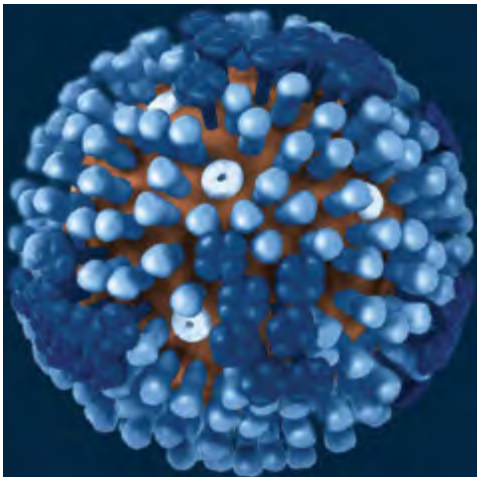

THE JORDAN REPORT

ACCELERATED DEVELOPMENT OF VACCINES **2012**

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

Images on cover, from the top: Courtesy of the US Centers for Disease Control and Prevention; istock.com; Courtesy of the National Library of Medicine; Courtesy of MedImmune

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ADDITIONAL RESOURCES

National Institute of Allergy and Infectious Diseases, www.niaid.nih.gov

Vaccines.gov: your best shot at good health, www.vaccines.gov

Centers for Disease Control and Prevention: Immunization Schedules, www.cdc.gov/vaccines/recs/schedules/

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Foreword to 2012 Jordan Report

Anthony S. Fauci, M.D., National Institute of Allergy and Infectious Diseases, National Institutes of Health

In 1981 the National Institute of Allergy and Infectious Diseases (NIAID) recognized the critical role vaccines have played in preserving human life and human health by initiating the Program for the Accelerated Development of Vaccines. The goal of this program was to build on 20th century vaccine triumphs against such important infectious diseases as diphtheria, measles, pertussis, poliomyelitis, tetanus, yellow fever, and others. Conceived of by NIAID scientist John R. Seal (1912–1984), the new program was ably directed for 6 critical early years by William S. (Bill) Jordan (1917–2008). After retirement in 1987, Dr. Jordan stayed involved with NIAID, and particularly in vaccine research, for another 20 years, time enough to teach and influence a new generation of NIAID scientists who today continue the tradition in support of the vaccine goals first articulated in 1981.

Progress in vaccine development is periodically reviewed and published by NIAID scientists in what has come to be called, with the affection and admiration of his colleagues, *The Jordan Report*. Drs. Seal and Jordan are sadly no longer with us, but after an eventful 30 years the initiative they started remains healthy and vigorous. As discussed in greater depth in this report, progress in vaccine development has moved continuously forward, sometimes leaping ahead while at other times seeming to crawl. Looking back at the challenges of 30 years ago, however, it is undeniable that there have been remarkable achievements. We now have licensed vaccines against *Haemophilus influenzae* type B and pneumococcal types that cause high childhood morbidity and mortality, against hepatitis A and B, against rotaviruses, and against varicella. We also have improved vaccines against such diseases as influenza and pertussis, and passive immunotherapy against respiratory syncytial virus (RSV) in newborns.

In addition to technological advances in making vaccines, including protein conjugation of bacterial polysaccharides, DNA vaccines, viral chimeras, viral vectors, and other novel platforms, the last 30 years also have been characterized by noteworthy advances in delivering vaccines to the developing world. Public health programs, supported by governments and energetic philanthropic foundations, have had extraordinary

Top: A grade school boy, held by a young woman wearing a safety patrol belt, is about to receive an immunization from a nurse (circa 1940). Courtesy of the National Library of Medicine

Bottom: Aerial view of a crowd awaiting polio immunization at a city auditorium in San Antonio, TX (1962). Courtesy of CDC/Mr. Stafford Smith

success in reducing childhood mortality. Poliomyelitis is now on the brink of elimination, and global measles mortality has been markedly reduced in recent years, with eradication a possibility. New vaccines against rotaviruses, which kill half a million children annually, are already having an important beneficial effect in the developing world and promise an enormous impact in reducing mortality in coming years. Vaccines against pneumococci and *Haemophilus influenzae* type B already have saved millions of lives. Clearly, the last 30 years have been a triumph for both vaccine technology and public health disease prevention programs that rely on these vaccines.

Many challenges remain, however. The last decade in particular has seen a discouraging resurgence of anti-vaccine sentiment in the United States and other countries. Fortunately, the proven importance of vaccines increasingly is being articulated in the media, on the Internet, and in other forums by leaders in medicine and public health and by other concerned and informed citizens. An effective HIV vaccine still eludes us. Yet we are making progress, achieving a modest level of protection with a two-step vaccine regimen in a large clinical trial and addressing fundamental issues in HIV vaccinology, such as the identification of neutralizing epitopes on the HIV envelope and use of these epitopes as immunogens through structure-based vaccine design. Progress on vaccines to prevent other high-burden diseases has been frustratingly slow, although here, too, we are moving forward with a growing pipeline of novel vaccines against dengue, malaria, and tuberculosis, among others.

Much has changed globally since the Program for the Accelerated Development of Vaccines was initiated 30 years ago. In that era, one could walk into almost any village in a poor country and see children crippled by poliomyelitis, as well as children only spottily vaccinated with a few intermittently available vaccines or, all too commonly, never vaccinated at all. Such situations still occur, but much less frequently, and the tide seems to be turning rapidly. Vaccines against infectious diseases have become a major component of personal and public health, and indeed of modern human existence, worldwide. In 2010, the Bill & Melinda Gates Foundation called for a Decade of Vaccines to support research, development, and delivery of lifesaving vaccines to the world's poorest nations. NIAID and other global health leaders have joined this initiative, which seeks to dramatically reduce child mortality and save millions of lives by 2020.

Some of the successes we have enjoyed and the challenges we now face are highlighted in this report. After 30 years, it has become clear that vaccines will remain critical to human health for the foreseeable future and that development and deployment of vaccines will remain a key challenge to research, public health, and clinical practice.

People standing in line at a polio immunization station outside a local grocery store in Columbus, Georgia (1961). Courtesy of CDC/Charles N. Farmer

Tribute

Carole A. Heilman, Ph.D., National Institute of Allergy and Infectious Diseases, National Institutes of Health

I would like to dedicate this edition of *The Jordan Report* to the memory of its beloved namesake, Dr. William S. Jordan, who passed away in 2008. With his passing, we lost a man of great vision, brilliance, and goodness. Dr. Jordan was tireless in his quest to improve human health through the development of new and improved vaccines for use against myriad diseases, including many that affect children. He leaves behind a lasting legacy that is boundless in its sheer impact. The change effected by Dr. Jordan has saved the lives of countless people worldwide. His commitment was unwavering as he advocated for the development of vaccines and treatments against all preventable diseases, including neglected tropical diseases and malaria. His leadership and enthusiasm were inspirational to those who were fortunate enough to know or work with him.

Dr. Jordan's distinguished career in the field of preventive medicine spanned more than 60 years as a practicing physician, dedicated teacher, and noted infectious disease researcher. "There are few names in vaccine research as recognizable, and few who have contributed as much to this life-saving field, as William Jordan," said Herman R. Shepherd, founder of the Sabin Institute, when presenting Dr. Jordan with the 2004 Sabin Award. Dr. Jordan's 32-year tenure at the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health, was one of great progress. He was the creator of and chief advocate for a new effort, which he dubbed the "Accelerated Development of Vaccines." He sensed that the advancing pace of discovery would yield many new ideas for vaccines of all kinds.

It was the synergy of new science and the practical application of that science in the form of new vaccines and other interventions that motivated Dr. Jordan and those around him. By creating *The Jordan Report*, Dr. Jordan established what is considered by many in the scientific community to be one of the most complete references available on vaccine research and development today. Simply stated, William S. Jordan was indeed a significant force behind what we now consider modern-day vaccinology. He will be missed.

This report is dedicated to the memory of Dr. William Jordan, a pioneer in vaccine research. Courtesy of Case Western Reserve University Medical School

BIOGRAPHY OF

WILLIAM S. JORDAN, M.D.

Bill had the unique ability to sense what was possible and create opportunities to move the field forward. He will be sorely missed.

—Anthony S. Fauci, M.D., *Director, National Institute of Allergy and Infectious Diseases, NIH*

Dr. William Jordan, a leading vaccine researcher and advocate and former Director of the NIAID Microbiology and Infectious Diseases Program, passed away on March 11, 2008.

Dr. Jordan had a distinguished career in preventive medicine as a physician, teacher, and researcher in infectious diseases. A graduate of the University of North Carolina and, in 1942, Harvard Medical School, Dr. Jordan devoted his professional life to advancing research on infectious diseases and gave impetus to national and global disease prevention strategies by promoting research on vaccine development. His medical research career began in 1947 at the Department of Preventive Medicine at Western Reserve University in Cleveland. There, he played a pivotal role in the landmark Cleveland Family Study, a comprehensive, long-term study that examined illness patterns in families and is considered an epidemiological classic. The study identified respiratory infections and viral gastroenteritis as the most common causes of illness in those families and noted the importance of the family setting on transmission, as summarized in the book *Illness in the Home.* Dr. Jordan's laboratory also contributed advances on pandemic influenza and adenoviruses.

In 1958, Dr. Jordan joined the University of Virginia, where he chaired the Department of Preventive Medicine. He was later honored by the University through the establishment of the William S. Jordan, Jr., Professorship of Medicine in Epidemiology. Dr. Jordan also served as the director of the Armed Forces Epidemiological Board's commission on acute respiratory diseases and later became dean of the University of Kentucky College of Medicine. He spent a sabbatical year at the London School of Hygiene and Tropical Medicine.

From 1976 to 1987, Dr. Jordan served as Director of the Microbiology and Infectious Diseases Program (now the Division of Microbiology and Infectious Diseases) at NIAID. Under Dr. Jordan's direction, vaccines for hepatitis B, *Haemophilus influenzae* type B, and pneumococcal pneumonia became available and major strides were made in influenza vaccine development. After serving as Program Director, Dr. Jordan remained a close and trusted advisor to NIAID for more than two decades. A key part of his mission at NIAID was stimulating vaccine research. He launched NIAID's Program for the Accelerated Development of Vaccines in 1981 and created an internal annual report to review progress in vaccine research—and the report evolved into what is now known as *The Jordan Report.*

IN MEMORY OF DR. ROBERT M. CHANOCK

Dr. Robert M. Chanock, world-renowned virologist and former chief of the Laboratory of Infectious Diseases at the National Institute of Allergy and Infectious Diseases (NIAID), died on July 30, 2010. He was 86 years old.

Dr. Chanock began his research career working under Dr. Albert Sabin at Children's Hospital Research Foundation in Cincinnati in the early 1950s. He joined the NIAID Laboratory of Infectious Diseases in 1957, where he and colleagues were the first to identify and characterize human respiratory syncytial virus (RSV), the most common cause of serious lower respiratory tract disease in infants and children worldwide. He and his research group subsequently developed and brought to Food and Drug Administration (FDA) licensure an antibody to prevent RSV disease in high-risk infants, and they were instrumental in the further development and licensure of the first nasal spray influenza vaccine.

Dr. Chanock and colleagues also discovered the four parainfluenza viruses (important causes of childhood respiratory disease), isolated new strains of rhinovirus and coronavirus (causes of the common cold), and isolated and characterized *Mycoplasma pneumoniae* (a cause of bacterial pneumonia). He and his colleagues helped develop an FDA-approved vaccine against the respiratory pathogen adenovirus and initiated studies on hepatitis viruses and gastroenteritis viruses that led to the development and licensure of vaccines for hepatitis A and rotavirus. Dr. Chanock also began an ambitious program in his laboratory to develop vaccines against dengue fever, which is still ongoing today.

"Dr. Chanock's innumerable contributions to the understanding of viral diseases helped make the world a healthier place for millions of people," said Dr. Anthony S. Fauci. "His work has had a profound impact on many in the scientific community."

IN MEMORY OF DR. EDWIN D. KILBOURNE

Dr. Edwin D. Kilbourne, a virologist who developed a reliable method to manufacture influenza vaccines, died on February 21, 2011. He was 90 years old. Dr. Kilbourne was a principal advisor to the U.S. government on influenza, and his innovations contributed to the development of the annual influenza vaccine.

In 1960, Dr. Kilbourne discovered that after he mixed different strains of influenza that grew readily in eggs, the strains would recombine and create an effective vaccine that would grow rapidly and be tailored to virus strains expected to circulate during a particular influenza season. Dr. Kilbourne's lab was a leader in this novel technology, which produced one of the first genetically engineered vaccines.

Dr. Kilbourne, who spent most of his career as a medical research scientist in New York, was involved in every aspect of preparing vaccines for the influenza season. He taught his pioneering vaccinology techniques to researchers at the National Institutes of Health and elsewhere. Without his efforts, the United States may not have had an annual influenza vaccine—or its development might have been delayed for years or even decades. His contributions to this field are truly immeasurable.

EXPERT ARTICLES

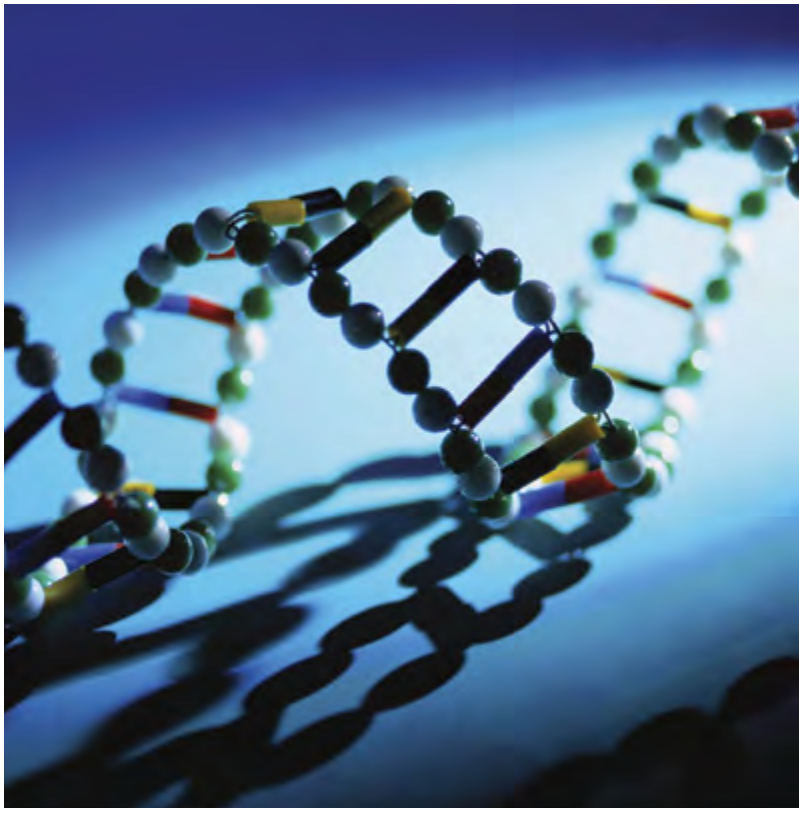
DISCLAIMER *The views expressed in this section are those of the authors and do not reflect the official policy or position of the National Institute of Allergy and Infectious Diseases, the National Institutes of Health, the Department of Health and Human Services, or the U.S. government.*

Vaccinomics and Personalized Vaccinology

Gregory A. Poland, M.D., Inna G. Ovsyannikova, Ph.D. and Robert M. Jacobson, M.D.

Abstract

Vaccines have historically been developed using an empiric approach characterized by an "isolate—inactivate—inject" paradigm. Unfortunately, such an approach has proven ineffective at developing vaccines for hypervariable viruses such as HIV, hepatitis C virus, rhinoviruses, and others that impose a large public health burden. In addition, immunization policy in the United States has, to date, been successful as a population-based approach characterized by a "one size fits all" paradigm. Increasingly it is becoming obvious that, as with drug therapy, interindividual variation in vaccine need, dosing, immunogenicity, and adverse reactions exist. These two issues may be effectively addressed by a new vaccinomics and personalized vaccinology approach we have developed by which new vaccines can be developed and delivered—informed by genotype-phenotype data and new high-dimensional throughput assays and bioinformatics tools that take into account individual and population-level genetic data.



Representation of DNA helix. Courtesy of the National Institute of Environmental Health Sciences

Introduction

The historically successful paradigm for delivering vaccines has been a population-centric public health approach. Because risk of infectious diseases was high, and the risk of vaccine-adverse events perceived to be low, *all* vaccines were essentially recommended to *all* members of the population who did not have a medical contraindication. While successful at a public health level, such a population-centric policy ignored considerations of individual risk of disease and adverse events, individual variations in immune response, and individual variations in dosing and method of administration. This approach mirrored that historically used for drug therapy. All members of the population with disease or symptom "x" were often treated with drug "y" at the same dose. However, pharmacogenomics revealed the need for an individualized approach to drug selection and dosing and, at least in referral centers, genetic testing is now commonly done to determine what oncologic or antidepressant medications to use and at what dose. Increasing amounts of data reveal significant individual variations in drug metabolism, and hence the need to carefully determine the need for, type of, and dosing of a given therapeutic agent. Similar data are now increasingly being generated demonstrating that what is true for drugs is also true for biologics—significant individual variation exists in risk of adverse events and in immune response to a given vaccine. The new biology and rapid advances in genetics and high-throughput technology are moving us toward a more patient-centric approach to the use and development of vaccines.

Our laboratory has termed the study of individual genetic, epigenetic, and other host-factor contributions to variations in immune responses to vaccines as "vaccinomics" [1, 2]. We believe that vaccinomics will lead to a more individualized or personalized approach to both the development and the delivery of vaccines, as explained later in this article. As genetic sequencing technologies generate more and more data at lower cost, databases of immune response and adverse-event vaccine

phenotypes will be studied in association with genotypes, thereby defining the effect of causal genetic variants on vaccine-induced responses. In turn, this information will drive new vaccine development as we better understand how to design and build vaccines at the molecular level, informed by knowing how antigen processing and other immune response gene polymorphisms affect the generation of immune responses. In the near future, it is increasingly likely that we will have advance knowledge of an individual's genotype, allowing us to predict susceptibility to infectious diseases, likelihood of vaccine response, dose(s) needed, best method of vaccine administration, and likelihood of a significant vaccine adverse event.

Why a New Approach?

We can best characterize the approach taken to vaccine development since the time of Edward Jenner, over the last 200 years, as an empirical approach, as contrasted with a new "directed" approach of personalized vaccinology (described later in this article). The empirical approach has worked but now is meeting obstacles that limit its utility. The empirical approach begins with testing presumed immunogenic candidates (often just the inactivated organism), which leads to identifying an agent that with proper formulation and dosing can lead to a host immune response mimicking a protective response to the infectious agent. Given before exposure to that agent, that immune response successfully protects against infection and its pathologic consequences [3]. The empirical approach succeeds when the targeted infectious agent results in such a protective immune response. Of note, this approach does not require us to fully understand the immunological processing and genetic activation/suppression and protein translation that proceed from antigen exposure to immune response [4]. The empirical approach has served us well in terms of eradicating smallpox, controlling rabies, and nearly eliminating poliovirus. The Centers for Disease Control and Prevention (CDC), for example, has recognized routine vaccination against infectious diseases as one of the top public health achievements of the 20th century [5, 6].

However, when the infectious agent fails to generate a durable, effective immune response, the empirical approach falters. Other situations similarly limit the empirical approach [3]. For example, it has failed to provide vaccines against malaria [4], schistosomiasis [7], HIV [8], respiratory syncytial virus (RSV) [8, 9], chlamydia [10, 11], herpes simplex [12], and other communicable diseases that significantly affect public health. A review of some of these failures identifies the following limits to the utility of the empirical approach to vaccine development:

- The natural disease does not provide immunity [3, 12].
- The infection cannot be controlled by neutralizing antibodies (e.g., requires T-cell immunity) [13].
- The period before latency is established is brief, occurring in days to weeks from infection and incorporation into host DNA, allowing little time for vaccination after infection has occurred [3].
- Natural immunity results only from repeated infection [4].
- The immunity resulting from natural disease prevents pathology but fails to prevent the spread of the disease [3].
- Exposure occurs at a time of developmental immunologic immaturity of the host [8].
- Passively transmitted maternal immunity interferes with vaccine response [8].
- The infectious agent and especially its antigens exhibit high levels of genetic variability [8, 13].
- Antibodies formed from vaccination result in non-neutralizing antibodies that fail to protect and may even cause harm. For example, use of inactivated measles and RSV vaccines actually led to more severe disease when exposure to wild virus occurred [14, 15].

Depending on the species of infectious agent, one or more of these barriers have, in some cases, prevented the empirical approach from leading to the development of a successful vaccine. To overcome these barriers, a variety of directed approaches to vaccine development, characterized by a shift in focus to the immunologic mechanisms that underlie host immune response and the genomics and proteomics of the infectious agents, have been devised. We call these directed approaches "vaccinomics" [1, 16].

Furthermore, although the empirical approach to vaccine development may generate serviceable vaccines for the majority of the population, it has become clear that subgroups of individuals will not benefit from a universal approach. Here a personalized vaccinology approach could emerge, and we envision that vaccinomics could provide the science base for it. The following are examples of situations in which universal vaccines developed through the empirical approach are insufficient:

- The individual lacks sufficient immunity to respond to a live, albeit attenuated, vaccine (e.g., infants suffering from malnourishment or HIV) [17].
- The individual lacks sufficient baseline immunity to safely receive a live, albeit attenuated, vaccine (e.g., infants suffering from malnourishment or HIV, leaving them at risk for unchecked infection from the licensed forms of measles vaccine).
- The individual has a condition other than an immunocompromising illness that is associated with poor or no response to particular vaccines (e.g., obese or nicotine-dependent individuals unresponsive to three doses of hepatitis B vaccine (HBV), genetic nonresponsiveness) [18, 19].
- The individual has a condition other than an immunocompromising illness that increases the risk for complications from the current, licensed form of vaccine—for example, scientists and technicians who wish to work with the vaccinia virus (gene therapy vector research, etc.), but because of a personal history of atopic dermatitis or eczema cannot receive the current, licensed form of smallpox vaccine [20].

New Tools for Vaccinomics

Vaccinomics is itself based on advancing science. With the completion of the Human Genome Project and the introduction of new sequencing technologies, the immunogenetic basis for vaccine variation can be explored in detail and, in turn, those understandings can inform the development of new vaccine candidates. To better understand the humoral and cellular immune responses elicited by vaccination, new technologies such as high-throughput genomic analysis (i.e., next-generation sequencing (NGS)), genome-wide linkage and association studies, and whole genome microarrays for transcriptome profiling can be successfully applied. As an example, full-length RNA-sequencing (RNA-Seq), which is a recently developed approach to transcriptome profiling that uses deep-sequencing technologies, has the potential to replace microarrays as the method of choice for transcriptome profiling. Of course, an important aspect of these tools is the concomitant bioinformatics approaches to understanding the data such that they inform our outcomes of interest [21].

As further examples, NGS technologies or platforms permit sequencing of DNA at unprecedented speed, allowing us to perform experiments that were previously not feasible [22]. The high-throughput capacity of NGS has now been used to sequence entire genomes from pathogens to humans. Paired-end sequencing of genomic subregions and genes has been used to map genomic structural variations together with deletions, insertions, and rearrangements. The genotyping data obtained using NGS technologies allow deep understanding of genotype-phenotype associations crucial to the development of the field of vaccinomics [1, 23].

Technology, experience, and better scientific insights into study design have led to the conclusion that the candidate gene approach has been surpassed by the genome-wide association studies (GWAS) approach, as this approach allows genotyping of thousands of single-nucleotide polymorphisms (SNPs) across the genome and is particularly useful to perform on polymorphisms with low allele frequencies. Such studies reveal that the most critical methodological issues for GWAS are sample size and power to detect allelic association. No GWAS population-based vaccine immunogenetic studies have yet been reported, although smallpox and measles, mumps, and rubella (MMR) vaccine GWAS are underway in our laboratory. Importantly, replication studies of initial genotype-phenotype (both single-SNP- and haplotype-based) associations are critical in separating true-positive from false-positive associations [24]. With better understanding of gene function and biological pathways, GWAS also may provide insights into the genetic basis for variation among vaccinated individuals and have the potential to inform new vaccine development.

Whole-genome microarrays are being widely used for measuring the expression pattern of thousands of genes in parallel, generating data on gene function that can identify appropriate targets for vaccines. This methodology was recently applied to a whole-transcriptome analysis of changes induced by live attenuated and inactivated influenza vaccines in children [25]. Results from this study show that the expression changes induced by the two vaccines differed significantly. Using similar microarray technology, our group studied differences in human leukocyte antigen (HLA) gene expression in measles-vaccine seropositive and seronegative individuals. There was more expression of the HLA class I B (p=0.0002), HLA class II cluster of DMA, DMB, TAP1, TAP2 (p=0.0007), and HLA–DR (p=0.0001) genes on day 7 or day 14 postvaccination in measles antibody seropositive subjects than among seronegative individuals [26]. This finding highlights an important approach to observing fine changes underlying the molecular, immunologic, and signaling mechanisms and pathways of vaccine-induced immune responses. Although considerable work is needed to fully apply these novel technologies to the field of vaccinomics, in terms of both bioinformatics and deeper scientific understanding, the potential for applying them to vaccine development is compelling.

Scientific Data for Personalized Vaccinology

Host genetic polymorphisms influence immune responses to vaccines [27]. Given the complexity of adaptive immune responses to vaccination, it can be inferred that the outcomes of vaccination are influenced or determined by multiple genetic and other contributing host factors. Immune responses to vaccines operate through numerous genetic networks interacting in functional pathways. For this reason, increasingly complex study designs are being used to identify both individual genes and gene pathways associated with vaccine-induced immune responses.

Population-based gene-association vaccine studies, such as those performed with hepatitis B, influenza A, MMR, and other vaccines, have been extensively described elsewhere [27–33]. As an example, we have identified polymorphisms in the HLA class I and class II alleles responsible for antigen presentation to CD8+ and CD4+ T helper cells, respectively, that are associated with responder and nonresponder phenotypes following hepatitis B, influenza A, and MMR vaccines [34–38]. Strong evidence exists that nonresponse to HBV is significantly influenced by HLA gene polymorphisms. Several HLA alleles have been associated with responder (DRB1*0101, DQB1*0501, DPB1*0402) and nonresponder (DRB1*0301, DRB1*0701, DQB1*0201) antibody phenotypes after full-dose HBV vaccination [39, 40]. In addition, other HLA (DRB1*07) and cytokine gene (IL2, IL4, IL12B) polymorphisms also have been found to be independently associated with responsiveness to HBV [41].

Host polymorphisms influence the immune response to influenza vaccine. Nonresponders to the trivalent influenza vaccine had altered frequencies of multiple HLA class II alleles (DRB1*0701, DQB1*0603–9/14, and DQB1*0303), compared with normal responders [42]. A recent influenza vaccine study demonstrates that HLA class I A*1101 (p=0.0001) and class II DRB1*1303 (p=0.04) alleles are associated with high and low circulating H1-specific antibody titers, respectively, following influenza A vaccine, suggesting that genetic polymorphisms may affect the development of humoral immune response in recipients of influenza vaccine [29].

Our population-based studies assessing associations between HLA genes and immune outcomes following a second dose of MMR demonstrated significant associations between HLA alleles and variations in immune responses to these vaccines. In regard to measles, the HLA haplotypes most strongly associated with low measles virus immunoglobulin G (IgG) antibody responses included DRB1*07–DQB1*03–DPB1*04 (p=0.001) and A*24–C*03–B*15 (p=0.04), whereas the DRB1*15/16–DQB1*06–DPB1*04 (p=0.02) haplotype was associated with high antibody levels [43]. We also found significant associations between the HLA–DQB1*0303 (p=0.04) alleles and low mumps vaccine-induced antibody levels [30]. Additionally, our data suggest that some HLA loci can be considered genetic determinants of rubella vaccine–induced immunity. Specifically, the DPA1*0201 (p=0.005) allele was associated with low rubella-induced antibodies, whereas the DPB1*0401 ($p\leq$0.001) allele was associated with increased antibody levels in two cohorts [44]. Furthermore, the association of DRB1*04–DQB1*03–DPB1*03 (p=0.01) and DRB1*15/16–DQB1*06–DPB1*03 (p=0.005) haplotypes with low rubella antibody levels was found in two separate studies [44]. These findings provide confirmatory support for an association between specific HLA alleles and haplotypes with rubella vaccine–specific antibody responses.

