

A number of groups have carried out clinical trials of AMA1-based vaccines. In addition to some of the structural issues involved in expressing AMA1, this antigen is also quite polymorphic. NIAID investigators have studied expression in a number of different systems to try to determine the optimal process for maintaining both conformation and yield. To address the issue of polymorphism, investigators are also assessing combinations of different variants of the antigens for the ability to elicit immune responses to multiple variants. In a recent Phase I trial of AMA1-C1 (a combination of two recombinant proteins based on the most divergent variants) formulated with Alhydrogel, antibody responses were detected in 92 percent of volunteers after three immunizations, with equal reactivity to both of the AMA1 components. Purified IgG obtained from these individuals also inhibited parasite

Public health campaigns, such as the distribution of insecticide-treated mosquito nets by the International Federation of Red Cross and Red Crescent Societies, are attempting to protect millions of children throughout developing countries. Courtesy of John Haskew / International Federation of Red Cross and Red Crescent Societies

growth *in vitro*. Subsequently, it was shown in preclinical studies that the addition of CpG oligodeoxynucleotide to this vaccine enhanced functional antibody responses. As a result, a Phase I study using AMA1-C1 adjuvanted with both Alhydrogel and CpG was conducted. The addition of CpG to the alum-based vaccine greatly enhanced the antibody response to AMA1-C1.

Other antigens that are being produced in recombinant protein expression systems include region II (RII, the red cell binding site) of the 175-kD erythrocyte binding antigen (EBA-175) of *P. falciparum*, and its paralog in *P. vivax*, the Duffy binding antigen (DBA). Material suitable for use in clinical trials has been produced for EBA-175 RII, and a Phase I clinical trial is under way. Clinical trial material for the DBA vaccine is also expected to be available in the near future. SE36, a recombinant protein based on serine repeat antigen, has also undergone a Phase I trial.

The structures of various recombinant protein-based candidate vaccines, e.g., MSP1 19, AMA1, EBA-175 RII, and DBA, have also recently been determined, and are yielding new insights about critical targets of protective immunity. For example, the recently published crystal structure of EBA-175 RII suggested that particular amino acids were involved in dimerization of the molecule and in binding to sugar residues on the red-cell surface molecule glycophorin A. Site-directed mutagenesis of these residues provided further supporting evidence for the roles of these residues. Since EBA-175 is a member of a family of parasite molecules that have been implicated in red cell invasion and are structurally related, the results of the crystal structure are likely to be informative for a number of molecules. Indeed, the structure of DBA has recently been determined, and was found to be similar to that of EBA-175. The availability of such structural features and their association with functional aspects may prove useful for targeted vaccine design in the future.

In addition to the studies with vaccines based on recombinant proteins, two clinical trials have been carried out recently with vaccines based on LSP versions of MSP3 and the glutamine-rich protein (GLURP). In a Phase I trial of MSP3, two different adjuvant systems, Montanide ISA 720 and aluminum hydroxide, were studied. Although both formulations were immunogenic, the Montanide formulation was found to be unacceptably reactogenic. The vaccines induced cytophilic antibody responses, and *in vitro* and *in vivo* assays for antibody-dependent cell-mediated inhibition (ADCI) of parasite growth suggested a sustained immune response eight months after the final vaccine dose.

The glycophosphotidylinositol (GPI) anchor of several malaria proteins has been identified as a putative malaria toxin responsible for several of the clinical manifestations of severe disease. As a result, it has been proposed that immune responses directed against the GPI anchor might underlie an "anti-disease" vaccine intended to limit the clinical pathology associated with malaria infection. A major obstacle to testing this hypothesis has been the inability to obtain the requisite amounts of GPI. Recent advances in automated carbohydrate synthesis, however, have made it possible to make large quantities of GPI reproducibly. Preclinical data obtained with such material supports the concept that anti-GPI responses elicited by a vaccine can limit severe disease, but have also demonstrated that in the absence of clinical signs and symptoms, high levels of parasites can appear in the blood. Additional preclinical studies are now under way to evaluate the safety and efficacy of these novel vaccine candidates.

Chimeric vaccines comprising different antigens are now beginning to undergo evaluation. Such combinations include GLURP-MSP3, PF CP-2.9 (a combination of AMA1 and MSP1 19-kD), and MSP1 and EBA-175.

Vaccines Against Sexual Stages of Malaria Parasites and Mosquito Vector Components (Transmission-Blocking Vaccines)

Transmission-blocking vaccines are designed to eliminate malaria from regions of the world with low transmission intensity. Antigens from the sexual stages of the malaria parasite that can induce transmission-blocking activity have been identified. Investigators at the NIAID MVDB have expressed a recombinant protein in yeast corresponding to a 25-kD molecule found in *P. falciparum* (Pfs25). Immunization with this molecule elicits transmission-blocking antibodies in animals; from these studies, however, it is clear that attaining and maintaining a high titer of transmission-blocking antibody is likely to be important for efficacy. Phase I clinical testing of this vaccine candidate formulated with alum has been conducted, and preliminary results indicate that improved formulation will be required. Experiments are under way to improve the preclinical profile and immunogenicity. A recombinant antigen corresponding to a similar 25-kD antigen found in *P. vivax* has also been produced by recombinant DNA technology by MVDB and shown to elicit transmission-blocking activity in monkeys. In a recent Phase I clinical trial, Pvs25H adsorbed to Alhydrogel elicited no serious adverse events and was shown to be immunogenic. In a membrane feed assay intended to evaluate transmission-blocking activity, functional antibody responses were observed. Unfortunately, the vaccine formulated with alum is poorly immunogenic.

Multicomponent Vaccines

Multicomponent vaccines directed against different antigens and different stages of the parasite life cycle may offer an advantage over single-component vaccines because they may provide multiple levels of protection. Such vaccines also may reduce the spread of vaccine-resistant strains, which can arise when the parasite changes a surface protein to avoid detection by the immune system.

Almost 10 years ago, a blood-stage vaccine (SPf66) developed in Colombia was reported to delay or suppress the onset of disease. In a randomized, double-blind trial conducted in Colombia, the vaccine was reported to have an overall efficacy of 40 percent. Two other clinical trials in South America reported similar results. These studies, however, were carried out in areas of low or seasonal malaria transmission, and thus the utility of this vaccine in areas of high transmission and in other geographic locations was questioned. To address these issues, randomized, double-blind, controlled clinical trials were carried out in Tanzania, The Gambia, and Thailand. In the Tanzanian study, the estimated efficacy of SPf66 was 30 percent, but with wide variability. In the Gambian and Thai studies, however, no significant efficacy was demonstrated. A later study in Brazil also did not demonstrate any efficacy of SPf66.

Future Directions

In recent years, the landscape for malaria vaccines has changed in remarkable ways. Certainly considerable progress has been made on various fronts. Promising results have been obtained with the RTS,S/AS02 candidate in a Phase II trial, although additional studies will be required to verify these results in younger children and in different epidemiologic settings. Additional improvements in the apparent efficacy of the vaccine may also be possible and are actively being investigated. Just as important, however, are significant scientific and technical advances that have led to the creation of a robust portfolio of candidate malaria vaccines supported by a number of research agencies and public-private partnerships, and the necessary elements of a defined clinical development pathway are being put in place.

Under its research plan for malaria vaccine development and its Global Health Research Plan for HIV/AIDS, Malaria, and Tuberculosis, NIAID has stimulated research in this area with recent initiatives and support activities. Novel vaccine targets, delivery systems, and alternative strategies to prime and boost protective immune responses differentially are being investigated. The Malaria Research and Reference Reagent Resource Center (MR4), a resource for the collection of malaria research and reference reagents, has been established at the American Type Culture Collection to provide a central source of quality-controlled, malaria-related reagents and information to the international malaria research community. NIAID also continues to collaborate with a number of partners in supporting large-scale sequencing of genomes of *Plasmodium* parasites. Such efforts are expected to result in the identification of new targets for potential vaccines and drugs. Finally, efforts are also in progress to expand capabilities to produce candidate malaria vaccines and to accelerate their evaluation domestically and internationally. In recent years, NIAID-supported investigators have carried out a number of studies and clinical trials in Mali and Ghana, and plans are now being made for additional clinical trials

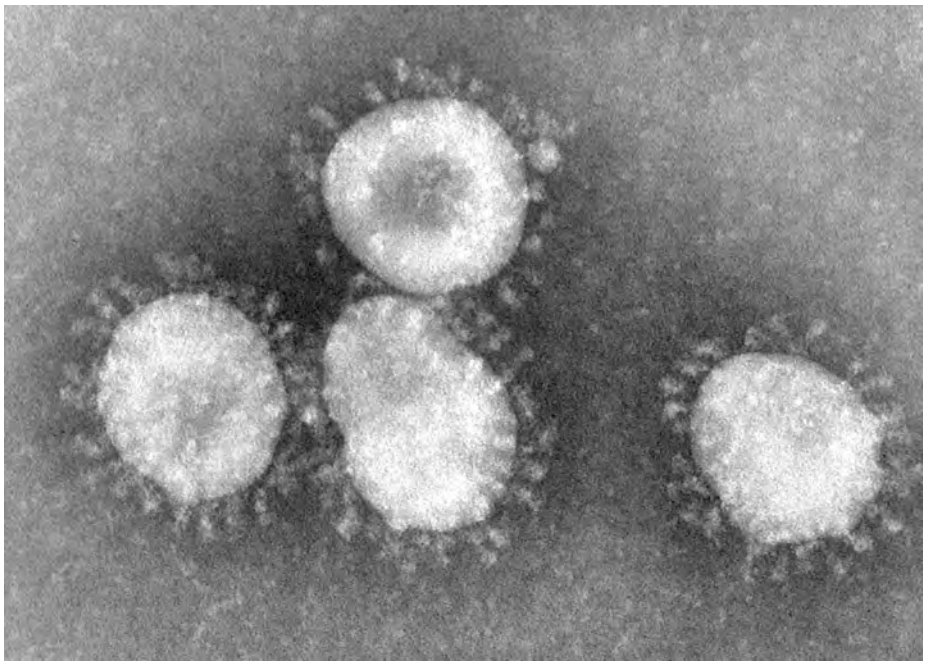
in the years to come.

Beginning in late 2004, a Malaria Vaccine Technology Roadmap initiative, organized by the MVI with support from the Wellcome Trust and the Bill and Melinda Gates Foundation, brought together investigators from both developed and developing countries as well as representatives of the broader malaria vaccine research and development community, including research funding agencies, economic development agencies, and philanthropies. Through a series of meetings and workshops, these groups were asked to identify challenges and hurdles confronting malaria vaccine development and to establish a set of shared priorities and objectives to further accelerate malaria vaccine development. The Roadmap thus offers a context in which to develop improved collaborative efforts, and these efforts are already under way. In late 2005, NIAID and the WHO Initiative for Vaccine Research jointly sponsored a meeting on standardization of assays for development of candidate malaria vaccines.

With an increasing number of groups becoming involved in malaria vaccine research and development, there are new opportunities for collaboration and synergy to accelerate the pace of malaria vaccine development. In addition, there are new malaria intervention and control efforts, including the Roll Back Malaria Partnership, the Intermittent Preventive Treatment in Infants Consortium, the Global Fund for AIDS, Tuberculosis and Malaria, and the U.S. President's Malaria Initiative. These activities are likely to impact future clinical and field studies of malaria vaccines, and raise both new opportunities and challenges. Effective control programs, for example, may call for adaptations in study design and sample sizes when evaluating malaria vaccines. On the other hand, the apparent efficacy of vaccines may be greater in areas in which control programs have been able to reduce but not eliminate malaria transmission.

Severe Acute Respiratory Syndrome (SARS)

Background



Coronaviruses are a group of viruses that have a halo, or crown-like (corona) appearance when viewed under a microscope. Courtesy of CDC / Dr. Fred Murphy

In the spring of 2003, the world first learned of an outbreak of a newly recognized atypical pneumonia that was named severe acute respiratory syndrome (SARS). Believed to have originated in the Guangdong province of China in late 2002, SARS quickly spread to Hong Kong, Taiwan, Singapore, Canada, and Vietnam and, ultimately, to a total of 29 countries. There were 74 probable cases in the United States. Overall, more than 8,000 patients were affected, with 774 fatalities in less than one year.

The speed with which the global health community responded to SARS was unparalleled. Shortly after SARS first emerged, the disease's etiological agent was identified as a novel coronavirus called SARS-CoV, which was determined to be phylogenetically distinct from previously known human and animal coronaviruses. Characterization of the virus indicated that it was a single stranded, positive sense RNA virus, with a large genome of 29.7 kilobases.

SARS-CoV was discovered to be primarily transmitted from person to person by close contact with large respiratory droplets. Symptoms of illness initially included flu-like symptoms, with fever, cough, body aches, and malaise after an incubation period ranging from 3 to 10 days. Most patients developed pneumonia and more than 60 percent of chest X-rays showed infiltrates. Up to 20 percent of individuals had diarrhea.

Epidemiological investigation showed that SARS disproportionately affected health-care workers and other close contacts of SARS-patients, such as family members. Higher mortality was observed in older patients, with more than 50 percent of fatalities occurring in people 65 years of age or older. Children were the least likely to acquire the disease.

The likely immediate sources of SARS-CoV that initiated the outbreak were exotic animals from Guangdong marketplaces. SARS-CoV-like viruses, with 99 percent identity to human strains, were isolated primarily from Himalayan palm civets as well as other marketplace animals. From two independent field studies, another animal species, the Chinese horseshoe bat, was subsequently found to harbor a SARS-CoV-like virus that was 93 percent identical to human SARS-CoV. Since SARS-CoV-like virus was not found in farm-raised palm civets, it is concluded that the horseshoe bat may serve as the natural reservoir of virus, with the civet serving as the intermediate host. Both animals are sold in Chinese marketplaces.

In the months after the disease first emerged, the clinical syndrome was characterized, the etiological agent was identified, diagnostic tests were devised, and the virus' genome was completely sequenced. The speed of scientific understanding and information exchange, combined with critical public health measures such as patient isolation and infection control, eventually led to outbreak containment. In July 2003, the World Health Organization officially declared the outbreak over. Since July 2003 there have been four separate laboratory-acquired SARS infections—one each in Singapore and Taiwan, and two in China. In addition, two individuals in southern China contracted SARS in December

Researchers have identified the *Rhinolophus macrotis* horseshoe bat as a natural reservoir of SARS Co-V and the civet as an intermediate host. Bat image courtesy of Dr. Tigga Kingston, Texas Tech University. Palm civet image courtesy of Dr. Wayne Marasco / Harvard Medical School

2003 after being exposed to the virus in a restaurant.

There have been no new SARS cases reported since April 29, 2004. While the 2003 outbreak has not been repeated, the threat has not disappeared. Because an animal reservoir of the precursor virus exists in nature, an effective vaccine and/or therapeutic is still needed should SARS reemerge. While the global health impact was substantial, that pales in comparison to the economic impact with respect to travel, tourism, and service industries.

Current Status of Science

Because it is not known which type of vaccine will be most effective against SARS CoV, the National Institute of Allergy and Infectious Diseases (NIAID) is supporting several different approaches to vaccine development.

In 2003, NIAID awarded contracts for the production of experimental, inactivated, whole virus SARS vaccines as well as for the production of a recombinant S protein subunit vaccine. S protein is used by the virus to attach to lung cells. A contract was also awarded to support the generation of a monoclonal antibody to the S protein, which has subsequently demonstrated both prophylactic and therapeutic properties in animals. After these experimental vaccines and the monoclonal antibody are manufactured and preclinical data generated and summarized, NIAID plans to test them in clinical trials conducted by its Vaccine and Treatment Evaluation Units.

In addition, NIAID-supported investigators are pursuing a SARS vaccine based on soluble S-protein expressed in mammalian cells, as well as a vaccine based on baculovirus-expressed S protein combined with a novel adjuvant for intranasal delivery. An alphavirus replicon vaccine against SARS is under development, as is a vaccine based on SARS proteins expressed in virus-like particles. Alternate strategies being developed include a peptide-based vaccine approach, and a rhabdovirus (rabies virus) genetically modified to express the SARS S protein.

Understanding SARS

NIAID-supported scientists have made significant advances in understanding SARS-CoV and its pathogenicity. For example, researchers discovered that the papain-like protease of SARS-CoV has a deubiquitinating enzyme activity that regulates location and stability of cellular proteins, and determined its three-dimensional structure. This work may lead to the design of small molecule inhibitors of this essential SARS enzyme.

Researchers have also identified and characterized the lung receptor molecule, ACE2, to which the S protein adheres. Regions of interaction between the S protein and ACE2 were mapped and characterized, and the domains of the S protein necessary for viral infection were determined. These findings are particularly important in the design of improved vaccines and therapeutics. Scientists have learned that the entry of SARS-CoV is blocked by inhibitors of the endosomal protease cathepsin L, and a secondary receptor that augments infection, L-SIGN, was also identified and characterized.

Researchers in NIAID's Laboratory of Infectious Diseases (LID) studied the replication of SARS-CoV in mice, hamsters, and non-human primates (NHPs) and established that intranasally administered SARS-CoV replicated efficiently in respiratory tissues. In BALB/c mice and hamsters, the virus replicated to levels that permit an evaluation of vaccines, immunotherapies, and antiviral drugs. In addition, further studies in mice and hamsters demonstrated that primary infection provides protection from re-infection and that antibodies alone can block against viral replication. This

work suggests that vaccines that induce neutralizing antibodies, and strategies for immunoprophylaxis or, perhaps, immunotherapy are likely to be effective in combating SARS. LID scientists have collaborated with scientists at academic institutions to demonstrate the efficacy of monoclonal antibodies against the spike protein of SARS-CoV in prevention and treatment of SARS-associated disease in hamsters.

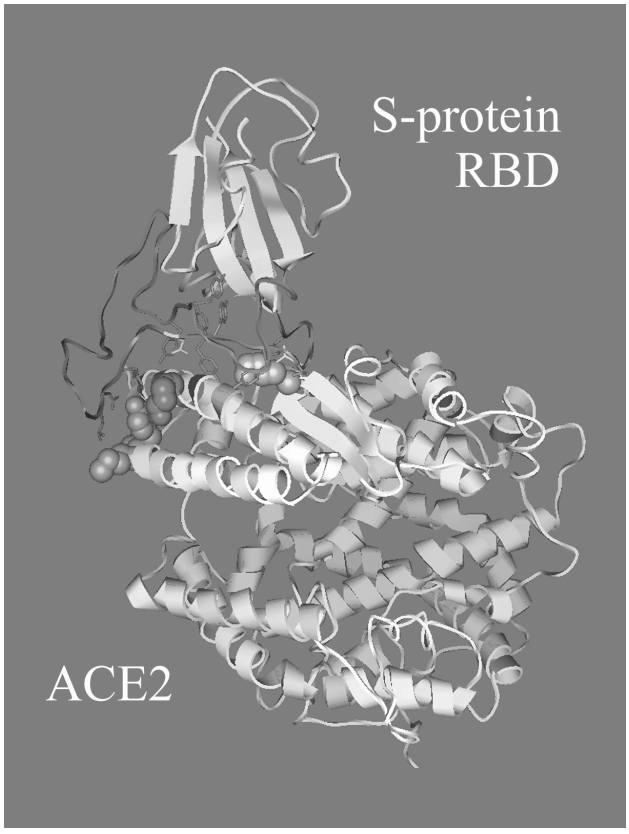
Further, while the LID investigators observed no clinical illness in young mice, hamsters, or NHPs, because advanced age has been associated with poorer outcomes, and greater mortality in SARS patients, the investigators examined whether aged mice might be susceptible to disease. They found that SARS-CoV-infected aged mice demonstrated signs of clinical illness that resolved by day 7 post-infection. The virus-infected aged mice mounted an adaptive immune response to infection; however, in contrast to young mice, they also mounted a proinflammatory cytokine response early post-infection. This work demonstrated in animals an age-related susceptibility to SARS that parallels the human experience.

The LID scientists have also collaborated with other scientists at the National Institutes of Health, as well as researchers at academic institutions and in industry, to evaluate a number of candidate SARS-CoV vaccines, including inactivated, subunit, vectored, and DNA vaccines, in animal models.