Identifying associations between variations in immunologic outcomes to vaccines enhances our understanding of vaccine adaptive immunity. Our data suggest that SNPs in cytokine (IL6) and cytokine receptor (IL12B, IL1R1, IL2RA, IL10RA) genes are associated with influenza hemagglutinin H1- and H3-induced antibody titers following receipt of the influenza A vaccine containing A/H1N1 New Caledonia/20/99 and A/H3N2 California/7/2004 influenza virus antigens [29]. Other studies have demonstrated that the -1082 (rs1800896) A allele in the IL10 promoter reduced the risk of developing adverse responses to inactivated influenza vaccine [33].

There are new genes and polymorphisms (SNPs) in key immune response genes, such as cytokine, cytokine receptor, Toll-like receptors, vitamin A and D receptors, signaling lymphocyte activation molecule (SLAM), antiviral effector, and innate immune response retinoic acid-inducible gene I (RIG–I) and tripartite motif 5 and 22 (TRIM5 and TRIM22) genes, that are associated with variations in MMR vaccine–induced immune responses [45–48]. In our recent rubella vaccine study, an increased carriage of minor alleles for the promoter SNPs (rs2844482, p=0.0002, and rs2857708, p=0.001) of the TNFA gene was associated with increase in rubella-induced antibodies [47]. Further, the TNFA haplotype AAACGGGGC (t=3.32) was associated (p<0.001) with high levels of rubella-specific IgG levels. Importantly, two TLR4 SNPs (rs1927907, p=0.0008, and rs11536889, p=0.0037) were successfully replicated in our two independent mumps vaccine studies. As an example, the minor allele for TLR4 SNP rs1927907 was associated with a 45 percent decrease in IgG antibody

response to mumps vaccine [30]. The role of vitamins A and D and their receptors in vaccine-induced immunity is a new and exciting area of inquiry. In our studies, minor alleles of rs4416353 (p=0.02) and rs6793694 (p=0.04) in the vitamin A receptor gene were associated with decreases in rubella vaccine antibody responses [45]. Notably, the nonsynonymous SNP rs3740996 (His43Tyr) in the TRIM5 gene was associated with variations in rubella antibodies (p=0.016). This SNP is known to affect the antiviral activity of TRIM5. Further replication studies are needed to confirm these data.

Many genes that encode receptors, including measles virus cellular receptors such as SLAM and CD46, have been associated with significant differences in immune response to vaccination. A novel nonsynonymous SNP (rs3796504) of the SLAM receptor gene was found to be significantly associated (p=0.01) with a 70 percent decrease in antibody response after measles vaccination [49]. Within CD46, the other measles virus cellular receptor, the minor allele for rs11118580 was associated ($p \leq 0.01$) with an allele dose-related decrease in measles antibodies. It is possible that these SNPs may hinder viral binding and thus limit infection and the subsequent generation of humoral immunity, but functional studies are currently pending to confirm this.

Conclusion

Given the data and concepts discussed above, vaccinologists and public health authorities must understand that a paradigm shift in vaccine science is occurring—away from a population-centric public health vaccine delivery approach to a patient-centric individualized approach through the application of vaccinomics. This shift will usher in a second golden age of both vaccine development and delivery [16, 21], particularly as the perceived risks of vaccine-preventable diseases (e.g., smallpox, rubella) diminish and the perceived risks of vaccine-induced side effects increase in the general public's mind—as we have seen in regard to many childhood vaccines (e.g., measles, human papillomavirus (HPV), varicella, rubella). Vaccinomics may address these concerns by providing increasingly accurate predictions of the likelihood of disease susceptibility and complications, along with the risks and benefits of receiving a given vaccine. Although some may see these ideas as too expensive or unrealistic, our collective work suggests that the benefits will be both real and useful to both practitioners and the public, and will, in the future, become economically viable as genetic sequencing and high-dimensional throughput assays decrease in cost. It is unlikely that individual prophylactic vaccines against infectious diseases will be developed (as is being done with cancer therapeutic vaccines), but it may well be the case that more than one type of vaccine against the same disease may be developed, informed by population-level gene HLA supertype and haplotype frequencies, and delivered on the basis of knowledge of individual genotypes.

We believe that vaccinomics also will inform new vaccine development, as illustrated in the examples above. This too will shift us away from the historic empirical approach to vaccine development and toward a new "directed" approach to vaccine development and design. Presumably, such improvements will lead to the ability to develop and test new vaccine candidates more quickly and inexpensively, and allow earlier "go/no go" decisions on vaccine development. This change may be particularly true as vaccinology now tackles more complex vaccine targets (e.g., malaria, Lyme disease, and others); hypervariable viruses (e.g., HIV, hepatitis C virus, West Nile virus, and others); and bacteria (e.g., *Mycobacterium tuberculosis*), for which traditional empirical approaches are too long, too expensive, and of low yield, as witnessed by our current progress for these vaccine targets using traditional empirical approaches. Thus, insights into how immunogenetics affects vaccine response is important to better understand variations in vaccine-induced immunity. The knowledge gained from such population-based vaccine immunogenetic studies has the potential to assist in designing new vaccines and to help us move toward a vaccinomics and personalized and predictive vaccinology approach [50].

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Sex Differences in Immune Responses to Vaccines

Col. Renata J. M. Engler, M.D. and Mary M. Klote, M.D.

Abstract

In an era of increasing appreciation of the need for personalized medicine, immunization practices continue to be a "one-size-fits-all" population-based delivery of disease preventive vaccines. Many clinicians remain unaware of the growing body of knowledge related to sex-based differences in immune responses to vaccines, as well as the differences in adverse events. Incorporation of sex-based population differences in future vaccine development and ongoing immunization programs may benefit vaccine safety, efficacy, and acceptability.

Introduction

The biology of immune responses to foreign antigens or infectious agents varies based on sex and may explain differences in disease incidence for autoimmunity, inflammatory conditions such as periodontal disease, and responses to vaccines [1–6]. Although growing evidence supports sex-based differences in both innate and adaptive immunity, attention to this confounder in study populations, particularly as related to vaccines, remains limited and in need of improvement, as there can be no doubt that men and women are different [7–8].

One of the criticisms of existing vaccine safety surveillance, with a focus on epidemiologic studies, is that these studies approach populations as if they were uniform and rarely report results by sex, even when disease incidence demonstrates significant sex-associated differences. There is a mounting body of literature relevant to sex-based differences in vaccine responses in both humoral and cellular immunity but with variations depending on the vaccine construct [5–6, 9]. Even from childhood, there appear to be sex-delineated immune response differences; further research is needed to clarify sex, age, nutritional, and environmental factors that affect immunity and potentially variations in vaccine efficacy and safety [10–12].

Each person has unique genetic variations that may influence how a particular vaccine will affect him or her. How genes are activated and/or inactivated (e.g., selective maternal or paternal X chromosome inactivation in women) and what environmental factors affect the host and level of immune reactivity (e.g., pregnancy, diet, and drugs/supplements) may all influence individual vaccine immune responses, efficacy, and risk for adverse events [1]. This multifactorial context adds to biodiversity and may explain some variations in published observations regarding sex-based differences. However, improved understanding of biologic sex differences may be the key to more effective vaccine constructs and administration guidelines that also reduce the severity and/or incidence of local and systemic side effects [13–15]. In the context of vaccine acceptability, if reduced-dose influenza vaccine in healthy young women can still provide efficacy along with improved acceptability through reduced side effects, then such a strategy enhances vaccine flexibility in delivery and options that respect patient-centric, individualized care [15]. With increased awareness of the broad range of sex-based biologic and immune response differences, it is hoped that the quality and clinical relevance of prelicensure vaccine studies and postlicensure safety, as well as efficacy study design and data reporting, will be enhanced.

Sex-Based Differences in Immunity

Beyond the obvious phenotypic differences and hormonal factors, the evidence points to tremendous complexity in the sex-based differences for both the levels of vaccine responses and adverse reaction rates. Table 1 outlines by vaccine type where data support a sex-based difference in immune responses and where responses appear to be sex neutral. It is noteworthy that the predominance of humoral immune responses as measured by specific antibody levels favors enhanced female responses [1–3, 5–6, 9, 13–15]. There are less clear definitions of sex-based differences in vaccine efficacy, since antibody levels have a broad range in terms of association with protection.

In the live virus yellow fever vaccine response model, remarkable differences exist in gene activation 2 to 10 days post-immunization in women (more than 500 genes), compared with men (fewer than 100 genes) [6]. In the 17D yellow fever vaccine studies, toll-like receptor-interferon signaling is substantially greater in women than men [6]. These and other studies suggest that intrinsic differences exist between the female and male immune systems when considering each of the major compartments: innate and adaptive

TABLE 1.

Sex differences in response to vaccines

Vaccine	Sex-Based Immune Response to Vaccine	Comments
Brucella	F>M	
Diphtheria	F>M	
Dengue virus, attenuated	F>M	
HSV–2 gD	F>M	Cell-mediated and antibodies
Hepatitis A	F>M	Rate of seroconversion, F=M
Hepatitis B	F>M	Rate of seroconversion, F=M
Human papillomavirus (HPV4)	M>F	Age 5–17 years
Influenza vaccines • Inactivated (TIV) • Live attenuated	F>M	Antibodies predominantly. Some smaller studies showed no differences or M>F. Adverse reactions: F>M for TIV
Japanese encephalitis virus, attenuated	F>M	Adverse reactions
Measles	M>F	
Meningococcal polysaccharide	M>F	Type A or C similar
MMR	F=M or F>M	Depending on study, age group. Adverse reactions: F>M; 1 study M>F
Pneumococcal polysaccharide	M>F	Normals, alcoholics, undernourished children
Rabies • HDCV • PCECV	F>M M>F	Infant study, F>M Adult intradermal, varied by study Adult intramuscular, M>F
Rubella	F>M	Strain RA27/3: M>F antibodies
Smallpox live attenuated	F>M	Antibody responses
Tetanus	F>M	
Venezuelan equine encephalitis	M>F	
Yellow fever vaccines • Virus strains 17DV and 17DD • BERNA–YF, RKI–YF, ARILVAX, YF–VAX	M>F F>M	17DV: Antibodies F>M Gene activation, cytokines Encephalitis reaction F>M with earlier vaccine

Abbreviations: ARILVAX—United Kingdom manufactured yellow fever vaccine; BERNA–YF—Flavimun (17D); F—female; HDCV—human diploid cell culture vaccine; HSV—herpes simplex virus; M—male; MMR—measles, mumps, and rubella; PCECV--purified chick embryo cell vaccine; RKI–YF—Robert Koch Institute yellow fever vaccine; TIV—trivalent influenza vaccine;
YF–VAX—U.S. manufactured yellow fever vaccine. **Source:** Adapted from references 5 and 6.

TABLE 2.

Sex differences in autoimmune disease incidence

Disease Predominance	Female>Male	Male>Female	Comments
Ankylosing spondylitis		M>F	
Arthritis, infection induced			Sex neutral
Autoimmune hemolytic anemia			Sex neutral
Biliary cirrhosis, primary	9:1		Antimitochondrial antibodies
Crohn's disease		M>F	Sex neutral
Diabetes type 1			Sex neutral
Drug-induced lupus		M>F	
Goodpasture's syndrome		M>F (1:0.2–1)	
Graves' disease	F>M		
Hashimoto's thyroiditis	5–50:1		
L-tryptophan induced eosinophilia-myalgia syndrome	F>M		
Lyme, chronic disease			Sex neutral
Multiple sclerosis	1.5–10:1		
Neurologic immune inflammatory disorders: e.g., Guillain-Barré syndrome		M>F 1.5:1	
Rheumatoid arthritis	2–3:1		
Scleroderma	3–12:1		
Scleroderma and contaminated cooking oil in Spain	F>M		
Scleroderma-like disease and silica exposure		M>F	
Sjogren's syndrome	>9:1		
Systemic lupus erythematosus	7–20:1		
Thrombocytopenic purpura	2–3:1		
Vasculitis	F>M		
Vitiligo			Sex neutral

Abbreviations: F—female; M—male.

Source: Adapted from references 23–26.

immunity. Klein et al. describe the hypothesized sex-associated quantitative differences in immune cell types and therefore levels of activation markers, cytokines, and humoral and/or cellular immunity after vaccination [6]. Modifying variables such as sex steroid hormones, sex chromosomal genes, and immunogene polymorphisms are believed to contribute to these differences between the sexes.

However, although hormonal and immune responses are attractive explanations for some of the observed sex-based differences, it must be noted that further research is needed to clarify all biologic sex-based differences that might affect immune response to vaccines (and drugs in general), as well as vaccine adverse reactions. For pain, as one example, published data suggest that there may be mechanisms other than immune response that account for sex-based differences in severity and impact, particularly in local reactions [16].

From a genetic perspective, it is noteworthy that the X chromosome contains approximately 1,100 genes, while the Y chromosome contains approximately 80. Although most of the different genes on the X chromosome support sex and reproductive functions, there are approximately 15 proteins produced that influence the immune response [1]. There are also some receptors and associated proteins clearly related to other biologic functions, such as the interleukin-1 receptor-associated kinase1 (IRAK–1) and interleukin-13 receptor 2 (IL–13Rα), both implicated in the risk for systemic lupus erythematosus [6, 17–18]. In addition, the IL–13Rα is a decoy receptor that can limit type 2 helper T cell (Th2) cytokine pattern responses [18]. These genes combined (IRAK–1 and IL–13Rα) result in risk ratios of about 1.5. This is not enough to explain the sex ratios of disease, but it suggests that sex chromosome differences may be relevant, nonetheless.

The recent discovery of *microchimerism*, the mechanism by which fetal cells persist in a mother for up to 40 years following the birth of a child, further challenges our understanding of immune system differences in women. Microchimeric cells have been characterized in the skin lesions of scleroderma, thyroid nodules, and the atrioventricular node in congenital heart block. What role these cells might play in vaccine immune responses and/or adverse reactions is unclear but further contributes to the complexity of the female immune system [19].

Destructive periodontal disease was recently recognized as a disease with a male predominance. It is theorized to originate from the male's heightened innate immune response to infection and the female's tendency to have higher antibody response offering protection against the chronic infection [4]. There is growing recognition that the response of the innate immune system at least to viral infection influences the cellular and humoral immune responses [20].

Recent literature documents a growing body of evidence that significant sex differences exist in drug responses in both pharmacodynamics and pharmacokinetics, coupled with the observation that adverse drug reactions in general are more frequent in women than men [21]. Sex-related or pregnancy-induced changes in drug absorption, distribution, metabolism, and elimination may have an impact on drug efficacy and safety, potentially requiring modified approaches and further driving the need for patient-centric and responsive medical practices [22]. Women have been less enrolled in clinical trials, and sex-specific analyses are usually not included in the evaluation of results [23], which is certainly true of vaccine-related studies.

Sex-Associated Differences in Autoimmunity

Sex-based differences in autoimmune disease incidence have been well documented, with some autoimmune disorders occurring more frequently in women than men, others more frequently in men than women, while some appear to be sex neutral [24–27]. If disease incidence is higher in women, as it is for most autoimmune disorders, then the current one-size-fits-all approach to vaccination may miss potential adverse reaction signals since many studies do not account for those differences [28].

Table 2 details examples of autoimmune diseases where there are published data regarding sex-based difference in incidence. Although disease severity may be affected by hormones, differences in disease incidence are not so easily explained by sex hormone differences alone. Complex environmental exposures are implicated in the development of autoimmune disease. Because vaccines are stimulants of the immune system with the markers of response focusing on antibody responses, it is not surprising that numerous citations raise concerns and questions about the role of vaccines and vaccine combinations (with potentially higher cumulative adjuvant concentrations) in potentially triggering autoimmune processes, particularly in genetically susceptible individuals [29]. It is noteworthy that the questions related to sex and autoimmune disorders and adverse reactions following vaccines remain an open challenge and part of the vaccine safety surveillance agenda prioritization [30].

Sex-Based Differences in Vaccine Responses: Adverse Events

Local reactions as well as systemic side effects are often higher or have more impact in women than men, particularly for such aluminum adjuvant containing vaccines as anthrax but also for the inactivated influenza vaccine [13–15]. There is a lack of prelicensure vaccine research detailing, by sex, potential differences in severity or frequency of side effects.

Adjuvants, used to enhance vaccine efficacy and potentially increase protective immune responses, further magnify the questions related to sex-based differences in vaccine immune responses and potential adverse reactions [31, 32]. There is a growing need for research that clarifies the roles of sex-based differences in optimum vaccine adjuvant dosing as well as in adverse reaction risk.

Quality improvement is needed in case definitions for ranking of side effect severity and functional impact stratified by sex, beyond simple incidence of events. Valuable and clinically useful information may be lost when data standardization and stratification are not part of research results reporting, particularly in relation to severity of side effects. There are very few published studies of vaccines that attempt to quantify the impact of post-immunization side effects, as was done in an anthrax vaccine study showing that 1–2 percent of individuals experienced symptoms like myalgias, arthralgias, headaches, and fatigue to a degree that interfered with "ability to perform and was not relieved by medications" [33]. These data can guide future research to address ways to reduce or manage subsets of individuals who refuse public health recommended vaccinations (also described as "refusers" in recent studies) [34].

Advances in Immunology

The science of immunology, immunogenetics, and molecular immunology with rapidly evolving technological approaches in research has grown in complexity, with a focus on systems biology and biodiversity. From sex-based differences in disease incidence to new technologies to study the immune system responses, these advances have led to further understanding of immune system functional dynamics and may need to be incorporated in future vaccine studies.

In the realm of new technology, "phosphoflow" or "phosflow" has been introduced to further our understanding of vaccine responses. With the ability to detect on the cellular level phosphorylated signaling molecules downstream of T cell receptor activation after vaccination, the potential to improve understanding of biodiversity in vaccine responses is becoming feasible for prelicensure studies and a way to clarify diversity of responses with possible correlations to degrees of efficacy and/or side effect severity. Although this methodology has limitations (e.g., weak phosphorylated signals and difficulty in identifying lymphocyte subsets), the ability to see multiple intracellular signaling molecules at the single-cell level (versus a population of cells) represents a powerful tool for clarifying the complexity of responses [35].

Implications of Sex-Based Differences on Vaccine Development and Immunization Health Care

There are many vaccine-related questions that require the vaccine community to conduct prospective, randomized controlled trials that stratify by sex looking for both immune response and adverse events differences. Timing, route, dose, and delivery systems, as well as delivery of multiple concomitant vaccines, may be significantly affected by sex [36]. In addition, more detailed information on biodiversity of responses empowers clinicians to personalize medicine for vaccines when indicated. Delivery systems for vaccines may contribute to the differences in the immune response to vaccination. New technologies, such as microneedles, thermal ablation, microdermabrasion, electroporation, and cavitational ultrasound, are being considered for vaccine delivery product lines and should take into account sex differences in the immune response of the cells of the stratum corneum [37].

The role of sex differences as related to mucosal vaccine delivery systems and mucosal immune responses remains to be defined. The mucosal immune system is a redundant system that produces large amounts of secretory immunoglobulin A (sIgA) and participates in cell-mediated immunity. Limited data exist on the sex differences in sIgA levels in saliva, but the available data demonstrate that women have lower levels [38].

Conclusion

In the evolution of patient-centric personalized medicine, sex-based differences in disease, risk for adverse drug reactions, and vaccine immune responses all merit closer attention in both pre- and postlicensure studies. The marginalization of vaccines in this regard is highlighted in a 2010 review of nonhormonal explanations for sex discrepancy in human illness in which the author states that "non autoimmune circumstances that engage the immune response system, such as infection, immunization and allergy, do not differ to any marked extent between the sexes" [39]. The current review highlights that considerable data exist about sex differences in

the context of immunization. These facts were highlighted in a letter we wrote to the journal *Lupus* in 2007 [40].

As the peer-reviewed literature expands in the area of sex-based differences in vaccine/drug responses, increased awareness and interest will hopefully influence future research study design and provide more granular data about immune responses and adverse events stratified by sex. Research regulatory hurdles (e.g., the complexities of research monitoring when adding women of childbearing potential into studies), while necessary to protect human subjects, may lead to protocol complexity, overall vaccine development costs, and hesitancy by sponsors. Despite Food and Drug Administration (FDA) guidance [8] and the priorities of the National Vaccine Advisory Committee [30] to design studies to look for these sex differences, the number of vaccine studies that stratify outcomes based on sex remains low.

The old rules regarding dose and route may not apply universally; this is a paradigm that must be accepted. From development of new vaccines, to delivery systems, to work with new adjuvants, all areas of vaccine research need to account for differences in immune response based on sex. Demonstrating a commitment to improved enrollment of women in vaccine development trials is crucial to quality immunization health care. Information gained may be used to develop clinical guidelines and options for addressing differences in vaccine safety and efficacy. Such guidelines and patient-centric responses also may significantly enhance immunization acceptability.

DISCLAIMER *The views expressed in this article are those of the authors and do not reflect the official policy of the Department of the Army, Department of Defense, or U.S. Government.*

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Immunization and Pregnancy

Flor M. Munoz, M.D.

Abstract

Prevention of infections in pregnant women and their newborns through maternal immunization is an underutilized public health intervention that has the potential to benefit a large, vulnerable segment of the U.S. population. The 2009 H1N1 influenza pandemic brought immunization of pregnant women to the forefront among priorities for health research and implementation. Identified barriers to the use of vaccines during pregnancy can be addressed through research, education, and targeted implementation interventions.

Introduction

Women who are pregnant and infants younger than 6 months of age are two of the most vulnerable populations, due to their susceptibility to infectious diseases and their potential to experience high morbidity and mortality from these diseases. A healthy mother who has received all recommended immunizations during childhood and adulthood can protect her newborn from infections. The natural process of active transplacental antibody transfer from the mother to the fetus during the second and especially the third trimesters of gestation, along with antibodies and other immunologic factors in breast milk, provide protection to infants in the first months of life while their immune system matures [1–4]. The strategy of vaccinating women during pregnancy takes advantage of this process to boost levels of maternal antibodies and protect infants against infectious diseases for which other preventive strategies are insufficient or unavailable. Routine prenatal and postpartum care provide an opportunity to ensure that women receive recommended immunizations and enjoy a healthy pregnancy and newborn.

Current Recommendations on Immunization of Pregnant Women

The Centers for Disease Control and Prevention's (CDC's) Advisory Committee on Immunization Practices (ACIP) and the American Congress of Obstetrics and Gynecology (ACOG) recommend immunization of pregnant women who have a high risk of exposure to a disease that poses a special risk to the mother and/or the fetus when there is an available vaccine that is unlikely to cause harm [5, 6]. These recommendations are based on the premise that the benefits of vaccinating pregnant women outweigh its potential risks, and that the risk for a developing fetus is only theoretical. There is no evidence of fetal injury or adverse pregnancy outcomes from vaccinating pregnant women with inactivated virus or bacterial vaccines or toxoids [5, 7]. Live vaccines are contraindicated during pregnancy because of the potential theoretical risk of transmission of the vaccine virus to the fetus. However, numerous reports of inadvertent administration of live vaccines to pregnant women (i.e., in women who were not yet aware of their pregnancy) have failed to show an association with fetal disease, anomalies, or other undesirable outcomes of pregnancy [8–16]. Maternal receipt of a live vaccine is not an indication to terminate the pregnancy.

Vaccines recommended for routine administration during pregnancy in the United States include tetanus and diphtheria toxoids (Td), if indicated, and trivalent inactivated influenza vaccines. Examples of live vaccines contraindicated for pregnant women include measles, mumps, and rubella (MMR), varicella (chickenpox), zoster (shingles), live attenuated influenza virus vaccines, smallpox (vaccinia), or Bacille Calmette-Guérin (BCG) vaccines. However, with the exception of smallpox, all these vaccines can be administered to postpartum and breastfeeding mothers if necessary [5]. For current recommendations, please refer to the CDC Web site at www.cdc.gov. Women who are pregnant or planning to become pregnant should consult their healthcare providers for additional information.

Protection of Mothers and Infants Through Vaccination

A unique aspect of maternal immunization is the potential to protect two individuals, the mother and her baby, against serious diseases, with one intervention. Although no vaccine has been specifically licensed for use during pregnancy, pregnant women have received immunizations against pertussis, tetanus, and influenza since vaccines first became available. Whole-cell pertussis (wDTP) vaccines were studied in pregnant women in the 1940s as a way to protect infants against this deadly disease [17–19]. However, associated local pain, swelling, and fever in mothers and a rapid drop in infant

titers after delivery precluded their routine administration. The resurgence of pertussis in the United States and elsewhere since the 1980s, with increasing infant mortality in the 21st century, prompted the development of less reactogenic acellular pertussis (Tdap) vaccines. Since 2006, Tdap vaccines have been recommended for all postpartum women not previously vaccinated to protect the woman and her newborn, and for all teens and adults, especially if they will be in close contact with an infant [19]. During recent outbreaks, pregnant women exposed to pertussis have received the Tdap vaccine, as vaccinating women during pregnancy is the most direct and immediate method of providing passive antibody protection to newborns who cannot receive active immunization until 6 weeks of age [20]. In 2009, the National Institutes of Health (NIH) sponsored a multiyear study to determine the safety and immunogenicity of Tdap vaccines in pregnancy and the effects of maternal immunization in infant protection and responses to active immunization [21]. In addition, Dalhousie University in Canada is supporting a study examining these issues [21].

The World Health Organization (WHO) strategy of routine tetanus immunization of women of childbearing age and pregnant women has resulted in a significant reduction of maternal and neonatal tetanus worldwide and its elimination (defined as a rate of less than 1 case per 1,000 live births) in 149 countries since the strategy's implementation in 1989 [22]. Although neonatal tetanus is rare in the United States (annual incidence <0.04 cases per 100,000 live births), poor adherence with the recommended decennial Td booster and incomplete primary immunization may result in increased susceptibility of women of reproductive age [23, 24]. Tetanus vaccination coverage within the preceding 10 years was reported to be up to date in 61.6 percent of adults in 2008, a decrease of 5 percentage points from 1999 [24]. In a 2003 survey of ACOG members, more than one-half of the respondents considered themselves the primary care providers for their patients, but only 32 percent offered the recommended Td booster during pregnancy, and just 10 percent offered all the vaccines recommended for women during pregnancy or after delivery [25]. Adult coverage with Tdap vaccine also remains low, reported at 5.9 percent nationwide in 2009 [24]. Coverage of adult women and protection of newborns against tetanus can improve with the routine use of the Tdap vaccine postpartum.

Inactivated influenza vaccine has been routinely administered to pregnant women since the 1950s, and since 1997 pregnancy has been included in the ACIP list of high-risk conditions indicating routine annual influenza vaccination

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[26]. The impact of influenza on pregnant women was documented during the 1918 and 1957 pandemics and in numerous reports of annual epidemics. The risk of hospitalization of otherwise healthy pregnant women with influenza in the third trimester of gestation is approximately five times higher than that of nonpregnant women [27]. The risk of severe manifestations and complications from influenza, need for medical attention, and mortality are also higher during pregnancy [27, 28]. In addition to the last trimester of pregnancy, the postpartum period is also a time of increased risk for influenza morbidity and mortality from seasonal and pandemic influenza [29, 30]. The safety of inactivated influenza vaccine has been documented in clinical studies and through routine surveillance of vaccine-related adverse events. A large prospective study of more than 2,000 women vaccinated from 1959 to 1965 [8, 9] and four clinical trials in which more than 100 women received monovalent or trivalent influenza vaccines from 1979 to 1993 [31–34] failed to identify significant adverse reactions to the vaccine, including local or systemic reactions, or fetal or pregnancy complications. Two retrospective database studies including 252 and 3,719 vaccinated pregnant women, respectively [35, 36], and two studies based on reports to the CDC Vaccine Adverse Event Reporting System (VAERS) from 1990 to 2009 that considered an estimated 11.8 million vaccinated women [16, 37], have provided additional support for the safety of inactivated influenza vaccines during pregnancy. Furthermore, two recent prospective studies and

one case-control study have confirmed these findings and documented the effectiveness of influenza vaccines in mothers and their infants. In Bangladesh, a substantial impact on laboratory-confirmed influenza and febrile respiratory illnesses was observed in vaccinated mothers (28 percent reduction) and their infants (41 percent reduction), compared with unvaccinated controls [38]. Transfer of maternal influenza antibodies to infants was documented, as well as infant protection against laboratory-confirmed influenza for the first 6 months of life [38, 39]. In the United States, among 1,160 Navajo and White Mountain Apache mother–infant pairs, a 41 percent reduction in the risk of laboratory-confirmed influenza virus infection and a 39 percent reduction in the risk of hospitalization for influenza-like illness were documented in infants born to mothers who had received influenza vaccine (*N*=573), compared with infants born to unvaccinated mothers (*N*=587) over three influenza seasons from 2002 to 2005 [40]. Finally, in an age-matched case-control study in New Haven, CT, from 2000 to 2009, receipt of influenza vaccine was documented in 2 of 91 (2.2 percent) infants younger than 6 months of age hospitalized for influenza, and 31 of 156 (19.9 percent) control subjects, for a 91.5 percent calculated effectiveness of maternal immunization in preventing hospitalization of infants for influenza in the first 6 months of life [41]. Despite these observations and established recommendations, the coverage of pregnant women with influenza vaccine has been very low, averaging 12–24 percent nationwide prior to 2009 [26].

The 2009 H1N1 Influenza Pandemic and Pregnancy

As with previous pandemics, the 2009 H1N1 influenza pandemic had a disproportionate impact on pregnant women. Pregnant women were at high risk of hospitalization, intensive care unit admission, mechanical ventilation, and death, particularly if they were in the third trimester of pregnancy or had an underlying condition in addition to pregnancy, such as asthma, that independently increased the risk for influenza complications [42]. Five percent of all reported 2009 H1N1 influenza deaths in the United States were in pregnant women, while only approximately 1 percent of the population was estimated to be pregnant. The median age of mothers who died was 25 years (range 14 to 43 years). Severe illness in the postpartum period and an increased rate of premature birth (30.2 percent) also were documented [30]. Pregnant women were promptly placed at the top of the priority list to receive the first available doses of 2009 H1N1 monovalent vaccine during the pandemic, and administration of seasonal influenza vaccine was highly encouraged [43]. At least five clinical trials evaluating seasonal and 2009 H1N1 influenza vaccines in pregnant women were carried out in the United States in 2009 and 2010 through the NIH, and many observational studies have been reported worldwide [44, 45]. These studies documented the safety and immunogenicity of different licensed seasonal trivalent influenza and monovalent 2009 H1N1 vaccines in pregnant women [46, 47]. With available research information and recommendations from the CDC, ACOG, American Medical Association, and other national organizations, the estimated vaccination coverage for pregnant women in 2009–2010 reached 50.7 percent for seasonal and 46.6 percent for 2009 H1N1 influenza vaccines, higher than in previous seasons, but not optimal, considering the potential benefits of maternal immunization [48].

Barriers to Maternal Immunization

Historically, the association of significant birth defects with exposure to specific medications or teratogenic agents during pregnancy has led to avoidance of any potential risks by pregnant women, including vaccines [49]. Therefore, concern about the safety of vaccines is one of the major issues for mothers and practitioners. Barriers to vaccination during pregnancy stem from both patient and provider knowledge, perceptions, beliefs, and motivations. Ultimately, lack of the physician's or healthcare provider's recommendation to receive the vaccinations and the mother's lack of knowledge about vaccine recommendations during pregnancy are key impediments to immunization of pregnant women [50]. Obstetric providers who are more knowledgeable about influenza vaccine, for example, are more likely to discuss vaccination with their patients, as are those who receive vaccinations themselves or whose clinic or multispecialty practice has an active program where healthcare personnel receive annual influenza vaccinations [25, 50–53]. Most women would accept influenza vaccine during pregnancy if their physician recommended it, particularly if they have received it before or experienced influenza disease before [51, 54]. This is true for acceptance of any vaccine. However, women might not know about recommended vaccinations, and some providers might not be aware of the most recent vaccine recommendations for pregnant women or might have inaccurate information [25, 51]. Organizational and implementation factors that interfere with vaccinating women during pregnancy include the ability of obstetric providers to receive adequate reimbursement from insurance carriers for vaccines and their administration;

to train and dedicate personnel and office space for the acquisition, storage, and administration of vaccines; and to incorporate patient education, consent, and documentation, all of which add more time to routine obstetric visits [50–56]. However, with obstetric providers' recognition of the important role that they play in providing primary and preventive health care to women, and the unique opportunity that prenatal care visits represent to administer immunizations, vaccination before, during, and after pregnancy can become part of the routine management of obstetric patients.

Working Toward Improving Immunization Coverage of Pregnant Women

The majority of obstetricians recognize the need to address vaccine-preventable diseases in their practices [25, 56]. To address liability and safety concerns, strong research-supported recommendations and up-to-date scientific information must be accessible to obstetric providers so that they can inform their patients and help them make decisions about immunizations. Women of childbearing age and pregnant women must be informed and have easy access to information that is objective and simple to understand. The CDC, ACOG, and other national and private organizations have Web sites with sections specifically dedicated to immunization of pregnant women to which providers and patients can refer. Any opportunity to disseminate this information should be encouraged, including through the lay media.

The support and collaboration of obstetric practice groups and delivery hospitals to make vaccines accessible to women are necessary for the successful implementation of routine immunization of pregnant women. Adding other vaccinations to established procedures for administration of Rh–IG during pregnancy or postpartum rubella vaccine would facilitate compliance with current recommendations. To achieve these goals, adequate reimbursement from insurance carriers to cover immunizations in pregnant and postpartum women is crucial [50–56]. With reimbursement, providers can work on specific strategies to support maternal immunization, including the logistics of offering the vaccines in their own offices, through a vaccine clinic within a multispecialty group, or at a pharmacy; requiring documentation of vaccination status of women during prenatal care; and authorizing designated personnel such as nurses, pharmacists, or other ancillary personnel to administer vaccinations to patients based on established standing orders or specific protocols designed to educate patients and improve compliance with immunization recommendations [53]. This is particularly important given the impact of influenza and pertussis epidemics experienced in the United States in the last 5 years, and because a number of potential vaccines that could be used to protect pregnant women and their infants currently exist or may become available in the future, including those to prevent infections caused by group B streptococcus, respiratory syncytial virus, *Streptococcus pneumoniae*, cytomegalovirus, herpes simplex virus, and HIV, among others.

Conclusion

Routine administration of vaccines to women of childbearing age and women who are pregnant or postpartum is a public health strategy that results in healthy mothers and infants and improves pregnancy outcomes. The ACIP recommends routine administration of tetanus and influenza vaccines during pregnancy and the administration of other available (nonlive) vaccines when pregnant women are at risk for infections that have the potential to cause significant morbidity and mortality for them or their newborn. Vaccine coverage in women of childbearing age and pregnant women remains low. The success of the WHO program for the elimination of maternal and neonatal tetanus worldwide and results of numerous contemporary studies of influenza vaccine during pregnancy support maternal immunization as a successful strategy for the prevention of certain infections in mothers and infants. Safe vaccines that can be administered to pregnant women are integral components for the control of outbreaks of influenza epidemics in the United States, as they were during the 2009 H1N1 pandemic [20, 26, 43]. Strategies for the control of other infectious diseases and epidemics could incorporate this intervention. Working toward the elimination of barriers for maternal immunization is a priority at multiple levels for the immediate future.

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She has authored/coauthored numerous peer-reviewed publications and textbook chapters dealing with maternal immunization, influenza, respiratory syncytial virus, pertussis, and other infectious diseases that affect infants and children. She has been invited to speak in numerous national and international venues. She is the Clinical Director of the pediatric outpatient Infectious Diseases Clinic at Ben Taub Hospital and of the Infectious Diseases Transplant Service at Texas Children's Hospital. She serves as a member of the Baylor College of Medicine Institutional Review Board and has served on the editorial board of the *Journal of Pediatric Infectious Diseases,* the *Infectious Diseases Journal of Obstetrics and Gynecology,* and UpToDate.

Second-Generation Malaria Vaccines: A Definitive End to Malaria-Related Deaths?

Vasee S. Moorthy, MRCP, Ph.D.

Abstract

Malaria vaccine development has entered a new stage. The scientific success of the RTS,S/AS01 program represents a proof-of-concept for development of vaccines for malaria and validates the human challenge model for improvement of pre-erythrocytic malaria vaccines. The longer term objective of a greater than 80 percent efficacy second-generation malaria vaccine with a major impact on malaria transmission is feasible if research and development funds are available and are used efficiently. An opportunity exists to re-examine approaches to development of malaria vaccines and increase the chances of success going forward. This article describes some key obstacles and possible ways to overcome them.

Background

Remarkable changes have occurred in malaria vaccine development in the past few years. A new vaccine, RTS,S/AS01, has emerged as a possible first-generation product that may receive a World Health Organization (WHO) recommendation for use in 2015, depending on the results of a large Phase III trial now ongoing in seven countries in sub-Saharan Africa. The manufacturer's target group for this vaccine is African infants resident in malaria-endemic countries, with immunization planned at an age of 6–14 weeks, given together with routine infant vaccines in the Expanded Programme on Immunization (EPI). The first of three sets of results from the Phase III trial were published in October 2011 in *The New England Journal of Medicine* [1–3]. The trial, including 15,460 infants and young children, showed that the vaccine reduced the incidence of clinical malaria by 55 percent when evaluated over 12 months following the third dose. This analysis was performed on data from the first 6,000 vaccinated children aged 5 to 17 months. Interestingly, malaria challenge trial efficacy of RTS,S/AS01 had been reported earlier as 50 percent in a trial with 102 study subjects [4]. The link between the immunology of RTS,S vaccination and reduction in morbidity in vaccinees is beginning to be understood [4–10]. In some ways, the scientific success of RTS,S/AS01 should be seen as the culmination of the many parallel revolutions that have occurred in subunit vaccination over the last 20 years: recombinant DNA technology; yeast and bacterial recombinant expression systems; polymeric particulate technology [11]; characterization of B- and T-cell immunity [12, 13] and harnessing molecular understanding of activation of innate immunity for adjuvant development, discoveries recognized by the 2011 Nobel Prize in Medicine [14]. The engagement of industry in a public-private partnership (PPP) to pursue licensure of a vaccine intended only for children in malaria-endemic countries and substantial funding from a private foundation were critical factors in developing what could become the first effective human anti-parasite vaccine.

Anopheles minimus mosquito, a malaria vector, feeding on a human host. *An. Minimus* is one of the mosquito species responsible for spreading the drug-resistant *P. falciparum* parasite in Thailand and Vietnam. Courtesy of CDC Public Health Image Library

This is against the background of substantial reductions in malaria disease burden associated with recent scaling up in long-lasting insecticidal nets, indoor residual spraying, prompt

diagnostic testing, and improved access to artemisinin-combination chemotherapies [15–17]. An estimated 1.1 million lives have been saved since 2000 through use of these measures, and there is an urgent imperative to achieve universal access and use.

It is now appropriate to talk of second-generation malaria vaccine development [18], and to reassess the prospects for the development of a malaria vaccine with efficacy of 80 percent or more.

On the one hand, we know that it is possible to confer partial efficacy against a complex multistage parasite through immunization with a vaccine containing fewer than 200 amino acids from 1 of more than 5,000 genes. On the other hand, it has taken decades and hundreds of millions of dollars to get this far. The question is no longer whether a higher efficacy malaria vaccine is technically possible, but whether the funds, momentum, and mechanisms can be found to successfully develop it. Discussed below are some of the obstacles and possible ways to overcome them.

Potential Return on Investment in Malaria Vaccine Development

The chances of developing an 80 percent efficacy malaria vaccine are high, but it will require substantial investment, which may not be available. Decisions about disbursement of donor agency funding for research and development (R&D) in global health are made on the basis of return on investment in terms of successful development of deployable public health tools. There are two key reasons why second-generation malaria vaccine development could represent an excellent return on investment: (1) the lowering of the technical risk that development of RTS,S represents and (2) the validation of surrogate efficacy measures that can be used to reduce costs and accelerate timelines.

The Importance of Optimizing Malaria Vaccine Candidates

This is not a time for complacency. Expensive and time-consuming field studies have been necessary in multiple centers. There are several reasons why it might be preferable to optimize future vaccines considerably before proceeding to large field trials that measure efficacy against morbidity. One important issue is that malaria transmission is dropping in many settings [17]. It should be noted that commentators disagree over the timeframe and sustainability of future reductions in malaria transmission. If falls in malaria transmission become widespread and sustained, three possibilities present themselves for altered trial design.

First, much greater emphasis could be placed on challenge trial efficacy [19]. Malaria has a well-developed clinical challenge model, which was central to development of RTS,S and allows optimization in adults [4]. Important optimization of vaccine construct, formulation, dose, route, and schedule can all be done in the challenge setting. Clinical challenge model capacity will need to be expanded, and standardization of trial conduct is highly desirable to facilitate comparability between centers and to protect safety of participants under conditions of artificial exposure. A collaborative, WHO-facilitated process of challenge trial standardization is underway. This process has demonstrated that the community of challenge trial centers, while appropriately competing in some senses, are able to cooperate to safeguard the highest standards and improve the utility of this evaluation technology for the global effort. What are the limitations of this challenge model?

Some key scientific strategic goals for second-generation malaria vaccine development

- Screening tools to identify new antigens for vaccine development
- Mechanisms to facilitate access to immunogenic formulations, formulation know-how, and particulate protein platforms
- Platforms to induce dual potent CD8 T cell and B cell responses in humans
- Qualified and validated key immunological readouts
- Standardized challenge and field efficacy trial designs
- Field-deployable high-throughput molecular methods for measurement of asexual and sexual parasitaemia
- Validated methods to quantify infectivity, transmission, exposure and immunity

Strain-transcendence and duration of efficacy will require field efficacy trials for their confirmation, though a preliminary indication of both is achievable with the challenge model. Age de-escalation and the effect of prior exposure to malaria cannot easily be taken into account in challenge trials.

Second, new types of field trials could be developed in which efficacy is tested using molecular methods, allowing for smaller sample sizes to counteract the decreased malaria transmission. An important example is available whereby an ultra-high sensitivity quantitative polymerase chain reaction (PCR) assay was used to detect subpatent malaria infection in a field efficacy trial with a reduced sample size [20]. In addition to supporting the challenge trial readout, RTS,S development provides strong support to field trials designed to measure efficacy against incidence of infection [21]. A debate in the scientific, regulatory, and public health communities about how malaria infection endpoints in field trials can be used to accelerate and streamline second-generation vaccine development is warranted. In these field trials measuring malaria infection rates, strain-transcendence questions can be addressed, although duration of efficacy is more difficult to assess as, generally, participants are censored at the diagnosis of first infection. If studies could be further optimized to include molecular force of infection by genotyping each incident infection, this would provide further information, also of use for model fitting [22].

Third and finally, more emphasis could be placed on correlates of immunity [5, 23]. Here, development of one or more validated functional assays will be critical to overcome antigen-specificity of some of the current immunoassays. Whether it will be possible to select appropriate functional assays and validate them remains questionable. Several candidate functional assays are available, though none have been validated in the regulatory sense. Development of international standard reagents and harmonized standard operating procedures for use in these assays will be beneficial; this is an area where WHO-facilitated approaches are often helpful [24].

Another limitation can be viewed as an opportunity. We are currently still in the era of clinical trials using a few antigens identified in the 1980s and 1990s. Development of a validated system to screen new antigens discovered in the postgenomic era and transition them to vaccine development would be of great utility. In practice, the validation would most likely stem from confirmation of protection in clinical efficacy trials, perhaps challenge trials, and so a level of risk is currently unavoidable with new antigens in malaria vaccine development, as preclinical or *in vitro* validation remains unproven for the time being.

Essential Components of Developing Next-Generation Malaria Vaccines

A new cohort of malaria product researchers and developers

Many leading scientists and malaria public health experts provided expertise that formed a vital part of the preclinical and clinical development of RTS,S. A new school of malaria vaccine scientists is beginning to emerge, many being natives of developing, malaria-endemic countries. Encouraged by the progress in the field, they are driven by a combination of intellectual interest and the ability to contribute to achievable and important public health goals. However, more initiatives to draw the brightest minds into the field and support them are needed. The issue of limited career opportunities for translational clinical researchers wishing to link lab, clinic, and field remains largely unresolved, particularly where the objective is product development rather than pure research goals.

The role of public-private partnerships

Substantially enlarged PPPs with increased industry engagement will be necessary to deliver a highly efficacious malaria vaccine. The current model by which not-for-profit PPPs bring academia, biotech, industry, and field centers together works but needs expanded industry involvement. New multilateral sources of funding will be necessary to achieve this scale-up in PPPs. As efficacy increases toward 80 percent, the potentially lucrative travel and military markets come into view, which could encourage increases in industry involvement.

Interagency coordination

Coordination between PPPs, leveraging synergies, avoiding inefficient overlap, and identifying gaps at the global level will be essential. There is an existing, functional Malaria Vaccine Funders Group forum, facilitated by WHO. This group meets twice a year with ad hoc interactions as necessary, allowing a global, interconnected perspective. If funders choose to coordinate studies, ensuring comparison between trials of related vaccine concepts by using comparable assays, and maximizing use of resources at the global level, the potential payoffs for timelines are substantial. But simple interagency mechanisms would be one prerequisite for successful coordination. There are two factors that currently may extend timelines in complex multipartner projects: contracting delays and the plurality of ethics committees reviewing the same protocol, in some cases

for a single site study. Possibilities exist for reform in both these areas without adversely affecting data quality and ethical standards. Another avenue for consideration is specialization of certain agencies and the need for further prioritizing based on chances of success according to each agency's strengths.

Metrics for Malaria Vaccine Development: Governance, Transparency, and Accountability

Progress has been made with governance of the agencies responsible for malaria vaccine R&D. However, the evaluation of previous funding to PPPs is challenging, as traditional parameters such as numbers of "vials and trials" are crude and can be misleading. Independent, external advisory bodies, when allied to transparent decision-making processes, can safeguard good governance. It is likely that metrics for organizations' governance, transparency, and accountability will receive more attention as agencies or philanthropists wish to evaluate between funding cycles. Lessons learned from the history of PPPs should increase efficiencies, such as the importance of considering where other mechanisms could pick up a project when it moves beyond the remit of an initial funder. An "easy win" could arguably be the requirement to publish R&D/clinical trial outcomes, particularly negative or inconclusive trials. These are often left unpublished unless there is a stimulus from funders. The National Institutes of Health, Wellcome Trust, and European Commission publication policies are evidence of major progress in this area in recent years.

Formulation: The Access and Know-How Bottlenecks

Progress has been made with the bottlenecks of access to immunogenic formulations for recombinant protein antigens and formulation expertise, notably with initiatives at the Infectious Disease Research Institute in Seattle and at University of Lausanne, Switzerland. A familiar story is a promising recombinant protein project that stalls at the Phase I stage due to lack of access to a sufficiently immunogenic adjuvant suitable for human use. Unfortunately, alum-adjuvantation has been inadequate in the malaria field to date, and water-in-oil formulations tend to yield promising preclinical results but are unlikely to lead to stable, consistent, final clinical formulations with acceptable reactogenicity. A linked issue is the scientific prerogative to develop polymeric approaches to overcome the immunogenicity deficiencies of monomeric proteins. Hepatitis B surface antigen and human papillomavirus particulate platforms are two licensed examples. Other potentially promising approaches include virosomal, protein conjugate, and nanoparticle technologies. It would be advantageous if such approaches increase immunogenicity to the point where novel adjuvants will not be necessary for a second-generation malaria vaccine.

Regulatory Pathways for International Use

Regulatory mechanisms to facilitate vaccine development for developing country target populations are also an area of progress. The European Medicines Agency has adopted its article 58 mechanism, whereby, with input from WHO, it can offer a scientific opinion (with the same procedural rigor as marketing authorization applications) for products exclusively intended for non-European customers. RTS,S/AS01 will be submitted under this mechanism [25], and some other pharmaceutical companies are considering this approach. Increasingly, well-resourced national regulatory agencies are placing international considerations within their focus, in addition to their core domestic scope. WHO is coordinating efforts to strengthen national regulatory authorities in the developing world with support to the Developing Countries' Vaccine Regulators Network (DCVRN) and the African Vaccine Regulatory Forum (AVAREF).

Goals for Next-Generation Malaria Vaccines

What is the aim for a second-generation vaccine? The malaria vaccine technology roadmap, endorsed by a group of major stakeholders, set a goal for an 80 percent efficacy vaccine by 2025. This goal still applies, and WHO works toward it. A refinement is that efficacy must be considered both in terms of reduction of direct morbidity and mortality and in terms of reduction of malaria transmission, as in low transmission settings some countries will wish to interrupt transmission using a combination of interventions.

Whether one is working within a framework focusing on malaria transmission or morbidity, there are two long-term aims. First, the much-discussed aim of global malaria eradication. This is a distant possibility requiring currently unavailable tools; most importantly, a suitable high-efficacy vaccine able to interrupt malaria transmission [26]. An alternative and highly laudable goal of a second-generation malaria vaccine is to reduce malaria-related deaths to zero or close to zero globally. Given the existence of the EPI infrastructure in all developing countries, the most feasible way of preventing all malaria deaths would be a malaria vaccine that induces sterile immunity of long duration, with substantial herd effects. These

twin goals are both crucial, and certain types of vaccine could satisfy both profiles.

A future where children and travelers no longer die from malaria is achievable through development of a second-generation vaccine. The key questions are whether the momentum will be generated to expand the current PPP landscape for malaria vaccine development, and whether the mechanisms can be put in place to ensure the dollars are well spent, incrementally moving toward the achievable 80 percent efficacy vaccine goal.

DISCLAIMER *The views expressed in this article are those of the author and do not necessarily represent the views, position, or stated policy of the World Health Organization.*

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Structural Biology and Other Resolution-Enhancing Technologies in the Design of an Effective HIV–1 Vaccine

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Abstract

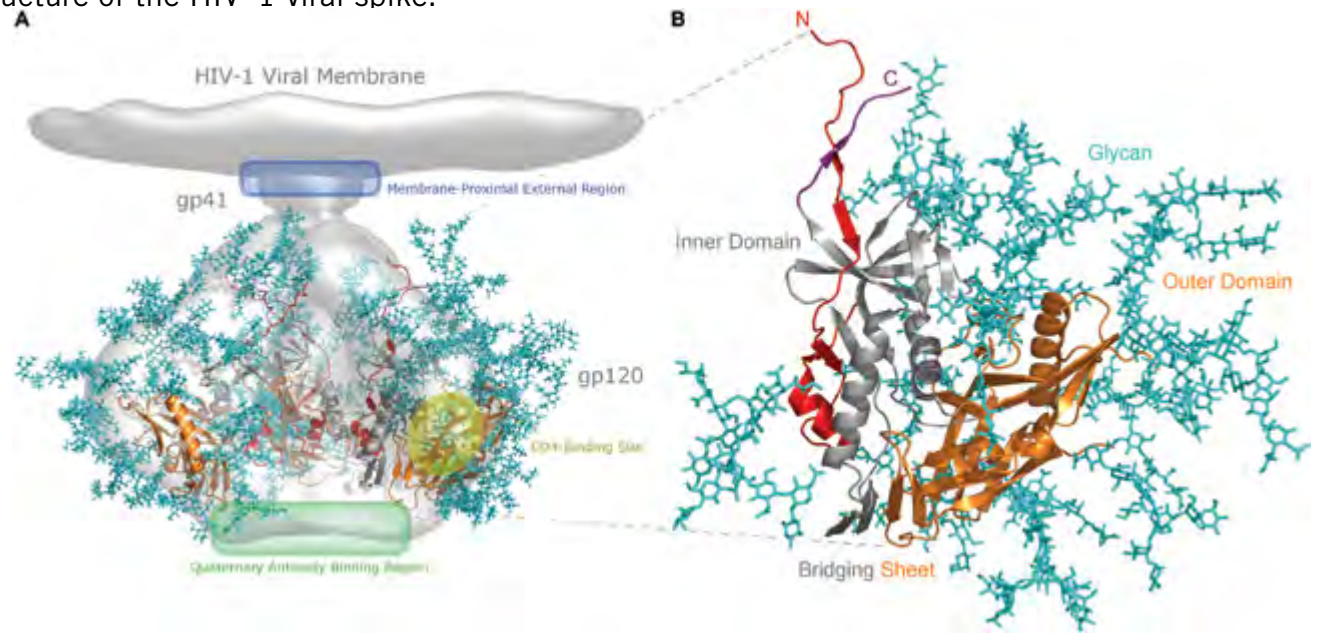
The successful development of an effective vaccine against HIV–1 will likely require novel approaches to vaccine design. At the Vaccine Research Center (VRC), part of the National Institute of Allergy and Infectious Diseases,, we have sought to harness structural biology and other informatics-related technologies in an effort to develop immunogens capable of eliciting neutralizing antibodies of exceptional breadth and potency against circulating strains of HIV–1.

Introduction

Francis Bacon's maxim "knowledge equals power," applies to many situations, including HIV–1 vaccine design. What critical information about the HIV–1 virus or about the human immune response might enable the development of an effective HIV–1 vaccine? At the National Institute of Allergy and Infectious Diseases' Vaccine Research Center (VRC), we have used resolution-enhancing technologies to (1) define relevant structures necessary for viral entry into host cells (Figure 1), (2) understand the elicitation of antibodies capable of neutralizing HIV–1, and (3) design immunogens that elicit targeted immune responses based on an atomic-level understanding of susceptible epitopes and the biology of antibody-elicitation

FIGURE 1

Structure of the HIV–1 viral spike.



A: Electron tomogram of the HIV–1 viral spike (shown as a grey envelope) and how it fits with atomic-level structure of the HIV–1 gp120 envelope glycoprotein. Polypeptide chains are displayed as backbone ribbons, with N-linked glycosylation shown as sticks. Sites of known vulnerability to neutralizing antibodies are shown. B: Crystal structure of the HIV–1 gp120 envelope glycoprotein in its CD4-bound conformation, with domain structure highlighted (inner domain, bridging sheet, and outer domain) and colored the same as in (A). The structure shown is missing two regions, the V1/V2 and V3 loops, but otherwise represents the entire mature form of gp120.

pathways. Overall, our structural and informatics-based approaches seek to incorporate information about virus-antibody interactions, assimilate feedback from antigenic and immunogenicity studies, and leverage recent advances in immunofocusing and computation biology.

Informatics and Vaccine Design

Structure-Based Approaches to Vaccine Development

Structural biology provides information about the three-dimensional organization and chemical structure of proteins. This information, and in particular an understanding of atomic-level structure, can be used to rationally design proteins that stimulate specific responses, thereby enabling atomic-level approaches to vaccine design.

One approach involves the structural definition of the functional viral spike (Figure 1A), which is used by the virus to enter host cells and is the target of all known virus-directed neutralizing antibodies. Atomic-level analysis of the spike facilitates immunogen designs that stabilize and help present potential sites of neutralization more optimally to the immune system. Unfortunately, the same strategies that allow the viral spike to evade an effective immune response also hinder structural analysis, and the entire HIV–1 spike has resisted and continues to resist atomic-level characterization.

Another approach seeks to bypass difficulties with the entire viral spike and focuses only on functionally critical sites the virus uses for entry. The virus cannot change these sites without hindering function. We and others have used this approach to elicit antibodies against the highly conserved site of co-receptor binding [1]. Unfortunately, the virus hides this site and reveals it only when the juxtaposition of viral and target cell membranes prevents antibody recognition [2]. Thus, in addition to functional importance and sequence conservation, an appropriate site of vulnerability needs to be accessible to the neutralizing antibody.