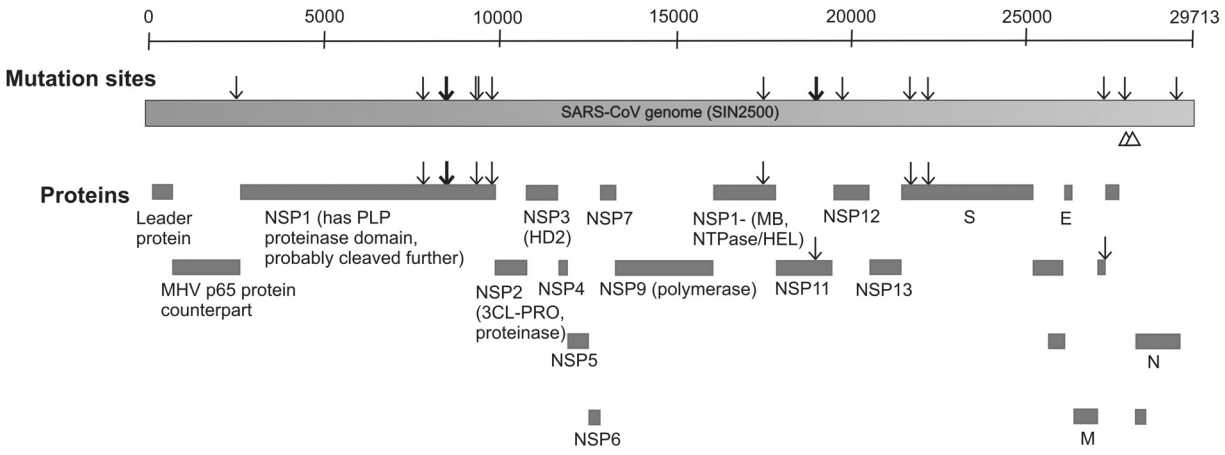
Researchers at NIAID's Dale and Betty Bumpers Vaccine Research Center are working in partnership with Vical, Inc., to manufacture an experimental SARS vaccine that has been shown to prevent the SARS-CoV from replicating in laboratory mice. Instead of using a weakened or inactivated virus, the new vaccine is composed of a modified piece of DNA that encodes the S protein of SARS-CoV and is expected to stimulate protective immunity in humans. A Phase I open-label clinical study to evaluate safety, tolerability, and immune response was initiated in December, 2004. The study enrolled 10 healthy volunteers, ages 18 to 50, and administered 4 mg DNA vaccinations at three one-month intervals. Interim study results indicate that the vaccine is well tolerated with no or mild systemic or local reactogenicity, and no serious adverse events.

NIAID-supported scientists have also made significant advances that have improved understanding of SARS-CoV. For example, researchers are elucidating the role of SARS-CoV non-structural proteins to enhance knowledge of the interaction of SARS-CoV proteins with the mediators of the innate immune system. In addition, NIAID contractors have screened 100,000 potential antiviral drugs and other compounds for activity against SARS-CoV. Several compounds have demonstrated antiviral activity and are being tested in animal model systems. Meanwhile, a full length cDNA clone of infectious SARS-CoV was generated by reverse genetics through a systematic assembly approach. This valuable tool and its variants were rapidly generated and utilized for replication and pathogenesis studies.

Several other efforts are ongoing throughout the world in private industry to advance the development of a SARS vaccine. For example, in May 2004, 36 volunteers in Beijing, China, received an inactivated SARS vaccine produced by a Beijing-based company, Sinovac Biotech. Most volunteers receiving this vaccine generated an antibody response and no obvious side effects were noted. A larger Phase II study is planned.



The receptor binding domain (RBD) of the SARS-CoV S protein depicted cocrystallized with human ACE2. Courtesy of Dr. Michael Farzan / Harvard Medical School



Genome map of SARS-CoV. Courtesy of Dr. Wayne Marasco / Harvard Medical School

Challenges and Opportunities

While the need is clear and the re-emergence of SARS likely, the cost, length of time for needed product development, and uncertain demand result in unfavorable economic conditions for commercial vaccine and therapeutic development.

Better understanding of several phenomena is needed, including how the SARS virus can infect animals without detrimental effect and how it passes from one animal species to another (horseshoe bat to civet) as well as from animal to human. Advances concerning these topics could also apply to the many other viruses that pass from animals to humans.

Preliminary *in vitro* and animal studies indicate that initial exposure to SARS-CoV could exacerbate disease resulting from a subsequent exposure; this phenomenon, called antibody-dependent enhancement, has been seen with respiratory syncytial virus, dengue virus, and Feline Infectious Peritonitis virus.

Improved small and large animal models for SARS are needed, particularly models that better mimic human disease with respect to clinical course and symptoms. Improved models will help to better understand the pathophysiology of disease, including innate and adaptive immune responses and immunopotentiation, and help to move vaccines and therapeutics through advanced development to licensure.

In the development of SARs therapeutics and next-generation vaccines, additional work is needed to determine the structure/function relationships of critical enzymes and structural proteins. Once these are better understood, design of improved small molecule and protein inhibitors is possible.

A long-term public health strategy that can control future SARS outbreaks will require effective vaccines and therapeutics, as well as plans to limit the impact on the transportation system, on health care and service workers, and on the elderly.

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Streptococcus Pneumoniae

Pneumococcus (*Streptococcus pneumoniae*) is a common bacterial pathogen in adults worldwide and one of the foremost causes of morbidity and mortality in infants and children in developing countries. Pneumococci are also responsible for many deaths from infectious diseases in the elderly, and cause the bulk of ear infections in young children. They are a significant cause of meningitis in young children and the elderly. In much of the developing world, *Streptococcus pneumoniae* is a leading cause of fatal respiratory infections in children less than 5 years of age, resulting in more than 800,000 deaths per year in this age group.

S. pneumoniae is a common bacterium that is present in the nasopharynx of many children and some adults, where it causes no harm to its host, but can be transmitted to others. If it moves beyond the nasopharynx, however, it can cause ear infections or invasive disease such as pneumonia or meningitis. Although ear infections in young children generally do not lead to meningitis or other serious pneumococcal diseases, they do result in costly clinic visits and much lost work for parents. Because of its ability to infect the very young, the very old, and the immunodeficient, pneumococcus has one of the largest public health and economic impacts of any infectious disease in the United States. Patients recovering from viral infections such as measles or influenza and those already afflicted with chronic diseases such as human immunodeficiency virus (HIV) constitute especially susceptible hosts in whom mortality is high.

There are more than 90 serotypes of *S. pneumoniae* based on variations in its capsular polysaccharide. Currently licensed vaccines are composed of capsular polysaccharide alone (containing 23 capsular serotypes) or in conjugated form where the polysaccharide is chemically linked to a protein carrier. Prevnar, a licensed pneumococcal conjugate vaccine, contains 7 of the more clinically important serotypes in the United States, but newer versions under evaluation contain up to 13 capsular types, many of which are responsible for invasive disease in developing countries. While these conjugate vaccines are proving to be efficacious against disease and are making a significant impact on public health, their success may be limited by economic and clinical factors.

In just 3 years following the widespread introduction of Prevnar in the United States in 2000, there was a 94 percent reduction in the rate of invasive pneumococcal disease among vaccinated children younger than 5 years of age for serotypes associated with the vaccine. Children receiving Prevnar showed reduced nasopharyngeal carriage of vaccine strains, especially for those strains that were most resistant to antimicrobial agents. A very interesting and unanticipated finding is the indirect (herd) effect of the vaccine in reducing the risk of nasopharyngeal colonization in unvaccinated children in close contact with immunized playmates. Because approximately 75 percent of children in the United States have been vaccinated with Prevnar, some of the decline in the overall infection rate appears to reflect an indirect effect due to the interruption of pneumococcal transmission. In fact, the herd immunity effect observed in 2003 was actually twice as large as the direct protective effect. Of the 29,599 vaccine-type infections prevented by Prevnar in 2003, just 9,140 were due to direct protective effects. The rest of the prevented infections were the result of herd immunity. This indirect effect has carried over into other populations as well. The greatest declines in both vaccine-type and overall pneumococcal infections occurred in individuals who were at least 65 years of age.

Between 1998 and 2003, pneumococcal invasive disease among adults over the age of 50 decreased by more than 50 percent for serotypes included in the Prevnar vaccine. In addition, the overall incidence of serious pneumococcal infections dropped 28 percent. Although there has been an increase in the use of the licensed 23-valent pneumococcal polysaccharide vaccine in the elderly over the past several years, it is unlikely that this would account for such marked declines. Rather, a good proportion of the decrease in invasive disease among adults can be attributed to the use of the Prevnar vaccine in children, further supporting the notion of a herd effect. Overall, the routine use of Prevnar has greatly decreased the incidence of pneumococcal disease caused by antibiotic-resistant pneumococci in the vaccinated population and reduced adult transmission.

There have been numerous articles in the literature citing other beneficial clinical effects following the licensure of Prevnar. One study that looked at 63,000 hospitalized adults showed that

vaccinated patients were less likely to die while in the hospital than unvaccinated patients or patients with unknown vaccination status. These vaccinated patients were less likely to develop respiratory failure, heart attacks, or kidney failure and their average hospital stay was two days shorter than that of unvaccinated patients. Another study involved a Phase III trial conducted in Finnish infants. Children receiving 3 doses of the 7-valent pneumococcal conjugate vaccine before 6 months of age and a booster dose before 15 months of age showed (1) a 7 percent reduction in the total number of episodes of middle ear infection; (2) a 34 percent reduction in pneumococcal acute otitis media; and (3) a 57 percent reduction in acute otitis media caused by the pneumococcal serotypes included in the Prevnar vaccine.

Even though antimicrobial therapy has resulted in reduced morbidity and mortality rates associated with invasive pneumococcal disease, the prevalence of antimicrobial resistance among *S. pneumoniae* continues to increase worldwide. Thus, the reduction in resistant infections following the use of Prevnar represents one of the most significant benefits of the vaccine, which translates into fewer complications due to antibiotic resistance and fewer treatment failures.

One of the major drawbacks or unwanted effects of the introduction of Prevnar into the routine immunization schedule has been the increased prevalence of colonization and disease caused by non-invasive strains not associated with the vaccine. This is referred to as "bacterial replacement." Several serotypes—in particular 11, 15, and 19A—have increased substantially and become prominent players in disease-related situations. This phenomenon has created much concern because these serotypes are capable of becoming resistant to antibiotics. Furthermore, the increased prevalence of non-vaccine serotypes, due to the selective pressure induced by the extensive use of the conjugate pneumococcal vaccine, may play a role in establishing highly virulent strains of pneumococci that cause more invasive forms of disease. Thus, there is a need to develop new pneumococcal vaccines that offer broader coverage and protection from all serotypes associated with the organism. Such an effort is best exemplified by the development of common surface protein vaccines that can provide complete protection to all pneumococcal serotypes and age groups. This work continues on many fronts with emphasis on such proteins as PspA, PspC, other choline-binding proteins, PsaA, autolysin, pneumolysin, neuraminidase, and IgA1 protease, along with several additional proprietary antigens. The limited number of serotypes that can be included in a single dose of conjugate vaccine, along with specialized needs for incorporating specific serotypes depending on where in the world the vaccine will be administered, poses certain restrictions for its general use on a global scale. A widely useful vaccine will probably have to contain a cocktail of several pneumoncoccal virulence factors, each of which will stimulate the immune system to block a specific function, such as attachment to human host cells or evasion of human immune responses.

Since 1989, the 23-valent pneumococcal polysaccharide vaccine has been routinely recommended for use in adults over the age of 65 in addition to individuals at high risk of pneumococcal infections as a result of certain chronic illnesses. This vaccine has varied efficacy against all manifestations of pneumococcal infections, but has been shown to be approximately 60 percent efficacious in preventing invasive disease in adults. One of the major concerns associated with this vaccine is its inability to induce a T-cell dependent memory response. It is for this reason that the vaccine is not recommended for use in children less than two years of age. Two-year-old children immunized first with a pneumococcal conjugate vaccine and then a pneumococcal polysaccharide vaccine demonstrated higher antibody concentrations to each of the serotypes contained in the conjugate vaccine than did children who received a *Haemophilus influenzae* type b (Hib) conjugate vaccine as a control. Infants primed with the pneumococcal polysaccharide vaccine had considerably lower antibody responses when subsequently immunized with the same vaccine. These results suggest that the pneumococcal conjugate vaccine is capable of effectively priming the immune system of infants to induce an anamnestic T-cell response to a subsequent dose of pneumococcal polysaccharide.

Along these same lines, the efficacy of the pneumococcal polysaccharide vaccine in immunocompromised adults is considerably less than that observed among immunocompetent individuals of a similar age. In addition, among adults 65 years of age and older who receive a recommended dose of the 23-valent pneumococcal polysaccharide vaccine, there is a steady decline in (1) the antibody response to the majority of the vaccine antigens over time; and (2) the efficacy of the vaccine with increasing age at the time of vaccination. Because of the waning efficacy post-immunization and the increased risk for disease, many physicians have routinely re-immunized their elderly patients with

the 23-valent vaccine approximately every 5 years. Unfortunately, these revaccinated individuals generally show a reduced immune response compared to their initial response when first immunized with the vaccine. To overcome this hypo-responsive state, several clinical trials were conducted to evaluate the benefit of first immunizing the elderly with a conjugate pneumococcal vaccine followed by revaccination with the 23-valent polysaccharide vaccine. Data from studies that use this approach look very promising and suggest that establishing a T-cell-dependent immune response early on, when the risk of disease is high, helps promote a more robust immune response and a reduced risk of hypo-responsiveness to subsequent immunization(s) with the 23-valent vaccine.

During the past year, an important set of results was presented from a vaccine efficacy trial conducted in The Gambia and supported by a broad coalition of international partners (see The Gambia Pneumococcal Vaccine Trial). The results revealed that deaths from pneumococcal infection in rural settings were preventable and that pneumococcal vaccination could prevent serious infections even under conditions where the burden of disease is high, offering great promise for improving the health and saving the lives of children in disadvantaged populations. Efforts to broaden the availability of these multivalent pneumococcal conjugate vaccines in developing countries are now under way and will require considerable support from the international community.

The Gambia Pneumococcal Vaccine Trial found there were 37 percent fewer cases of pneumonia in children who received the vaccine. Courtesy of PneumoADIP / Photograph by Selena Haylock

THE GAMBIA PNEUMOCOCCAL VACCINE TRIAL

Each year, pneumococcal pneumonia and meningitis cause 800,000 to 1 million deaths in children under the age of 5. Ninety percent of these deaths occur in developing countries, where pneumococcal meningitis kills or disables 40 percent to 75 percent of the children who contract it.

From 2000-2004, a team of scientists led by the British Medical Research Council vaccinated and followed more than 17,000 young children in The Gambia to determine whether a pneumococcal conjugate vaccine could substantially reduce death and serious illness from *Streptococcus pneumoniae*. This randomized, double-blind, placebo-controlled trial was supported by the National Institute of Allergy and Infectious Diseases along with a coalition of international partners.

The primary objective of the trial was to examine the ability of a nine-valent pneumococcal conjugate vaccine to reduce the morbidity and mortality from pneumococcal diseases in a rural setting where child mortality was high and access to healthcare was limited.

This was the first major randomized, controlled vaccine clinical trial in nearly 20 years to show significant reduction in overall child mortality.

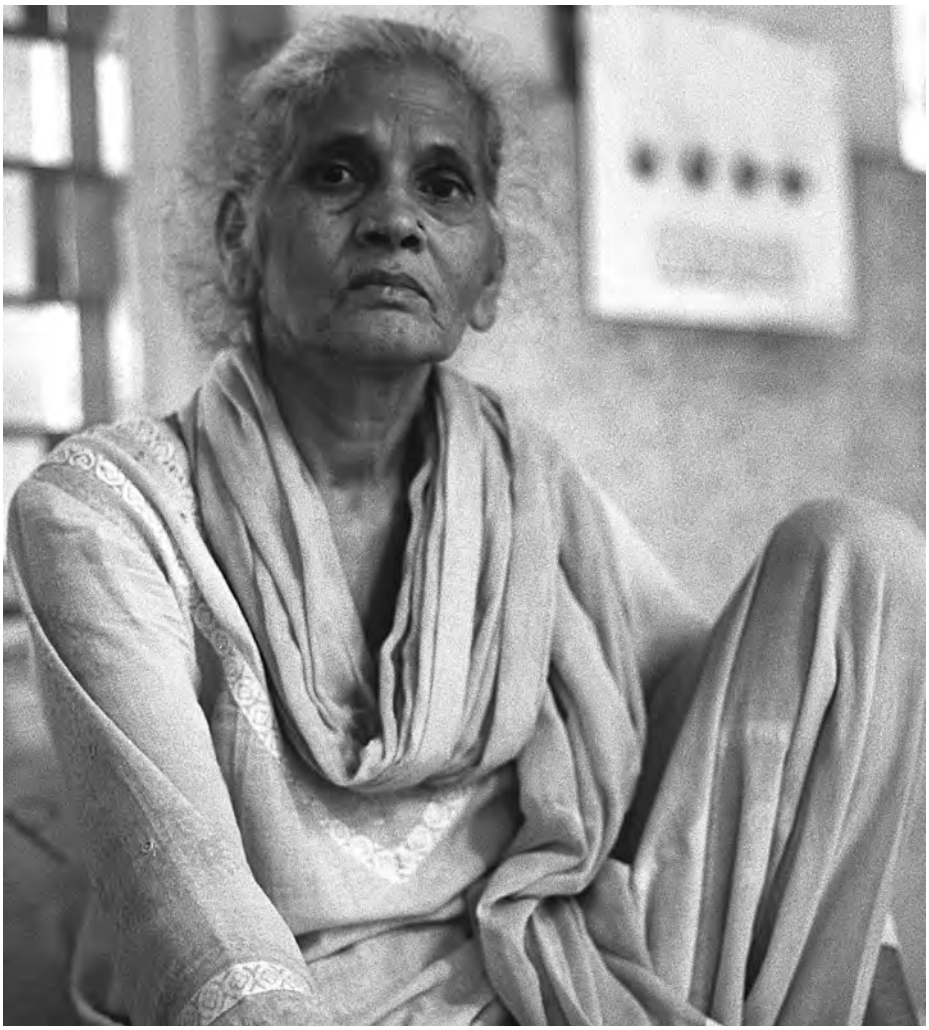
- The vaccine used was 77 percent effective in preventing invasive pneumococcal infections caused by the vaccine serotypes.
- There were 37 percent fewer cases of pneumonia in the children who received the vaccine.
- The vaccine significantly reduced the need for hospitalization: children receiving the pneumococcal vaccine had 15 percent fewer hospital admissions than those who did not.
- The vaccine reduced childhood mortality by 16 percent in children who received the pneumococcal conjugate vaccine.

Article: Cutts FT et al., Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia: randomised, double-blind, placebo-controlled trial, *Lancet* 2005;365:1139-1146.

Tuberculosis

Background

Despite significant advances in tuberculosis (TB) research and improvement in treatment strategies worldwide, the disease remains one of the leading killers in infectious disease. Although TB is usually curable, tuberculosis continues to spread across the globe and claims close to two million lives each year. Recent increases in drug resistant TB and the appearance of strains of *Mycobacterium tuberculosis* that are resistant to many of the available drugs to treat TB have increased awareness that this ancient disease has the potential to re-surge in many countries, including the United States and lead to significant public health issues. Failure to contain this disease can be attributed to a number of factors including the need for a more comprehensive tuberculosis treatment and care infrastructure in endemic, resource-limited countries; the lack of integration of TB and human immunodeficiency virus (HIV) healthcare services in areas where the spread of tuberculosis is closely linked to the HIV co-epidemic; the lack of availability of rapid and sensitive diagnostics; the development of multi-drug resistant (MDR) and extensively drug resistant (XDR) TB that are difficult to treat and remains transmissible; and the lack of a highly effective vaccine.



Sadhne, a 60-year-old tuberculosis patient in an East Delhi clinic. According to the World Health Organization, the largest number of new TB cases in 2004 occurred in Southeast Asia, which accounted for 33 percent of cases globally. © World Lung Foundation / Photograph by Gary Hampton

In most cases, infection with *M. tuberculosis* results in an asymptomatic colonization that is controlled by the immune system (latent or persistent infection). Weakening of the immune system, as is the case in persons also infected with HIV, can result in reactivation of bacterial growth and progression from latent infection to active tuberculosis. Patients with active tuberculosis are treated with combination chemotherapy for six to nine months. The length of this regimen, combined with drug-related adverse events, frequently leads to noncompliance and treatment failures, which in turn can result in the development and spread of drug-resistant tuberculosis. Proactive identification of patients with active TB and rapid identification of MDR and XDR TB, followed by directly observed drug treatment with the most appropriate drug regimen (directly observed treatment, short course [DOTS]), combined with effective vaccination schedules, are considered the most likely means by which tuberculosis could be eliminated as a global public health burden.

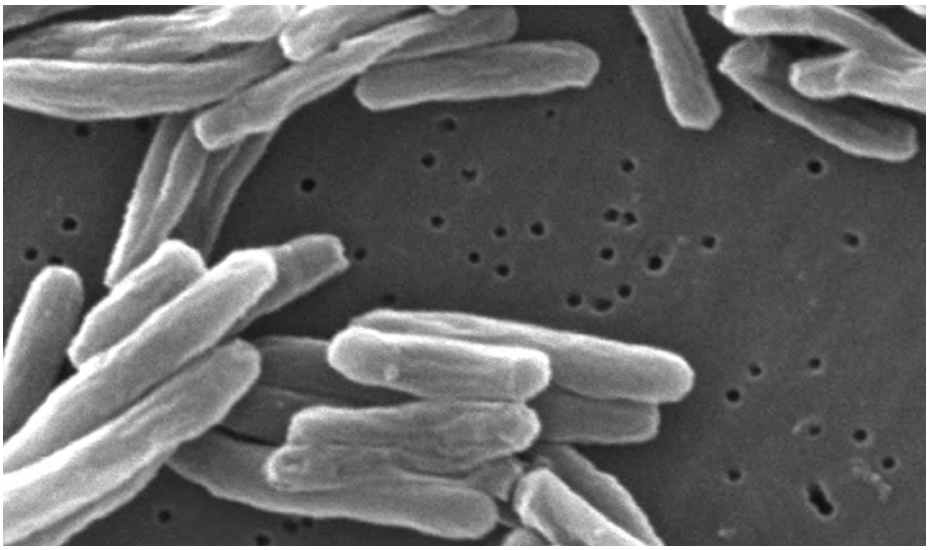
The currently available tuberculosis vaccine, *Mycobacterium bovis* Bacille Calmette-Guérin (BCG), was developed

almost 100 years ago. Worldwide, a variety of BCG strains are available and widely delivered under the World Health Organization (WHO) Expanded Programme on Immunization. One BCG vaccine strain (Tice) is licensed in the United States against tuberculosis but is not recommended for general use. Despite its lack of consistent, reproducible efficacy in clinical trials to prevent adult pulmonary tuberculosis in various regions of the globe, BCG is the most widely administered vaccine worldwide and appears to protect against childhood complications and death in children, although few controlled clinical studies have been conducted to confirm this observation. Although vaccination with BCG has been insufficient to prevent adult pulmonary tuberculosis, which is primarily responsible for the continued spread of the disease, it is not known how many cases of tuberculosis would occur globally without BCG vaccine.

Development of more effective vaccines to prevent either infection with *M. tuberculosis* or progression to active disease remains a priority for the National Institute of Allergy and Infectious Diseases (NIAID). Since 1998 when the U.S. Department of Health and Human Services' Advisory Council for Elimination of Tuberculosis (ACET), the U.S. National Vaccine Program Office, and NIAID convened a workshop to develop the Blueprint for Tuberculosis Vaccine Development, several promising vaccine candidates have been identified, some of which are now being evaluated in humans in clinical trials.

State of the Science in Tuberculosis Vaccine Development

Until the early 1980s, tuberculosis in the United States had been steadily declining.



A scanning electron micrograph (SEM) depicting some of the ultrastructural details seen in the cell wall configuration of a number of Gram-positive *Mycobacterium tuberculosis* bacteria. Courtesy of CDC / Janice Carr

A sudden spike in new cases was reported between 1986 and 1992. This resurgence of tuberculosis was attributable largely to deteriorating public health infrastructure and was also coincident with the HIV epidemic. In 1993, tuberculosis was declared a global health emergency by the WHO. Following these events, awareness of the global impact of tuberculosis increased and led to the realization that improving our understanding of tuberculosis pathogenicity and host-pathogen interaction is a prerequisite for identifying better ways to diagnose, prevent, and treat this disease. Research funding has steadily increased since 1992, and NIAID has developed a comprehensive research program to stimulate and support all aspects of tuberculosis science and product development. This funding has contributed significantly to the current advances in tuberculosis vaccine development, specifically in the areas of TB immunology, pathogenicity, and molecular aspects of host-pathogen interaction. Research was also boosted in recent years by publication of the genome sequence of *M. tuberculosis* and other mycobacterial species, and development of microbiologic and genetic tools that helped dissect the interaction of the pathogen with the host immune response and aided in vaccine development and evaluation. Readily available microarrays have facilitated the investigation of differential expression of host and pathogen genes at various stages of infection and disease, and have contributed to our understanding of the mechanisms driving tuberculosis pathogenicity. These efforts have also been aided by the establishment of structural genomics consortia and collection of data using a systems biology approach, activities that have been funded by NIAID and through National Institutes of Health (NIH) initiatives.

Significant effort has been expended to develop relevant animal models of *M. tuberculosis* infection that more closely mimic human disease, in order to predict vaccine efficacy from animal studies. From that effort it became clear that the pathogenesis of tuberculosis varies among different animal species and that

a number of dynamic immunological factors modulate disease outcome after infection with *M. tuberculosis*. Through the development and refinement of these models, which now extend from rodents (mice and guinea pigs) to rabbits and non-human primates, researchers continue to gain insight into immunological and microbiologic factors that are involved in developing active disease versus asymptomatic infection. This enhanced understanding of small animal models of tuberculosis has enabled the testing of more than 100 new vaccine candidates and approaches over the past nine years. These candidates are representatives of a diverse set of vaccine classes and include recombinant BCG and live attenuated *M. tuberculosis* strains; various other live vectors (bacterial and viral); and DNA, protein, and peptide subunit vaccines. It is recognized that due to the benefit that BCG provides against pediatric TB, as well as its collateral efficacy against leprosy, viable vaccination strategies will likely have to include neonatal BCG vaccination boosted with novel vaccines at a later stage in life with the intent to reactivate immunological memory and protect against primary infection and/or reactivation of latent infection. Because about one-third of the world's population is thought to harbor asymptomatic infection with *M. tuberculosis*, and may therefore serve as a reservoir for new cases of tuberculosis, prevention of reactivation of disease may prove critical to curb the spread of tuberculosis. This consideration is especially important in regions where there is a high prevalence of HIV co-infection, which increases the chances of developing active disease from 1 in 10 over the course of a person's life to 1 in 10 per year.

Several candidates that demonstrated protection against infection with *M. tuberculosis* in small animal models equally well or better than BCG have entered human clinical trials. These are the first studies of new, engineered tuberculosis vaccine candidates since the introduction of BCG in 1921, with the exception of current studies using *Mycobacterium vaccae* as a vaccine to prevent disseminated tuberculosis in AIDS patients. This new generation of clinical candidates includes a recombinant BCG vaccine expressing the 30 kD major secretory protein of *M. tuberculosis*, Ag85B (rBCG30); a fusion protein composed of immuno-dominant *M. tuberculosis* peptides (Mtb72f); and a boost strategy using Ag85A expressed from a viral vector after primary BCG vaccination. Additionally, clinical studies are being planned to better define the immune protection elicited by BCG in pediatric populations, as well as whether alternative means of administering BCG would enhance the spectrum of immune response elicited by this vaccine. Overall, the research community is developing a comprehensive approach to designing improved vaccination strategies for tuberculosis. Currently, it is estimated that combination approaches will be needed to produce protective immune responses in adult populations.

Challenges and Opportunities for Developing a Vaccine for Tuberculosis

The majority of research toward new and improved vaccines has only occurred during the last decade. Hence, little historical experience in tuberculosis vaccinology is available that can be used as guidance for developing or improving new tuberculosis vaccines. Although tuberculosis vaccine research has made tremendous advances over the last 10 to 15 years, a number of critical questions remain to be answered. These answers will likely provide the keys to faster tuberculosis vaccine development.

- Why are some individuals able to contain infection with *M. tuberculosis* and contain this pathogen as a latent, asymptomatic infection while others progress to active disease, and what does it mean from the standpoint of bacterial physiology and host response? To answer this question, longitudinal human studies of *M. tuberculosis* infection are needed to define parameters that can then be modeled in animals to derive approaches and solutions to preventing progression to active disease.
- What factors can serve as markers of immunoprotection in humans to allow assessment of immunogenicity in clinical trials? Only with the aid of data from human vaccine trials will researchers be able to refine animal models and identify which immune parameters need to be established for further vaccine development. For these reasons, it is critical that vaccine candidates are quickly evaluated for safety and efficacy in human trials, and any subsequent findings used to devise more targeted vaccine strategies.
- What is the importance of co-infections and co-morbidities in patients at high risk for *M. tuberculosis* infection and progression to active disease? Will a vaccine that was developed in laboratory animals be effective in these real-life settings? How will persons already infected with *M. tuberculosis* respond to vaccination?
- What role will diagnostics play in the development of tuberculosis vaccines? Since delayed-type hypersensitivity

testing is not a reliable measure of infection or cure, identification of the appropriate patient population remains a challenge. For this reason, diagnostics development needs to remain closely coupled with immunology and vaccinology research to produce, in parallel, essential tools for the successful conduct of clinical evaluation of candidate vaccines.

- How does BCG work in children? This is a currently understudied but important aspect of vaccine development. Little is known about general or tuberculosis-specific differences in immune response and vaccine efficacy among infants, children, and adults. It is recognized that the clinical presentation of tuberculosis is different in young children from that in adults and that BCG efficacy differs significantly in these populations.
- Because it will not be ethical to conduct a placebo controlled clinical trial with an experimental vaccine, what treatment regimens will the patient populations receive during this trial? How will this influence the ability to assess efficacy of the vaccine or even the outcome measures of the trial? How can studies be designed to minimize the sample size and study duration? Who will fund such challenging and time-consuming studies and commercialize a vaccine?

NIAID-Supported Tuberculosis Vaccine Research

To answer the above questions, NIAID is funding not only investigator-initiated research, but solicited research on tuberculosis immunology, pathology, pathogenesis, vaccine development, target antigen identification, diagnostics, development of improved tools for epidemiological studies, and development of markers of immunoprotection. In order to to stimulate product development against this re-emerging disease, applied research in tuberculosis is included under Category C of NIAID's Biodefense Research Program. In addition, NIAID provides resources through its genomics and bioinformatics programs that are also available to the tuberculosis research community. It is recognized that the resurgence of tuberculosis, and especially MDR TB and now also XDR TB, places a tremendous economic burden on affected countries, with the potential to re-emerge in the United States.

NIAID's Tuberculosis Research Materials and Vaccine Screening contract at Colorado State University (www.cvmbs.colostate.edu/microbiology/tb/top.htm) provides high-quality research reagents and vaccine testing services in small animal models to researchers worldwide. The NIAID-supported Tuberculosis Animal Research and Gene Evaluation Taskforce (TARGET) at Johns Hopkins University (http://webhost.nts.jhu.edu/target) bridges the gap between identification of genes that may play a role in interaction between host and pathogen and actual determination of the biological function of these genes. Resources for researchers working on infectious agents under Category C of NIAID's Biodefense Research Program are available to help advance promising preclinical candidates, as are public-private partnership opportunities to enhance product development for Category C agents. (For more information, please see www.niaid.nih.gov/ research/resources and www.niaid.nih.gov/biodefense/research/funding.htm.) NIAID's Tuberculosis Research Unit at Case Western Reserve University (www.tbresearchunit.org) and NIAID's Vaccine and Treatment Evaluation Units provide clinical trials infrastructure that is accessible to TB projects that evaluate vaccine candidates and conduct studies on surrogate markers of protection.

Knowledge gained from research over the past 14 years has matured to the level where tangible product candidates are entering clinical trials. Development needs for an implementable vaccine are being discussed in the StopTB Partnership's Global Plan to Stop TB, 2006–2015. This publication not only attests to the continued need for new vaccines against TB but also recognizes the need for continued funding for and contributions from fundamental and translational science, both of which are heavily supported by NIAID. Although the field of tuberculosis vaccine development has produced a rich array of potential candidates and many donors are continuing to support preclinical research, a clear funding and "interest" gap continues to exist for non-academic production, safety assessment, and readying of vaccine candidates for clinical development.

Despite the many challenges remaining in tuberculosis vaccine development, a new sense of optimism is permeating the tuberculosis research and public health communities as recent research advances result in novel vaccine candidates entering human trials.

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Varicella-Zoster Virus

Background

Primary infection with varicella-zoster virus (VZV) is manifested as chickenpox (varicella) and results in a lifelong latent infection. Reactivation of the latent virus leads to shingles (zoster).

Varicella

Prior to the introduction of the live attenuated vaccine, approximately 4 million cases of varicella occurred annually, primarily in young children, with more than 90 percent of the U.S. population becoming seropositive [1]. Chickenpox was estimated to cost about $400 million each year, much of this representing the cost to parents of lost income from work [2]. With increasing vaccine coverage, varicella disease has declined dramatically in areas subject to surveillance [3]. As the use of the vaccine expands, it will lead to changes in the epidemiology and costs of this childhood illness in the United States.

Varicella can be complicated by a variety of serious conditions, including skin infections that can progress to systemic infections, infections of the brain, and pneumonia [4]. Prior to introduction of the vaccine, complications of varicella were responsible for as many as 9,300 hospitalizations and 100 deaths annually. The risk of these complications is highest in adults. While less than 5 percent of varicella cases occur in adults more than 20 years of age, 55 percent of the deaths occur in this age group [5]. As would be expected, the number of hospitalizations due to the complications of varicella has declined significantly since the introduction of the vaccine [6].

Zoster

Zoster typically involves large areas of skin that ulcerate and require several weeks to heal. The skin eruption itself is very painful, and it is often followed by postherpetic neuralgia (PHN), a pain syndrome that may persist for many months or years and that can be very disabling. There is no established therapy for PHN. The incidence and severity of zoster and its complications increase with age. The incidence among people aged 50–59 appears to be between two and four cases per 1,000 persons per year, and it more than doubles by the age of 80 years. More than one-half of all cases occur in persons 60 years of age and older [7]. PHN is the major complication of zoster in the immunocompetent host. Rare in individuals less than 40 years of age, PHN is estimated to occur in 25 to more than 50 percent of patients with zoster who are more than 59 years of age [8].

Current Status and Key Issues in Research and Development

Humoral and cellular immune responses are elicited early in primary VZV infections, and their relative contribution to protection from disease is not well understood. The impact of active humoral immunity appears to be limited, but preexisting antibody has been shown to provide some level of protection. Passively acquired maternal antibody affords some protection to infants, and postexposure administration of VZV immunoglobulin (VZIG) to immunocompromised children reduces disease severity [9]. In children receiving the live attenuated Oka vaccine, the incidence and severity of breakthrough infection are inversely correlated with antibody titer to VZV glycoproteins, and possibly with the level of T-cell responses as well [10, 11]. Conversely, it is clear that cellular responses play the primary role in preventing disease associated with reactivation of latent VZV. While decreases in humoral immunity are not associated with increased risk of zoster, the age-related decline in cell-mediated responses to VZV antigens is proportional to the age-related increase in the incidence and severity of zoster [12, 13, 14, 15], suggesting that this loss is a causative factor.

The role of viral immune evasion mechanisms in VZV infection is not well defined. For example, VZV is similar to herpesviruses (HSV) in that its glycoprotein gE forms a complex with gI and can act as an Fc receptor, but it is not known whether the similarity to HSV extends to providing protection from virus-specific antibody [16]. Efforts are currently under way to identify VZV genes that may be associated with evasion of major histocompatibility complex (MHC) class I- and class II-mediated immune responses [17].

Oka, a live attenuated varicella vaccine, was developed in Japan in the early 1970s [18]. In the United States, this vaccine (Varivax) is produced by Merck. It was licensed for use in healthy individuals by the U.S. Food and Drug Administration (FDA) in 1995 and is now recommended for universal use in early childhood by the Centers for

Disease Control and Prevention (CDC) Advisory Committee for Immunization Practices, the American Academy of Pediatrics, and the American Academy of Family Physicians [19, 20]. The use of Varivax in the United States has been increasing steadily: in 2004 coverage among children 19 to 35 months of age was estimated at 87.5 percent [21]. All states ordered the vaccine for use in their immunization programs, and by June 2004, a total of 44 states had implemented elementary school or child care entry requirements for varicella vaccination. Post-licensure surveillance in day-care centers indicates that the vaccine is generally well tolerated, leads to a lower attack rate, and protects from severe disease [22, 23]. However, there remain instances of breakthrough infection, and evidence has accumulated that a single dose of varicella vaccine is not sufficiently immunogenic when given to some children from 1 to 12 years of age [24]. Vaccine-induced immunity appears to be durable. Studies of individuals immunized up to 20 years previously show persistence of antibodies and protection against serious disease [25, 26, 27]. Further studies will establish whether immunization will provide protection as durable as that from natural infection, or whether boosting will be required to maintain protection through adulthood. The expanding use of this vaccine will undoubtedly alter the epidemiology and costs of varicella in the United States, and it affords the opportunity to study in greater detail the correlates of protection against infection and disease, and the viral functions associated with virulence and attenuation.

It remains to be demonstrated whether the VZV vaccine will be effective in other populations, such as immunosuppressed transplant patients.

In addition to further studies on the live attenuated virus, there are continuing efforts to evaluate alternate vaccines. Inactivated virus showed some efficacy in protecting bone marrow transplant recipients from shingles, although this strategy also has been associated with a weaker MHC class I-restricted cytotoxic response and reduced protection from varicella when compared to the live attenuated vaccine [28, 29, 30]. Other strategies being pursued include disabled virus and plasmid DNA.

Recent Accomplishments

The availability of a live attenuated VZV vaccine that is safe, effective, and FDA-licensed for the prevention of varicella presented an opportunity to determine whether the same vaccination strategy might be effective for preventing zoster in the elderly. In a collaboration between the Veteran's Administration, the National Institute of Allergy and Infectious Diseases (NIAID), and Merck & Co., Inc., a Phase III clinical trial was conducted to assess whether administration of a higher-titer version of the varicella vaccine in adults 60 years of age and older can reduce the incidence and/or severity of zoster and its complications. The Shingles Prevention Study was conducted over five years at 22 study sites across the United States and enrolled a total of 38,546 volunteers. The study was completed late in 2004 [31]. Use of the vaccine reduced the burden of illness due to zoster by 61.1 percent, reduced the incidence of post-herpetic neuralgia by 66.5 percent, and reduced the incidence of zoster by 51.3 percent. The vaccine was also found to be safe and well-tolerated. Based on the results of this study, licensure was approved by the FDA in May 2006.

Next Steps and Challenges Ahead

Although the current varicella vaccine is highly effective in preventing disease, outbreaks of varicella are still occasionally reported. Furthermore, the dynamics of protection are likely to change as the incidence of varicella declines further, and vaccinated children receive less of a boosting effect through natural exposure. It has been demonstrated that a second dose of the varicella vaccine can significantly decrease the rate of varicella breakthrough illness and increase vaccine efficacy, and some authorities now feel that a two-dose regimen should be recommended for this age group, as it is for adolescents and adults [24].

The development of a VZV vaccine containing virus incapable of becoming reactivated, or of a subunit vaccine, will require much more basic research. Studies of the antigenic components most important for developing an immune response in humans, and of novel methods for presenting viral antigens to cells of the immune system, are in progress. Other populations at risk for severe VZV disease—e.g., pediatric renal transplant recipients—are also candidates for studies evaluating the safety and efficacy of the live attenuated vaccine.

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West Nile Virus

The identification of West Nile virus (WNV) in New York in the summer of 1999 was the first time the mosquito-borne microbe had been detected in the Western Hemisphere. Until then, the virus had been found chiefly in Africa, Eastern Europe, the Middle East, and Asia. Since 1999, WNV has been reported in an ever-growing area, and human cases have been reported from coast to coast in the United States, as well as in Canada and Mexico. Between 1999 and 2005, WNV caused 19,602 cases of the disease in the United States, including 785 deaths, according to the Centers for Disease Control and Prevention.

Although infection with WNV usually causes only mild symptoms in humans, it can spread to the central nervous system and cause encephalitis, a potentially deadly brain inflammation, most common among the elderly. Currently, no treatment is available for WNV encephalitis, and no licensed vaccine exists to prevent the human form of the disease. Mosquito control has been the only available strategy to combat the rapid spread of this emerging disease, but effective spraying is difficult to carry out in urban areas.

Faced with the potential for a serious WNV epidemic, National Institute of Allergy and Infectious Diseases (NIAID)-supported researchers began development of a vaccine to prevent infection. Basic research on newly emerging microbes has enabled rapid progress in the development of a WNV vaccine. In addition, WNV vaccine development has benefited from the fact that the virus belongs to a group known as flaviviruses, which have many characteristics in common. These similarities have allowed scientists to build on earlier discoveries about other flaviviruses that are closely related to WNV, including Japanese encephalitis virus, St. Louis encephalitis virus, yellow fever virus, and dengue virus.