A third approach focuses on effective antibody responses [3, 4]. Through analysis of monoclonal antibodies selected for their ability to neutralize HIV–1 effectively, one gains an understanding of effective immune responses. Working backward from monoclonal antibody to recognized epitope, one creates mimics of the epitope with the hope of using these mimics to elicit the original template antibody. Unfortunately, many of the identified monoclonal antibodies that neutralize HIV–1 effectively appear to have unusual properties, which make their elicitation difficult or unlikely, suggesting that this approach needs to include information about the frequency and elicitation pathway of the template antibody.

At the VRC, we have used resolution-enhancing technologies to increase our understanding of both the viral spike and the human immune response. Rather than rely on any particular approach for vaccine design, our resolution-enhancing approach seeks to provide the necessary knowledge base from which relevant hypotheses can be formed and tested [5]. Because of the ability of structural biology to provide detailed atomic-level information required for precise manipulation, we have focused on (1) maximizing the application of structural methods of definition (e.g., of the functional viral spike), (2) using structural techniques to interrogate the natural response to HIV (e.g., in the use of epitope-specific probes to identify specific monoclonal antibodies), and (3) incorporating structural feedback (e.g., of the immunogen and for the elicited response).

HIV–1 Viral Spike

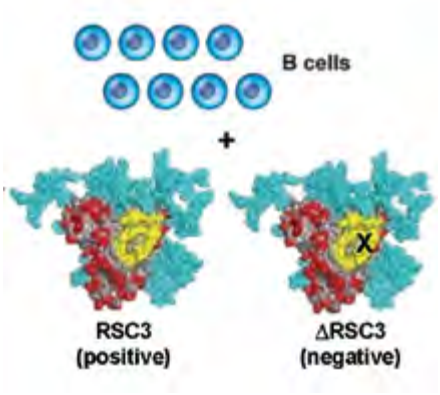
HIV–1 is an enveloped virus, with a host-derived lipid membrane that surrounds the viral core structural proteins. The only viral proteins that protrude through the protective lipid are the gp120-envelope and the gp41-transmembrane glycoproteins (Figure 1). Both are targets of neutralizing antibody, which either bind to the spike and prevent cell or receptor attachment, or bind and prevent conformational changes required for virus-cell entry.

The functional viral spike is made up of three gp120s, which associate noncovalently with the ectodomains of three gp41s. Despite extensive efforts by several groups worldwide, the trimeric spike has thus far resisted atomic-level determination. Low-resolution cryoelectron microscopy studies [6, 7], however, have provided insight into gp120–gp41 arrangements. Such information includes structures of the viral spike prior to receptor encounter, intermediate states of the virus during entry, and postfusion states. We and others have obtained atomic-level structural information on individual gp120 and gp41 components. For gp41, only postfusion structures have been determined. For gp120, the crystal structures of a number of states for a conserved core have been determined, including antibody-bound conformations, though the best characterization comes from the CD4-bound state.

The structure of the core gp120 in its CD4-bound state is arranged in an inner domain, an outer domain, and a four-stranded bridging sheet minidomain, the latter of which is composed of two β-hairpins, which extend from the inner

FIGURE 2

Use of rationally designed Env probes to identify broadly neutralizing antibodies against HIV–1.



Structure-based design produced selective probes that expose the primary receptor binding site (yellow) while masking all other potentially interfering surfaces by changes to non-HIV residues (red) and retaining glycan camouflage (cyan). These selective probes are labeled and used to identify B cells that express broadly neutralizing antibodies. (RSC3, resurfaced stabilized core 3.)

(β2–β3) and outer (β20–β21) domains, respectively (Figure 1B) [8, 9]. The outer domain is extensively glycosylated, and antigenic analysis and fitting into the viral spike reveals the glycan surface to cover most of the exposed surface of the spike and to be immunologically silent [10]. Multiple mechanisms of evasion, including the already mentioned glycan shielding, as well as variable loop divergence and extensive conformational change succeed in preventing either the elicitation or the binding of most antibodies.

Human Immune Responses to HIV–1

Most vaccines seek to mimic the immune response generated during natural infection with the corresponding pathogen. For example, polio and influenza vaccines generate specific antibodies that circulate throughout the body [11]. These antibodies inactivate the invading virus during the earliest stages of infection, thus preventing illness in the vaccinated individual. During HIV infection, there is a strong antibody response to the viral envelope glycoproteins (Env), but most of these antibodies are unable to neutralize or inactivate HIV. Among the many known monoclonal antibodies against HIV, only a few display a combination of potent neutralization and breadth of reactivity [4, 12, 13]. The limited natural examples of HIV-neutralizing antibodies have made it difficult to understand how an HIV vaccine might generate an effective antibody response [14]. However, new high-throughput assays have improved our ability to measure large panels of sera for HIV neutralization, and this has led to an appreciation that about 25 percent of HIV-infected individuals make relatively broadly reactive neutralizing antibodies during the course of HIV infection [15]. At the VRC, we have been studying the sera and the antibody secreting B cells from infected donors to understand how such antibodies arise during natural HIV infection. This information can then be used to inform the design of HIV vaccines and vaccination strategies that would elicit similar neutralizing antibodies.

Our understanding of the antibody response against HIV has been facilitated by several resolution-enhancing technologies. These include (1) the ability to dissect the types of antibodies in sera and to determine what regions of the HIV Env are targeted [16], (2) the ability to isolate neutralizing antibodies from individual B cells [17, 18], and (3) the ability to determine the atomic-level structure of neutralizing antibodies bound to HIV Env [19–21]. We used knowledge of the structure of the HIV Env to design protein probes that expose various regions of the HIV Env (Figure 2). These probes were then used to evaluate the regions of the HIV Env that are targeted by serum-neutralizing antibodies. One such region is the CD4-binding site of gp120; CD4 is the primary cellular receptor for HIV, and antibodies that bind to the CD4-binding site can block HIV infection of CD4+ T cells. To further define the characteristics of neutralizing antibodies to the CD4-binding site, a specific protein probe was designed to expose the CD4-binding site of gp120, while other regions of HIV were altered to be unrecognizable to HIV antibodies. This epitope-specific probe, along with a knockout mutant version, was used to identify B cells making antibodies to the CD4-binding site. After such B cells were isolated by flow cytometry, the genes encoding the antibody heavy and light chain variable regions could be amplified by polymerase chain reaction, and the full immunoglobulin G (IgG) monoclonal antibody could be expressed in tissue culture. With the monoclonal antibody in hand, its ability to neutralize HIV could be verified and studied in detail. Using this technology, we recently isolated three CD4-binding site neutralizing monoclonal antibodies called VRC01, VRC02, and VRC03 [22]. Importantly, the crystal structure of the VRC01 bound to HIV gp120 has provided an atomic-level footprint showing the precise region of HIV gp120 that is vulnerable to neutralizing antibodies [21]. This structural information can be used to

make new vaccine immunogens that are designed to teach the immune system to generate antibodies similar to VRC01.

Deciphering the Elicitation Pathway

Elicitation of a particular antibody requires three steps: recombination from appropriate precursors, deletion of autoreactive clones, and antigen-driven affinity maturation. Despite substantial quantities of gp120 in HIV–1 infected individuals, it takes the human immune system several years to make antibodies against the CD4-binding site that are effective at neutralizing primary isolates of HIV–1 [16, 23].

Detailed analysis of antibody VRC01 provides insights into which of these steps might be responsible for the reduced elicitation of VRC01 [21]. Recognition of gp120 by VRC01 primarily involves regions of the antibody derived from the heavy chain variable gene (V_H) and the kappa light chain variable gene (V_κ), and does not appear to be dependent on specific joining events. VRC01 is highly affinity matured and does not appear to be autoreactive. The putative genomic precursors, moreover, appear to have low (mM or weaker) affinity for gp120, a level unlikely to drive antibody maturation. Thus, a key barrier to eliciting VRC01-like antibodies appears to be reduced affinity of likely genomic precursors to the gp120 immunogen. A potential path to eliciting VRC01-like antibodies might involve bypassing this barrier by creating altered gp120s able to bind to genomic precursors.

Design of Immunogens Based on the Structure of the Epitope and the Biology of Elicitation

Our understanding of the interactions of broadly neutralizing antibodies, particularly the b12 and VRC01 antibodies directed to the CD4-binding site of HIV Env, provides the conceptual basis for the development of four strategies to elicit antibodies with similar specificities. First, we have generated trimeric forms of the HIV–1 Env by including the gp41 trimerization sites in the absence of the transmembrane domain. This form of the protein can be further stabilized through the use of trimerization sequences from heterologous proteins, such as the fibritin protein from phage lambda. It is therefore possible to generate stable trimers using site-specific mutations to fix the core structure. The variable domains of these proteins are deleted because they might otherwise divert immune responses to strain-specific determinants.

A second strategy focuses on stabilized-core Env proteins that are further modified using structure-based design [22]. With the knowledge of bioinformatics and computer-assisted design, we have introduced mutations that eliminated HIV residues on the surface of gp120 and replaced them with those of SIV Env, which shows minimal serologic cross-reactivity with HIV–1. By progressively modifying the surface of the constrained Env core protein and by subsequently covering this region with glycans, we have been able to use the resultant engineered molecules not only as probes to analyze complex antisera for the presence of broadly neutralizing antibodies, but also as prototype immunogens to elicit antibodies directed to the highly conserved CD4-binding site.

A third approach aims to eliminate irrelevant immunologic determinants. We have been able to generate a subdomain of the HIV–1 Env, the outer domain that contains the initial CD4 binding loop, by eliminating considerable additional protein sequence that is not relevant to the generation of the desired immune response to the β15 loop. Previous studies have shown that a soluble form of the outer domain that contains the β15 loop was not able to bind to b12 with high affinity. By including a transmembrane domain [24] or by further site-directed mutagenesis based on the VRC01/Env structure, we have devised ways to stabilize this interaction, possibly by providing additional hydrophilic surfaces that may improve folding or stabilize additional contacts of the VRC01 antibody. In addition, we have recently generated additional mutations in the outer domain region that preserve high-affinity binding by decreasing the off-rate in binding as determined by surface plasmon resonance spectroscopy. These vectors are currently under evaluation for their ability to elicit broadly neutralizing antibodies and also for their ability to characterize these complex antisera.

The fourth approach to immunogen development focuses on the use of scaffolds designed by probing the structural database and transplanting critical epitopes, for example the β15 loop, onto heterologous scaffolds. Although several scaffolds have been identified that bind to these antibodies, they remain of low affinity. This approach remains a topic of continued investigation.

A number of concerns related to fundamental B cell biology must be considered in generating a robust neutralizing antibody response to HIV. These include the need to trigger the appropriate germ line rearrangements, the ability to generate antibodies that are not autoreactive and can escape clonal deletion, and the necessity of generating somatic mutations to facilitate affinity maturation of the appropriate specificity. Immunogen design efforts must take these factors into account and address these basic aspects of B cell development and antibody production. Critical to their success is the ability of immunogens to engage the appropriate low-affinity germ line

precursors that give rise to high-affinity antibodies. This task will likely be facilitated by the addition of suitable adjuvants and/or delivery matrices. As these efforts progress, it will be important to identify which reagents have the safety and immunogenicity profiles suitable for advanced development. A variety of such compounds have been compared systematically in rodent (mouse and guinea pig) and nonhuman primate (NHP) immunogenicity studies. These studies include collaborative efforts to evaluate alum, RIBI, ASO1A and B, ASO2, MF59, nanoparticles, and multimeric viral carriers, such as Qb. Successful candidates will require evaluation in challenge studies in the NHP and potentially also in improved humanized mouse models with CCR5-tropic HIV–1 strains.

Conclusion

HIV–1 hides behind a host-derived envelope and uses a viral spike, replete with molecular trickery, to evade the immune response. Standard approaches at vaccine design have failed, and it has become unclear what hypotheses to test. Instead we have tried an information-based approach, which seeks to bring each of the three major players—(1) HIV–1 virus, (2) human immune response, and (3) immunogen design—into atomic-level focus. Such a resolution-enhancing approach may have utility not only with HIV–1, but also with other viruses that resist standard approaches to vaccine design.

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Peter D. Kwong, Ph.D.

Dr. Kwong is Chief of the Structural Biology Section and the Structural Bioinformatics Core Section in NIAID's VRC. By combining the power and efficiency of computation with atomic-level structural analysis of the HIV-1 envelope glycoproteins and related molecules, Dr. Kwong seeks to apply structural biology to HIV-1 vaccine development. His efforts focus on understanding the atomic-level structure of the HIV-1 envelope gp and how this allows HIV-1 to evade the immune system. This has important ramifications for AIDS vaccine research. Dr. Kwong's achievements form the foundation for the structure-based design of modified HIV-1-envelope vaccines that will be better able to generate a protective antibody response against HIV/AIDS. His work is being applied to accelerate scientific design and development of better HIV-1/AIDS vaccine immunogens.

In 2003, Dr. Kwong was awarded the Presidential Early Career Award for Scientists and Engineers, the highest honor bestowed by the U.S. government on outstanding scientists and engineers beginning their independent careers. Recently, Dr. Kwong determined the atomic-level molecular structure of the broadly neutralizing antibody VRC01, caught in the act of recognizing HIV-1 gp120. Dr. Kwong's iterative process of rational vaccine design and hypothesis testing is likely to be a key component of a successful effort to produce an effective AIDS vaccine.

John R. Mascola, M.D.

Dr. Mascola is the Deputy Director of the VRC and Chief of the BSL-3 Core Virology Laboratory of NIAID. His research focuses on understanding antibody-mediated protective immune responses against HIV-1 via studies of both the plasma antibody compartment and the B-cell compartment. The goal of these studies is to elucidate mechanisms of virus neutralization and the viral epitopes targeted by neutralizing antibodies, and to translate this information into novel strategies for vaccine design. In collaboration with his VRC colleagues, Dr. Mascola's laboratory has worked closely with other VRC investigators to isolate new monoclonal antibodies that neutralize diverse strains of HIV-1.

Dr. Mascola is a Fellow of the American College of Physicians and a member of the Infectious Diseases Society of America, the American Society for Clinical Investigation, and the American Association for the Advancement of Science. He also holds concurrent appointments as Professor of Medicine at the Uniformed Services University of the Health Sciences and as a Staff Physician in the Division of Infectious Diseases at the Walter Reed Army Medical Center.

Gary J. Nabel, M.D., Ph.D.

The VRC was established in 1999 under Dr. Nabel's leadership by President Clinton to assist in the development of a vaccine against AIDS. As Director of the VRC, Dr. Nabel provides overall direction and scientific leadership for basic, clinical, and translational research activities, and guides development of novel vaccine strategies against HIV and other emerging and re-emerging infectious diseases, including Ebola/Marburg hemorrhagic fevers, Chikungunya, influenza, and other viruses. Dr. Nabel also serves as Chief of the VRC's Virology Laboratory, which examines molecular regulation of HIV replication, optimization of immune responses to gene-based vaccination, and development of improved HIV envelope immunogens. Dr. Nabel recently led a discovery team that collaborated with other VRC teams to identify several broadly neutralizing human antibodies against multiple HIV strains and developed a two-step immunization approach to elicit antibodies that attacked a diverse array of influenza virus strains.

In recognition of his expertise at the forefront of virology, immunology, gene therapy, and molecular biology, Dr. Nabel was elected as a member of the Institute of Medicine of the National Academy of Sciences in 1998, and in 2010 was honored as a Fellow of the American Academy of Arts and Sciences.

New Methods for Analyzing Vaccine Responses

Mark M. Davis, Ph.D. and John D. Altman, Ph.D.

Abstract

A revolution is brewing in how vaccine responses are being analyzed. For many decades the only laboratory assays considered valid were simple measures of antibody responses to pathogens, but now a variety of high-throughput, information-rich assays that cover a much broader range of immune responses are being employed. This enables a much more comprehensive picture of how a particular vaccine formulation triggers various parts of the immune system. One such assay involves using "tetramers" and other multivalent forms of antigens to label specific lymphocytes, providing a much clearer picture of how an adaptive immune response develops and proceeds through various stages toward achieving protective immunity.

Introduction

Vaccination with killed or live attenuated versions of infectious organisms has been by far one of the most successful types of medical intervention in the modern era, saving hundreds of millions of lives. And yet, even a standard vaccine such as influenza has limited efficacy for older adults, and we have had an extremely frustrating time trying to develop a vaccine for HIV and other pathogens, showing that we still have a lot to learn about designing the right type of vaccine for these more difficult, highly mutable infectious organisms. This experience has led to a general re-examination of how we formulate and characterize vaccines in general. It is also providing the raw material with which we will be able to define "metrics" of immunological health [1] using a simple blood test, much like the way that cholesterol tests are used today to monitor cardiovascular health. In this article, we focus on the very dramatic changes occurring in how we are evaluating vaccines, both those that are a standard and effective part of our repertoire as well as those still being developed. This sea change in how vaccines are being evaluated is being driven by our desire to make better and more effective vaccines as well as our more sophisticated knowledge of the immune system and its cells and molecules. It is also being greatly aided by a wealth of new technology—much of it deriving from the Human Genome Project—that allows us to measure many different parameters at one time.

The Immune System and Vaccination

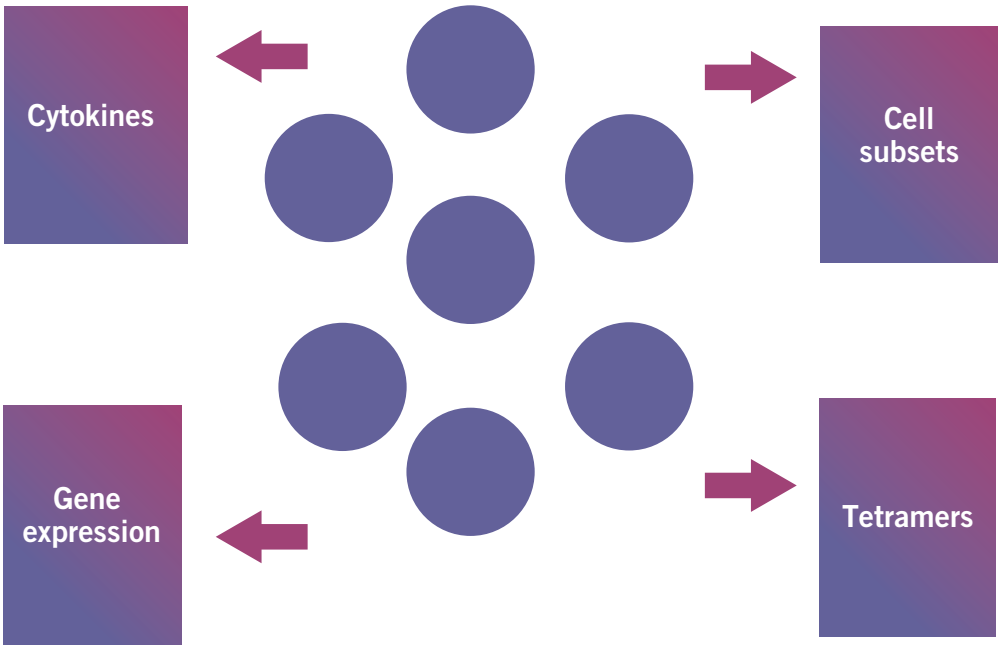
The immune system consists of a dozen or more different types of cells in the blood, lymph nodes, and spleen that respond in different ways to foreign entities and communicate with each other through a series of secreted factors or cell-surface molecules. These cells are known as white blood cells to distinguish them from red blood cells, which carry oxygen. It is now thought that the immune response has two major arms, starting with the innate response pathway, in which pathogens first trigger an inflammatory response through pattern recognition receptors or pathogen-associated molecular patterns (PAMPs). This arm involves the detection of something characteristic of bacteria or pathogens (e.g., highly methylated DNA, which is characteristic of bacterial DNA). The innate response creates a local condition of inflammation that attracts other immune cells to the "scene of the crime," including two types of white blood cells, the B and T lymphocytes, that initiate an adaptive immune response (the second arm of immune response). This process involves triggering the activation of very specific (but also very rare) B and T cells that can recognize specific antigens on the pathogen. B cells do this through their immunoglobulin molecules, also known as antibodies, which bind tightly to various molecules on the pathogen and target it for destruction. T cells also express a very diverse molecule on their surface, called the T cell receptor, which in most cases recognizes a fragment of a protein antigen (called a peptide) bound to a major histocompatibility complex molecule. T cells that are specific for a particular pathogen can either kill infected cells directly or "help" B cells to proliferate and make more effective antibodies.

For more than 50 years, the standard way to evaluate vaccines has been to measure the concentration of antibodies in blood that is sufficient to neutralize the pathogen (i.e., the antibody titer). Although this has generally been a good indicator of a vaccine's effectiveness, more and more evidence [2, 3] suggests that measuring other aspects of immune response, particularly the innate response and the T cell response, may be equally or more relevant to efficacy (Figure 1). This interest in measuring more of the immune response than just

FIGURE 1.

Analyzing the whole immune system

Assaying the whole immune response. Although classical methods focused solely on the antibody response to vaccination, new technologies allow us to analyze many other aspects of an immune response as well: gene expression analysis of the blood cells; the levels of dozens of cytokines in the blood; changes in the many types of white blood cells; and the antigen specificities of responding T and B cells using tetramers or other probes.



the standard has been greatly aided by the development of a number of new technologies that allow many aspects of the immune system to be measured at one time in a single blood sample, including gene expression microarrays, multiplex cytokine assays, and FACS (fluorescence activated cell sorting) analysis.

Gene Expression Microarrays

Nanofabrication techniques have allowed probes for all the expressed genes in the human genome (more than 25,000) to be synthesized on a single silicon chip, and this chip can then be used to analyze the expression of any of these genes in white blood cell RNA. This technology was the principal method used in two landmark papers, by Sekaly and colleagues [4] and Pulendran and colleagues [5], to analyze the response to yellow fever vaccine, one of the most successful vaccines known. In these papers, the authors showed numerous significant gene expression patterns that correlated with the response to this vaccine across multiple immune cell types. These studies developed valuable clues as to what makes a successful immune response and have provided a roadmap for future studies.

Multiplex Cytokine Assays

More than 100 cytokines and other molecules that allow the immune system to communicate with itself are present in the blood. To assay these factors, antibodies specific to these molecules are attached to beads and then analyzed for their binding to 50 or more of the different cytokines found in the blood; their relative concentrations are then measured. The rise and fall of these molecules can signal the onset or decline in an immune response and other types of activity.

FACS Analysis

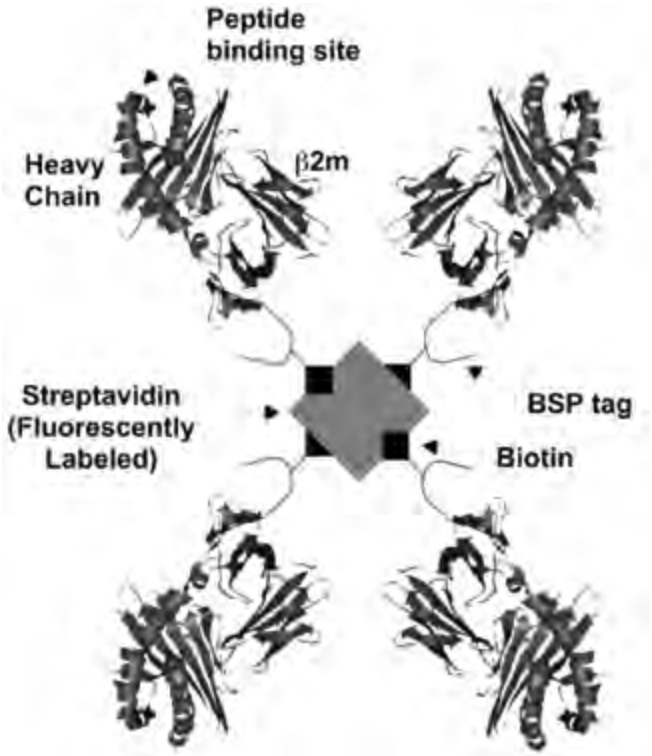
Cells of the immune system can express any of the 350 known cell-surface molecules, called CD antigens, or secrete one or more of 100+ possible cytokines. The fluorescence-based flow cytometer can catalog many of these molecules, and the new mass spectrometry–based machine, which uses lanthanide metal labels, can provide significantly more information about cell types in the blood, their relative activation state, and their frequency and functional attributes (e.g., what cytokines they are secreting).

Peptide-MHC Tetramers and Other Antigen-Specific Labels

T lymphocytes play many roles in the immune system, not the least of which is to regulate many of the other components. Finding the particular cells contributing to a specific response has been difficult because the main determinant of their specificity, the T cell receptor for antigen (TCR), has a very low affinity for its typical ligand, an antigenic peptide bound to a major histocompatibility complex molecule (pMHC). Our solution to this problem was to make a tetramer of a particular pMHC using a biotinylation site on the MHC and the tetrameric nature of streptavidin, in which each of the four subunits has its own biotin binding site (Figure 2). These multiple pMHCs provide much-needed stability to the tetramers when they bind T cells, because when one falls off briefly, at least two others are still bound. This simple labeling format has now worked for thousands of different pMHCs, has fueled a great deal of both clinical and basic research studies in the almost 15 years since reported [6], and continues to be useful. This work also benefited from the creation of a research facility, established by the National Institute of Allergy and Infectious Diseases (NIAID) and led by one of us (JDA), which has provided reagents and related products to thousands of investigators over the years and contributed to at least 1,100 scientific publications. This concept also has been applied to B cell ligands, where multimers of HIV [7] or flu antigens [8] have been used to track the development of a B-cell response from its early, low-affinity form of surface antibody to its higher affinity form later in development. Recent work on T cells has shown that even very rare (1 in 1 million, or fewer) naive cells (i.e., those that have never seen their specific antigen) can be identified with tetramer labels and an enrichment technique [9]. This new ability to follow a B- or T-cell response from its early beginnings to full-blown antibody or effector T-cell activity will give us an unprecedented view of the way a successful vaccine works and provide important clues when it does not work.

FIGURE 2.

Peptide-MHC tetramers.



This figure shows the structure of a tetramer, with four MHC molecules bound to a fluorescently labeled streptavidin molecule. As many as three of the peptide-MHC (pMHC) molecules can be bound to T-cell receptors on a T-cell surface at one time, greatly increasing the stability of binding.

A Novel Plan to Make Peptide-MHC Tetramers Available to Researchers at the National Institutes of Health

After we introduced MHC tetramer technology, it was licensed for commercial manufacture and reagents became available for sale in the United States. However, in contrast to typical antibody reagents sold for flow cytometry and related applications, MHC tetramer reagents are inherently customized with respect to both the MHC allele and the peptide bound to it, limiting the market size for any one reagent. In the early days, the manufacturer focused on a relatively small subset of high-demand tetramer reagents, leaving researchers in an enormous swath of research areas without an option for purchasing appropriate MHC tetramer reagents. At the Keystone Symposium on Viral Immunology in 1998, the first big wave of tetramer results were announced, and it became clear that this promising technology should be more widely available to the research community. The National Institutes of Health (NIH) established the NIH Tetramer Core Facility to manufacture and distribute tetramer reagents for the research community. At the outset, the facility focused on class I MHC reagents (for which robust production technologies were already in place). In recent years, novel technologies have enabled expanded production of class II peptide complexes as well as CD1d tetramers for the detection of natural killer T (NKT) cells, the current most popular single reagent offered by the facility.

Tetramer Studies to Date

MHC tetramers have transformed the conduct of research on, and our understanding of, adaptive cellular immunity. In animal models, they have led to a radical reassessment of the magnitude of T-cell responses to systemic viral infections [10] and were essential tools in the discovery of T-cell exhaustion in the face of high-level persistent viral infections [11], including studies in HIV-infected humans [12]. In rhesus macaques, tetramer analyses have influenced the development of novel heterologous prime-boost approaches to vaccination [13]. In humans, they have been applied to studies of responses to a wide variety of viral infections, including influenza [14], the hepatitis viruses [15, 16], the herpes viruses (cytomegalovirus, Epstein-Barr virus) [17–19], the retroviruses HIV–1 [20, 21] and human T-lymphotropic virus 1 (HTLV–1) [22], and the South American Andes hantavirus [23]. In human vaccine clinical trials, tetramer analyses have had the most impact in epitope-targeted vaccines, such as those designed to elicit responses to well-defined tumor antigens [24]. However, because of production and detection bottlenecks, measurement of T-cell responses to candidate antiviral vaccines tends to be done by ELISPOT or intracellular cytokine staining assays, which can include many more epitopes in a single test [25]. The obstacles that have prevented more widespread use of tetramer technology in human vaccine trials are now being addressed with new advances in their synthesis and use, as described below.