Applying mosquito repellent to the skin helps prevent mosquitos from biting, thereby, preventing many arboviral infections, such as West Nile virus. Courtesy of CDC / James Gathany

There has been great success controlling yellow fever and Japanese encephalitis with well-organized vaccination campaigns centered on an efficacious vaccine. Therefore, the National Institutes of Health (NIH) encouraged similar WNV vaccine development programs.

Importantly, NIAID-supported basic research studies discovered that hamsters and mice were good models for West Nile disease. NIH-supported researchers at the University of Texas Medical Branch, Galveston, conducted a series of preliminary experiments to learn more precisely the degree of protection that candidate WNV and other licensed flavivirus vaccines might have against WNV. Researchers found that hamsters were completely protected by prototype WNV vaccines, and surprisingly, at least partially protected by Japanese encephalitis and yellow fever vaccines. Thus, these new models are an important resource that could be used in the development of WNV vaccines to test the efficacy of a new vaccine candidate (or a new antiviral medicine).

NIAID is supporting a number of vaccine approaches. One of the earliest began in 1999 when NIAID funded a fast-track project to develop a candidate WNV vaccine with Acambis, Inc. The vaccine is constructed using a vaccine licensed for preventing yellow fever as the backbone. To create the WNV vaccine, researchers substituted the surface protein of WNV for the deleted yellow

fever virus protein using chimeric technology developed at NIAID in the early 1990s. This method of creating chimeric flavivirus vaccines is also being applied to developing a vaccine for dengue and Japanese encephalitis virus. The Acambis, Inc., vaccine has undergone successful preclinical evaluations in hamsters, mice, monkeys, and horses, and has yielded encouraging results in a recently completed Phase I clinical trial. In December, 2005, the vaccine was moved into Phase II clinical trial evaluation, making Acambis, Inc., the first company to enter Phase II testing of a WNV vaccine. The randomised, double-blind, placebo-controlled trial is being conducted in more than 200 volunteers in the United States. The safety, tolerability, and immunogenicity of the vaccine at different dose levels will be evaluated first in healthy young adults and the optimal dose will subsequently be tested in healthy elderly subjects.

The *Ochlerotatus triseriatus* mosquito, commonly referred to as the "treehole mosquito," is a known vector of West Nile virus. Courtesy of CDC / James Gathany

NIAID intramural scientists, with early assistance from collaborators from the Walter Reed Army Institute of Research (WRAIR), capitalized on advances in recombinant DNA technolgy and previous research on dengue virus to produce a new candidate WNV vaccine. The NIAID team already had successfully tested a strategy that used the new technology to replace key genes of different flaviviruses with those of dengue virus type 4 (DEN4). Unlike many flaviviruses, DEN4 does not cause disease in the brain. The resulting attenuated virus strains were safe for use in a vaccine but still protective. The NIAID team then used this strategy to combine genes from WNV and DEN4. This hybrid virus did not infect the brain, but still stimulated a strong immune response with even a single dose. This WNV/DEN4 chimeric virus was further attenuated by deleting 30 nucleotides from its 3' untranslated region. The WNV/DEN4 candidate vaccine with the 30-nucleotide deletion has been tested in monkeys with promising results and is currently being tested in humans in Phase I clinical trials that began in 2005.

Meanwhile, NIAID scientists at the Dale and Betty Bumpers Vaccine Research Center have developed a DNA-based vaccine against WNV in collaboration with the San Diego-based biotechnology company Vical, Inc. The vaccine is based on an existing codon-modified gene-based DNA plasmid vaccine platform designed to express WNV proteins. In April 2005, following pre-clinical safety studies and viral challenge studies, the VRC initiated a Phase I clinical trial to evaluate safety, tolerability, and immune responses of this recombinant DNA vaccine in human volunteers.

In addition to pursuing replicating chimeric vaccines, researchers have made advances in the development of non-replicating subunit vaccines. Scientists at Hawaii Biotech, Inc., supported by the National Institute of Neurological Disorders and Stroke, are using genetically engineered viral proteins that cannot cause disease. Two formulations of the vaccine have been tested in the golden hamster animal model, with promising results. At L2 Diagnostics, LLC, NIAID-supported researchers have developed a recombinant subunit vaccine found to induce virus-neutralizing antibodies in mice, rabbits, and horses; these antibodies prevent infection in a murine model of WNV infection. Researchers are now developing a manufacturing process for the vaccine, with the goal of producing a vaccine suitable for Phase I clinical trials.

APPENDIXES

APPENDIX A: Status of Vaccine Research and Development, 2006

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Ancyclostoma duodenale	Recombinant protein	+	+	+		
Bacillus anthracis	Recombinant subunit	+	+	+	+	
Bordetella pertussis	*B. pertussis* surface protein expressed by vector (e.g., *Salmonella and Vibrio cholerae*)	+	+			
	PT peptides-CRM conjugates	+	+			
	Purified adenylate cyclase	+	+			
Blastomyces dermatitidis	Purified yeast cell proteins (e.g., WI-1)	+	+			
	Recombinant proteins (e.g., WI-1)	+				
	WI-1 DWA	+	+			
	Live attenuated strain	+	+			
Borrelia burgdorferi	Recombinant Osp A	+	+	+	+	+
	Osp A-based DNA vaccine	+	+			
	BCG-expressed Osp A	+	+			
	Purified Osp B, Osp C	+	+	+		
	Osp C (14 valent)	+	+	+	+	
	DbpA	+				
	DbpB	+				
Brugia malayi	Purified parasite antigens (paramyosin, etc.)	+	+			
Calicivirus	Norwalk VLP's in transgenic potato	+	+	+		
	Norwalk VLP's orally delivered	+	+			
Campylobacter jejuni	Inactivated whole cell with mutant *E. coli* labile toxin (mLT) adjuvant, oral vaccine	+	+	+		
	Whole cell (intact)	+	+	+	+	
Chlamydia trachomatis	Major outer membrane protein (MOMB) viral vectors	+	+			
	Purified major outer membrane protein	+				
	Polymorphic membrane protein D	+				
	Chlamydia-secreted Protease Factor (CPAF)	+				
Clostridium botulinum	Toxoid	+	+	+	+	
	Recombinant AB vaccine	+	+	+		
	Recombinant heavy chain	+	+			
Clostridium difficile	Formalin-inactivated toxins A and B	+	+	+		
Clostridium tetani	Recombinant toxin	+	+			
	Salmonella vector	+	+	+		
	Microencapsulation	+	+			
	Transcutaneous immunization	+	+			
Candida albicans	Cell surface oligomannosyl epitope	+	+			
	Recombinant Als1p surface protein	+	+			

Note: This list outlines publicly available information concerning the status of vaccines in the research and development pipeline and should not be considered inclusive of all ongoing vaccine research and development.

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Candida albicans (continued)	Recombinant Als3p surface protein	+	+			
Chikungunya virus	Live attenuated	+	+	+	+	
Coccidioides immitis	Formalin-killed spherules	+	+	+	+	+
	Recombinant protein for Ag2, rAg2 (PRAg2)	+	+			
	Spherule homogenate (27kxg)	+	+			
	C-ASWS (Ag2)	+	+			
	Urease (recombinant and cDNA) (rURE)	+	+			
	Spherule outer wall glycoprotein (SOWgp)	+	+			
	PMP-1	+	+			
Corynebacterium diphtheriae	Recombinant toxin	+	+			
	Salmonella vector	+	+	+		
	Transcutaneous immunization	+	+			
Coxiella burnetii	Formalin inactivated	+	+	+	+	
	Antigen immunization	+				
	DNA vaccine	+				
Cryptococcus neoformans	Partially purified capsular polysaccharide	+	+			
	Glycoconjugate of capsular polysaccharide with tetanus toxoid	+	+	+		
Cytomegalovirus (CMV)	Live attenuated strains (conventional)	+	+	+	+	
	Live attenuated strains (engineered)	+	+	+		
	Glycoprotein subunit vaccine	+	+	+	+	
	Multiprotein subunit vaccine	+				
	Nucleic acid (DNA) vaccines	+	+			
	Canarypox vectored	+	+	+		
	VEE-vectored	+	+			
	Peptide	+				
	DNA prime + Inactivated boost	+				
Dengue virus	Purified rDNA-expressed viral proteins	+	+			
	Infectious clone	+	+			
	Yellow fever/dengue chimeric virus	+	+	+	+	
	Inactivated whole virus particle	+	+	+		
	VEE replicon vector	+				
	Naked DNA	+				
	Vaccinia vector (live)	+	+			
	Vaccinia subunit	+	+			
	Baculovirus subunit	+	+			
	Synthetic peptide	+	+			
	Micelle/ISCOM	+	+			

Note: This list outlines publicly available information concerning the status of vaccines in the research and development pipeline and should not be considered inclusive of all ongoing vaccine research and development.

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Dengue virus (continued)	Yeast subunit	+	+			
	Recombinant envelope (baculovirus and drosophila expression systems)	+	+			
	Live attenuated dengue virus (monovalent)	+	+	+	+	
	Live attenuated dengue virus (combined quadrivalent)	+	+	+	+	
Eastern Equine Encephalitis virus	Inactivated whole virus particles	+	+	+	+	
	VEE virus Replicon Particle	+	+			
	Recombinant Eastern/Western encephalitis virus chimera	+				
Ebola virus	Recombinant protein subunit (various virus and eukaryotic expression and delivery systems)	+	+			
	VEE virus Replicon Particle	+	+			
	Kunjin virus Replicon Particle	+				
	Plasmid DNA prime /Adenovirus-expressed protein boost	+	+			
	Plasmid DNA	+	+			
	Virus-Like Particle	+				
	Recombinant subunit expressed in irradiated *Brucella abortus*	+				
Endotoxin (Gram-negative sepsis)	Detoxified lipopolysaccharide from *E. coli* 0111:B4, Rc (J5)	+	+			
Entamoeba histolytica	Yeast subunit	+	+			
	Recombinant galactose-binding protein	+	+			
	Galactose-binding proteins expressed in *Salmonella*	+	+			
Enterohemorrhagic *Escherichia coli (EHEC) [Shiga* toxin-producing *E. coli (STEC)]*	Nontoxic mutant toxins	+	+			
	Intimin	+	+			
	LPS conjugates	+	+			
	Intimin expression in plants	+	+			
	Stx-1 beta-subunit in *Vibrio cholerae* vector	+	+			
Enterotoxigenic *Escherichia coli* (ETEC)	Killed cells and beta-subunit of cholera toxin	+	+	+	+	
	Nontoxigenic ETEC derivative, live attenuated	+	+	+	+	
	Salmonella and *Shigella* vectored CFAs	+	+			
	Subunit synthetic toxoid (ST) and B subunit of heat-labile toxin (LT)	+	+			
	LTB expressed in potatoes	+	+	+		
	CFA II microencapsulated	+	+			
Epstein-Barr virus (EBV)	Glycoprotein subunit (gp350)	+	+	+	+	
	Vaccinia recombinant virus expressing gp350	+	+	+		
	Peptide induction of CTL	+	+	+		

Note: This list outlines publicly available information concerning the status of vaccines in the research and development pipeline and should not be considered inclusive of all ongoing vaccine research and development.

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Escherichia coli (urinary tract)	Anti-FimH adhesin	+	+			
Francisella tularensis	Live attenuated	+	+	+		
Group A streptococcus	Glycoconjugate Group A polysaccharide with tetanus toxoid	+	+			
	M protein, multivalent type-specific epitopes	+	+	+		
	M protein conserved epitope expressed in a commensal vector (*S. gordonii)*	+	+			
	M protein conserved epitope in combination with M serotype epitopes	+	+			
	Cysteine protease	+	+			
	C5a peptidase	+	+			
	Fibronectin-binding protein Sfb1	+	+			
	Streptococcal pyrogenic exotoxins	+	+			
	Surface protein(s)	+	+			
Group B streptococcus	Glycoconjugate vaccines of type Ia, Ib, II, III, and V polysaccharides linked to carrier proteins	+	+	+	+	
	Surface protein(s)	+	+			
Haemophilus ducreyi	Outer membrane proteins	+	+			
	Hemolysin/cytotoxin	+	+			
	Hemoglobin receptor	+	+			
Haemophilus influenzae (nontypeable)	Recombinant protein subunit containing either P1, P2, or P6 proteins to serve as carriers in conjugate vaccine preparations	+	+			
	Recombinant protein subunit containing P4 and P6	+	+			
	P4 and P6	+	+			
	Subunit Hi nontypeable 47 OMP (adjuvanted)	+	+			
	Subunit lipoprotein D (nonacylated)	+	+	+		
	Subunit detoxified lipooligosaccharide conjugate to tetanus toxoid	+	+			
	Subunit detoxified lipooligosaccharide conjugated to HMW protein from *H. influenzae* (nontypeable)	+	+			
	OMP HiN47	+	+	+	+	
	Pili (HifE)	+	+			
Haemophilus influenzae type b (Hib)	Glycoconjugate of Hib PRP with CRM197	+	+	+	+	+
	Glycoconjugate of Hib PRP with diphtheria toxoid	+	+	+	+	+
	Glycoconjugate of Hib PRP with tetanus toxoid	+	+	+	+	+
	Hib-IPV-HBV	+	+	+	+	+
	Glycoconjugate of Hib PRP with meningococcal type B outer membrane protein	+	+	+	+	+
	Glyconjugate Hib with meningococcal type A and/or C	+	+	+		
Hantaan virus	Vaccinia vector	+	+	+	+	

Note: This list outlines publicly available information concerning the status of vaccines in the research and development pipeline and should not be considered inclusive of all ongoing vaccine research and development.

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Hantaan virus (continued)	Recombinant subunit	+				
	RNA replicons	+	+			
	Non-replicating adenovirus vector	+				
	Naked DNA	+				
Helicobacter pylori	Recombinant *H. pylori* urease and cholera toxin-oral vaccine	+	+	+		
	Whole cell vaccine with mutant *E. coli* heat-labile toxin (LT) adjuvant	+	+	+	+	
	H. pylori antigens and mutant CT or LT	+	+	+		
	Killed whole cells	+	+			
	Salmonella vectored *H. pylori* antigens	+	+			
	Multi-epitope DNA vaccine	+				
Hepatitis A virus (HAV)	Inactivated HAV particles	+	+	+	+	+
	Live attenuated HAV	+	+	+	+	+
	Virosome-formulated inactivated HAV	+	+	+	+	+
	Viral proteins expressed by vectors (baculovirus or vaccinia virus)	+	+			
Hepatitis B virus (HBV)	HBV core protein expressed by rDNA	+	+			
	HBV proteins expressed in yeast cells by rDNA	+	+	+	+	+
	Salmonella vector	+	+	+		
	Variants	+	+			
	Generation of cytotoxic T lymphocytes	+	+	+	+	
	DNA vaccines	+	+			
	rDNA, plants	+	+	+		
Combined HAV/HBV vaccine	Combined inactivated components	+	+	+	+	+
Hepatitis C virus (HCV)	rDNA-expressed surface proteins and epitopes	+	+	+	+	
	Generation of cytotoxic T lymphocytes	+	+			
	Nucleocapsid	+	+			
	DNA vaccines	+	+			
	Core Ag + immunostimulatory complex	+	+	+	+	
	MVA-based rVac w/3 NS protein genes	+	+			
	Recombinant viruses carrying HCV non-structural genes: adenovirus	+	+			
	Recombinant viruses carrying various HCV genes: vesicular stomatitis virus (VSV); adeno-associated virus (AAV); Measles virus (MV); Equine herpesvirus (EHV-1); WHV cores	+				
	Bacterial recombinants with HCV proteins: *Listeria monocytogenes*	+				
	Cell based vaccines; yeast	+				
	Plants systems for HCV protein expression	+				

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Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Hepatitis C virus (HCV) (continued)	Human dendritic cells (matured *in vitro* with HCV peptides), for autologous transfer	+				
Hepatitis D virus (HDV)	Synthetic peptides	+	+			
	Baculovirus	+				
Hepatitis E virus (HEV)	Expressed proteins	+	+	+	+	
Herpes simplex virus types 1 and 2	gD2 recombinant protein	+	+	+	+	+
	Inactivated virus	+	+	+		
Histoplasma capsulatum	Purified yeast cell proteins (e.g., His-62)	+	+			
	Recombinant proteins (e.g., His 62, H antigen, hsp-70)	+	+			
Human immunodeficiency virus, HIV-1	**See DAIDS appendix**					
Human papillomavirus (HPV)	Bivalent VLP L1 (HPV-11, HPV-16)	+	+	+	+	+
	Quadrivalent recombinant VLP L1 (from HPV-6, HPV-11, HPV-16, and HPV-18)	+	+	+	+	+
Influenza virus	Inactivated (interpandemic)	+	+	+	+	+
	Inactivated (pandemic)	+	+	+	+	+
	Cold-adapted live attenuated (interpandemic)	+	+	+	+	+
	Cold-adapted live attenuated (pandemic)	+	+	+		
	Purified viral HA subunit	+	+	+		
	Liposome-containing viral HA	+	+	+	+	
	Purified CTL specific peptides	+	+	+		
	Microencapsulated inactivated vaccine	+	+	+		
	Purified, inactivated viral neuraminidase	+	+	+		
	Baculovirus expressed recombinant HA subunit	+	+	+	+	
	Baculovirus expressed nucleoprotein	+	+	+		
	Inactivated viral vaccines with novel adjuvants	+	+	+	+	
	M2e vaccines with novel adjuvants	+	+			
	Cell culture derived influenza vaccine	+	+	+	+	+
	DNA Vaccines	+	+	+	+	
	Recombinant vector vaccines (i.e., adenoviral vectors, VEE vectors)	+	+			
Japanese encephalitis virus	Whole, inactivated virus particles, mouse brain derived	+	+	+	+	+
	Whole, inactivated virus particles, Vero cell derived	+	+	+	+	+
	Infectious clone	+	+			
	Purified DNA expressed protein	+	+			
	Live attenuated virus (SA-14-14-2)	+	+	+	+	+
	Inactivated SA-14-14-2 plus novel adjuvant	+	+	+	+	+
	Vaccinia vector (live)	+	+	+		
	Chimeric live attenuated Yellow Fever/Japanese Encephalitis virus	+	+	+	+	

Note: This list outlines publicly available information concerning the status of vaccines in the research and development pipeline and should not be considered inclusive of all ongoing vaccine research and development.

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Junin virus (Argentine hemorrhagic fever)	Live attenuated (Candid 1)	+	+	+	+	
Lassa virus	Chimeric live reassortant Mopeia/Lassa virus	+				
	DNA vaccine	+				
	Viral-Like Particles	+				
	Recombinant subunit expressed in irradiated *Brucella abortus*	+				
Legionella pneumophila	Attenuated mutant	+	+			
	Purified bacterial surface protein	+	+			
Leishmania major	Attenuated or killed whole parasites	+	+	+	+	+
	Deletion mutagenized, attenuated parasite	+	+			
	Recombinant trivalent polypeptide	+	+	+	+	
Leishmania amazonensis	Killed whole parasites	+	+	+	+	
Multiple Leishmania spp.	Leishmanial surface antigens (gp63, 46 kD, and lipophosphoglycan)	+	+			
Listeria monocytogenes	cytoLLO/cytoPFO vaccine strains	+				
Marburg virus	VEE virus Replicon Particle	+				
	Baculovirus-expressed protein subunit	+				
	Various virus-vectored vaccines	+				
	Virus-Like Particle	+				
Measles virus	rDNA HA and fusion proteins	+	+	+		
	ISCOM	+	+			
	Live attenuated	+	+	+	+	+
	High-titer live (multiple strains)	+	+	+	+	+
	Poxvirus vector (live)	+	+	+		
Moraxella catarrhalis	High molecular weight, outer membrane proteins CD, E, B1, and LBP for use in conjugate vaccines	+	+			
	Detoxified LOS conjugated to either tetanus toxoid or high MW proteins from nontypeable *H. influenzae*	+	+			
	Subunit derived from type IV pilin protein	+				
Mycobacterium leprae	*Mycobacterium bovis* BCG (Bacillus Calmette Guérin)					+
	BCG + heat killed *Mycobacterium leprae* (HKML)					+
	BCG + killed *Mycobacterium vaccae*					+
	Killed *Mycobacterium vaccae*					+
	Killed *Mycobacterium habana*				+	
	ICRC bacilli, heat killed (Indian Cancer Research Center strain)					+
	Mycobacterium welchii, killed					+
	Secretory proteins of *Mycobacterium habana*	+				
	BCG over-expressing Ag85A,B and MPB51	+				

Note: This list outlines publicly available information concerning the status of vaccines in the research and development pipeline and should not be considered inclusive of all ongoing vaccine research and development.