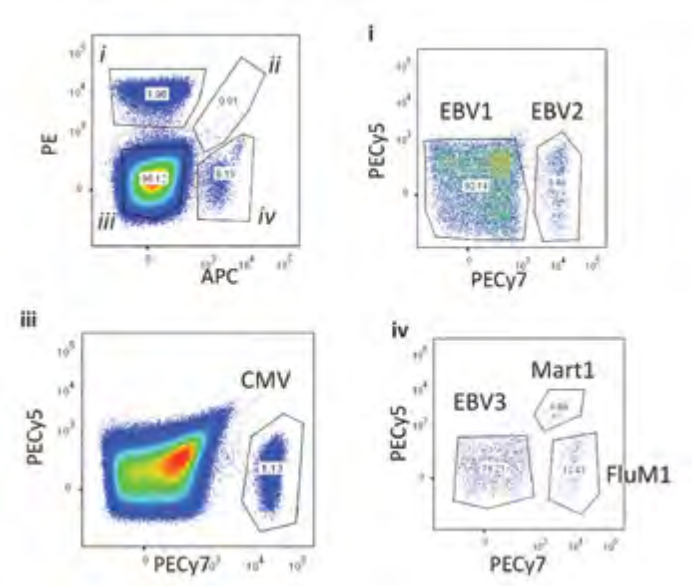
New Developments in Tetramers

A number of recent technical advances have increased the ease with which tetramers can be made and have expanded their use significantly. In particular, it has been difficult to make tetramers in laboratories that lack biochemical expertise and specialized equipment, thus limiting production to one or a few at a time. This situation has now changed radically with readily exchangeable peptide systems. One can now make a single pMHC complex in which the bound peptide is exchangeable with peptides in solution. This system employs the use of modified peptides, which can be degraded by ultraviolet light, enabling peptides in solution to occupy the newly vacated groove of the MHC molecule [26]. For such modified pMHC combinations, one need only produce and purify a particular pMHC complex and then use ultraviolet light to quickly exchange into the MHC binding site hundreds of different peptides in separate reaction wells, thus making hundreds or even thousands of different tetramers in a few hours. But how could one use so many tetramers? Two groups have come up with very similar solutions, using different combinations of colors to create a large number of different tags. Traditionally, four different fluorescent dyes would be used to label just four different tetramers. But in this new combinatorial color scheme [27], these four colors can be combined in different ways to create 15 different labels, thus greatly expanding the number of tetramers that can be surveyed at once (Figure 3). A similar scheme using Q dot labels was developed by Schumacher and colleagues and works by the same principle [28].

Another important innovation is the use of simple enrichment schemes that give us the ability to detect rare populations [9]. This method can be as simple as adding magnetic beads coated with an antifluorophore antibody to a crude preparation of tetramer labeled T cells, but it results in a big (fiftyfold to one hundredfold) enrichment for the cells of interest. This approach has made possible the detection of the very rare naive T cells, which may be less than 1 in 1 million CD4+ or CD8+ T cells, allowing us to characterize a person's pre-immune repertoire. That is, does the individual have the right T cells to respond to a particular antigen or not? And if so, do those cells develop in the right way when exposed to that antigen, either as a component of a vaccine or during an infection?

FIGURE 3.

Combinatorial tetramer staining with different epitopes



Combinatorial tetramer staining allows many specificities to be analyzed at a time. In this figure, a 15-tetramer mixture was used to reveal six distinct populations of T cells in a human blood sample—populations that recognize peptides from three viruses (influenza, cytomegalovirus (CMV), and Epstein-Barr virus (EBV)) and one common skin cell antigen (melanoma-associated antigen recognized by T cells (MART–1)).

New Developments in Cell Analysis

Lastly, another technology that is starting to have an impact on T-cell analysis and tetramers is a new mass spectroscopy-based cell analysis method called CyTOF (cytometry time of flight) [29]. Because the readout is spectral lines with little or no overlap between the different metal labels, many labels can be assessed with no danger of overlap or confusion. With the current instrument, we are using 32 different channels, allowing many more labels to be used than in fluorescence-based studies, in which 12 colors are the typical limit. This method delivers a wealth of information that will redefine lymphocyte subset analysis and allow us to follow vaccine responses in much greater depth. Because there are potentially more than 1 billion different combinations of 30 independent markers, the complexity of a CyTOF panel may soon approach that of a gene array chip, depending on how many of these possible contributions are used.

A Key Role for Bioinformatics

As more studies are done with these high-throughput, information-intensive assays, developing the appropriate computational and statistical analyses becomes essential, just as they have been in the Human Genome Project when datasets became larger than the human eye could handle. In many of our experiments today, we are collecting 30,000 data points per blood sample. In the near future, this number could easily be much larger.

In addition, many unique challenges exist in dealing with immunological data of the types discussed here. One is that, unlike genomic data, there are different technology platforms to integrate (e.g., cytokines, gene expression, cell subsets) so that one can link them together and back to a particular individual or response group. Currently, this can be done ad hoc by experts, but a general user-friendly software package would be very welcome.

Another challenge is that there is a great deal of white blood cell subset variation in people, such that one person may have three times the number of B cells as another (healthy) person, or 10 times the number of NKT cells. This variation means that gene expression in blood cells is fraught with "noise," which can easily obscure important results, such as differences in gene expression between B cells in different patient groups. Fortunately, a new statistical method has been developed that allows one to simultaneously analyze information about a group's subset variation and gene expression patterns and directly compare that group's average gene expression pattern with that of another group for the different cell types [30]. In a test case, this method, called csSAM (cell specific significance analysis of microarrays), found hundreds of genes that were expressed differently in a group of patients who rejected their kidney transplants versus those who tolerated their graft. In this group of 24 patients, conventional analysis had failed to find any consistent gene expression differences between the two groups.

Other important bioinformatic advances involve the use of gene expression "modules" to organize sets of genes important for immune function and to determine their relationships and hierarchies [31]. This approach has already had success in finding commonalities between responses to autoantigens and pathogens, and has helped refine the definition of an interferon signature found in certain types of autoimmunity. In summary, bioinformatics will play a critical role in analyzing the complex datasets that are beginning to emerge in vaccine studies and in relating that information to an overall picture of the immune response in health and disease. Significant work remains to be done in integrating the different datasets and using them to develop conclusions about likely vaccine efficacy or patient prognosis.

Conclusion

Applying these many new analysis methods to vaccine research is rapidly changing how vaccines are evaluated. We are now able to obtain a much more complete view of the immune response to a given vaccine, providing a more reliable way to assess and improve efficacy, allowing new methods to be tested quickly on smaller numbers of people, shortening the development time and expense, and increasing the success rate. We believe that tetramers and other probes for specific populations of lymphocytes will become increasingly important parts of this analysis, as they will reveal the antigenic and functional breadth of the T- and B-cell responses. Thus we can look forward to a highly productive new era in vaccine research.

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Mark M. Davis, Ph.D.

Dr. Davis is a professor at the Stanford University School of Medicine in the Department of Microbiology and Immunology. He also has served as the Director of the Stanford Institute for Immunity, Transplantation and Infection since 2004, and, since 2007, he has held the title of the Burt and Marion Avery Family Professor of Immunology. Additionally, he is an investigator at the Howard Hughes Medical Institute.

Dr. Davis is well known for his identification in the 1980s of the elusive T-cell antigen receptor genes, which allow T lymphocytes to fight disease-causing microbes. He has been an international leader in the T-cell receptor area since that time, including the development of peptide-MHC tetramer reagents with Dr. John Altman. In addition, Dr. Davis has led the field in many other aspects of T-cell biology and in human immunology. He has published more than 250 research articles.

Dr. Davis received his B.A. in molecular biology from Johns Hopkins University with departmental honors and his Ph.D. in molecular biology from the California Institute of Technology, where he was the recipient of the Milton and Francis Clauser Doctoral Prize. He spent 3 years as a postdoctoral and staff fellow at the National Institutes of Health (NIH) before moving to Stanford in 1983.

Dr. Davis has received numerous honors and awards, including the Behring-Heidelberger Award from the American Association of Immunologists, the Alfred P. Sloan Prize from the General Motors Cancer Research Foundation, and the King Faisal International Prize in Medicine. He was a Pew Scholar for 4 years and is a member of the National Academy of Sciences, the American Academy of Arts and Sciences, and the Institute of Medicine. He has served as a member of the NIH Allergy and Immunology Study Section and a variety of other review panels.

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Dr. Altman pioneered the development of tetramer reagents. He has developed several research programs centered around the use of this technology to investigate many aspects of CD8+ T cell mediated immune responses to viral infections. His research also focuses on new ways to prevent or cure HIV infections.

Dr. Altman received his B.S. from the Massachusetts Institute of Technology and his Ph.D. in pharmaceutical chemistry from the University of California, San Francisco. He completed his postdoctoral training at Stanford University, where he worked with Dr. Mark Davis. Dr. Altman was a 1999 Pew Scholar.

Developing Vaccines for the Neglected Tropical Diseases

David J. Diemert, M.D., FRCP(C) and Saman Moazami, B.A.

Abstract

Neglected tropical diseases (NTDs) such as hookworm and schistosomiasis rank among the most important health problems in developing countries. Although vaccines for these infections do not currently exist, their development could significantly reduce the global disability associated with these helminthiases. Recent progress in the development of vaccines for the NTDs is described in this article.

Introduction

The neglected tropical diseases (NTDs) consist of a group of parasitic and other infections that are some of the most common diseases of the world's poorest people. The most prevalent NTDs are the soil-transmitted helminth infections, which include hookworm, ascariasis, and trichuriasis; schistosomiasis; liver fluke infections; protozoan infections such as leishmaniasis and Chagas disease; and bacterial infections such as trachoma (Table 1). In addition, NTDs such as leptospirosis and amebiasis are estimated to be highly prevalent, although insufficient data exist to support these claims [1].

FIGURE 1.

Geographic overlap of the major neglected tropical diseases[94]

Figure created by Molly Brady, Emory University.

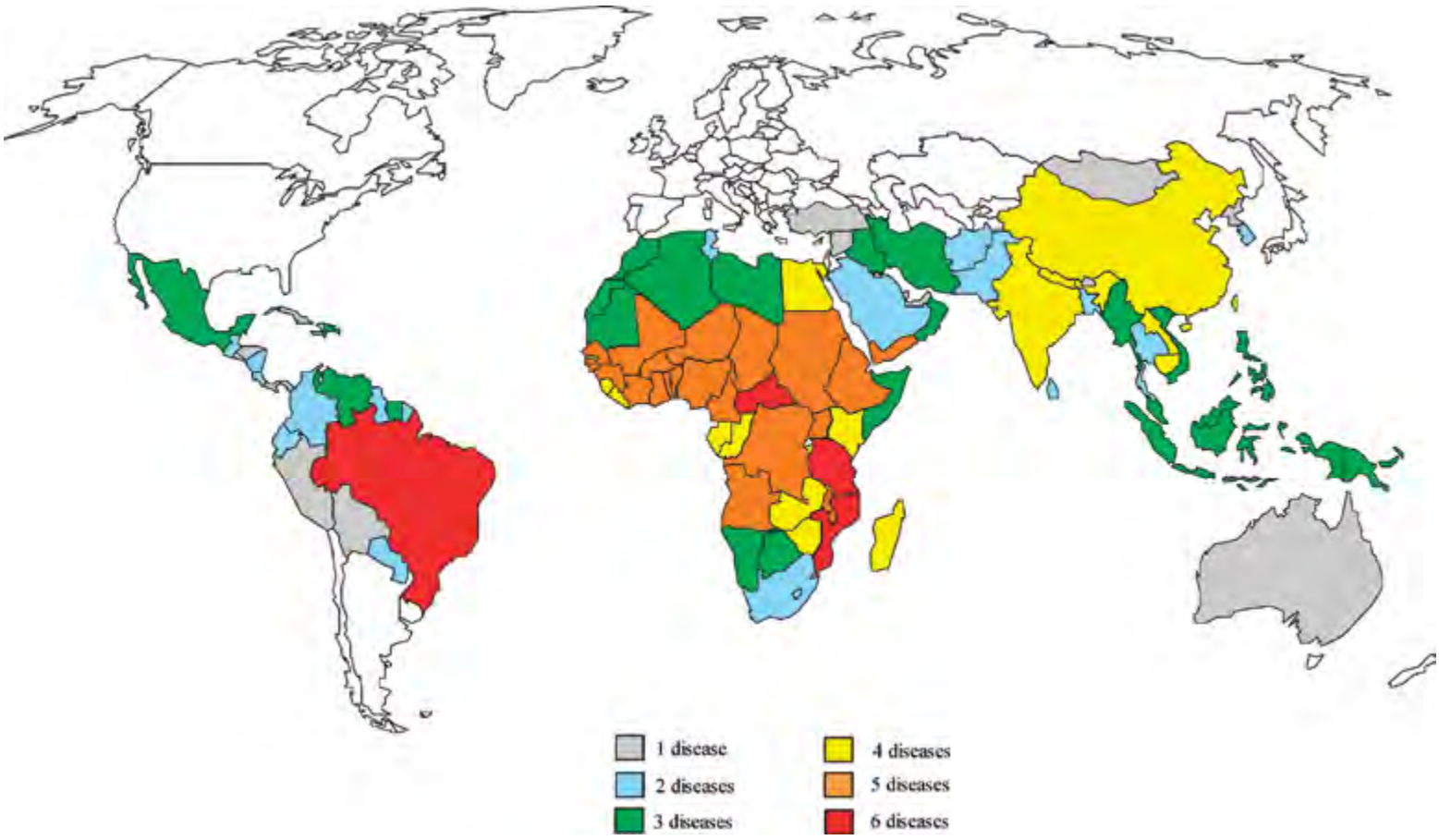


TABLE 1.

The principal neglected tropical diseases

Disease	Predominant Organism(s)	Prevalence (Millions)
Helminth Infections		
Ascariasis	*Ascaris lumbricoides*	800
Hookworm	*Necator americanus* *Ancylostoma duodenale*	600–700
Trichuriasis	*Trichuris trichiura*	600
Schistosomiasis	*Schistosoma mansoni* *Schistosoma haematobium* *Schistosoma japonicum* *Schistosoma intercalatum/mekongi*	200–400
Lymphatic filariasis	*Wuchereria bancrofti* *Brugia timori/malayi*	120
Strongyloidiasis	*Strongyloides stercoralis*	30–100
Clonorchiasis/opisthorchiasis	*Clonorchis sinensis* *Opisthorchis viverrini*	20
Onchocerciasis	*Onchocerca volvulus*	20
Loiasis	*Loa loa*	<13
Cysticercosis	*Taenia solium*	NA
Echinococcosis	*Echinococcus granulosus* *Echinococcus multilocularis*	NA
Protozoan Infections		
Amebiasis	*Entamoeba histolytica*	500
Leishmaniasis	*Leishmania spp*	12
American trypanosomiasis (Chagas' disease)	*Trypanosoma cruzi*	8–9
African trypanosomiasis	*Trypanosoma brucei gambiense* *Trypanosoma brucei rhodesiense*	0.05
Toxoplasmosis	*Toxoplasma gondii*	NA
Cryptosporidiosis	*Cryptosporidium parvum*	NA
Giardiasis	*Giardia intestinalis*	NA
Bacterial Infections		
Trachoma	*Chlamydia trachomatis*	60
Leptospirosis	*Leptospira interrogans*	NA
Leprosy	*Mycobacterium leprae*	NA

NA = Not available

The NTDs share several features that distinguish them from better known infectious diseases. For instance, NTD pathogens do not usually result in acute mortality but, more frequently, they cause chronic infections lasting for years. Over this period they can result in considerable morbidity, such as chronic anemia and inflammation, malnutrition, disfigurement, and blindness. When measured in terms of the disability-adjusted life years (DALYs) lost, it has been argued that the NTDs carry a global health burden equivalent to that of malaria or HIV [2, 3].

Children and women of childbearing age are disproportionally affected by the NTDs. For example, growing children are especially susceptible to the anemia and malnutrition caused by the most common NTDs worldwide, especially hookworm and schistosomiasis [4, 5]. As a result, such children experience stunted growth and cognitive delays [6, 7]. Chronic hookworm infection in childhood has been associated with reduced future wage earnings [8, 9], presumably partly as a result of these effects. Moreover, the anemia and inflammation associated with schistosomiasis and hookworm result in increased maternal morbidity and adverse pregnancy outcomes [10]. In addition, some of the NTDs, such as genital tract schistosomiasis, can result in infertility, and there is evidence that female genital schistosomiasis increases the risk of transmission of HIV [11], while the stigma of disfigurement resulting from lymphatic filariasis, onchocerciasis, and other NTDs also disproportionately affects young women [12].

Currently, there are no licensed vaccines for any of the NTDs. Instead, control efforts are based mostly on periodic mass administration of medications (known as mass drug administration or MDA) targeting one or more of these infections. Cost-effective MDA programs are currently aiming to control or eliminate the soil-transmitted helminths, lymphatic filariasis, onchocerciasis, trachoma, and other NTDs using drugs donated by pharmaceutical companies or low-cost generic drugs [13]. Furthermore, due to the extensive geographic overlap among many of the NTDs (Figure 1), efforts are being made to combine administration of several drugs into a low-cost package to concomitantly control multiple NTDs [13].

Unfortunately, however, MDA is not a magic bullet, and there is a need for new control tools such as vaccines. Due to high rates of drug failure with existing drugs and rapid rates of re-infection following treatment, effective control through MDA has remained elusive for some of the most common NTDs such as hookworm and schistosomiasis [14–17]. In addition, there are other NTDs, such as leishmaniasis and Chagas disease, for which MDA is neither feasible nor possible and development of vaccines represents the most promising strategy for control.

Various species of snails serve as the intermediate host of schistosomes. From left, *Bulinus truncatus truncatus* (host for *S. haematobium*), *Biomphalaria glabrata* (host for *S. mansoni*), and *Oncomelania hupensis hupensis* (intermediate host for the Chinese isolate of *S. japonicum*). Courtesy of Biomedical Research Institute/Fred A. Lewis, Ph.D.

Why are there currently no licensed vaccines for the NTDs? Unfortunately, because the NTDs affect almost exclusively the world's poorest people, no commercial market exists for such vaccines. In addition, important scientific barriers have hampered vaccine development, including the complex genomes of many of the NTDs, the absence of *in vitro* systems to propagate organisms in the laboratory, and the lack of appropriate animal models. Recently, however, the availability of genomes and proteomes for NTD pathogens, access to new adjuvants, and increased financial support from sources such as the Bill & Melinda Gates Foundation have made it possible to expand research and development efforts for NTD vaccines.

In terms of their global health impact, hookworm and schistosomiasis are two of the most important NTDs [17, 18]. When the chronic morbidities associated with these two parasites are tabulated based on the number of DALYs lost, hookworm and schistosomiasis together rank among the most consequential diseases in developing countries, resulting in the annual loss of between 4.5 and 92 million DALYs [3, 4, 19]. As mentioned above, current efforts to control hookworm and schistosomiasis are inadequate, and new tools are needed. The remainder of this article will focus primarily on the status of efforts to develop vaccines to combat hookworm infection and schistosomiasis, with an emphasis on disease due to *Necator americanus*, the most prevalent hookworm, and *Schistosoma*

mansoni, the principal cause of intestinal schistosomiasis. These efforts are being coordinated by the nonprofit Sabin Vaccine Institute located in Washington, DC, working with partners throughout the world, including the George Washington University (United States), the Fundação Oswaldo Cruz (Fiocruz) and Instituto Butantan (Brazil), James Cook University (Australia), and the London School of Hygiene and Tropical Medicine (United Kingdom).

Vaccine Development for Hookworm

Hookworm infection is caused by the soil-transmitted nematodes *N. americanus* and *Ancylostoma duodenale*. Between 600 and 700 million people are currently infected, mostly in the poor rural communities of sub-Saharan Africa, Southeast Asia, and tropical regions of the Americas [20, 21]. The majority of infections are caused by *N. americanus* [22]. Like most NTDs, hookworm does not directly account for substantial mortality, but instead causes chronic anemia and protein malnutrition, which in turn result in impaired physical and cognitive development in children and poor outcomes for pregnant women and their newborns. Current global control efforts rely on the repeated mass administration of a benzimidazole drug (albendazole or mebendazole), particularly to children, although as outlined above, concern regarding the sustainability of this strategy has prompted the search for new approaches to disease control, including the development of a hookworm vaccine [23].

In endemic areas, hookworm infection occurs when infective third-stage larvae (L3) come into contact with the skin, which they actively penetrate. Larvae then migrate within the vasculature to the lungs, where they ascend the pulmonary tree to the pharynx, are swallowed, and molt to become adult hookworms that burrow into the mucosa and submucosa of the small intestine [5]. Hookworms feed by rupturing capillaries and arterioles to ingest blood; lysis of erythrocytes is followed by enzymatic digestion of host hemoglobin [24–27]. Female hookworms mate with males in the small intestine and produce eggs that are expelled from the body in feces. Eggs hatch in warm, moist soil, resulting in a new generation of larvae that continue the life cycle.

Iron-deficiency anemia is the hallmark of hookworm disease and results from intestinal blood loss as a consequence of the feeding of adult worms at the site of parasite attachment in the gut [5, 28]. Protein malnutrition also results from intestinal blood loss [29]. Hookworm is a substantial contributor to the global burden of iron-deficiency anemia, disproportionally affecting children and pregnant women [10, 29–32]. For both children and women, anemia is far more likely to be present in those with moderate to heavy hookworm infections [10, 30], defined based on quantitative fecal egg counts, compared with those with no or light infection.

The failure of individuals living in endemic areas to develop protective immunity despite frequent infection suggests that successful vaccine development will be more challenging than it has been for existing vaccines. However, proof of concept that a human hookworm vaccine is feasible was shown with the 1970s development of a commercial canine hookworm vaccine consisting of irradiation-attenuated L3 that resulted in significant—although incomplete—protection against challenge infection [33–35]. Studies of the immunological basis of protection obtained by vaccinating with irradiated L3 indicated the importance of antibodies directed against antigens secreted by invading larvae [36]. Furthermore, passive transfer of antibodies obtained from dogs immunized with irradiated L3 resulted in protection of nonvaccinated dogs [37].

Due to these results, the first antigens to be explored as potential vaccine components were those associated with invading L3. Incubating hookworm L3 *in vitro* with serum leads to the release of three main products, two of which are members of the pathogenesis-related protein superfamily: *Ancylostoma* secreted protein (ASP)–1 and ASP–2 [38–40]. ASP–2 was chosen as the most promising potential larval component of a hookworm vaccine and advanced into clinical development based on several pieces of evidence, including studies demonstrating that ASP–2 is the predominant antigen to which the antibody response to the irradiated L3 *A. caninum* vaccine is directed [41]. Additionally, when recombinant *A. caninum* ASP–2 (*Ac*-ASP–2) or *A. ceylanicum* ASP–2 (*Ay*-ASP–2) were used to vaccinate dogs or hamsters, respectively, high levels of protection after challenge with live L3 were elicited in terms of reduced adult worm burdens, fecal egg counts, and host blood loss, when compared with control animals [42–44]. Anti-ASP–2 antibodies from vaccinated animals also were able to inhibit the *in vitro* migration of larvae through tissue [42, 45]. Finally, studies in hookworm-endemic areas of Brazil and China demonstrated that anti-ASP–2 antibodies are associated with reduced likelihood of having a heavy hookworm infection [42]. ASP–2 based vaccines likely protect by eliciting antibodies that inhibit larval invasion or development, thereby preventing their maturation into adult worms that inhabit the host's intestine, resulting in reduced worm burdens and intestinal blood loss [23].

N. americanus ASP–2 (*Na*-ASP–2) was produced as a recombinant protein expressed in *Pichia pastoris* yeast cells and was formulated with Alhydrogel (aluminum hydroxide) adjuvant. In a Phase I trial in healthy volunteers in the United States, this vaccine formulation was found to be safe and induced significant and sustained antigen-specific immunoglobulin G (IgG) and cellular immune responses [46]. However, in a second Phase I trial conducted in a hookworm-endemic area of Brazil, several adult volunteers experienced generalized urticaria (hives) immediately upon vaccination [47], leading to the study being halted. Subsequently, it was found that the individuals who developed urticaria had high levels of prevaccination immunoglobulin E (IgE) against *Na*-ASP–2, likely due to previous exposure and infection.

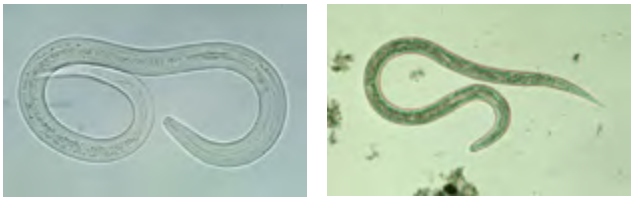
The finding that volunteers living in an endemic area had preexisting levels of IgE to *Na*-ASP–2 that resulted in a serious safety issue with the vaccine led to a more extensive assessment of how prevalent such antibodies might be in the general population. A large sero-epidemiological study was conducted in which sera from more than 800 adults and children living in hookworm-endemic areas of Brazil were tested for IgE antibodies to *Na*-ASP–2 as well as other hookworm antigens being developed as vaccines. The results of this study indicate that a significant proportion of individuals, even young children, have detectable IgE antibodies not only to *Na*-ASP–2, but also to other larval-stage antigens [48].

Because of this significant safety issue associated with larval-stage antigens, their further development has been abandoned. Instead, the vaccines that are currently being developed target the nutritional and metabolic requirements of the *adult* hookworm. The approach has been to identify essential proteins involved in parasite blood feeding, to produce them as recombinant proteins, and then to combine them to elicit protective antibodies upon vaccination [23].

N. americanus depends on host hemoglobin and serum proteins for survival. Following ingestion of blood, erythrocytes are lysed to release hemoglobin that is degraded by a series of hemoglobinases located in the brush-border membrane of the parasite digestive tract (Figure 2) [24, 25]. First, intact hemoglobin is cleaved by an aspartic protease (*Na*-APR–1), followed by further proteolysis through the action of several cysteine proteases and metalloproteases that yield peptides and free amino acids, which serve as the worm's source of energy [49]. After cleavage from digested globin, both free heme and hematin-containing iron can generate oxygen radicals that may damage parasite structures [50]. Hookworms have developed mechanisms to detoxify and transport heme, such as the glutathione S-transferase (GST) molecule of *N. americanus* (*Na*-GST–1) that can bind both heme and hematin, thereby putatively neutralizing their toxicity (Figure 2) [26, 51–53].

Candidate Hookworm Vaccines

Na-GST–1 and *Na*-APR–1 are the lead hookworm vaccine antigens that have been selected for clinical development based on criteria such as efficacy in animal trials, data from epidemiological studies in individuals resident in endemic areas, and the feasibility of protein expression and manufacture using low-cost protein expression systems [23, 29]. Both antigens are involved in parasite blood feeding, and it is thought that each will induce antibodies that will inhibit worm survival by interfering with the function of the respective protein. Importantly, no detectable levels of IgE to either *Na*-GST–1 [54] or *Na*-APR–1 [55] have been found in individuals living in hookworm-endemic areas of Brazil, thus permitting their continued development.

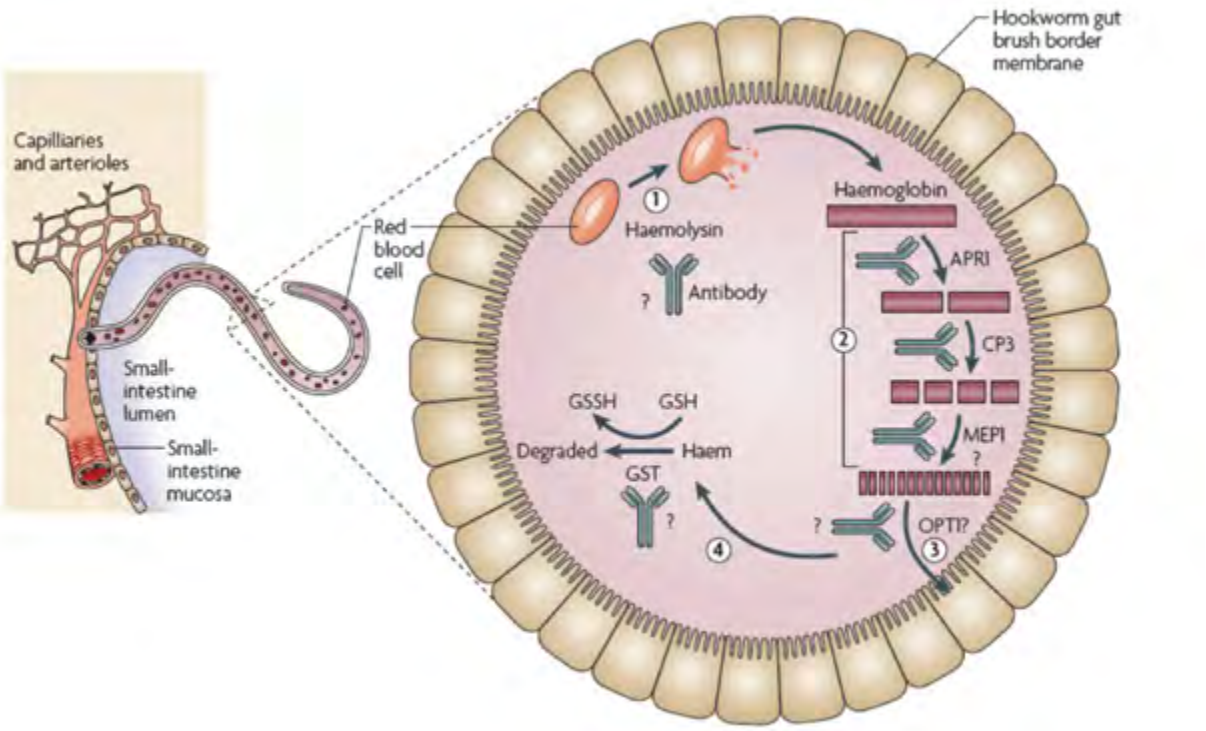


Filariform (L3) hookworm larvae are found in the environment and infect the human host by penetration of the skin. Courtesy of CDC

Na-GST–1 is a 24-kDa recombinant protein expressed in genetically engineered *P. pastoris*. The protein belongs to the Nu class of nematode GSTs that are characterized by reduced peroxidase activity relative to other classes of GSTs but elevated binding capacity for heme and related products [26, 51, 56]. *Na*-GST–1 forms homodimers in solution, creating atypically large binding cavities accessible to a diversity of ligands, including heme. In dogs, vaccination with the recombinant GST–1 homologue from *A. caninum* resulted in significantly lower worm burdens and fecal egg counts following challenge with infective larvae, compared with controls [26]. Similarly, vaccination of hamsters with recombinant *Na*-GST–1 followed by homologous larval challenge resulted in substantially lower worm burdens [52]. Because of these encouraging results, recombinant *Na*-GST–1 (formulated with Alhydrogel) was produced according to current good manufacturing practice

FIGURE 2.

Degradation of host blood by *Necator americanus* hemoglobinases lining the adult worm's brush border membrane, followed by detoxification of free heme and absorption of free amino acids



Erythrocytes are lysed in the gut of the adult worm (step 1), followed by digestion of host hemoglobin by an ordered cascade of hemoglobinases (step 2). Released globin and free amino acids are absorbed by gut cells, putatively transported by OPT1 (step 3), while free heme is detoxified by glutathione S-transferase (GST) (step 4). Question marks indicate processes that have not been experimentally confirmed.

APR1, an aspartic protease; CP3, a cysteine protease; GSH, glutathione; GSSH, glutathione disulphide; GST, glutathione S-transferase; MEP1, a metalloproteinase; OPT1, oligopeptide transporter-1.

Source: Reproduced from Hotez PJ, Bethony JM, Diemert DJ, Pearson M, Loukas A. Developing vaccines to combat hookworm infection and intestinal schistosomiasis. Nat Rev Microbiol. 2010 Nov;8(11):814-26 [95].

(GMP) and successfully underwent preclinical toxicology testing. An Investigational New Drug Application was submitted to the Food and Drug Administration in January 2011, and a Phase I trial of this candidate vaccine is scheduled to begin in Brazil.

Na-APR–1 is a 45-kDa recombinant protein that has had its protease activity inactivated by mutation of the catalytic aspartic acid residues to alanines [55]. In dogs vaccinated with either recombinant *Na*-APR–1 or *Ac*-APR–1, antigen-specific antibodies were induced that inhibited protease activity *in vitro* and were associated with substantial protection from infection and anemia following challenge with *A. caninum* larvae [55, 57]. Vaccination with *Ac*-APR–1 also resulted in a significant reduction in worm burdens in hamsters challenged with *N. americanus,* compared with controls [58]. Following vaccination, anti-APR–1 antibodies are ingested by the parasite during blood feeding and localize to the parasite digestive tract, where they are thought to inhibit hookworm feeding by neutralizing enzyme activity (Figure 2) [57, 58]. Several systems have been evaluated to express recombinant *Na*-APR–1, with *Escherichia coli* [55] and tobacco plants [59] producing the highest yields. Other molecules involved in hookworm blood feeding have been identified [60], including putative orthologs of the extracellular domain of a peptide transporter that is essential for nutrient uptake and growth in *Caenorhabditis elegans* [49]

and a prolyl-carboxypeptidase (contortin) that protects sheep against *Haemonchus contortus* [61].

Ultimately, the aim is to combine *Na*-GST–1 and *Na*-APR–1 in a single vaccine formulation with the goal of preventing the moderate and heavy hookworm infections that are associated with significant intestinal blood loss. Protective immunity would manifest as diminished hookworm-related blood loss and reduced numbers of hookworms in the intestine, compared with unvaccinated people. Because hookworm-related morbidity is proportional to the number of worms harbored by individuals, a fully sterilizing vaccine is not considered an absolute requirement, and one that prevents moderate and heavy infections would be sufficient to have a major impact on the worldwide burden of disease. Such a vaccine could be administered to very young children prior to exposure to infective larvae in the environment or to older children who may have already been exposed and infected, following administration of an anthelminthic drug [62].

Vaccine Development for Schistosomiasis

Approximately 200 million people are affected by schistosomiasis [53]. In Africa, *S. haematobium* causes urinary tract schistosomiasis, whereas *S. mansoni* is the principal cause of intestinal schistosomiasis. *S. mansoni* also causes schistosomiasis in Latin America, with most of the cases occurring in Brazil, whereas *S. japonicum* and *S. mekongi* cause fewer than 1 million cases of intestinal schistosomiasis in Asia. Humans become infected upon contact with fresh water containing microscopic cercariae, which directly penetrate the skin, enter the vasculature, and eventually migrate to the venous system, where they become sexually mature adults, pair, and mate. *S. haematobium* adult schistosomes migrate to the venous plexus that drains the bladder and reproductive organs, while *S. mansoni* and *S. japonicum* inhabit the mesenteric veins draining the intestine. Most of the pathology associated with schistosomiasis is related to the immune response to parasite eggs deposited in host tissues such as the liver or bladder, with the resulting granulomatous lesions leading to fibrosis and end-organ dysfunction [19, 64, 65]. In addition, anemia is a key manifestation of this chronic infection, with children and pregnant women being especially vulnerable, as with hookworm [66–70]. Schistosomiasis-associated anemia has been attributed to several different mechanisms, including iron deficiency due to blood loss in the intestine or urine, splenic sequestration and destruction of erythrocytes, autoimmune hemolysis, and the chronic inflammatory response to schistosome eggs [66, 71].

Schistosoma mansoni adult. Courtesy of the National Cancer Institute(NCI)/Bruce Wetzel and Harry Schaefer

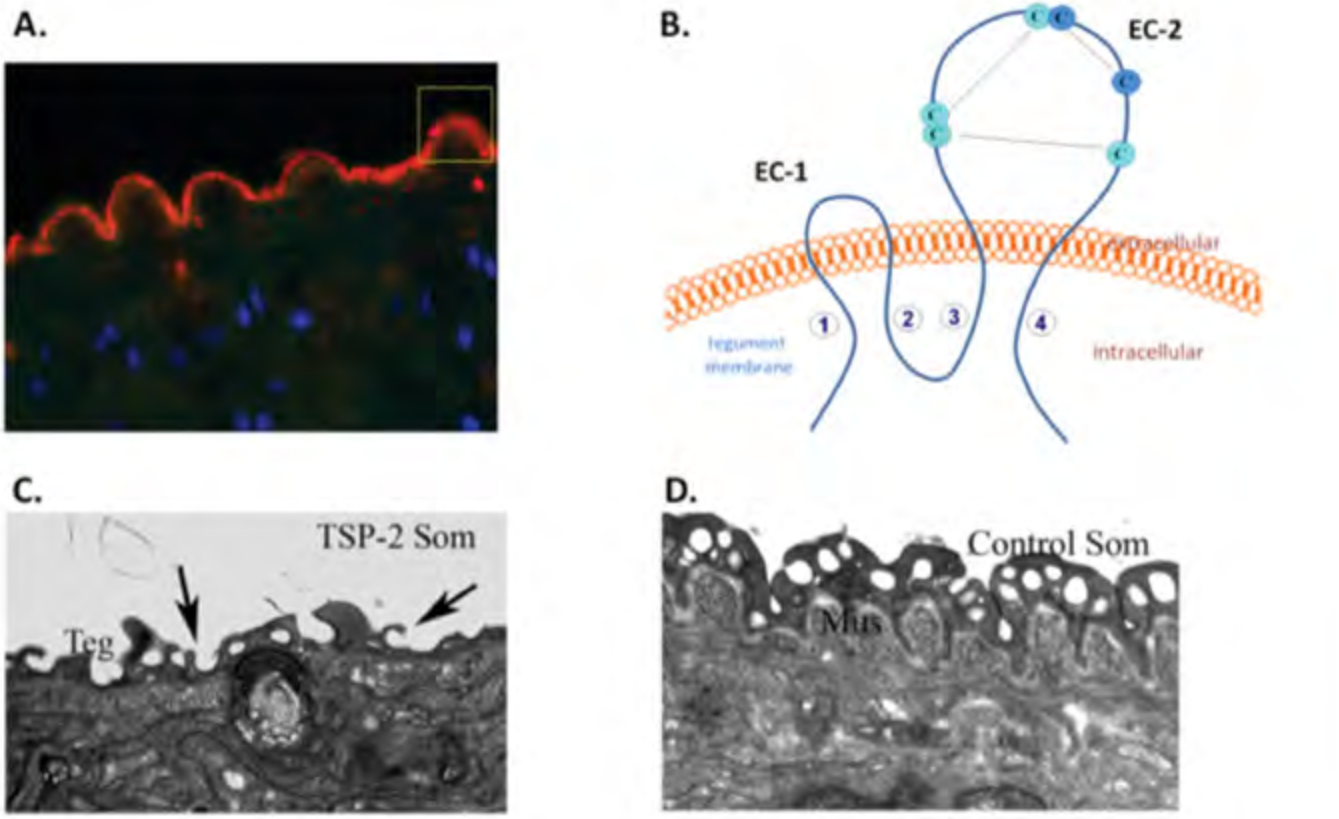
Unlike with hookworm infection, individuals residing in endemic areas can become resistant or partially immune to re-infection with schistosomiasis over time [72]. Furthermore, irradiated cercariae can elicit high levels of protective immunity in laboratory animals, and several recombinant protein vaccines have been shown to elicit comparable levels of protective immunity in immunized animals that were subsequently challenged with cercariae [73].

Candidate Schistosomiasis Vaccines

To date, one vaccine for urinary schistosomiasis has entered clinical trials. A recombinant 28-kDa GST from *S. haematobium* formulated with aluminum hydroxide adjuvant has undergone Phases I and II clinical testing in Europe and West Africa and has been reported to be immunogenic and safe [73,

FIGURE 3.

Tegument of an adult male *Schistosoma mansoni* worm



Mus = muscle; Som = schistosomula; Teg = tegument.

Panel A: Fluorescence micrograph of the tegument probed with mouse anti-Sm-TSP–2 antibody (red); blue represents nuclei stained with 4',6-diamidino-2-phenylindole (DAPI). **Panel B:** Schematic representation of Sm-TSP–2 in the tegument plasma membrane; extracellular (EC) loops are shown, with colored circles containing a "C" indicating cysteine residues and lines between them denoting disulfide bonds; numbers inside circles indicate the transmembrane domains from N- to C-termini. **Panel C:** Tegument (Teg) of *S. mansoni* schistosomula (Som) incubated for 7 days with Sm-TSP–2 double-stranded RNAs. Digitate extensions (arrows) are more abundant on the tegument surface. **Panel D:** Tegument of *S. mansoni* schistosomula incubated for 7 days with luciferase control double-stranded RNAs.

Sources: Panel A: Loukas A, Tran M, Pearson MS. Schistosome membrane proteins as vaccines. Int J Parasitol. 2007 Mar;37(3-4):257-63 [81] (© 2007 Elsevier, reproduced with permission). Panel C: Tran MH, Freitas TC, Cooper L, Gaze S, Gatton ML, Jones MK, et al. Suppression of mRNAs encoding tegument tetraspanins from Schistosoma mansoni results in impaired tegument turnover. PLoS Pathog. 2010;6(4):e1000840 [85]. Panel D: Hotez PJ, Bethony JM, Diemert DJ, Pearson M, Loukas A. Developing vaccines to combat hookworm infection and intestinal schistosomiasis. Nat Rev Microbiol. 2010 Nov;8(11):814-26 [95].

74]. In addition, several candidate vaccines for intestinal schistosomiasis caused by *S. mansoni* will soon be ready for clinical testing [75]. Sm-p80, the large subunit of a calcium-dependent neutral protease, is the basis of a DNA vaccine that provides levels of protection in baboons comparable to that provided by irradiated cercariae [76, 77]. Another *S. mansoni* vaccine potentially moving into clinical development is Sm14, a 14-kDa fatty acid binding protein that also elicits protection in experimental animals [78, 79]. Finally, the *S. japonicum* molecule paramyosin is undergoing pilot-scale manufacture in Asia, potentially as a transmission-blocking vaccine administered to water buffaloes [80].

The Sabin Vaccine Institute, in partnership with Instituto Butantan and Fiocruz, also is developing *S. mansoni* vaccines. The primary targets of this schistosomiasis vaccine development program are proteins found on the outer surface, or tegument, of adult *S. mansoni* worms [81]. Schistosome tegument is thought to be a dynamic layer involved in critical physiologic processes, including evasion of host immune responses, worm nutrition, and osmoregulation [81]. A family of tegumental proteins called "tetraspanins" (TSP) has been

identified that contains four transmembrane domains with two extracellular loops that are predicted to interact with exogenous proteins or ligands (Figure 3) [81, 82].

The second extracellular domain fragment of a schistosome tetraspanin known as *Sm*-TSP–2 has been selected for development as a vaccine antigen. Recombinant *Sm*-TSP–2 provides high levels of protection in vaccinated mice upon challenge with *S. mansoni* cercariae [83]. In addition, putatively resistant individuals who are repeatedly exposed but remain uninfected have elevated antibody responses to *Sm*-TSP–2, compared with chronically infected individuals [84]. Given these data, *Sm*-TSP–2 is being developed as a recombinant protein expressed in *P. pastoris* and adjuvanted with Alhydrogel. GMP manufacture at Instituto Butantan is planned, with clinical testing to start in Brazil in 2012.

Sm-TSP–2 is thought to play a critical role in tegument development, maturation, or stability [85]. Treatment of adult worms or schistostomula with *Sm*-TSP–2 double-stranded RNA (dsRNA) results in a vacuolated and thinner tegument, compared with controls [85], while mice injected with schistosomula pretreated with *Sm*-TSP–2 dsRNA develop significantly fewer worms recovered in their mesenteric veins, compared with mice injected with untreated schistosomulae [85]. Other tegument tetraspanins are also potential candidate vaccines, such as *Sm*-TSP–3, a protein highly expressed by maturing schistosomula, a developmental stage that is susceptible to attack by human immune responses [86, 87]. Finally, Sj23 is a tegument tetraspanin that is being developed as an *S. japonicum* DNA vaccine for water buffaloes in China [88].

Vaccine Development for Other Neglected Tropical Diseases

Although vaccines for hookworm and schistosomiasis are the most advanced, candidate vaccines also are being developed for other NTDs such as onchocerciasis and leishmaniasis, to name a few. More than 37 million people in Africa, South America, and the Arabian Peninsula are infected with *Onchocerca volvulus*, the cause of river blindness. Vaccine development activities have focused on identification of specific L3 antigens, because this stage seems to be the target of protective responses in putatively immune individuals who are chronically exposed but remain uninfected [89]. Using sera from such individuals, more than 20 specific immunoreactive antigens have been identified, with *Ov*-CPI–2 (*O. volvulus* cystatin, or onchocystatin) being the most immunodominant [89]. This antigen is currently the lead candidate vaccine being developed for *O. volvulus* infection.

Leishmaniasis is a protozoan parasitic infection that currently affects 12 million people globally, with approximately 2 million new cases annually [90]. For centuries, inoculation with live *Leishmania major* (leishmanization) has been effective in providing lifelong protection against cutaneous leishmaniasis. However, given the safety concerns of such an approach, alternative vaccination strategies are being pursued [90]. Given that *L. major* dwells within macrophages, vaccine development has focused on stimulation of type 1 T helper cell (Th1) cellular immune responses to promote killing and control of intracellular replication. Because recombinant proteins alone induce poor T-cell responses, incorporation of adjuvants such as Toll-like receptor agonists is being explored to efficiently induce predominantly Th1 responses. Multiple recombinant parasite antigens have been tested in animal studies and clinical trials with a combination of LmST11 (*L. major* homologue to eukaryotic stress-inducible protein) and TSA (thiol-specific-antioxidant protein), showing the most promising efficacy in nonhuman primates [91]. Additionally, sand fly salivary antigens have shown promise as transmission-blocking candidate vaccines [92]. The prospect of developing a successful vaccine against leishmaniasis has been strengthened by the facts that protective antigens are shared between *L. major* species, that vaccine development can be pursued in both dogs (an important reservoir host) and humans, and that vaccines can potentially have both prophylactic and therapeutic uses [93].

Conclusion

Vaccines for two of the most important NTDs—hookworm and schistosomiasis—are being developed to reduce the major parasite-induced morbidities, including intestinal blood loss, chronic inflammation, and fibrosis [17]. Administered in early childhood, such vaccines are anticipated to prevent the major pediatric sequelae of these infections, which include anemia, malnutrition, and impaired physical and cognitive maturation. Such vaccines also may have a significant impact on poverty reduction because of their potential effect on improving child and maternal health and development.

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The Public Health Need for a *Staphylococcus aureus* Vaccine

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Abstract

An effective *Staphylococcus aureus* vaccine could substantially reduce morbidity and mortality resulting from *S. aureus* disease. As candidate vaccines and the optimal implementation strategies to maximize their public health impact are evaluated, the analysis should include considerations related to patients seeking health care in a broad variety of settings.

Introduction

Staphylococcus aureus colonizes the skin or mucous membranes of roughly 30 percent of the human population [1]. It has long been recognized as a major cause of localized and invasive infections, resulting in a diverse set of clinical syndromes along a wide spectrum of illness severity that includes skin and soft tissue infections (SSTIs), muscle and visceral abscesses, septic arthritis, osteomyelitis, pneumonia, pleural empyema, bloodstream infections, endocarditis, and toxin-mediated syndromes, including toxic shock syndrome, scalded skin syndrome, and food poisoning. In addition, *S. aureus* is a major cause of healthcare-associated infections, including surgical site infections, infections associated with the use of invasive devices, and pneumonia. The emergence of methicillin-resistant strains as a major cause of *S. aureus* infections, first in health care and more recently in community settings, has had an important public health impact. First, methicillin-resistant *S. aureus* (MRSA) strains that have recently emerged in the community have spread rapidly and now have become the most common cause of community-associated purulent SSTIs [2]. Second, infections caused by MRSA have fewer effective treatment options, especially for the most serious infections. Studies suggest that patients with healthcare-associated MRSA bloodstream infections are almost twice as likely to die from the infections, compared with patients with infections caused by methicillin-susceptible strains [3]. One potential explanation for this observation is decreased effectiveness of anti-staphylococcal agents that are frequently used in treating MRSA infections. For example, infections caused by MRSA strains with a vancomycin minimum inhibitory concentration of 2 mcg/ml, which are considered susceptible according to current testing standards, have been associated with clinical failure and worse outcomes following vancomycin therapy [4]. In addition, 12 strains of *S. aureus* that are fully resistant to vancomycin have now been reported [5]. Furthermore, recent reports of resistance to newer anti-staphylococcal agents such as linezolid and daptomycin raise concern about the future durability of these agents, and few additional anti-staphylococcal antibiotics are currently in the drug development pipeline. These limitations in the availability of effective therapy for serious *S. aureus* infections highlight the importance of implementing effective prevention strategies. Current prevention strategies appear to have significant limitations; the addition of a safe and effective *S. aureus* vaccine to current prevention strategies has the potential for great public health benefit.

Burden of Disease

Measuring the absolute burden of *S. aureus* disease is extremely challenging because of the infection's diverse clinical manifestations, the different levels of care required for treatment, and the resulting variability in morbidity. A 2001 estimate of the frequency of hospitalizations in the United States associated with any type of *S. aureus* infection was 292,000 discharges, 20 percent of which may have been associated with an invasive procedure or surgery [6]. Using 2005 data and a similar methodological approach resulted in an estimated 477,927 *S. aureus* associated hospitalizations; of these, 103,300 were classified as *S. aureus* septicemias [7]. Most of the increase observed since 1999 was attributable to the increasing frequency of MRSA-associated SSTIs among nonhospitalized patients requiring inpatient therapy [7]. On the pediatric side, a specialized evaluation of 33 U.S. children's hospitals identified a twofold increase in *S. aureus* associated hospitalizations between 2002 and 2007, when it reached 35 per 1,000 admissions [8]. Limitations in the use of administrative data to estimate burden of disease have been highlighted elsewhere and include, most importantly, the data's lack of sensitivity

as well as a lack of specificity in the ability for researchers to accurately classify types of infection [9, 10].

Dedicated surveillance systems to measure incidence of specific types of *S. aureus* disease allow for more accurate estimates of these types of infections. Since 2005, annual population estimates of invasive MRSA infections have been conducted as part of the Centers for Disease Control and Prevention's (CDC's) Emerging Infections Program activities. Most invasive infections among persons with obvious healthcare exposures—those in which MRSA has been cultured from a normally sterile site—occur within the first few days of hospital admission (about 60 percent) or later during hospitalization (25 percent) [11]. In 2008, an estimated 89,785 invasive MRSA infections occurred in the United States, reflecting a decrease from the 105,222 estimated in 2005 [12]. This overall decline was accounted for by decreases in hospitalized and recently discharged persons (i.e., healthcare-onset or -associated disease) [13]. Although the reason for the decrease was not systematically determined, investigators suspect it occurred as a result of hospital-based MRSA bloodstream infection prevention efforts. Despite this overall decline, an estimated 15,249 persons died with invasive MRSA infections during their hospitalization in 2008. Although this population-based system focuses on MRSA, other data sources suggest that these burden estimates reflect about half of all invasive *S. aureus* infections in the United States. National prevalence assessments have estimated the proportion of MRSA positive *S. aureus* isolates cultured from blood to range between 52 percent and 59 percent [7, 14, 15]. By assuming that 55 percent of all invasive *S. aureus* infections are MRSA and extrapolating from the 2008 MRSA-specific estimates, an estimated 163,000 persons developed invasive *S. aureus* infections, with an associated 27,000 deaths.

Invasive disease represents the most serious of *S. aureus* infections, as reflected by the fact that roughly 88 percent of these infections are bloodstream infections [13]. However, many other severe infections are not captured by these estimates, including some surgical site infections, pneumonia, or necrotizing fasciitis without associated bloodstream infections. Therefore, these estimates should not be used to describe the complete burden of severe *S. aureus* disease, but rather to develop a conceptual framework to identify those populations most at risk and potential vaccination strategies.

Populations at Risk Relevant to *S. aureus* Vaccine

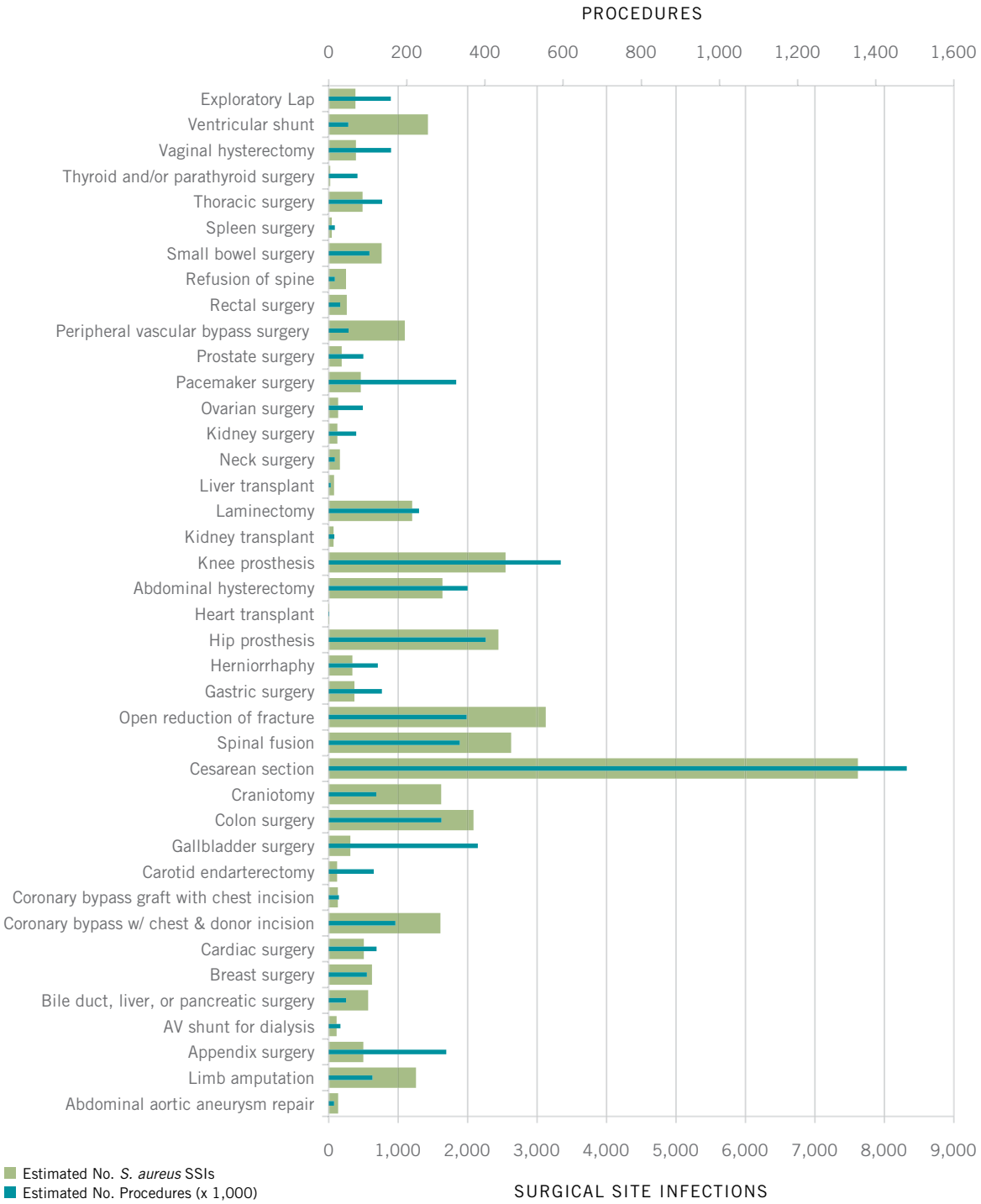
Hidden within these large population estimates are groups of people who share characteristics placing them at high risk for severe infections with *S. aureus*. Identifying these populations is critical to outlining a vaccine prevention strategy. Hemodialysis patients are known to be at highest risk of infection, with rates of invasive MRSA estimated to be as high as 45.2 per 1,000 population (about one-hundredfold higher than the general population) [16]. Assuming that these rates would double if they include methicillin-susceptible *S. aureus* infections, roughly 30,000 invasive *S. aureus* infections would be likely to occur among the 350,000 hemodialysis patients each year in the United States [13]. An effective *S. aureus* vaccine would therefore result in significant benefits for this patient population.