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Mycobacterium leprae (continued)	Adjuvanted Ag85A, B and C proteins	+				
	DNA subunit vaccine of *Mycobacterium leprae* 35KDa protein	+				
	DNA subunit vaccine of *Mycobacterium tuberculosis* Ag85B	+				
	Mycobacterium leprae complex cellular fractions	+				
	Adjuvanted *Mycobacterium leprae* MLSA and MLCwA	+				
	Recombinant, adjuvanted *Mycobacterium leprae* hsp65-CpG DNA vaccine	+				
Mycobacterium tuberculosis	Mycobacterium bovis BCG (Bacillus Calmette Guérin)					+
	BCG homologous and heterologous boosting	+	+			+
	BCG delivered orally	+		+		
	Mycobacterium vaccae, heat killed				+	
	Recombinant BCG over-expressing Ag85A (rBCG30)	+	+	+		
	*Reco*mbinant BCG with endosome escape, overexpressing several key antigens (rBCG-Aeras 403)	+	+			
	Recombinant BCG with endosome escape (rBCGΔUre:CHly+)	+	+			
	Recombinant BCG with re-introduced RD1 region (BCG::RD1)	+				
	Superoxide dismutase (SOD) diminished BCG	+	+			
	Live attenuated *Mycobacterium tuberculosis* strains	+	+			
	Modified vaccinia virus expressing *Mycobacterium tuberculosis* Ag85A (MVA-85A)				+	
	Ag85B + ESAT6 (Hybrid-1) subunit vaccine in IC3 adjuvant		+	+		
	Ag85B + TB10.4 (Hybrid-4) subunit vaccine in IC3 adjuvant		+			
	Mtb72f 9(Mtb39 + Mtb32) subunit vaccine in adjuvant AS02A and AS01B adjuvant		+	+		
	Nascent BCG protein associated with heat shock proteins as subunit vaccines	+				
	Hsp65 DNA vaccine	+	+			
	Non-replicating Adenovirus 35 expressing multiple proteins of *Mycobacterium tuberculosis*	+				
	Double stranded RNA capsids encoding *Mycobacterium tuberculosis* antigens	+				
	Various adjuvanted protein antigens of *Mycobacterium tuberculosis*	+				
	Various *Mycobacterium tuberculosis* antigens as DNA vaccines	+				
Mycoplasma pneumoniae	Recombinant membrane-associated proteins	+	+			
	Purified outer membrane protein	+	+			
	Inactivated (heat-killed) oral vaccine	+	+	+		

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Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Neisseria gonorrheae	Por (protein I)	+	+			
	Recombinant Por protein	+	+			
	Iron-binding protein (BPs)	+				
	LPS anti-idiotype	+				
Neisseria meningitidis (Group A)	Glycoconjugate with tetanus toxoid	+	+			
	Group A LOS	+				
Neisseria meningitidis (Group B)	Native outer membrane vesicle (NOMV)-intranasal route	+	+	+		
	OMP-dLPS liposome	+	+			
	Recombinant PorA outer membrane protein in liposomes	+	+			
	Recombinant factor H binding protein	+	+			
	Membrane vesicle-based vaccine (containing over-expressed proteins normally expressed in low amounts)	+				
	Polysaccharide derivative	+				
	Outer membrane vesicles (OMV), high MW proteins, and C polysaccharide	+	+	+	+	+
	Hexvalent PorA outer membrane vesicle vaccine	+	+	+	+	
	Outer membrane vesicles (deoxycholate extracted)	+	+	+	+	+
	Recombinant transferrin binding protein (TBP1 and TBP2)	+	+			
	Recombinant low MW (NspA) outer membrane protein	+	+			
	Glycoconjugate modified polysaccharide with recombinant PorB protein	+	+			
	LOS micelle-based vaccine	+				
	Genome-derived Neisserial Antigen (Universal)	+				
Neisseria meningitidis (Group C)	Glycoconjugate with tetanus toxoid	+	+	+	+	+
Neisseria meningitides A and C	Glycoconjugate A and C with CRM197	+	+	+	+	
	Glycoconjugate A and C with DT	+	+	+		
Neisseria meningitides A, B, and C	Combination glycoconjugate with recombinant PorB	+	+			
Neisseria meningitides A, B, C, and W-135	Glycoconjugate with DT	+	+	+		
Nipah virus	Poxvirus vectors expressing F and G glycoproteins	+				
	Soluble F and G glycoproteins	+				
Newcastle Disease Virus	Recombinant Newcastle Disease virus expressing foreign HN protein	+	+			
Norwalk Virus	VLPs	+	+	+		
	VLPs in transgenic potatoes	+	+	+		
	VLPs in transgenic tomatoes (lyophilized)	+	+			
Onchocerca volvulus	Recombinant proteins	+	+			
Paracoccidioides brasiliensis	Purified yeast cell proteins	+	+			

Note: This list outlines publicly available information concerning the status of vaccines in the research and development pipeline and should not be considered inclusive of all ongoing vaccine research and development.

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Paracoccidioides brasiliensis (continued)	Recombinant proteins	+	+			
	Synthetic peptide or multipeptide construction (P10, MAP-10)	+	+			
	DNA plasmid with gp43 gene	+	+			
Parainfluenza virus	Cold-adapted PIV3 attenuated virus	+	+	+	+	
	Purified HN and F protein subunit vaccine	+	+			
	Bovine attenuated PIV3 vaccine	+	+	+	+	
Plasmodium falciparum	Circumsporozoite antigen-based peptide or recombinant protein	+	+	+	+	
	Circumsporozoite antigen fused to hepatitis B surface antigen viral-like particle (RTS, S)	+	+	+	+	
	Circumsporozoite antigen epitopes in viral-like particles	+	+	+	+	
	Circumsporozoite antigen expressed in various vectors	+	+	+	+	
	Circumsporozoite antigen-based DNA vaccine	+	+	+	+	
	Noncircumsporozoite, pre-erythrocytic antigen-based constructs	+	+	+	+	
	Merozoite surface protein-1 (MSP-1) based recombinant protein	+	+	+		
	Non-MSP-1 asexual blood stage antigens	+	+	+		
	25-kD gametocyte antigen recombinant protein (TBV25H)	+	+	+		
	Other sexual stage antigens	+	+			
	Multivalent viral vector-based combination vaccines incorporating different stage-specific antigens (e.g., NYVAC Pf7)	+	+	+	+	
	DNA-based combination vaccines incorporating different stage-specific antigens	+	+			
	Combination vaccines incorporating different stage-specific antigens (e.g., SPf 66)	+	+	+	+	+
	Purified irradiated sporozoites	+	+			
	Genetically attenuated sporozoite	+	+			
Plasmodium vivax	Circumsporozoite antigen-based peptide or recombinant protein	+	+	+		
	Asexual erythrocytic antigens	+	+			
Poliovirus	Reversion-stable attenuated OPV	+				
	Live (nonreverting)	+	+			
	Chimeric virus	+	+			
Pseudomonas aeruginosa	Purified bacterial proteins, including flagellar Ag, LPS-O, porins, several inactivated bacterial toxins, and high MW polysaccharide antigen and glycoconjugate	+	+	+		
	Inactivated whole bacteria-oral preparation	+	+	+		
	Synthetic peptides	+	+	+		

Note: This list outlines publicly available information concerning the status of vaccines in the research and development pipeline and should not be considered inclusive of all ongoing vaccine research and development.

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Pseudomonas (Burkholderia) cepacia	Purified bacterial proteins, LPS	+				
Pythium insidiosum	Sonicated hyphal antigens	+	+			
	Culture filtrate antigens	+	+			
	Purified proteins (e.g., 28, 30, 32 kD)	+	+			
Rabies virus	rDNA vaccinia virus recombinant for use in sylvatic rabies (veterinary vaccine)	+	+	+	+	+
	Inactivated mammalian brain	+	+	+	+	+
	Inactivated cell culture	+	+	+	+	+
	Replication-defective adenovirus vector	+				
	Live attenuated	+				
Respiratory Syncytial virus (RSV)	Purified F protein subunit vaccine	+	+	+	+	
	Co-purified F, G, and M vaccine	+				
	RSV live attenuated strains	+	+	+	+	
	Recombinant Sendai virus expressing RSV G protein	+	+	+		
	Recombinant attenuated parainfluenza virus type 3 expressing RSV F protein	+	+	+		
	Recombinant Sendai virus	+	+	+		
Ricin Toxin	Recombinant inactivated toxin	+	+	+		
Rickettsia rickettsii	Subunit vaccine containing major surface proteins (155 and 120 kD)	+	+			
Rift Valley Fever virus	Inactivated	+	+	+	+	
	Live attenuated virus (MP-12)	+	+	+		
	VEE virus Replicon Particle	+				
	Sindbis virus Replicon Particle	+				
	Virus-Like Particle	+				
	Live attenuated recombinant virus	+				
Rotavirus	Attenuated human rotavirus strain 89-12 P1A[8], G1—ROTARIX® (GlaxoSmithKline)	+	+	+	+	+
	Salmonella expressing VP4, VP7, or both	+	+			
	Attenuated bovine/human virus reassortants (G1-WC3; G2-WC3; G3-WC3; GA-WC3; P1A[8]-WC3—ROTATEQ® Merck	+	+	+	+	+
	Human nursery strains	+	+	+	+	
	Purified rotavirus proteins rDNA-derived virus-like particles (VLPs)	+	+			
	Vaccina virus recombinant expressing VP4, VP7, or both	+	+			
	DNA vaccines	+	+			
Rubella Virus	Live attenuated	+	+	+	+	+
	Infectious clone	+				
	Synthetic peptide	+				

Note: This list outlines publicly available information concerning the status of vaccines in the research and development pipeline and should not be considered inclusive of all ongoing vaccine research and development.

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Salmonella typhi	Vi carbohydrate	+	+	+	+	+
	Live attenuated Ty21a vaccine	+	+	+	+	+
	Live attenuated auxotrophic mutants	+	+	+	+	
	rPAE-Vi conjugate vaccine	+	+	+	+	+
	Ty800 live attenuated strain	+	+	+	+	
	Live attenuated CVD908-htrA and CVD 909	+	+	+	+	
Schistosoma mansoni	Purified larval antigens	+	+			
	Recombinant antigens	+	+			
	Multiple antigen peptides (MAP)	+	+			
	DNA vaccines	+				
Schistosoma haematobium	Recombinant Sh28 GST (*S. haematobium* glutathione-S-transferase)	+	+	+		
Schistosoma japonicum	Recombinant larval antigens	+	+			
	DNA vaccine	+				
Sendai Virus	Recombinant Sendai Virus	+	+	+		
	Sendai virus for gene therapy and vaccination	+	+			
Severe Acute Respiratory Syndrome (SARS Co-V)	DNA plasmid expressing S protein	+	+	+		
	Inactivated viral vaccines	+	+	+		
	Baculovirus expressed S protein	+	+			
	CHO cell expressed S protein	+	+			
	Baculovirus expressed S protein with novel adjuvant, intranasally delivered	+	+			
	Alphavirus replicon vaccine	+	+			
	Virus-like particle vaccine	+				
	Rhabdovirus (rabies) expressing S protein	+	+			
	Modified vaccinia Ankara (MVA) expressing S protein	+	+			
	Adenovirus vector expressing S1 or N	+	+			
	B- and T-epitope peptide-based vaccine	+				
Shigella dysenteriae	Live auxotrophic, attenuated mutants	+	+	+		
	Polysaccharide-protein conjugate	+	+	+	+	
Shigella flexneri	*E. coli* hybrids	+	+	+	+	
	Polysaccharide-protein conjugate	+	+	+	+	
	Live attenuated oral vaccines	+	+	+	+	
	LPS proteosome (intranasal)	+	+			
	LPS-invasin proteins complex	+	+			
Shigella sonnei	Live attenuated (WRSS1) oral vaccine	+	+			
	LPS proteosome (intranasal)	+	+			
	Polysaccharide-protein conjugate	+	+	+	+	
	Nucleoprotein	+	+			

Note: This list outlines publicly available information concerning the status of vaccines in the research and development pipeline and should not be considered inclusive of all ongoing vaccine research and development.

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Staphylococcus aureus	Clumping Factor B	+				
	rAls3p-N	+				
	Polymeric N-acetylglucosamine			+		
	Staphylococcus aureus protein/polypeptide antigen expressed in yeast surface proteins IsdA, IsdB, SdrD, SdrE	+				
Staphylococcal enterotoxin B	Recombinant toxin	+	+			
Streptococcus pneumoniae	Glycoconjugate vaccine (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F) conjugated to CRM197	+	+	+	+	+
	23-valent licensed vaccine with novel adjuvants (Quil A, QS21, MPL)	+	+	+		
	Glycoconjugate multivalent vaccine with novel adjuvants (e.g., MPL)	+	+	+		
	PspA	+	+	+		
	PsaA	+	+			
	Pneumolysin	+	+			
	Autolysin	+	+			
	Neuraminidase	+	+			
	Glycoconjugate vaccine (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F) linked to either tetanus or diphtheria toxoid carrier	+	+	+	+	+
	Phospholcholine	+	+			
	Synthetic peptide epitopes and capsular polysaccharide combined	+	+			
	Genetic fusions (PspA-IL2 and PspA-GM-CSF)	+	+			
	CpG motifs cross-linked with 7-valent pneumococcal vaccine	+	+			
	PGCvax (a fusion protein)	+	+	+		
Tick-borne Encephalitis virus	DNA vaccine	+	+			
	Inactivated, alum adjuvant	+	+	+	+	
	Recombinant subunit vaccine	+				
	Chimeric live attenuated dengue/TBE virus	+				
	Recombinant Vaccinia virus	+				
Toxoplasma gondii	Recombinant parasite surface protein (p30)	+	+			
	Live attenuated parasites	+	+			
	Parasite surface protein expressed in viral vector	+	+			
Treponema pallidum	Membrane proteins	+				
Trypanosoma cruzi	Recombinant peptide	+	+			
Varicella zoster virus	Live, attenuated vaccine	+	+	+	+	+
	Subunit, glycoproteins	+				
	Vaccinia-vectored glycoprotein	+				

Note: This list outlines publicly available information concerning the status of vaccines in the research and development pipeline and should not be considered inclusive of all ongoing vaccine research and development.

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Venezuelan Equine Encephalitis	Inactivated, whole virus particles	+	+	+	+	
	Live attenuated virus strain (TC-83)	+	+	+	+	
	Live attenuated mutagenized virus (V3526)	+	+	+		
	Infectious clones	+	+			
	VEE virus Replicon Particle	+	+			
Vibrio cholerae	Killed bacteria plus toxin B subunit	+	+	+	+	+
	Live recombinant O1	+	+	+	+	+
	Live recombinant O139	+	+	+	+	
	Conjugate lipopolysaccharide (LPS)	+	+			
Yellow Fever virus	Live attenuated	+	+	+	+	+
	Infectious clone	+	+			
Western Equine Encephalitis virus	Inactivated, whole virus particles	+	+	+	+	
	VEE virus Replicon Particle	+	+			
West Nile virus	Chimeric live attenuated Yellow Fever/ West Nile virus	+	+	+	+	
	Chimeric live attenuated dengue/West Nile virus	+	+	+		
	DNA vaccine	+	+	+		
	Drosophila and Baculovirus-expressed recombinant protein subunit	+	+			
Yersinia pestis	Recombinant subunit	+	+			

Note: This list outlines publicly available information concerning the status of vaccines in the research and development pipeline and should not be considered inclusive of all ongoing vaccine research and development.

APPENDIX B: Vaccines Licensed for Immunization and Distribution in the US*

Product Name	Trade Name	Sponsor
Anthrax Vaccine Adsorbed	BioThrax	BioPort Corp[1]
BCG Live	TICE BCG	Organon Teknika Corp
BCG Live	Mycobax	Aventis Pasteur, Ltd[2]
Diphtheria & Tetanus Toxoids Adsorbed	No Trade Name	Aventis Pasteur, Inc[3]
Diphtheria & Tetanus Toxoids Adsorbed	No Trade Name	Aventis Pasteur, Ltd[2]
Diphtheria & Tetanus Toxoids & Acellular Pertussis Vaccine Adsorbed	Tripedia	Aventis Pasteur, Inc[3]
Diphtheria & Tetanus Toxoids & Acellular Pertussis Vaccine Adsorbed	Infanrix	GlaxoSmithKline
Diphtheria & Tetanus Toxoids & Acellular Pertussis Vaccine Adsorbed	DAPTACEL	Aventis Pasteur, Ltd
Diphtheria & Tetanus Toxoids & Acellular Pertussis Vaccine Adsorbed, Hepatitis B (recombinant) and Inactivated Poliovirus Vaccine Combined	Pediarix	SmithKline Beecham Biologicals
Haemophilus b Conjugate Vaccine (Diphtheria CRM197 Protein Conjugate)	HibTITER	Lederle Lab Div, American Cyanamid Co
Haemophilus b Conjugate Vaccine (Meningococcal Protein Conjugate)	PedvaxHIB	Merck & Co, Inc
Haemophilus b Conjugate Vaccine (Tetanus Toxoid Conjugate)	ActHIB	Aventis Pasteur, SA[4]
Haemophilus b Conjugate Vaccine (Meningococcal Protein Conjugate) & Hepatitis B Vaccine (Recombinant)	Comvax	Merck & Co, Inc
Hepatitis A Vaccine, Inactivated	Havrix	GlaxoSmithKline
Hepatitis A Vaccine, Inactivated	VAQTA	Merck & Co, Inc
Hepatitis A Inactivated and Hepatitis B (Recombinant) Vaccine	Twinrix	GlaxoSmithKline
Hepatitis B Vaccine (Recombinant)	Recombivax HB	Merck & Co, Inc
Hepatitis B Vaccine (Recombinant)	Engerix-B	GlaxoSmithKline
Influenza Virus Vaccine	FluLaval	ID Biomedical Corp of Quebec
Influenza Virus Vaccine, Live, Intranasal	FluMist	MedImmune Vaccines, Inc
Influenza Virus Vaccine, Trivalent, Types A and B	Fluarix	GlaxoSmithKline Biologicals
Influenza Virus Vaccine, Trivalent, Types A and B	Fluvirin	Evans Vaccines[5]
Influenza Virus Vaccine, Trivalent, Types A and B	Fluzone	Aventis Pasteur, Inc[3]
Japanese Encephalitis Virus Vaccine Inactivated for Microbial Diseases of Osaka University	JE-Vax	Research Foundation
Measles Virus Vaccine, Live	Attenuvax	Merck & Co, Inc
Measles and Mumps Virus Vaccine, Live	M-M-Vax	Merck & Co, Inc (not available)
Measles, Mumps, and Rubella Virus Vaccine, Live	M-M-R II	Merck & Co, Inc
Measles, Mumps, Rubella and Varicella Virus Vaccine Live	ProQuad	Merck & Co, Inc
Meningococcal Polysaccharide (Serogroups A, C, Y and W-135) Diphtheria Toxoid Conjugate Vaccine	Menactra	Aventis Pasteur, Inc

Product Name	Trade Name	Sponsor
Meningococcal Polysaccharide Vaccine, Groups A, C, Y and W-135 Combined	Menomune-A/C/Y/W-135	Aventis Pasteur, Inc[3]
Mumps Virus Vaccine Live	Mumpsvax	Merck & Co, Inc
Pneumococcal Vaccine, Polyvalent	Pneumovax 23	Merck & Co, Inc
Pneumococcal 7-valent Conjugate Vaccine (Diphtheria CRM 197 Protein)	Prevnar	Lederle Lab Div, American Cyanamid Co
Poliovirus Vaccine Inactivated (Human Diploid Cell)	Poliovax	Aventis Pasteur, Ltd[2] (not available)
Poliovirus Vaccine Inactivated (Monkey Kidney Cell)	IPOL	Aventis Pasteur, SA[4]
Quadrivalent Human Papillomavirus (Types 6, 11, 16, 18) Recombinant Vaccine	GARDASIL	Merck & Co., Inc.
Rabies Vaccine	Imovax	Aventis Pasteur, SA[4]
Rabies Vaccine	RabAvert	Chiron Behring GmbH & Co
Rabies Vaccine Adsorbed	No Trade Name	BioPort Corp[1] (not available)
Rotavirus Vaccine, Live, Oral, Pentavalent	RotaTeq	Merck & Co., Inc.
Rubella Virus Vaccine Live	Meruvax II	Merck & Co, Inc
Smallpox Vaccine, Dried, Calf Lymph Type	Dryvax	Wyeth Laboratories, Inc (available only thru CDC or DoD programs)
Tetanus & Diphtheria Toxoids Adsorbed for Adult Use	No Trade Name	Massachusetts Public Health Biologic Lab
Tetanus & Diphtheria Toxoids Adsorbed for Adult Use	DECAVAC	Aventis Pasteur, Inc[3]
Tetanus & Diphtheria Toxoids Adsorbed for Adult Use	No Trade Name	Aventis Pasteur, Ltd (not available)
Tetanus Toxoid	No Trade Name	Aventis Pasteur, Inc[3]
Tetanus Toxoid Adsorbed	No Trade Name	Massachusetts Public Health Biologic Lab
Tetanus Toxoid Adsorbed	No Trade Name	Aventis Pasteur, Inc[3]
Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Adsorbed	Adacel	Aventis Pasteur, Ltd
Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Adsorbed	Boostrix Biologicals	GlaxoSmithKline
Typhoid Vaccine Live Oral Ty21a	Vivotif	Berna Biotech, Ltd
Typhoid Vi Polysaccharide Vaccine	TYPHIM Vi	Aventis Pasteur, SA[4]
Varicella Virus Vaccine Live	Varivax	Merck & Co, Inc
Yellow Fever Vaccine	YF-Vax	Aventis Pasteur, Inc[3]
Zoster Vaccine, Live, (Oka/Merck)	Zostavax	Merck & Co., Inc.

*United States Food and Drug Administration, Center for Biologics Evaluation and Research (Information updated 10/11/2006)

Footnotes

1. BioPort Corporation acquired product ownership on November 12, 1998 from the Michigan Biologic Products Institute, formerly under the Michigan Department of Public Health.
2. Aventis Pasteur, Ltd obtained product ownership from Connaught Laboratories, Ltd, effective February 24, 2000.
3. Aventis Pasteur, Inc obtained product ownership from Connaught Laboratories, Inc effective December 9, 1999.
4. Aventis Pasteur, SA is the new corporate name for Pasteur Merieux Serums et Vaccins, SA effective February 4, 2000.
5. Powderject obtained product ownership from Evans Medical Ltd. Effective 2001
6. Wyeth Laboratories, Inc is the new corporate name for Wyeth-Ayerst, Inc, effective July 1, 1980.

APPENDIX C: HIV VACCINE CANDIDATES IN PRECLINICAL DEVELOPMENT
January 2007

Vaccine *	HIV Subtype	Preclinical Partners**	Manufacture
MVA	C	UCT, SAAVI, Therion	Therion
DNA	C	UCT, SAAVI	Althea
DNAs + IL-12 or IL-15 DNA	B	U. Penn (D. Weiner), Wyeth	Althea
Multiepitope DNA + MVA	Multi-epitope	Pharmexa Epimmune	Althea/Barvarian-Nordic
VEE replicons	C	Alphavax	Alphavax
Novel Adenoviral Vectors	A	Harvard (D. Barouch)	CruceII
Novel Adenoviral Vectors	A	VRC	GenVec
VSV vector	B	Wyeth Vaccines	Henagen
Env protein	C	Chiron	Chiron
AAV-based vectors	A	Children's Hospital of Philadelphia, Targeted Genetics	Targeted Genetics

APPENDIX D: CLINICAL TRIALS OF HIV VACCINE CANDIDATES IN HIV-UNINFECTED ADULTS *February 16, 2007*

Protocol	Type of Vaccine	Vaccine/Antigens	HIV Clade	Adjuvant and/or Formulation	Developer	Manufacturer	Conducting Trial	Phase
DNA								
—	DNA	DNA plasmid	BC	—	—	—	Guangxi CDC	Phase I China
C060301	DNA	GTU®MultiHIV B clade, DNA plasmid. expressing nef, rev, tat, gag, pol, env, and CTL epitopes	B	—	FIT Biotech, IAVI	FIT Biotech	FIT Biotech	Phase I Finland
HVTN 060	DNA	Gag DNA	B	IL-12 DNA	Wyeth	Wyeth	NIAID/HVTN	Phase I United States, Thailand
HVTN 063	DNA	Gag DNA	B	IL-12 DNA IL-15 DNA	Wyeth	Wyeth	NIAID/HVTN	Phase I United States
DNA plus Peptide								
HVTN 064	DNA	EP HIV-1090 DNA vaccine, expressing 21 CTL epitopes from gag, pol, env, nef, rev, vpr, and the universal HTL epitope PADRE®	Conserved across multiple clades	Polyvinylpyrrolidone (PVP, Plasdone® povidone)	Pharmexa-Epimmune	Pharmexa-Epimmune	NIAID/HVTN	Phase I United States
	Peptide	EP-1043: gag, pol, and vpu peptides		Aluminum hydroxide (Alhydrogel®)				
DNA plus Protein								
HVTN 049	DNA	Env DNA and gag DNA vaccines as PLG microparticles	B	polylactide coglycolide (PLG). microparticles	Chiron/Novartis	Chiron/Novartis	NIAID/HVTN	Phase I Unikted States
	Protein	gp140 (oligomeric, V2-deleted)	B	MF-59				
DNA plus Live Vector: Modified Vaccinia Ankara (MVA)								
HVTN 065	DNA	pGA2/JS7: Env, gag, protease, reverse transcriptase, Rev, Tat, and Vpu	B	—	GeoVax	Strathmann Biotech Quiagen	NIAID/HVTN	Phase I Untied States
	Live vector	MVA62(2), expressing gag, pol, env	B	—		BioReliance Ltd		
HIVIS 02	Live vector	MVA-CMDR, MVA expressing HIV-1 CRF01A_E	AE	—	WRAIR	NIAID, WRAIR	Karolinska Institute	Phase I Sweden
	DNA	gp160, and rev DNA vaccines and p17/p24, and rt DNA vaccines	A, B, C; B A and B; B	+/- sargramostim (rGM-CSF) —	Swedish Institute for Infectious Disease Control (SMI)	Karolinska Institute, SMI, Vecura		

Protocol	Type of Vaccine	Vaccine/Antigens	HIV Clade	Adjuvant and/or Formulation	Developer	Manufacturer	Conducting Trial	Phase
DNA plus Live Vector: Adenovirus 5 (Ad5)								
HVTN 068	DNA	VRC HIVDNA009-00-VP: multiclade env, gag, pol and nef DNA vaccine	A, B, C	—	NIAID VRC	Vical	NIAID/HVTN	Phase I United States
	Live vector	VRC HIVADV014-00-VP: adenoviral vectors expressing multiclade env, and a gag-pol polyprotein	A, B, C	—		Molecular Medicine, Genvec Inc		
HVTN 069	DNA	VRC HIVDNA009-00-VP: multiclade env, gag, pol and nef DNA vaccine	A, B, C	—	NIAID VRC	Vical	NIAID/HVTN	Phase Ib United States
	Live vector	VRC HIVADV014-00-VP: adenoviral vectors expressing multiclade env, and a gag-pol polyprotein	A, B, C	—		Molecular Medicine, Genvec Inc		
RV 172	DNA	VRC-HIVDNA016-00-VP: multiclade env, gag, pol, and nef DRV158NA vaccine	A, B, C	—	NIAID VRC	Vical	WRAIR, NIAID VRC	Phase II Kenya, Tanzania, Uganda
	Live vector	VRC-HIVADV014-00-VP: adenoviral vectors expressing multiclade env, and a gag-pol polyprotein	A, B, C	—		Molecular Medicine, Genvec Inc		
VRC 011	DNA	VRC-HIVDNA016-00-VP: multiclade env, gag, pol, and nef DNA vaccine	A, B, C	—	NIAID VRC	Vical	NIAID VRC	Phase I United States
	Live vector	VRC-HIVADV014-00-VP: adenoviral vectors expressing multiclade env, and a gag-pol polyprotein		—		Molecular Medicine, Genvec Inc		
HVTN 204	DNA	VRC-HIVDNA016-00-VP: multiclade env, gag, pol, and nef DNA vaccine	A, B, C	—	NIAID VRC	Vical	NIAID/HVTN	Phase II United States, Brazil, Haiti, Jamaica, S. Africa
	Live vector	VRC-HIVADV014-00-VP: adenoviral vectors expressing multiclade env, and a gag-pol polyprotein	A, B, C	—		Molecular Medicine, Genvec Inc		
RV 156, RV156A	DNA	VRC HIVDNA009-00-VP: multiclade env, gag, pol and nef DNA vaccine	A, B, C	—	NIAID VRC	Vical	WRAIR, NIAID VRC	Phase I Uganda
	Live vector	VRC-HIVADV014-00-VP: adenoviral vectors expressing multiclade env, and a gag-pol polyprotein	A, B, C	---		Molecular Medicine, Genvec Inc		
IAVI V001	DNA	VRC-HIVDNA016-00-VP: multiclade env, gag, pol, and nef DNA vaccine	A, B, C	---	NIAID VRC	Vical	IAVI, NIAID/HVTN	Phase I Kenya, Rwanda
	Live vector	VRC-HIVADV014-00-VP: adenoviral vectors expressing multiclade env, and a gag-pol polyprotein	A, B, C	—		Molecular Medicine, Genvec Inc		
Live Vector: Vaccinia								
—	Live vector	PolyEnv1 vaccinia	B	—	St. Jude Children's Research Hospital	St. Jude Children's Research Hospital	St. Jude Children's Research Hospital	Phase I United States

Protocol	Type of Vaccine	Vaccine/Antigens	HIV Clade	Adjuvant and/or Formulation	Developer	Manufacturer	Conducting Trial	Phase
Live Vector: MVA								
—	Live vector	MVA HIV vaccine	BC	—	—	—	Guangxi CDC	Phase I China
DHO-0586	Live vector	ADMVA: MVA expressing env/gag-pol and nef-tat fusion protein.	C	--	ADARC	ADARC	ADARC, IAVI	Phase I United States
IAVI D001	Live vector	TBC-M4: MVA expressing env, gag, tat-rev, and nef-RT	C	---	Therion, IAVI	Therion	IAVI, Indian Council of Medical Research, and the National AIDS Control Org. of India	Phase I India
RV 158	Live vector	MVA-CMDR, modified vaccinia Ankara, expressing HIV-1 CM235 gp160, CM240 gag/pol	E, A	--	WRAIR	WRAIR	WRAIR	Phase I United States
IAVI C002	Live vector	ADMVA: MVA expressing env/gag-pol and nef-tat fusion protein.	C	---	Aaron Diamond AIDS Research Center	Impfstoffwerk Dessau-Tornau GmbH	IAVI	Phase I United States
Live Vector: MVA and Fowlpox								
HVTN 055	Live vector	rFPV-HIV env/gag (TBC-F357), rFPV-HIV tat/rev/nef-RT (TBC-F349), rMVA-HIV env/gag (TBC-M358), rMVA-HIV tat/rev/nef-RT (TBC-M335)	B	---	Therion	Therion	NIAID/HVTN	Phase I United States, Brazil
Live Vector: Adeno-Associated Virus (AAV)								
IAVI A002	Live vector	tgAAC09: AAV capsid enclosing DNA expressing gag, protease, and RT	C	---	Targeted Genetics, Columbus Children's Research Institute, Children's Hospital of Philadelphia, IAVI	Targeted Genetics	IAVI	Phase II South Africa, Uganda, Zambia
Live Vector: Ad5								
Merck 018/ HVTN 050	Live vector	MRKAd5 HIV-1 gag	B	--	Merck	Merck	Merck, NIAID/HVTN	Phase I United States, Puerto Rico, Peru, Haiti, South Africa, Malawi, Dominican Republic, Brazil, Thailand
Merck 023/ HVTN 502	Live vector	MRKAd5 vectors expressing HIV-1 gag, pol, nef	B	---	Merck	Merck	Merck, NIAID/HVTN	Phase IIb United States; Puerto Rico, Canada, Dominican Republic, Haiti, Jamaica, Brazil, Peru, Australia

Protocol	Type of Vaccine	Vaccine/Antigens	HIV Clade	Adjuvant and/or Formulation	Developer	Manufacturer	Conducting Trial	Phase
HVTN 503	Live vector	MRKAd5 vectors expressing HIV-1 gag, pol, nef	B	---	Merck	Merck	NIAID/HVTN	Phase IIb South Africa
Live Vector: Ad 5 and Adenovirus 6 (Ad6)								
Merck 001	Live vector	MRKAd5 vectors and MRKAd6 vectors expressing HIV-1 gag, pol, nef	B	---	Merck	Merck	Merck	Phase I United States
Live Vector: Canarypox plus Protein								
RV 144	Live Vector	ALVAC-HIV vCP 1521, expressing env (E), gag/pol (B)	E/B	—	Sanofi Pasteur	Sanofi Pasteur	Thai MOH, DoD/WRAIR, NIAID	Phase III Thailand
	Protein	AIDSVAX gp120 B/E	B/E	Alum	VaxGen	VaxGen		
Peptide								
ANRS VAC18	Peptide	LIPO-5: mix of 5 lipopeptides comprising CTL epitopes from gag, pol, nef	B	Lipopeptides	ANRS	Sanofi Pasteur	ANRS	Phase II France
Protein								
C86p1	Protein	gp140 V2 loop-deleted protein, administered intranasally (IN) and by intramuscular injection (IM)	B	LTK63 (heat-labile enterotoxin from E. coli.) (for IN administration) MF59 (for IM administration)	Novartis Vaccines	Novartis Vaccines	St George's Vaccine Institute, University of London, Richmond Pharmacology Ltd, Commission of the European Union	Phase I U.K.
HVRF-380-131004	Protein	VIRCHEPOL: chimeric recombinant env (gp41), gag (p17, p24) protein	B	Polyoxidonium	Ivanovsky Institute of Virology	Moscow Institute of Immunology	Russian Federation Ministry of Education and Science	Phase I Russia

ANRS: National Agency for Research on AIDS (France)
CDC: Centers for Disease Control and Prevention
DoD: Department of Defense
IAVI: International AIDS Vaccine Initiative
NIAID/HVTN: National Institute of Allergy and Infectious Diseases/HIV Vaccine Trials Network
NIAID VRC: National Institute of Allergy and Infectious Diseases Vaccine Research Center
Thai MOH: Thai Ministry of Health
WRAIR: Walter Reed Army Institute of Research

APPENDIX E

MMWR™
QuickGuide

Recommended Immunization Schedules for Persons Aged 0–18 Years — United States, 2007

Weekly January 5, 2007 / Vol. 55 / Nos. 51 & 52

The Advisory Committee on Immunization Practices (ACIP) periodically reviews the recommended immunization schedule for persons aged 0–18 years to ensure that the schedule is current with changes in vaccine formulations and reflects revised recommendations for the use of licensed vaccines, including those newly licensed.

The changes to the previous childhood and adolescent immunization schedule, published January 2006 (*1*), are as follows:

- The new rotavirus vaccine (Rota) is recommended in a 3-dose schedule at ages 2, 4, and 6 months. The first dose should be administered at ages 6 weeks through 12 weeks with subsequent doses administered at 4–10 week intervals. Rotavirus vaccination should not be initiated for infants aged >12 weeks and should not be administered after age 32 weeks (*2*).
- The influenza vaccine is now recommended for all children aged 6–59 months (*3*).
- Varicella vaccine recommendations are updated. The first dose should be administered at age 12–15 months, and a newly recommended second dose should be administered at age 4–6 years (*4*).
- The new human papillomavirus vaccine (HPV) is recommended in a 3-dose schedule with the second and third doses administered 2 and 6 months after the first dose. Routine vaccination with HPV is recommended for females aged 11–12 years; the vaccination series can be started in females as young as age 9 years; and a catch-up vaccination is recommended for females aged 13–26 years who have not been vaccinated previously or who have not completed the full vaccine series (*5*).
- The main change to the format of the schedule is the division of the recommendation into two schedules: one schedule for persons aged 0–6 years (Figure 1) and another for persons aged 7–18 years (Figure 2). Special populations are represented with purple bars; the 11–12 years assessment is emphasized with the bold, capitalized fonts in the title of that column. Rota, HPV, and varicella vaccines are incorporated in the catch-up immunization schedule (Table).

The recommended immunization schedules for persons aged 0–18 years and the catch-up immunization schedule for 2007 have been approved by the Advisory Committee on Immunization Practices, the American Academy of Pediatrics, and the American Academy of Family Physicians. The standard *MMWR* footnote format has been modified for publication of this schedule.

Suggested citation: Centers for Disease Control and Prevention. Recommended immunization schedules for persons aged 0–18 years—United States, 2007. MMWR 2006;55(51&52):Q1–Q4.

Vaccine Information Statements

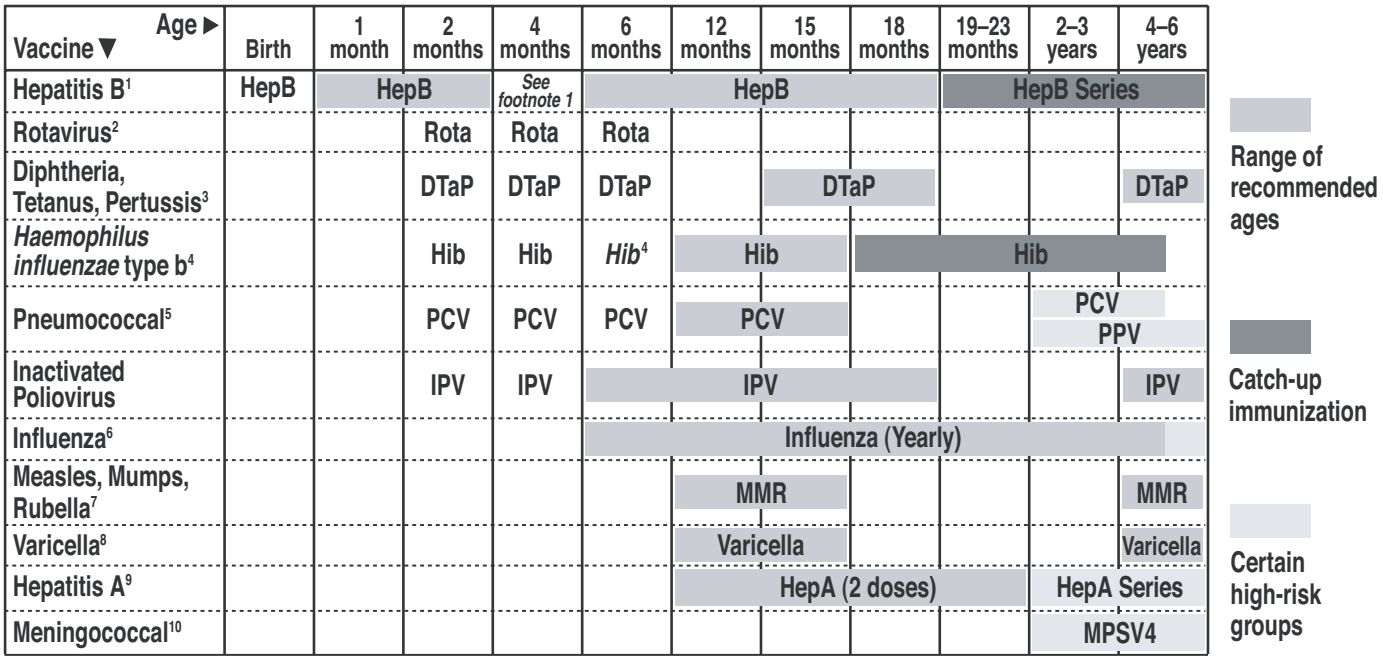
The National Childhood Vaccine Injury Act requires that health-care providers provide parents or patients with copies of Vaccine Information Statements before administering each dose of the vaccines listed in the schedule. Additional information is available from state health departments and from CDC at http://www.cdc.gov/nip/publications/vis.

Detailed recommendations for using vaccines are available from package inserts, ACIP statements on specific vaccines, and the *2003 Red Book* (*6*). ACIP statements for each recommended childhood vaccine are available from CDC at http://www.cdc.gov/nip/publications/acip-list.htm. In addition, guidance for obtaining and completing a Vaccine Adverse Event Reporting System form is available at http://www.vaers.hhs.gov or by telephone, 800-822-7967.

References

1. CDC. Recommended childhood and adolescent immunization schedule—United States. MMWR 2006;54(52):Q1–Q4.
2. CDC. Prevention of rotavirus gastroenteritis among infants and children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 2006;55(No. RR-12):1–13.
3. CDC. Prevention and control of influenza. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 2006;55(No. RR-10):1–42.
4. CDC. ACIP provisional recommendations for the prevention of varicella. Available at http://www.cdc.gov/nip/vaccine/varicella/varicella_acip_recs_prov_june_2006.pdf.
5. CDC. ACIP provisional recommendations for the use of quadrivalent HPV vaccine. Available at http://www.cdc.gov/nip/recs/provisional_recs/hpv.pdf.
6. American Academy of Pediatrics. Active and passive immunization. In: Pickering LK, ed. 2003 red book: report of the Committee on Infectious Diseases. 26th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2003.