Another population at high risk for invasive *S. aureus* infections is surgical patients, particularly those undergoing cardiac, orthopedic, and spinal procedures. For example, among procedures reported to CDC's National Healthcare Safety Network (NHSN), about 2–5 percent of patients undergoing cardiac surgery develop surgical site infections, of which roughly 33 percent are caused by *S. aureus* [14, 17]. The frequency and type of postoperative invasive *S. aureus* infection varies significantly across procedure types [14, 18]. *S. aureus* accounts for roughly one-third of surgical site infections following obstetrical and gynecological procedures (28 percent); higher proportions are reported for major orthopedic procedures (48 percent) and neurologic procedures (51 percent), and lower proportions for abdominal procedures (13 percent) [14]. Considering how frequently these procedures are performed in U.S. hospitals, approximately 40,000 patients are expected to develop surgical site infections with *S. aureus* within 30 days of the procedure (or within 1 year if an implant is left in place) (Figure 1). Data from NHSN demonstrate that about half of these would be superficial surgical site infections [19]. Patients undergoing elective surgical procedures could be an appropriate target population for preoperative vaccination. Some populations will be difficult to include in any immunization program, most notably those undergoing emergency procedures such as cesarean sections, open reduction of fractures, and potentially amputations. Infections from these three procedure types, which are likely out of reach of a typical vaccine prevention strategy, account for about 30 percent of the estimated 40,000 *S. aureus* surgical site infections.

Investigation of the use of *S. aureus* vaccine in surgical populations has focused, to date, primarily on elective cardiac

FIGURE 1.

Estimate of number of surgical procedures performed in the United States each year and the corresponding estimated number of *Staphylococcus aureus* surgical site infections, calculated using unadjusted rates reported to the National Healthcare Safety Network



Sources: Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, et al. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. Infect Control Hosp Epidemiol. 2008 Nov;29(11):996-1011. Edwards JR, Peterson KD, Mu Y, Banerjee S, Allen-Bridson K, Morrell G, et al. National Healthcare Safety Network (NHSN) report: data summary for 2006 through 2008, issued December 2009. Am J Infect Control. 2009 Dec;37(10):783-805. CDC. National Center for Health Statistics. FastStats: inpatient surgery [Internet]. Atlanta, GA: CDC; 2010 [updated 2010 Jan 18; cited 2010 Oct 14]. Available from: www.cdc.gov/nchs/fastats/insurg.htm

and orthopedic surgical patient populations. More than 1 million adults undergo coronary artery bypass graft surgery or major orthopedic procedures each year in the United States [20]. Based on data reported to NHSN, we estimate that fewer than 5,000 of these procedures are complicated by deep tissue or organ space *S. aureus* surgical site infections [14, 17]. Although targeting elective cardiac or joint replacement surgical populations with an effective *S. aureus* vaccine would provide significant morbidity and mortality benefit to these populations, particularly because *S. aureus* surgical site infections following these procedures require additional surgical procedures with additional morbidity to the patients, limiting a vaccination program to these procedures would, again, be expected to prevent only a small fraction of serious *S. aureus* infections (Figure 2).

Although not necessarily relevant to an active immunization program, but very relevant when considering passive immunization as a therapeutic agent or treatment adjuvant, *S. aureus* is a particular burden among newborns admitted to neonatal intensive care units. Between 1990 and 2004, the incidence of *S. aureus* infections among neonates admitted to high-risk nurseries reported to CDC increased 13 percent; this increase was mostly due to increases in MRSA infections, especially prominent beginning in 1999 [21]. In 2002, the Neonatal Research Network supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), reported that 1.7 percent of infants with birth weights <1,500 grams develop *S. aureus* sepsis during their stay in the intensive care unit [22]. The 2006 national estimates from the National Center for Health Statistics include 63,000 births of infants weighing <1,500 grams. Applying these published infection rates, approximately 2 percent of the newborns in this risk group, or 1,200, infants would develop *S. aureus* sepsis each year, and roughly 17 percent of those will die [8, 22].

Expanding the Notion of Preventable *S. aureus* Infections

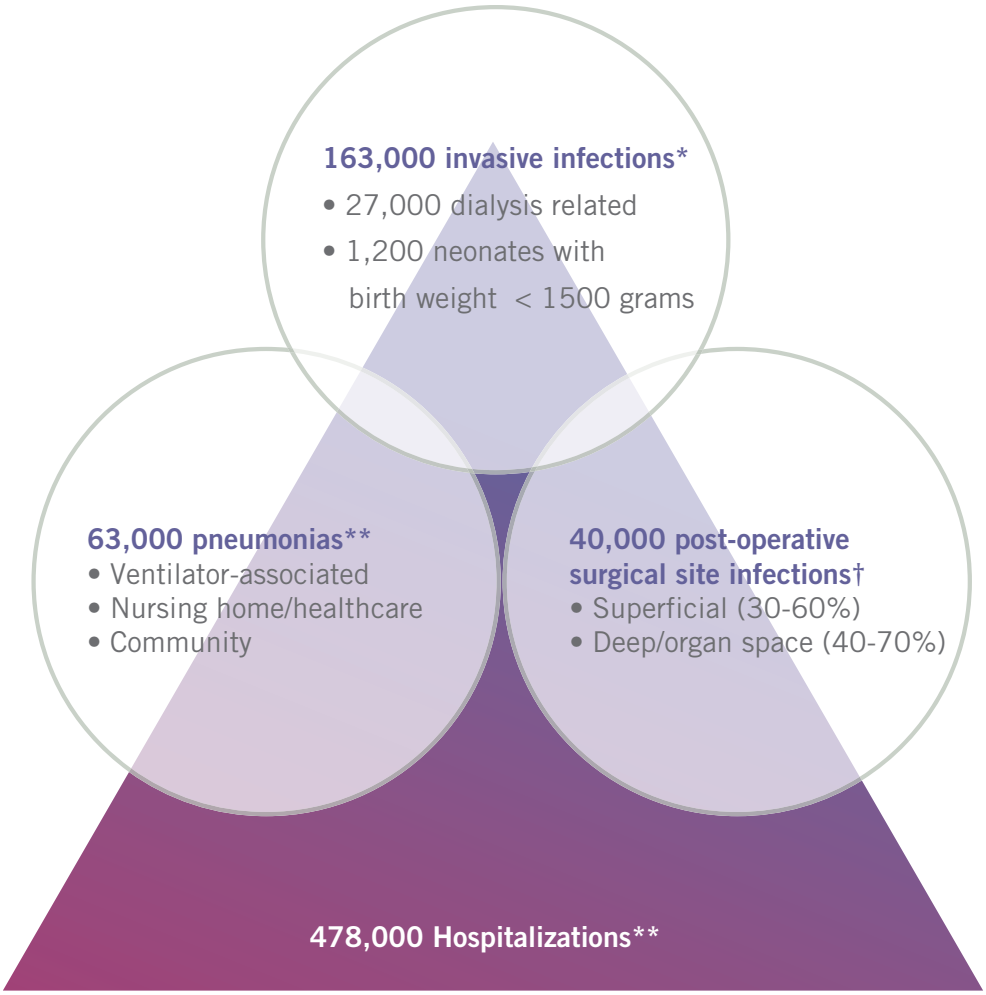
Although certain high-risk patient populations would likely benefit from an effective *S. aureus* vaccine, to have a more substantial impact on the national burden of invasive *S. aureus* infections, more comprehensive vaccination strategies are worth exploring. We recently performed an exploratory analysis on the potential impact of an *S. aureus* vaccine on the estimated national burden of invasive MRSA infections in the United States using a national population-based surveillance program [23]. If extrapolating on these published data to account for methicillin-susceptible *S. aureus* (again, assuming 55 percent of *S. aureus* are MRSA), then the use of a theoretical *S. aureus* vaccine, conferring 1 year of protection among persons 65 years of age and older, could prevent about 24,000 invasive *S. aureus* infections in the year subsequent to immunization. The estimated number needed to vaccinate (NNV) to prevent one case of invasive *S. aureus* infection in this age group would be about 1,000, somewhat lower than the estimated NNV to prevent a case of invasive pneumococcal infection (3,000–5,000) [24, 25], but similar to estimates of NNV to prevent hospitalizations related to influenza (800) [25]. By using a more expansive strategy, vaccinating persons ≥15 years of age at the time of hospital discharge and all those ≥65 years of age annually, approximately 34,000 cases of invasive *S. aureus* could be prevented. Patients being discharged from the hospital represent an important vaccine target group, given their propensity to develop invasive *S. aureus* infections. Although compliance with vaccine administration at hospital discharge may be challenging, identifying and overcoming the barriers will be essential to this type of approach.

Moving Beyond Practice Change to Prevent *S. aureus* Infection

Much progress has been made in recent years in preventing many types of healthcare-associated infections due to *S. aureus*; notable among these successes is marked reduction in the incidence of central line-associated bloodstream infections with either MRSA or methicillin-susceptible *S. aureus* [26]. Efforts aimed at reducing *S. aureus* infections (e.g., pneumonia, bloodstream infections), however, focus on prevention efforts applied to hospitalized persons, where changing the behavior of healthcare personnel, although difficult, has been associated with dramatic reductions in incidence of healthcare-associated infections. Expanding these types of prevention approaches to the postdischarge setting will be challenging but necessary: the majority of invasive infections (about 60 percent) occur among persons outside the acute care setting but with a recent exposure to healthcare delivery [11]. Considering this, the potential impact for prevention though vaccination strategies in the postdischarge setting is very attractive [13]. Although dialysis or surgical patients are attractive primary targets of candidate vaccine trials (e.g., easily identified and consented, repeated visits by same provider and follow-up, high attack rates), broader vaccine strategies will have a larger public health impact. If the vaccine research and development efforts

FIGURE 2.

Estimates of the burden of *Staphylococcus aureus* infections in the United States, from divergent sources and methodology



Sources: ** Noskin GA, Rubin RJ, Schentag JJ, Kluytmans J, Hedblom EC, Smulders M, et al. The burden of *Staphylococcus aureus* infections on hospitals in the United States: an analysis of the 2000 and 2001 Nationwide Inpatient Sample Database. Arch Intern Med. 2005 Aug 8-22;165(15):1756-61.

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lead to candidate vaccines that are effective at providing protection for even a few months, there is potential enormous public health impact by providing protection around the time of healthcare delivery, across a variety of age groups and patient settings. Along similar lines, with the largest burden of *S. aureus* disease remaining in the noninvasive infection types, other groups at high risk for noninvasive community-acquired infections (e.g., athletes, inmates) represent additional potential targets for vaccination worth exploring as vaccine efficacy trials get underway.

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DISCLAIMER *The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry.*

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Adjuvants—Past, Present, and Future

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Abstract

Following the serendipitous discovery that addition of foreign material could enhance immune response to vaccines, alum (aluminum sulfate salts) was identified in 1926 as a potent adjuvant. For many years subsequently, alum remained the only adjuvant in general use for vaccine formulation. As whole pathogens are being replaced by pathogen subunits for vaccine use and significant progress is being made in manufacturing and biotechnology, it is possible to produce large amounts of highly purified subunit vaccines. However, the resulting lots are observed to be less immunogenic, and larger vaccine dose amounts are required to achieve protective vaccine effects. Efforts to address these challenges through adjuvant development have been slow. Recent advances in the fields of immunology and molecular biology, such as the identification and characterization of host pattern recognition receptors, have led to the discovery of new adjuvants and the potential for even more. Ideally, these newer adjuvants should activate specific signal pathways that will safely direct and amplify host immune response to vaccines. To meet the increasing worldwide need for vaccination, this newer approach to adjuvant development and others like it will need to be more vigorously pursued. Ideas for facilitating these approaches are discussed.

Introduction

The concept of vaccination was preceded in the 10th century in China and the 16th century in Africa by inoculation with infectious fluids from smallpox-infected individuals into naive individuals to protect them against the disease. This inoculation procedure (called variolation) was brought to Europe and the Americas around 1720. Vaccination began to replace variolation in 1798, when Edward Jenner published an influential paper on protection from smallpox by inoculation with cowpox materials. Decades later, vaccination led to development of vaccines against other infectious agents with live-attenuated or killed pathogen-based vaccines, or by inactivated toxins [1]. New approaches have followed, such as split pathogens or purified antigens extracted from the pathogen or produced through recombinant technologies. Because pathogens cannot always be grown in the quantities needed to produce vaccines, the vast majority of today's vaccines use purified antigens manufactured under large-scale manufacturing conditions that are compliant with good manufacturing practices (GMP). Purified antigens may lack many features of the original pathogens, including the inherent ability to appropriately stimulate one of the first lines of defense, known as the innate immune response. In target populations with impaired immune systems, or when the targeted pathogen is complex, this feature may take on added significance due to the inability to trigger early protective immune responses. The combination of reduced immunogenicity of purified antigens and an increased awareness of the fact that a subset of the general population that is intended to benefit from vaccination may be inherently unequipped to do so has led to recognition of the need for safe and potent immunologic adjuvants that can act as replacements for the original pathogens' danger signals to trigger, direct, and enhance vaccine-specific immunity.

Gaston Ramon discovered in 1925 that adding substances such as bread crust or tapioca to diphtheria toxoid in a vaccine formulation increased immune responses against the toxoid. One year later, in 1926, Alexander Glenny reported that administering diphtheria toxoid formulated with potassium aluminum sulfate (alum) induced better antibody responses than soluble antigen alone.

Ever since, aluminum salts have been the most widely used vaccine adjuvant approved for human use. More than 70 years passed before a vaccine containing a new adjuvant (MF59) was introduced in several countries in an influenza vaccine. When used as adjuvants, aluminum salts can be safe and effective vaccine components. Since the introduction of aluminum salts in vaccines, increased knowledge in immunology and host-pathogen interaction, as well as access to new production technologies, has led to a more accurate selection of the appropriate antigen(s); development of a theoretical framework for the mode of action of several adjuvants, such as Toll-like receptor (TLR) agonists and aluminum salts; and a better understanding of host-pathogen interaction. The knowledge gained and the recognition of the fact that different adjuvants may be required to elicit a specific immune enhancement have led to a resurgence of interest in adjuvants.

TABLE 1.

Examples of adjuvants used in licensed vaccines

Adjuvant	Pathogen/(Vaccine)
Mineral (aluminum) salts	Pneumococcal conjugate vaccine (*Prevnar*); Hepatitis A (*Havrix*); Hepatitis B + *Haemophilus influenzae* type b (Hib) (*COMVAX*); Human papillomavirus (*Gardasil*); Hepatitis A + Hepatitis B (*Twinrix*)
AS04	Hepatitis B (*Fendrix*) Human papilloma virus (*Cervarix*)
RC529	Hepatitis B (*Supervax*)
MF59	Influenza (*Fluad*)
Virosomes	Influenza (*Inflexal V*)
Cytokine/growth factor	Sipuleucel-T (*Provenge*)

Different Classes of Adjuvants

Over the last three decades, and as a result of research carried out across different disciplines, additional classes of adjuvants have been identified. One of the central reasons has been our improved understanding of the innate immune system and its activation. Although this improved understanding has resulted in regulatory approval of vaccines formulated with new adjuvants, other adjuvants known to be potent immunostimulators are not yet widely used in vaccine formulations due to theoretical safety concerns. Examples of adjuvants in licensed vaccines or those that are in advanced development are discussed below and presented in Table 1.

Mineral Salts

Mineral salts represent the oldest and most frequently used class of vaccine adjuvants. They consist of different salts of aluminum, sometimes collectively referred to as alum. These compounds have been in use since 1926. Alum is licensed in many market regions, including the United States, and is used with a variety of vaccine antigens, including diphtheria, tetanus, hepatitis, *pneumococcal pneumoniae*, and human papillomavirus [1]. Although still widely used and expected to continue to be used, their mode of action is still not yet fully understood and extensive work is being undertaken to establish it [2, 3].

Emulsions/Surfactants

Emulsions are mixtures of two immiscible substances (water and oil), stabilized by the presence of emulsifier or surfactants. The oldest example of this class of adjuvants was developed by Le Moignic and Pinoy in 1916 and consisted of inactivated *Salmonella typhimurium* in an emulsion of water in Vaseline oil. Later, Jules Freund developed two more widely used examples of this group, known as complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA). Both consist of water-in-mineral oil emulsion with mannide monooleate emulsion; they differ in that heat-killed *Mycobacterium tuberculosis* is added to CFA [4]. IFA induces type 2 helper T-cell (Th2) responses, while CFA induces cell-mediated responses as well. Due to some cases of sterile abscess induction, plus the fact that they are relatively unstable, neither of these adjuvants is now being used in humans.

Perhaps the most widely known and widely used adjuvant in this class is MF59, which is an emulsion of 4.3 percent squalene in water stabilized by nonionic surfactants (Tween 80 and Span 85) in low ionic strength citrate buffer. Squalene is a natural hydrocarbon primarily obtained from shark liver oil. In MF59, the squalene droplets are <250 nanometers (nm) in diameter. The emulsion is stabilized by microfluidization and filter-sterilized before being combined with the antigen being investigated [5]. Although it is currently not a component of a Food and Drug Administration (FDA)-licensed vaccine, MF59 has been widely used in clinical trials of vaccines in the United States and in licensed products in other parts of the world (Table 1) [6–8]. The adjuvant effect is believed to be based on early leukocyte recruitment. MF59 is also believed to stimulate the local muscle fibers to produce immune factors that activate local dendritic cells (DCs). MF59 adjuvant effects are therefore believed to be based on enhanced antigen presentation and enhanced antibody production.

Saponins

Saponins have been known and tested in veterinary vaccines for more than 40 years in the partially purified form known as Quil A [9, 10]. They are a heterogeneous group of sterol glycosides and triterpene glycosides found in plants. *Quillaja saponaria,* a plant native to South America, continues to be the main source of most saponins used as adjuvants. Saponins have

been shown to stimulate humoral and cytotoxic T lymphocyte (CTL) responses against T-cell dependent and independent antigens in animal models and in some clinical trials [11]. Local toxicity due to their lytic activity has led to the development of specific adjuvants, such as immune-stimulating complexes (ISCOMs), or to the selection of the saponin fraction that presents the best balance between adjuvant effect and lytic activity. This fraction, QS–21, is used in human vaccine formulations as such [8, 9] or in formulations abrogating their lytic activity [12]. Highly purified QS–21 promotes type 1 helper T-cell (Th1) responses when injected in combination with antigens.

Toll-Like Receptor Agonists

Increased understanding of the innate immune response and its impact on adaptive immunity, as well as use of whole human genome sequencing, has allowed us to build on existing adjuvants and has led to the design of new ones. We now understand pathogen-associated molecular patterns and TLRs, which play key roles in the early steps of immune system activation. Upon binding and activating the corresponding TLR or pattern recognition receptors (PRRs), soluble mediators such as cytokines and chemokines are expressed, and antigen-presenting cells (APCs) are activated. This leads to the stimulation of the innate immune system, which in turn shapes and directs the subsequent adaptive immune response (Figure 1) [3, 11, 13]. The range of TLR agonists is illustrated in Table 2. TLR agonists are the most advanced immunoenhancers to date, and several have already progressed to human clinical trials (TLR9 agonists: CpG, IC31) or are already being used in licensed vaccines. For example, monophosphoryl lipid A (MPL) is a TLR4 agonist used in hepatitis B and human papillomavirus vaccines with worldwide distribution [1].

Mucosal Adjuvants

The mucosal surface presents ample opportunities for pathogen entry to the body. Although it is endowed with natural defense features such as an epithelial barrier, production of defense molecules such as mucins, and an elaborate lymphoid tissue system, the mucosal surface continues to be successfully targeted by pathogens such as HIV/AIDS and hepatitis. Therefore, concerted efforts to develop effective adjuvants for use in vaccines intended to act through mucosal immunization are needed. Bacterial toxins such as cholera toxin, or CT (elaborated by *Vibrio cholera*), and the heat labile enterotoxin of *Escherichia coli,* LT, have been extensively tested in the context of intranasal vaccines. Their use must be carefully monitored, however, as the potential for toxicity is high. Indeed, the first intranasal adjuvanted influenza vaccine registered had to be withdrawn from the market due to serious adverse events observed post-registration. There are presently no licensed adjuvanted mucosal vaccines [14–17].

Particulate Antigen Delivery Systems

Virus-Like Particles

Many antigens owe a significant portion of their vaccine effect to the way they are packaged and delivered. The choice of delivery system provides the option to move the vaccine preparation from a purely liquid to a particulate phase. In this context, while viral vectors are powerful tools for targeting a vaccine or therapeutic agent, their use also results in the agent being delivered in a particulate form, which is associated with enhanced uptake by APCs and the activation of cell-mediated immunity. Theoretical risks associated with their use (reactogenicity as well as decreased efficacy with increased number of doses) have motivated research for alternatives such as virus-like particles (VLPs), which are particulate viral entities displaying the conformationally complete viral antigens on their surface but lacking the genetic material necessary for viral replication [18, 19]. Null VLPs by themselves do not always provide adjuvant function [20–22], but when combined with more than one adjuvant they may produce increased humoral and cell-mediated immunity, as demonstrated in the recently licensed human papillomavirus vaccines [23].

Immune Stimulating Complexes (ISCOMs and ISCOMATRIX)

The advent of ISCOMs as adjuvants is fairly recent (within two decades). ISCOMs are particles in the 40 nm range consisting of saponins (Quil A), lipids, cholesterol, and antigen. The complex is held together by hydrophobic interactions between the saponin, lipid, and cholesterol. ISCOMs increase the efficiency of antigen presentation to B cells and the uptake of antigens by APCs. They have been shown to engage the major histocompatibility complex (MHC) class I pathway, thereby activating CD8+ CTLs. The net effect is that they can provide immunoenhancement by inducing Th1/Th2 and direct CTL responses in the host. Interestingly, tomatine, a related plant alkaloid, was recently identified as having similar adjuvant properties [24, 25]. Immune stimulating complex matrix (ISCOMATRIX) adjuvants are similar to ISCOMs in composition except that they lack the antigen. ISCOMATRIX adjuvants

TABLE 2.

Pattern recognition receptors targeted by different adjuvants

PRR	Cellular location of PRR	Natural ligand	Adjuvant
TLR1/TLR2 (Heterodimer)	Cell surface	Bacterial triacylated lipoproteins	*Escherichia coli* heat-labile enterotoxin (B subunit)
TLR2/TLR6 (Heterodimer)	Cell surface	Lipoteichoic acids, bacterial diacyl lipoproteins, fungal zymosan	Macrophage-activating lipopeptide-2
TLR3	Endosome/lysosome	Double-stranded RNA	Poly (I:C)
TLR4	Cell surface	Gram-negative bacterial liposaccharide	Monophosphoryl lipid A (MPL)
TLR5	Cell surface	Flagellin	Flagellin fusion proteins
TLR7, TLR8	Endosome/lysozyme	Single-stranded RNA	Imiquimod, resiquimod
TLR9	Endosome/lysosome	Bacterial (unmethylated) CpG DNA	CpG oligonucleotides
NOD1	Cytoplasm	Bacterial peptidoglycan	Diaminopimelic acid (DAP)
NOD2	Cytoplasm	Bacterial peptidoglycan	Muramyl dipeptide (MDP)

Poly (I:C), polyinosinic:polycytidylic acid; PRR, pattern recognition receptor; TLR, Toll-like receptor.

are made by combining an antigen with ISCOMs. Like ISCOMs, ISCOMATRIX adjuvants enhance the efficiency of antigen presentation to B cells and uptake by APCs. However, unlike ISCOMs, which also elicit Th1 and CTL responses, ISCOMATRIX adjuvants elicit only a Th2 response in the host [26].

Virosomes

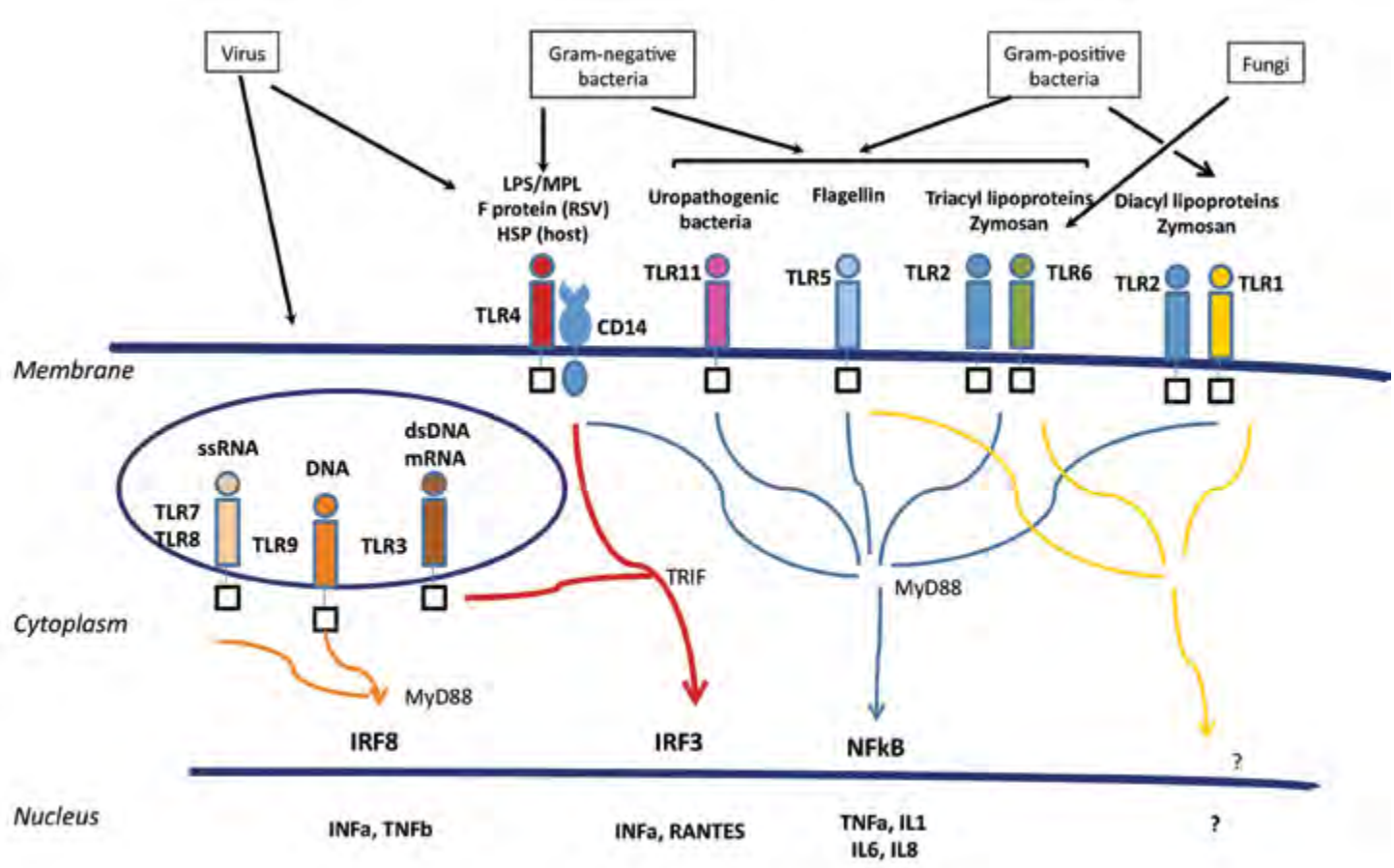
Virosomes are reconstituted viral envelopes that display desired vaccine antigens but lack the viral genome. Their mode of action has been described as being through endosomal fusogenic properties that enable them to present antigens in the cytosol in the context of the MHC class I antigen presentation system. Therefore, they can directly stimulate CD8+ T cell activity, in addition to stimulating a humoral response and enhanced antigen presentation [19]. They are components of two licensed vaccines (seasonal influenza and hepatitis B) and are being tested alone and in combination with other adjuvants. Virosomes also have been used with considerable success as adjuvants for plasmid DNA vaccines.