FIGURE 1. Recommended immunization schedule for persons aged 0–6 years — United States, 2007



This schedule indicates the recommended ages for routine administration of currently licensed childhood vaccines, as of December 1, 2006, for children aged 0–6 years. Additional information is available at http://www.cdc.gov/nip/recs/child-schedule.htm. Any dose not administered at the recommended age should be administered at any subsequent visit, when indicated and feasible. Additional vaccines may be licensed and recommended during the year. Licensed combination vaccines may be used whenever any components of the combination are indicated and other components of the vaccine are not contraindicated and if approved by the Food and Drug Administration for that dose of the series. Providers should consult the respective Advisory Committee on Immunization Practices statement for detailed recommendations. Clinically significant adverse events that follow immunization should be reported to the Vaccine Adverse Event Reporting System (VAERS). Guidance about how to obtain and complete a VAERS form is available at http://www.vaers.hhs.gov or by telephone, 800-822-7967.

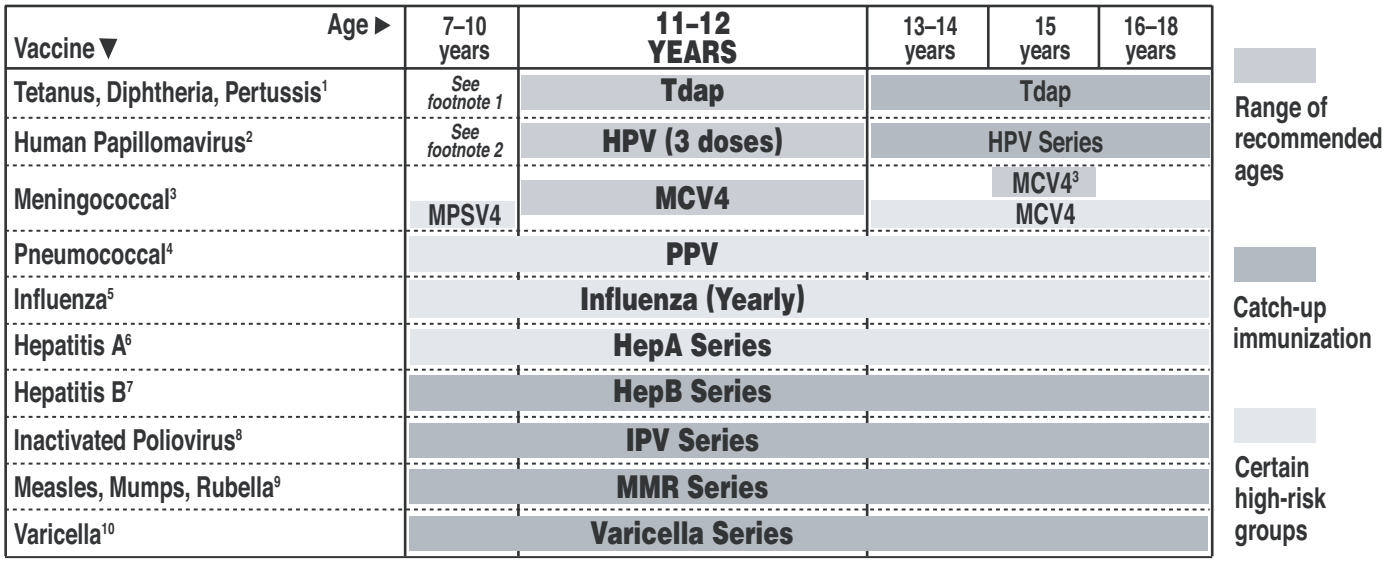
1. Hepatitis B vaccine (HepB). (Minimum age: birth)
   At birth:
   - Administer monovalent HepB to all newborns before hospital discharge.
   - If mother is hepatitis surface antigen (HBsAg)-positive, administer HepB and 0.5 mL of hepatitis B immune globulin (HBIG) within 12 hours of birth.
   - If mother's HBsAg status is unknown, administer HepB within 12 hours of birth. Determine the HBsAg status as soon as possible and if HBsAg-positive, administer HBIG (no later than age 1 week).
   - If mother is HBsAg-negative, the birth dose can only be delayed with physician's order and mothers' negative HBsAg laboratory report documented in the infant's medical record.

   After the birth dose:
   - The HepB series should be completed with either monovalent HepB or a combination vaccine containing HepB. The second dose should be administered at age 1–2 months. The final dose should be administered at age ≥24 weeks. Infants born to HBsAg-positive mothers should be tested for HBsAg and antibody to HBsAg after completion of ≥3 doses of a licensed HepB series, at age 9–18 months (generally at the next well-child visit).

   4-month dose:
   - It is permissible to administer 4 doses of HepB when combination vaccines are administered after the birth dose. If monovalent HepB is used for doses after the birth dose, a dose at age 4 months is not needed.
2. Rotavirus vaccine (Rota). (Minimum age: 6 weeks)
   - Administer the first dose at age 6–12 weeks. Do not start the series later than age 12 weeks.
   - Administer the final dose in the series by age 32 weeks. Do not administer a dose later than age 32 weeks.
   - Data on safety and efficacy outside of these age ranges are insufficient.
3. Diphtheria and tetanus toxoids and acellular pertussis vaccine (DTaP). (Minimum age: 6 weeks)
   - The fourth dose of DTaP may be administered as early as age 12 months, provided 6 months have elapsed since the third dose.
   - Administer the final dose in the series at age 4–6 years.
4. Haemophilus influenzae type b conjugate vaccine (Hib). (Minimum age: 6 weeks)
   - If PRP-OMP (PedvaxHIB® or ComVax® [Merck]) is administered at ages 2 and 4 months, a dose at age 6 months is not required.
   - TriHiBit® (DTaP/Hib) combination products should not be used for primary immunization but can be used as boosters following any Hib vaccine in children aged ≥12 months.
5. Pneumococcal vaccine. (Minimum age: 6 weeks for pneumococcal conjugate vaccine [PCV]; 2 years for pneumococcal polysaccharide vaccine [PPV])
   - Administer PCV at ages 24–59 months in certain high-risk groups. Administer PPV to children aged ≥2 years in certain high-risk groups. See MMWR 2000;49(No. RR-9):1–35.
6. Influenza vaccine. (Minimum age: 6 months for trivalent inactivated influenza vaccine [TIV]; 5 years for live, attenuated influenza vaccine [LAIV])
   - All children aged 6–59 months and close contacts of all children aged 0–59 months are recommended to receive influenza vaccine.
   - Influenza vaccine is recommended annually for children aged ≥59 months with certain risk factors, health-care workers, and other persons (including household members) in close contact with persons in groups at high risk. See MMWR 2006;55(No. RR-10):1–41.
   - For healthy persons aged 5–49 years, LAIV may be used as an alternative to TIV.
   - Children receiving TIV should receive 0.25 mL if aged 6–35 months or 0.5 mL if aged ≥3 years.
   - Children aged <9 years who are receiving influenza vaccine for the first time should receive 2 doses (separated by ≥4 weeks for TIV and ≥6 weeks for LAIV).
7. Measles, mumps, and rubella vaccine (MMR). (Minimum age: 12 months)
   - Administer the second dose of MMR at age 4–6 years. MMR may be administered before age 4–6 years, provided ≥4 weeks have elapsed since the first dose and both doses are administered at age ≥12 months.
8. Varicella vaccine. (Minimum age: 12 months)
   - Administer the second dose of varicella vaccine at age 4–6 years. Varicella vaccine may be administered before age 4–6 years, provided that ≥3 months have elapsed since the first dose and both doses are administered at age ≥12 months. If second dose was administered ≥28 days following the first dose, the second dose does not need to be repeated.
9. Hepatitis A vaccine (HepA). (Minimum age: 12 months)
   - HepA is recommended for all children aged 1 year (i.e., aged 12–23 months). The 2 doses in the series should be administered at least 6 months apart.
   - Children not fully vaccinated by age 2 years can be vaccinated at subsequent visits.
   - HepA is recommended for certain other groups of children, including in areas where vaccination programs target older children. See MMWR 2006;55(No. RR-7):1–23.
10. Meningococcal polysaccharide vaccine (MPSV4). (Minimum age: 2 years)
    - Administer MPSV4 to children aged 2–10 years with terminal complement deficiencies or anatomic or functional asplenia and certain other high-risk groups. See MMWR 2005;54(No. RR-7):1–21.

The Recommended Immunization Schedules for Persons Aged 0–18 Years are approved by the Advisory Committee on Immunization Practices (http://www.cdc.gov/nip/acip), the American Academy of Pediatrics (http://www.aap.org), and the American Academy of Family Physicians (http://www.aafp.org).

FIGURE 2. Recommended immunization schedule for persons aged 7–18 years — United States, 2007



This schedule indicates the recommended ages for routine administration of currently licensed childhood vaccines, as of December 1, 2006, for children aged 7–18 years. Additional information is available at http://www.cdc.gov/nip/recs/child-schedule.htm. Any dose not administered at the recommended age should be administered at any subsequent visit, when indicated and feasible. Additional vaccines may be licensed and recommended during the year. Licensed combination vaccines may be used whenever any components of the combination are indicated and other components of the vaccine are not contraindicated and if approved by the Food and Drug Administration for that dose of the series. Providers should consult the respective Advisory Committee on Immunization Practices statement for detailed recommendations. Clinically significant adverse events that follow immunization should be reported to the Vaccine Adverse Event Reporting System (VAERS). Guidance about how to obtain and complete a VAERS form is available at http://www.vaers.hhs.gov or by telephone, 800-822-7967.

1. Tetanus and diphtheria toxoids and acellular pertussis vaccine (Tdap). (Minimum age: 10 years for BOOSTRIX® and 11 years for ADACEL™)
   - Administer at age 11–12 years for those who have completed the recommended childhood DTP/DTaP vaccination series and have not received a tetanus and diphtheria toxoids vaccine (Td) booster dose.
   - Adolescents aged 13–18 years who missed the 11–12 year Td/Tdap booster dose should also receive a single dose of Tdap if they have completed the recommended childhood DTP/DTaP vaccination series.
2. Human papillomavirus vaccine (HPV). (Minimum age: 9 years)
   - Administer the first dose of the HPV vaccine series to females at age 11–12 years.
   - Administer the second dose 2 months after the first dose and the third dose 6 months after the first dose.
   - Administer the HPV vaccine series to females at age 13–18 years if not previously vaccinated.
3. Meningococcal vaccine. (Minimum age: 11 years for meningococcal conjugate vaccine [MCV4]; 2 years for meningococcal polysaccharide vaccine [MPSV4])
   - Administer MCV4 at age 11–12 years and to previously unvaccinated adolescents at high school entry (at approximately age 15 years).
   - Administer MCV4 to previously unvaccinated college freshmen living in dormitories; MPSV4 is an acceptable alternative.
   - Vaccination against invasive meningococcal disease is recommended for children and adolescents aged ≥2 years with terminal complement deficiencies or anatomic or functional asplenia and certain other high-risk groups. See MMWR 2005;54(No. RR-7):1–21. Use MPSV4 for children aged 2–10 years and MCV4 or MPSV4 for older children.
4. Pneumococcal polysaccharide vaccine (PPV). (Minimum age: 2 years)
   - Administer for certain high-risk groups. See MMWR 1997;46(No. RR-8):1–24, and MMWR 2000;49(No. RR-9):1–35.
5. Influenza vaccine. (Minimum age: 6 months for trivalent inactivated influenza vaccine [TIV]; 5 years for live, attenuated influenza vaccine [LAIV])
   - Influenza vaccine is recommended annually for persons with certain risk factors, health-care workers, and other persons (including household members) in close contact with persons in groups at high risk. See MMWR 2006;55 (No. RR-10):1–41.
   - For healthy persons aged 5–49 years, LAIV may be used as an alternative to TIV.
   - Children aged <9 years who are receiving influenza vaccine for the first time should receive 2 doses (separated by ≥4 weeks for TIV and ≥6 weeks for LAIV).
6. Hepatitis A vaccine (HepA). (Minimum age: 12 months)
   - The 2 doses in the series should be administered at least 6 months apart.
   - HepA is recommended for certain other groups of children, including in areas where vaccination programs target older children. See MMWR 2006;55 (No. RR-7):1–23.
7. Hepatitis B vaccine (HepB). (Minimum age: birth)
   - Administer the 3-dose series to those who were not previously vaccinated.
   - A 2-dose series of Recombivax HB® is licensed for children aged 11–15 years.
8. Inactivated poliovirus vaccine (IPV). (Minimum age: 6 weeks)
   - For children who received an all-IPV or all-oral poliovirus (OPV) series, a fourth dose is not necessary if the third dose was administered at age ≥4 years.
   - If both OPV and IPV were administered as part of a series, a total of 4 doses should be administered, regardless of the child's current age.
9. Measles, mumps, and rubella vaccine (MMR). (Minimum age: 12 months)
   - If not previously vaccinated, administer 2 doses of MMR during any visit, with ≥4 weeks between the doses.
10. Varicella vaccine. (Minimum age: 12 months)
    - Administer 2 doses of varicella vaccine to persons without evidence of immunity.
    - Administer 2 doses of varicella vaccine to persons aged ≤13 years at least 3 months apart. Do not repeat the second dose, if administered ≥28 days after the first dose.
    - Administer 2 doses of varicella vaccine to persons aged ≥13 years at least 4 weeks apart.

The Recommended Immunization Schedules for Persons Aged 0–18 Years are approved by the Advisory Committee on Immunization Practices (http://www.cdc.gov/nip/acip), the American Academy of Pediatrics (http://www.aap.org), and the American Academy of Family Physicians (http://www.aafp.org).

TABLE. Catch-up immunization schedule for persons aged 4 months–18 years who start late or who are ≥1 month behind — United States, 2007

The table below provides catch-up schedules and minimum intervals between doses for children whose vaccinations have been delayed. A vaccine series does not need to be restarted, regardless of the time that has elapsed between doses. Use the section appropriate for the child's age.

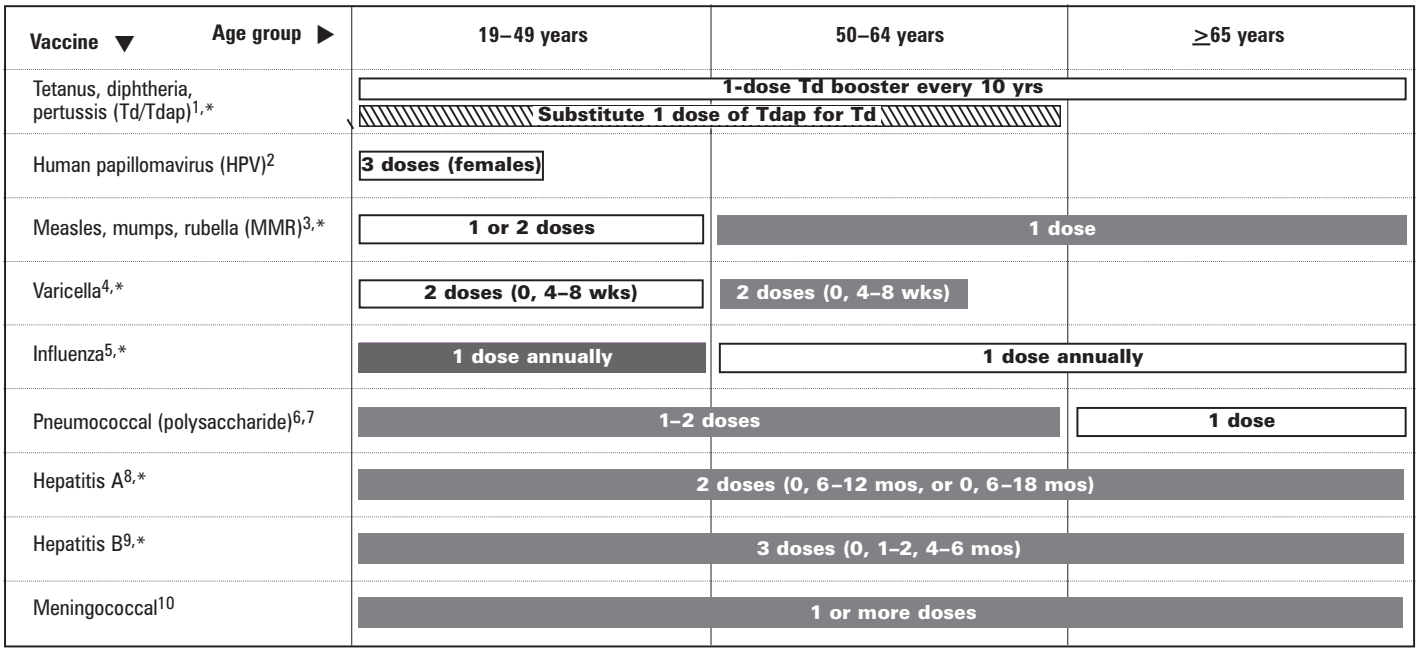
Vaccine	Minimum age for Dose 1	Minimum interval between doses			
		Dose 1 to Dose 2	Dose 2 to Dose 3	Dose 3 to Dose 4	Dose 4 to Dose 5
CATCH-UP SCHEDULE FOR PERSONS AGED 4 MONTHS–6 YEARS					
Hepatitis B[1]	Birth	4 weeks	8 weeks (and 16 weeks after first dose)		
Rotavirus[2]	6 weeks	4 weeks	4 weeks		
Diphtheria, Tetanus, Pertussis[3]	6 weeks	4 weeks	4 weeks	6 months	6 months[3]
Haemophilus influenzae type b[4]	6 weeks	4 weeks if first dose administered at age <12 months; 8 weeks (as final dose) if first dose administered at age 12–14 months; No further doses needed if first dose administered at age ≥15 months	4 weeks[4] if current age <12 months; 8 weeks (as final dose)[4] if current age ≥12 months and second dose administered at age <15 months; No further doses needed if previous dose administered at age ≥15 months	8 weeks (as final dose) This dose only necessary for children aged 12 months–5 years who received 3 doses before age 12 months	
Pneumococcal[5]	6 weeks	4 weeks if first dose administered at age <12 months and current age <24 months; 8 weeks (as final dose) if first dose administered at age ≥12 months or current age 24–59 months; No further doses needed for healthy children if first dose administered at age ≥24 months	4 weeks if current age <12 months; 8 weeks (as final dose) if current age ≥12 months; No further doses needed for healthy children if previous dose administered at age ≥24 months	8 weeks (as final dose) This dose only necessary for children aged 12 months–5 years who received 3 doses before age 12 months	
Inactivated Poliovirus[6]	6 weeks	4 weeks	4 weeks	4 weeks[6]	
Measles, Mumps, Rubella[7]	12 months	4 weeks			
Varicella[8]	12 months	3 months			
Hepatitis A[9]	12 months	6 months			
CATCH-UP SCHEDULE FOR PERSONS AGED 7–18 YEARS					
Tetanus, Diphtheria/ Tetanus, Diphtheria, Pertussis[10]	7 years[10]	4 weeks	8 weeks if first dose administered at age <12 months; 6 months if first dose administered at age ≥12 months	6 months if first dose administered at age <12 months	
Human Papillomavirus[11]	9 years	4 weeks	12 weeks		
Hepatitis A[9]	12 months	6 months			
Hepatitis B[1]	Birth	4 weeks	8 weeks (and 16 weeks after first dose)		
Inactivated Poliovirus[6]	6 weeks	4 weeks	4 weeks	4 weeks[6]	
Measles, Mumps, Rubella[7]	12 months	4 weeks			
Varicella[8]	12 months	4 weeks if first dose administered at age ≥13 years; 3 months if first dose administered at age <13 years			

1. Hepatitis B vaccine (HepB). (Minimum age: birth)
   - Administer the 3-dose series to those who were not previously vaccinated.
   - A 2-dose series of Recombivax HB® is licensed for children aged 11–15 years.
2. Rotavirus vaccine (Rota). (Minimum age: 6 weeks)
   - Do not start the series later than age 12 weeks.
   - Administer the final dose in the series by age 32 weeks. Do not administer a dose later than age 32 weeks.
   - Data on safety and efficacy outside of these age ranges are insufficient.
3. Diphtheria and tetanus toxoids and acellular pertussis vaccine (DTaP). (Minimum age: 6 weeks)
   - The fifth dose is not necessary if the fourth dose was administered at age ≥4 years.
   - DTaP is not indicated for persons aged ≥7 years.
4. Haemophilus influenzae type b conjugate vaccine (Hib). (Minimum age: 6 weeks)
   - Vaccine is not generally recommended for children aged ≥5 years.
   - If current age <12 months and the first 2 doses were PRP-OMP (PedvaxHIB® or ComVax® [Merck]), the third (and final) dose should be administered at age 12–15 months and at least 8 weeks after the second dose.
   - If first dose was administered at age 7–11 months, administer 2 doses separated by 4 weeks plus a booster at age 12–15 months.
5. Pneumococcal conjugate vaccine (PCV). (Minimum age: 6 weeks)
   - Vaccine is not generally recommended for children aged ≥5 years.
6. Inactivated poliovirus vaccine (IPV). (Minimum age: 6 weeks)
   - For children who received an all-IPV or all-oral poliovirus (OPV) series, a fourth dose is not necessary if third dose was administered at age ≥4 years.
   - If both OPV and IPV were administered as part of a series, a total of 4 doses should be administered, regardless of the child's current age.
7. Measles, mumps, and rubella vaccine (MMR). (Minimum age: 12 months)
   - The second dose of MMR is recommended routinely at age 4–6 years but may be administered earlier if desired.
   - If not previously vaccinated, administer 2 doses of MMR during any visit with ≥4 weeks between the doses.
8. Varicella vaccine. (Minimum age: 12 months)
   - The second dose of varicella vaccine is recommended routinely at age 4–6 years but may be administered earlier if desired.
   - Do not repeat the second dose in persons aged <13 years if administered ≥28 days after the first dose.
9. Hepatitis A vaccine (HepA). (Minimum age: 12 months)
   - HepA is recommended for certain groups of children, including in areas where vaccination programs target older children. See MMWR 2006;55(No. RR-7):1–23.
10. Tetanus and diphtheria toxoids vaccine (Td) and tetanus and diphtheria toxoids and acellular pertussis vaccine (Tdap). (Minimum ages: 7 years for Td, 10 years for BOOSTRIX®, and 11 years for ADACEL™)
    - Tdap should be substituted for a single dose of Td in the primary catch-up series or as a booster if age appropriate; use Td for other doses.
    - A 5-year interval from the last Td dose is encouraged when Tdap is used as a booster dose. A booster (fourth) dose is needed if any of the previous doses were administered at age <12 months. Refer to ACIP recommendations for further information. See MMWR 2006;55(No. RR-3).
11. Human papillomavirus vaccine (HPV). (Minimum age: 9 years)
    - Administer the HPV vaccine series to females at age 13–18 years if not previously vaccinated.

Information about reporting reactions after immunization is available online at http://www.vaers.hhs.gov or by telephone via the 24-hour national toll-free information line 800-822-7967. Suspected cases of vaccine-preventable diseases should be reported to the state or local health department. Additional information, including precautions and contraindications for immunization, is available from the National Center for Immunization and Respiratory Diseases at http://www.cdc.gov/nip/default.htm or telephone, 800-CDC-INFO (800-232-4636).

APPENDIX F

Recommended Adult Immunization Schedule, by Vaccine and Age Group
UNITED STATES • OCTOBER 2006–SEPTEMBER 2007



*Covered by the Vaccine Injury Compensation Program. NOTE: These recommendations must be read with the footnotes (see reverse).

For all persons in this category who meet the age requirements and who lack evidence of immunity (e.g., lack documentation of vaccination or have no evidence of prior infection)

Recommended if some other risk factor is present (e.g., on the basis of medical, occupational, lifestyle, or other indications)

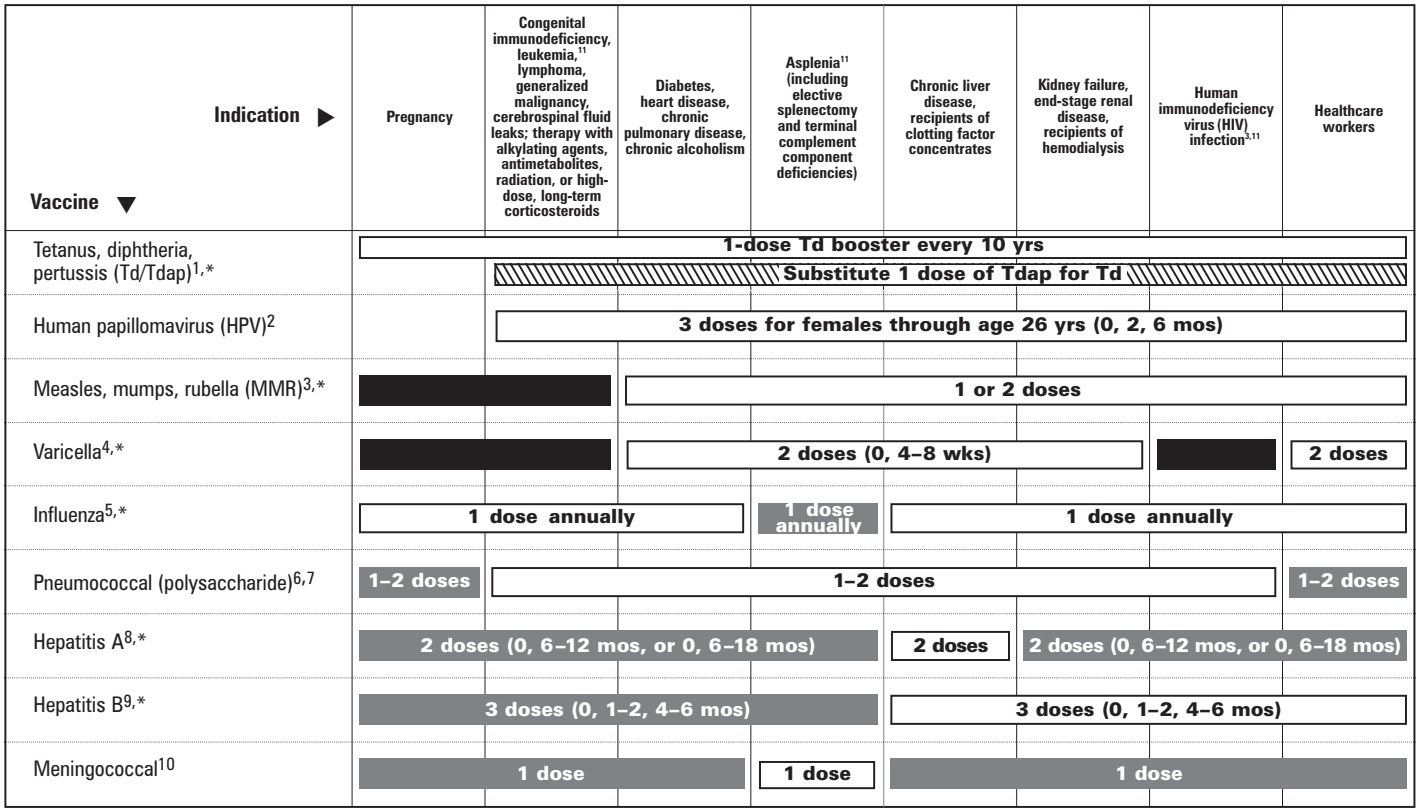
This schedule indicates the recommended age groups and medical indications for routine administration of currently licensed vaccines for persons aged ≥19 years, as of October 1, 2006. Licensed combination vaccines may be used whenever any components of the combination are indicated and when the vaccine's other components are not contraindicated. For detailed recommendations on all vaccines, including those used primarily for travelers or that are issued during the year, consult the manufacturers' package inserts and the complete statements from the Advisory Committee on Immunization Practices (www.cdc.gov/nip/publications/acip-list.htm).

Report all clinically significant postvaccination reactions to the Vaccine Adverse Event Reporting System (VAERS). Reporting forms and instructions on filing a VAERS report are available at www.vaers.hhs.gov or by telephone, 800-822-7967.

Information on how to file a Vaccine Injury Compensation Program claim is available at www.hrsa.gov/vaccinecompensation or by telephone, 800-338-2382. To file a claim for vaccine injury, contact the U.S. Court of Federal Claims, 717 Madison Place, N.W., Washington, D.C. 20005; telephone, 202-357-6400.

Additional information about the vaccines in this schedule and contraindications for vaccination is also available at www.cdc.gov/nip or from the CDC-INFO Contact Center at 800-CDC-INFO (800-232-4636) in English and Spanish, 24 hours a day, 7 days a week.

Recommended Adult Immunization Schedule, by Vaccine and Medical and Other Indications
UNITED STATES • OCTOBER 2006–SEPTEMBER 2007



*Covered by the Vaccine Injury Compensation Program. NOTE: These recommendations must be read with the footnotes (see reverse).

For all persons in this category who meet the age requirements and who lack evidence of immunity (e.g., lack documentation of vaccination or have no evidence of prior infection)

Recommended if some other risk factor is present (e.g., on the basis of medical, occupational, lifestyle, or other indications)

Contraindicated

Approved by
the Advisory Committee on Immunization Practices,
the American College of Obstetricians and Gynecologists,
the American Academy of Family Physicians,
and the American College of Physicians

DEPARTMENT OF HEALTH AND HUMAN SERVICES
CENTERS FOR DISEASE CONTROL AND PREVENTION

Footnotes
Recommended Adult Immunization Schedule • UNITED STATES, OCTOBER 2006–SEPTEMBER 2007

1. Tetanus, diphtheria, and acellular pertussis (Td/Tdap) vaccination. Adults with uncertain histories of a complete primary vaccination series with diphtheria and tetanus toxoid–containing vaccines should begin or complete a primary vaccination series. A primary series for adults is 3 doses; administer the first 2 doses at least 4 weeks apart and the third dose 6–12 months after the second. Administer a booster dose to adults who have completed a primary series and if the last vaccination was received ≥10 years previously. Tdap or tetanus and diphtheria (Td) vaccine may be used; Tdap should replace a single dose of Td for adults aged <65 years who have not previously received a dose of Tdap (either in the primary series, as a booster, or for wound management). Only one of two Tdap products (Adacel® [sanofi pasteur]) is licensed for use in adults. If the person is pregnant and received the last Td vaccination ≥10 years previously, administer Td during the second or third trimester; if the person received the last Td vaccination in <10 years, administer Tdap during the immediate postpartum period. A one-time administration of 1 dose of Tdap with an interval as short as 2 years from a previous Td vaccination is recommended for postpartum women, close contacts of infants aged <12 months, and all healthcare workers with direct patient contact. In certain situations, Td can be deferred during pregnancy and Tdap substituted in the immediate postpartum period, or Tdap can be given instead of Td to a pregnant woman after an informed discussion with the woman (see www.cdc.gov/nip/publications/acip-list.htm). Consult the ACIP statement for recommendations for administering Td as prophylaxis in wound management (www.cdc.gov/mmwr/preview/mmwrhtml/00041645.htm).

2. Human papillomavirus (HPV) vaccination. HPV vaccination is recommended for all women aged ≤26 years who have not completed the vaccine series. Ideally, vaccine should be administered before potential exposure to HPV through sexual activity; however, women who are sexually active should still be vaccinated. Sexually active women who have not been infected with any of the HPV vaccine types receive the full benefit of the vaccination. Vaccination is less beneficial for women who have already been infected with one or more of the four HPV vaccine types. A complete series consists of 3 doses. The second dose should be administered 2 months after the first dose; the third dose should be administered 6 months after the first dose. Vaccination is not recommended during pregnancy. If a woman is found to be pregnant after initiating the vaccination series, the remainder of the 3-dose regimen should be delayed until after completion of the pregnancy.

3. Measles, mumps, rubella (MMR) vaccination. *Measles component:* adults born before 1957 can be considered immune to measles. Adults born during or after 1957 should receive ≥1 dose of MMR unless they have a medical contraindication, documentation of ≥1 dose, history of measles based on healthcare provider diagnosis, or laboratory evidence of immunity. A second dose of MMR is recommended for adults who 1) have been recently exposed to measles or in an outbreak setting; 2) have been previously vaccinated with killed measles vaccine; 3) have been vaccinated with an unknown type of measles vaccine during 1963–1967; 4) are students in postsecondary educational institutions; 5) work in ahealthcare facility; or 6) plan to travel internationally. Withhold MMR or other measles-containing vaccines from HIV-infected persons with severe immunosuppression.

Mumps component: adults born before 1957 can generally be considered immune to mumps. Adults born during or after 1957 should receive 1 dose of MMR unless they have a medical contraindication, history of mumps based on healthcare provider diagnosis, or laboratory evidence of immunity. A second dose of MMR is recommended for adults who 1) are in an age group that is affected during a mumps outbreak; 2) are students in postsecondary educational institutions; 3) work in a healthcare facility; or 4) plan to travel internationally. For unvaccinated healthcare workers born before 1957 who do not have other evidence of mumps immunity, consider giving 1 dose on a routine basis and strongly consider giving a second dose during an outbreak. *Rubella component:* administer 1 dose of MMR vaccine to women whose rubella vaccination history is unreliable or who lack laboratory evidence of immunity. For women of childbearing age, regardless of birth year, routinely determine rubella immunity and counsel women regarding congenital rubella syndrome. Do not vaccinate women who are pregnant or who might become pregnant within 4 weeks of receiving vaccine. Women who do not have evidence of immunity should receive MMR vaccine upon completion or termination of pregnancy and before discharge from the healthcare facility.

4. Varicella vaccination. All adults without evidence of immunity to varicella should receive 2 doses of varicella vaccine. Special consideration should be given to those who 1) have close contact with persons at high risk for severe disease (e.g., healthcare workers and family contacts of immunocompromised persons) or 2) are at high risk for exposure or transmission (e.g., teachers of young children; child care employees; residents and staff members of institutional settings, including correctional institutions; college students; military personnel; adolescents and adults living in households with children; nonpregnant women of childbearing age; and international travelers). Evidence of immunity to varicella in adults includes any of the following: 1) documentation of 2 doses of varicella vaccine at least 4 weeks apart; 2) U.S.-born before 1980 (although for healthcare workers and pregnant women, birth before 1980 should not be considered evidence of immunity); 3) history of varicella based on diagnosis or verification of varicella by a healthcare provider (for a patient reporting a history of or presenting with an atypical case, a mild case, or both, healthcare providers should seek either an epidemiologic link with a typical varicella case or evidence of laboratory confirmation, if it was performed at the time of acute disease); 4) history of herpes zoster based on healthcare provider diagnosis; or 5) laboratory evidence of immunity or laboratory confirmation of disease. Do not vaccinate women who are pregnant or might become pregnant within 4 weeks of receiving the vaccine. Assess pregnant women for evidence of varicella immunity. Women who do not have evidence of immunity should receive dose 1 of varicella vaccine upon completion or termination of pregnancy and before discharge from the healthcare facility. Dose 2 should be administered 4–8 weeks after dose 1.

5. Influenza vaccination. *Medical indications:* chronic disorders of the cardiovascular or pulmonary systems, including asthma; chronic metabolic diseases, including diabetes mellitus, renal dysfunction, hemoglobinopathies, or immunosuppression (including immunosuppression caused by medications or HIV); any condition that compromises respiratory function or the handling of respiratory secretions or that can increase the risk of aspiration (e.g., cognitive dysfunction, spinal cord injury, or seizure disorder or other neuromuscular disorder); and pregnancy during the influenza season. No data exist on the risk for severe or complicated influenza disease among persons with asplenia; however, influenza is a risk factor for secondary bacterial infections that can cause severe disease among persons with asplenia. *Occupational indications:* healthcare workers and employees of long-term–care and assisted living facilities. *Other indications:* residents of nursing homes and other long-term–care and assisted living facilities; persons likely to transmit influenza to persons at high risk (e.g., in-home household contacts and caregivers of children aged 0–59 months, or persons of all ages with high-risk conditions); and anyone who would like to be vaccinated. Healthy, nonpregnant persons aged 5–49 years without high-risk medical conditions who are not contacts of severely immunocompromised persons in special care units can receive either intranasally administered influenza vaccine (FluMist®) or inactivated vaccine. Other persons should receive the inactivated vaccine.

6. Pneumococcal polysaccharide vaccination. *Medical indications:* chronic disorders of the pulmonary system (excluding asthma); cardiovascular diseases; diabetes mellitus; chronic liver diseases, including liver disease as a result of alcohol abuse (e.g., cirrhosis); chronic renal failure or nephrotic syndrome; functional or anatomic asplenia (e.g., sickle cell disease or splenectomy [if elective splenectomy is planned, vaccinate at least 2 weeks before surgery]); immunosuppressive conditions (e.g., congenital immunodeficiency, HIV infection [vaccinate as close to diagnosis as possible when CD4 cell counts are highest], leukemia, lymphoma, multiple myeloma, Hodgkin disease, generalized malignancy, or organ or bone marrow transplantation); chemotherapy with alkylating agents, antimetabolites, or high-dose, long-term corticosteroids; and cochlear implants. *Other indications:* Alaska Natives and certain American Indian populations and residents of nursing homes or other long-term–care facilities.

7. Revaccination with pneumococcal polysaccharide vaccine. One-time revaccination after 5 years for persons with chronic renal failure or nephrotic syndrome; functional or anatomic asplenia (e.g., sickle cell disease or splenectomy); immunosuppressive conditions (e.g., congenital immunodeficiency, HIV infection, leukemia, lymphoma, multiple myeloma, Hodgkin disease, generalized malignancy, or organ or bone marrow transplantation); or chemotherapy with alkylating agents, antimetabolites, or high-dose, long-term corticosteroids. For persons aged ≥65 years, one-time revaccination if they were vaccinated ≥5 years previously and were aged <65 years at the time of primary vaccination.

8. Hepatitis A vaccination. *Medical indications:* persons with chronic liver disease and persons who receive clotting factor concentrates. *Behavioral indications:* men who have sex with men and persons who use illegal drugs. *Occupational indications:* persons working with hepatitis A virus (HAV)–infected primates or with HAV in a research laboratory setting. *Other indications:* persons traveling to or working in countries that have high or intermediate endemicity of hepatitis A (a list of countries is available at www.cdc.gov/travel/diseases.htm) and any person who would like to obtain immunity. Current vaccines should be administered in a 2-dose schedule at either 0 and 6–12 months, or 0 and 6–18 months. If the combined hepatitis A and hepatitis B vaccine is used, administer 3 doses at 0, 1, and 6 months.

9. Hepatitis B vaccination. *Medical indications:* persons with end-stage renal disease, including patients receiving hemodialysis; persons seeking evaluation or treatment for a sexually transmitted disease (STD); persons with HIV infection; persons with chronic liver disease; and persons who receive clotting factor concentrates. *Occupational indications:* healthcare workers and public-safety workers who are exposed to blood or other potentially infectious body fluids. *Behavioral indications:* sexually active persons who are not in a long-term, mutually monogamous relationship (i.e., persons with >1 sex partner during the previous 6 months); current or recent injection-drug users; and men who have sex with men. *Other indications:* household contacts and sex partners of persons with chronic hepatitis B virus (HBV) infection; clients and staff members of institutions for persons with developmental disabilities; all clients of STD clinics; international travelers to countries with high or intermediate prevalence of chronic HBV infection (a list of countries is available at www.cdc.gov/travel/diseases.htm); and any adult seeking protection from HBV infection. Settings where hepatitis B vaccination is recommended for all adults: STD treatment facilities; HIV testing and treatment facilities; facilities providing drug-abuse treatment and prevention services; healthcare settings providing services for injection-drug users or men who have sex with men; correctional facilities; end-stage renal disease programs and facilities for chronic hemodialysis patients; and institutions and nonresidential daycare facilities for persons with developmental disabilities. *Special formulation indications:* for adult patients receiving hemodialysis and other immunocompromised adults, 1 dose of 40 μg/mL (Recombivax HB®) or 2 doses of 20 μg/mL (Engerix-B®).

10. Meningococcal vaccination. *Medical indications:* adults with anatomic or functional asplenia, or terminal complement component deficiencies. *Other indications:* first-year college students living in dormitories; microbiologists who are routinely exposed to isolates of *Neisseria meningitidis*; military recruits; and persons who travel to or live in countries in which meningococcal disease is hyperendemic or epidemic (e.g., the "meningitis belt" of sub-Saharan Africa during the dry season [December–June]), particularly if their contact with local populations will be prolonged. Vaccination is required by the government of Saudi Arabia for all travelers to Mecca during the annual Hajj. Meningococcal conjugate vaccine is preferred for adults with any of the preceding indications who are aged ≤55 years, although meningococcal polysaccharide vaccine (MPSV4) is an acceptable alternative. Revaccination after 5 years might be indicated for adults previously vaccinated with MPSV4 who remain at high risk for infection (e.g., persons residing in areas in which disease is epidemic).

11. Selected conditions for which *Haemophilus influenzae* type b (Hib) vaccine may be used. Hib conjugate vaccines are licensed for children aged 6 weeks–71 months. No efficacy data are available on which to base a recommendation concerning use of Hib vaccine for older children and adults with the chronic conditions associated with an increased risk for Hib disease. However, studies suggest good immunogenicity in patients who have sickle cell disease, leukemia, or HIV infection or who have had splenectomies; administering vaccine to these patients is not contraindicated.

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