Polysaccharides

Advax, a crystalline fructose polymer, is a derivative of delta inulin, which has been successfully used in human trials as an adjuvant with influenza H1N1 antigen. It showed up to threefold enhancement in immune response (both humoral and cell mediated) and was well tolerated. It has shown similar effects with other vaccines in animal studies. Its mechanism of action is not fully understood but does not appear to be receptor-mediated [27, 28].

FIGURE 1.

Signaling pathway for Toll-like receptors



Immune cells have evolved to recognize various danger signals through their Toll-like receptors (TLRs). These can be extracellular (TLR1, 2, 4, 5, 6) or intracellular (TLR3, 7, 8, 9) to allow for recognition of both extra- and intracellular pathogens. Their expression patterns vary from one species to another and differ depending on the immune cell considered. Monocytes express TLRs 1/2, 4, 5, 2/6, 7, 8. Myeloid dendritic cells express 1/2, 3, 4, 5, 2/6, 7. Plasmacytoid dendritic cells express TLR9. B cells express TLR9. CD4+ T cells express TLR 1/2, 5, 2/6, 7. CD8+ T cells express TLRs 1/2, 3, 2/6. Natural killer cells express TLRs 3, 5, 7, 8. Treg cells express TLRs 1/2, 5, 2/6, 8.

Adjuvant Combinations

With increased knowledge and understanding of the principles underlying the immunopotentiating effects of the different classes of adjuvants, it has become logical to explore the possibility of designing customized adjuvant combinations that should maximize host immune response to a particular vaccine antigen target. Using this approach, several vaccines designed to elicit varying degrees of cell-mediated immune response alongside humoral antibody response have received FDA and European regulatory approval. Examples include Cervarix and Fendrix against human papillomavirus and hepatitis B viruses, respectively. Both of these adjuvants contain the adjuvant system AS04 [13, 29, 30]. Adjuvant systems are designed to elicit specific responses that should optimize the vaccine effect of the test antigen. AS04 is based on a specific form of MPL, a derivative of *S. minnesota* lipopolysaccharide that stimulates both cell-mediated and humoral immune responses. MPL is combined with alum in this system to obtain a combined adjuvant effect through the binding and activation of TLR4 by aluminum and MPL. AS04 allows for both arms of the immune system to be engaged in the host response to the vaccine. Another member of the adjuvant systems family, AS03, is based on a combination of an oil-and-water emulsion

and tocopherol. This well-known immune enhancer has been licensed in Europe and internationally [29, 30].

Modes of Action

Although adjuvants have been in use for more than 70 years, it is only within the last few years that their mechanisms of action are being understood. The adjuvants described below are those for which new data recently became available.

Aluminum Salts

Specific receptors for aluminum salts have not been identified in the host and, consequently, the known adjuvant effect of alum compounds was believed to be based on the enhancement of the physical interaction between the antigen and immune competent cells, resulting in prolonged availability of the antigen. (This is known as the "depot effect.") The adjuvant effects of aluminum salts were thus traditionally considered receptor independent [1, 2]. However, more recent work [3, 4] has demonstrated that alum is a powerful inducer of uric acid production in the host, suggesting that MYD88 (a key adaptor protein in the TLR signaling cascade, see Figure 1) plays a role in the adjuvant effect of alum. Intracellular NOD-like receptors (NLRs) are able to bind uric acid and other small molecules generated during cellular damage to activate the NALP3, which in turn activates the inflammasome and caspase-1 system. This system regulates the cleavage and release of pro-inflammatory cytokines such as interleukin-1 beta (IL–1β) or interleukin-18 (IL–18). These cytokines in turn promote the recruitment and maturation of inflammatory DCs and CD+ T cell activation. More work remains to be done, however, to fully delineate alum's mode of action, and new hypotheses on its mode of action continue to emerge [31].

MF59

The adjuvant effect of emulsions is believed to be based on early leukocyte recruitment and on stimulation of local muscle fibers to produce immune factors that activate local DCs. MF59 adjuvant effects are therefore thought to be based on enhanced antigen presentation and enhanced antibody production. The exact mechanism of action of this oil-and-water emulsion, however, is not yet fully delineated, and the involvement of cell receptor(s) or other types of mechanisms is not yet known [32].

Toll-Like Receptor Agonists

Current knowledge suggests that TLR agonists differ from the adjuvants previously described in this article. TLR agonists employ a directed receptor-mediated mechanism through specific signaling, leading to activation of APCs (Figure 1) [30]. The combination of APC activation and antigen presentation leads to adaptive immune response. As such, the nature of APC activation will define the extent and quality of the adaptive immune response induced. Current understanding of TLRs is attributable to the discovery of PRRs, exemplified by TLRs and NLRs and their interaction with various ligands primarily of microbial origin, to subsequently activate a generalized short-lived innate immune response (called the danger alarm response). Further downstream, the ligand/receptor interactions activate a cascade of signal pathways that ultimately result in the engagement of the adaptive immune system and the activation of other biological processes involved in the immune response [13, 32, 33].

Although TLR engagement leads to favorable immunopotentiation when deployed in this manner, the potential also exists for undesirable side effects that may result from the activation of the innate immune response machinery, causing the release of inflammatory cytokines such as interleukin-1 (IL–1), interleukin-6 (IL–6), tumor necrosis factor-alpha (TNF-alpha), neutrophil chemoattractants, and antimicrobial peptides. Therefore, to take full advantage of the immunoenhancing potential of TLRs in vaccinology and immunotherapy, strategies have to be developed to either ensure confinement of the effect (i.e., site of administration) or "down-modulate" the innate immune response that accompanies the desirable adaptive immune response.

How To Select an Adjuvant

From their initial introduction, adjuvants have been selected through both empirical observation and rational design based on analysis of the immune system itself. They can be used for a variety of purposes linked to the pathogen and the target population: (1) to enhance the immunogenicity of highly purified or recombinant antigens, (2) to allow a broader immune response that may be required for more complex pathogens such as HIV or malaria, (3) to improve the vaccine efficacy in newborns, elderly, or immune-compromised populations, or (4) to reduce the amount of antigen or number of doses needed to achieve protective immunity.

An understanding of the host-pathogen interaction, the selection and production of protective antigens, and the availability of adequate immunological tools to evaluate/establish potential correlates of protection are needed to select the most appropriate adjuvant. Typically, single antigens by themselves

or in combination with classical adjuvants such as aluminum salts have not been sufficient to induce a protective immune response beyond antibodies. Therefore, alternative adjuvants need to be evaluated. To be a potential candidate for the vaccine considered, the adjuvant needs to be compatible with the antigen, be stable over time, induce the immune response deemed necessary for protection, and have a safety/reactogenicity profile acceptable for the target population.

New-generation adjuvants improve on the first generations that were developed and tested in animals or humans. These adjuvants have established the ability of emulsions to strongly affect humoral immune response, and the ability of molecules such as MPL and CpG, now known as TLR agonists, to affect humoral and cell-mediated immunity. Their design involves a rigorous selection process to identify adjuvants that provide both the suitable physicochemical properties required for long-term stability and the necessary compatibility with the antigen(s). Their ability to induce the appropriate immune response is evaluated in preclinical animal models and confirmed in animal challenge models when available. Upon definition of the adjuvant composition and establishment of the immune profile induced, the adjuvant is further developed and produced for use in Phase I clinical trials. When first tested in humans, the adjuvanted vaccine is typically compared with the antigen alone or antigen combined with alum to establish its safety profile and its superiority over antigen alone or antigen with alum. Dose-finding studies will establish the amount of adjuvant required for the target population to attain maximum protection with an acceptable safety profile. When human challenge models exist, such as in the case of malaria, they can support the formulation selection and its use in Phase III efficacy studies. The vaccine candidate will then proceed to Phase III efficacy studies according to the same rules and principles as for any other nonadjuvanted vaccine, with special attention to safety evaluation and a particular emphasis on rare events of immune origin.

How To Evaluate Safety

The benefit of adding adjuvants to a vaccine to enhance immune response must be weighed against the risk that these agents may induce adverse reactions. Safety is an integral part of every step of vaccine development. The potential risk posed by adjuvants is evaluated throughout the development process, including during preclinical and clinical testing.

Preclinical Evaluation

The safety evaluation of a vaccine, adjuvanted or not, starts from the selection of the antigen and continues through the whole life cycle of the vaccine. Antigens are selected for their recognized ability to induce a protective immune response. Protein adjuvants are also evaluated for their potential homology with human proteins. This is readily achieved through bioinformatic analyses. Antigen sequences that could theoretically lead to autoimmune response, known as antigen mimicry, can be identified and further scrutinized for selection as a final candidate antigen. In those cases where the antigen sequence is determined to have a high theoretical likelihood for homology, thereby potentially triggering an autoimmune response in humans, the antigen is not selected.

The use of immunoenhancers in vaccine formulations may create additional safety concerns that need to be addressed during the course of vaccine development. In addition to classical clinical safety evaluation, the European Medical Authority and the World Health Organization have issued guidelines for the specific preclinical safety evaluation of adjuvanted vaccines. This evaluation should be performed in *in vitro* test systems or appropriate animal models (chosen according to species and physiological status) and should support the selected route of administration. It should aim at assessing the impact of any new adjuvant, and antigen-adjuvant combination, on local and systemic immune response, including adverse immune events such as hypersensitivity and autoimmune disease.

One limitation of preclinical testing is that the prediction of human autoimmune response through the use of animal models is not yet established. This is due in part to the number of autoimmune diseases and the complexity of etiologies, but also to the lack of appropriate or relevant animal models for these diseases [10–15]. Consequently, when adjuvants are being evaluated for the development of new vaccines, nonclinical studies must be carefully designed to ensure that safety signals, particularly those that may affect human health, are identified for follow-up in subsequent clinical studies as applicable.

Clinical Safety Evaluation

Clinical trials in humans are conducted in series (Phase I to Phase IV)—from first-in-human safety evaluation to efficacy assessment and postmarketing surveillance. Through each phase, an assessment of safety is performed. Once the vaccine safety profile has been evaluated and efficacy demonstrated in suitable study populations, the vaccine can be submitted for

licensing. Following approval, Phase IV trials or postmarketing surveillance is put in place to assess and monitor the safety of the vaccine in the general population under conditions of routine use. Clinical trials may not be large enough to detect rare adverse events that may become apparent during large-scale use. Sometimes, integrated safety analysis or meta-analysis regrouping different studies involving the same adjuvanted vaccine are performed to evaluate the frequency of rare events, such as those related to autoimmunity in persons receiving the vaccines versus those in comparison groups. However, these analyses should only be undertaken if data are collected in a manner that allows meaningful comparison and interpretations, e.g., through clinical trials appropriately designed to be pooled (same inclusion and exclusion criteria and randomization rate, same data collection and interpretation, etc.).

Currently, nonclinical and clinical evaluations provide the safety information package for a new vaccine licensure. A good understanding of the adjuvant's mode of action defining the nature of the effect (local or systemic, short- or long-lived, as demonstrated in the case of AS04 [34]) as well as the precise mechanism (target cells, identification of receptor or pathway) can complement these evaluations and bring a valuable insight to the candidate vaccine safety profile.

The Way Forward

The immune system has evolved by developing a wide array of mechanisms to respond to infectious diseases. The ideal vaccine will provide protection against the original pathogen but also against mutations or the pathogen's escape strategies over a long period of time. This will require orchestrated immune responses similar to those seen during natural infection.

Today, some but not all single adjuvants can induce all the immunoenhancement required for a given vaccine. The use of adjuvant combinations, which capitalize on the additive or synergistic effect of each component, as well as strategies to combine various primary and booster approaches, may hold the key to the development of vaccines for challenging diseases such as HIV and tuberculosis and may open the door to new therapeutic approaches for diseases such as allergies, addiction, autoimmune diseases, or cancers.

Understanding host-pathogen interactions and the induction and maintenance of protective immune response will be crucial for future progress in the field. Defining markers for innate and adaptive immune response [35] that provide correlates for safety and efficacy profiles of new vaccines and adjuvant strategies will be key for the progression of adjuvants to the next level of development.

Conclusion

The more recent advancements in vaccine research illustrate a new approach in vaccine/adjuvant design. They represent a coalescence of significant findings from various research fields, particularly in the area of innate immunity and how it influences the adaptive immune response. In the new approach, the objective is to select an adjuvant or design a combination of adjuvants that will achieve certain defined immunologic objectives. These objectives are defined by an understanding of the candidate vaccine antigen and what type of host response is required to achieve maximum and long-lasting protection with the vaccine. This approach was used to successfully launch recent vaccines targeting infectious diseases such as human papillomavirus. It is noteworthy that the same principles appear to be just as valid in other disease disciplines as well. For example, the recently approved prostate cancer vaccine, sipuleucel-T, was designed to include an adjuvant that provides durable immunoenhancement by significantly improving the interaction between the vaccine antigen and the homologous DCs, thereby improving antigen uptake, processing, and presentation by the APCs to the host effector cells [36]. We predict that this approach of selectively applying adjuvants or adjuvant combinations based on an understanding of the immunologic needs of the vaccine antigen as well as the target population will likely continue to yield similar successes in all disciplines involving the use of adjuvants. In an effort to promote the realization of this goal, the National Institute of Allergy and Infectious Diseases supports the discovery, development, and evaluation of new candidate vaccine adjuvants. This and similar efforts in the public and private sectors should facilitate the delivery of novel adjuvants for commercial vaccine development.

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Dr. Garçon joined SmithKline Beecham Biologicals (now GlaxoSmithKline Biologicals) in 1990, where she set up and led the vaccine adjuvant and formulation group. She moved from this position to head of technologies, head of research, head of global research and North America research and development; to her current position as head of the global adjuvants and delivery systems center for vaccines. She provides leadership within GlaxoSmithKline Biologicals in the field of adjuvants, from discovery to registration and commercialization of adjuvanted vaccines. Dr. Garçon's expertise in vaccinology extends from research to manufacturing, in particular immunology, adjuvant and formulation technologies, analytical methods, animal experimentation, and toxicology/safety evaluation and testing. She has authored more than 40 papers and book chapters and holds more than 200 patents.

Progress, Promises, and Perceptions: The National Vaccine Plan— A Path Forward for the Coming Decade

Bruce G. Gellin, M.D., M.P.H. and Sarah R. Landry, M.A.

Abstract

The 20th century could be considered the century of vaccines. In the United States during that time, the average lifespan increased by more than 30 years and mortality from infectious diseases decreased fourteenfold. A child born in the U.S. today has the potential to be protected against 17 serious diseases through immunization. Thanks to vaccines, we have witnessed the eradication of smallpox worldwide and, in the United States, the elimination of polio and the near elimination of measles and rubella. Globally, vaccination saves 2 to 3 million lives per year. A recent economic analysis indicated that vaccination of each U.S. birth cohort with the recommended childhood immunization schedule prevents approximately 42,000 deaths and 20 million cases of disease, with a net savings of nearly $14 billion in direct costs and $69 billion in societal costs [1].

Vaccines have the unique quality of protecting both individuals and communities. Because they have been so effective for many years in preventing and eliminating a number of serious infectious diseases, the significant contributions vaccines make to our society and its health may have faded from public consciousness. Before the development and widespread use of safe and effective vaccines, infectious diseases threatened the lives of millions of children and adults in this country and abroad. What were once referred to as the common diseases of childhood are now vaccine-preventable diseases. In the United States, we no longer see crippling cases of polio or children dying from infections such as diphtheria or *Haemophilus influenza* type B (Hib). Vaccines also prevent cancers caused by human papillomavirus (HPV) and hepatitis B virus.

As we look to the future, the National Vaccine Plan will serve as a roadmap for the U.S. vaccine and immunization enterprise for the next decade. The plan articulates a comprehensive strategy to enhance all aspects of vaccine and immunization efforts, including research and development, supply, financing, distribution, safety, informed decision-making among consumers and healthcare providers, vaccine-preventable disease surveillance, vaccine effectiveness and use monitoring, and global cooperation.

The National Vaccine Plan

In the last century, we witnessed the worldwide eradication of natural infection from smallpox and the complete elimination of polio in the United States. During that same period, the average lifespan of Americans increased by more than 30 years, and mortality from most vaccine-preventable diseases decreased in the United States by 99 percent [2]. The routine series of vaccines given to each birth cohort of children in the United States is estimated to save nearly $14 billion in direct costs and $69 billion in societal costs [1]. As a result of the tremendous progress in developing vaccines, and of including them as a standard of care in our national immunization program, a baby born in the United States today has the benefit of vaccines to protect him or her against 17 serious infectious diseases.

The United States has made tremendous progress in scientific research and in the licensing of new and improved vaccines. At the same time, new challenges exist, particularly in implementing vaccine policy, integrating new technologies and vaccines within the current immunization schedule, and addressing the public's perceptions of the value of vaccines. Vaccines are one of the best prevention tools we have. Vaccines are different from other medical products because they are given to healthy individuals to prevent diseases they may or may not encounter. In addition, schools often mandate recommended vaccines to ensure community protection, and immunization programs have a relatively large public financing component. Furthermore, federal and state government health agencies set policies on how to use vaccines to protect the public health and fund activities to strengthen implementation of immunization delivery programs.

Bill Gates has declared this the "decade of vaccines" [3], and the agencies within the U.S. Department of Health and Human Services (HHS) have collaboratively developed a new National Vaccine Plan to ensure a robust and integrated immunization system (www.hhs.gov/nvpo/vacc_plan/). This 10-year vision for the nation outlines strategies and programmatic steps to more effectively prevent infectious diseases and reduce adverse reactions to vaccines. This document is important not only for use in planning by federal partners, but because it is national in scope, it also requires coordinated implementation by vaccine and immunization stakeholders. In addition to federal, state, and local policymakers, these groups include healthcare providers, manufacturers, insurers, investors, innovators, academia, and the public. Of note, the plan also includes a goal to increase global vaccination.

The 2010 plan is the first update of the nation's vaccine strategy since the original National Vaccine Plan was issued in 1994, and it includes strategies for advancing vaccine research and development, safety, communications, delivery, and global cooperation. The plan aims to achieve five broad goals:

1. Develop new and improved vaccines.
2. Enhance the vaccine safety system.
3. Support communications to enhance informed vaccine decision-making.
4. Ensure a stable supply of recommended vaccines and achieve better use of existing vaccines to prevent disease, disability, and death in the United States.
5. Increase global prevention of death and disease through safe and effective vaccines.

Progress, Promises, and Perceptions

Since the initial National Vaccine Plan was written, the vaccine and immunization environment has changed considerably, and progress has been made in many areas.

Tremendous advances also have been made recently in basic areas of science underlying vaccinology, and such advances are likely to continue to drive vaccine development. For example, in 1994 microbial genomic sequencing was in its infancy, and that information was not available to allow researchers to identify epitopes of importance for immune protection. Since then researchers have completed hundreds of genomic sequences for disease-causing organisms, including those for the pathogens responsible for malaria, tuberculosis, chlamydia, and seasonal and pandemic influenzas. Recently, the National Institute of Allergy and Infectious Diseases (NIAID)-supported Structural Genomics Centers for Infectious Diseases accomplished a significant milestone by determining their 200th 3–D protein structure—information that could provide researchers with critical knowledge for developing new vaccines. Likewise, our understanding of host immunity has grown tremendously. In 1994 scientists were only beginning to understand the importance of the innate immune system and its involvement and importance for adaptive immunity. Now, with an increasing emphasis on and understanding of how the human immune system works and responds to antigens, we may be able to identify correlates of protection using systems-biology approaches. In the future, scientific advances in pinpointing genetic and environmental risk factors for disease may enable researchers to focus prevention strategies more effectively and target vaccines to those populations at highest risk. At an individual level, scientists may one day be able to predict the likelihood of vaccine response and the number of doses needed to achieve protection. Some researchers speculate that eventually we may be able to predict who will have an adverse reaction to vaccination on the basis of their genetic makeup, or even know the dose needed to produce the desired immunologic effect [4]. Studies of yellow fever and smallpox vaccines are already showing such progress [5].

Since 1994, vaccines against an additional eight infectious diseases have been licensed, and many new formulations or updated recommendations for existing vaccines have been made. In total, 19 new vaccines have been licensed since 1994 (see Table 1). With the licensing of the rotavirus vaccines, human papillomavirus (HPV) vaccines, and an influenza vaccine for the elderly, we are now moving into an era in which multiple vaccines are being developed against the same disease or infection and marketed on the basis of individual clinical differences among products.

Despite the inclusion of these additional vaccines, coverage rates have continued to increase during this time. For example, in 1994, just 70 percent of 2-year-olds had been adequately vaccinated against measles, mumps, rubella, polio, diphtheria, tetanus, and pertussis [6]. Fifteen years later, a 2009 survey of children aged 19 to 35 months found that vaccine coverage against poliovirus, measles, mumps, rubella, hepatitis B, and varicella was greater than 90 percent [7].

As the number of vaccines has increased and the scope of the immunization program has expanded, new challenges have emerged. The increasing cost of vaccines, vaccine shortages, new population groups (adolescents and adults), and the

TABLE 1.

U.S. licensed vaccines against bacterial and viral disease agents by recommended age cohorts

Routinely Recommended Vaccines			
Age Cohort	**1989[a]**	**1995[b]**	**2010[c,d]**
3–5 years	Diphtheria, tetanus, pertussis	Diphtheria, tetanus, pertussis	Diphtheria, tetanus, pertussis
	Poliovirus MMR	Poliovirus MMR	Inactivated poliovirus MMR
	Hib	Hib	Hib
		Hepatitis B	Hepatitis B
			Rotavirus
			Influenza
			Pneumococcus
			Varicella
			Hepatitis A
			Meningococcal
7–18 years	Tetanus toxoid	Tetanus toxoid	Tdap
		MMRa	HPV
			Meningococcal
			Influenza
18+ years	Tdap	Tdap	Tdap
	MMR	MMR	MMR
	Influenza	Influenza	Influenza
	Pneumococcus	*Pneumococcus*	*Pneumococcus*
			HPV
			Herpes zoster

Abbreviations: Hib—*Haemophilus influenzae* type B; HPV—human papillomavirus; MMR—measles, mumps, rubella; Tdap—tetanus, diphtheria, pertussis

a Source: Advisory Committee on Immunization Practices (ACIP), Centers for Disease Control and Prevention. General recommendations on immunization [Internet]. Atlanta (GA): Centers for Disease Control and Prevention; 1989. Table 2. Recommended schedule for active immunization of normal infants and children. Available from: www.cdc.gov/vaccines/pubs/images/schedule1989s.jpg; b Source: Centers for Disease Control and Prevention. Recommended childhood immunization schedule—United States, January 1995. MMWR Morb Mortal Wkly Rep. 1995 Jan 6;43(51-52):959-60.; c Source: Centers for Disease Control and Prevention. Recommended immunization schedules for persons aged 0 through 18 years—United States, 2010. MMWR Morb Mortal Wkly Rep. 2010 Jan 8;58(51-52):1-4.; d Source: Centers for Disease Control and Prevention. Recommended adult immunization schedule—United States, 2011. MMWR Morb Mortal Wkly Rep. 2011 Feb 4;60(4):1-4.

complexity of the vaccination schedule have become concerns of public health officials and providers. Recent increases in the number and costs of vaccines routinely recommended for children and adolescents have raised issues about the ability of the current public vaccine financing and delivery systems to maintain access to recommended vaccines without financial barriers. Vaccine financing through public funding has not kept pace with the introduction of new vaccines [8]. Some groups believe that two programs funded by the Centers for Disease Control and Prevention (CDC)—the Section 317 Immunization Grant Program and the Vaccines for Children Program—are inadequately financed at present and are unable to support vaccines that have already been licensed for several years [9]. From 2005 to 2011, the cost to vaccinate a child up to age 18 according to the recommended immunization schedule increased from $545 to $1,332 for a boy and $1,620 for a girl [8].

The Patient Protection and Affordable Care Act, which was signed into law on March 23, 2010, is the most recent national policy change for immunizations. It aims to provide affordable, stable, and near-universal healthcare coverage. As a result of this law, nearly all Americans will have healthcare coverage in 2014. With its emphasis on disease prevention and community-based medical services, there is optimism that this law will help address financial barriers to immunization. Under this law, both individual and group plans must offer vaccines recommended by the Advisory Committee on Immunization Practices (ACIP) at no cost to the patient.

Although coverage rates for most vaccines have increased since the last plan was written, parents continue to report that they are worried about the total number of vaccines children get and the safety of vaccines overall. A 1999 survey found that the vast majority (87 percent) of parents thought immunizations were important to keep their child healthy. Despite this, 25 percent believed their child's immune system could be weakened by immunizations, and 23 percent thought that children received too many vaccines [10]. Ten years later, a 2009 study by Freed surveyed parents on their vaccine-related attitudes and beliefs. Again, the vast majority of parents (89 percent) continued to vaccinate their children but many raised doubts or concerns about the safety of vaccines. More than half of the parents were concerned about the potential for serious adverse events that they perceived could be connected with vaccines, and a quarter reported they believed vaccines cause autism in some healthy children, despite overwhelming evidence to the contrary [11]. These beliefs have led some parents to "opt out" of vaccination, and in a few states, the rate of personal belief exemptions from school requirements has increased. In some cases, this increase has led to new outbreaks of measles [12]. Focus groups and surveys conducted between 1999 and 2010 indicate that 1 to 11 percent of parents each year refused to have their children receive at least one recommended vaccine [13, 14].

Future Needs for the Decade of Vaccines

In the next decade we can anticipate that a strong scientific base, with increasing knowledge in areas such as bioinformatics, immunology, and genomics, will drive the development of new and improved vaccines. Unfortunately, there is evidence that scientific data, repeated demonstrations of vaccine effectiveness, widespread support from medical organizations and advisory panels, and even immunization mandates may not be sufficient to ensure widespread use of recommended vaccines. For example, a 2011 paper by Kennedy and colleagues showed that 36 percent of parents in a national survey believed that children already receive too many vaccines [12]. Moreover, public health services are stretched to administer and deliver the currently recommended vaccines, and a sustained and steady supply of vaccines continues to be a problem. New opportunities and advances in healthcare technology could help address many of the challenges that exist with immunization.

As the routine immunization schedule continues to expand, the U.S. immunization program will be challenged to integrate new vaccines within its current structure. Furthermore, the effects of newer vaccines will be more difficult to calculate because many of them will be more important for minimizing illness rather than preventing death. This change in focus will have a tremendous influence on how we measure the societal impact of vaccines [15]. For some newer vaccines, such as meningococcal conjugate, or those in development against West Nile or dengue virus, it may be increasingly difficult from a societal public health perspective to justify a recommendation for routine use. Many of the new vaccines will likely be competing against each other, which will create policy and implementation challenges.

Additional work is needed in immunizing adults and adolescents and in addressing the health disparities that exist in the uptake of many vaccines. A recent survey on immunization of teenagers aged 13–17 years old found increased coverage in adolescents over the previous year: 50 percent of teens in this survey had received a tetanus, diphtheria, and pertussis (Tdap) vaccine and a meningococcal vaccine.

But more work needs to be done with HPV vaccines—only 44 percent of girls surveyed had received one dose, and just 26 percent had received all three doses [16]. Among adults, only 36.1 percent were vaccinated against the seasonal flu in 2008, and just 2.1 percent who were due for a booster had had the tetanus, diphtheria, and whooping cough vaccine in the previous 2 years; only 10 percent of eligible adult women had received the HPV vaccine [17]. In the coming decade we will be using the Healthy People 2020 plan [18], which sets out ambitious objectives of 80–90 percent coverage for most vaccines, as a benchmark for progress.

We also need to consider new vaccinees and venues for immunization and the policy needs that accompany expanding in these different directions. For example, the 2009–2010 H1N1 influenza pandemic demonstrated the critical importance of influenza vaccination in protecting both the mother and her baby. A study in Bangladesh showed a 63 percent reduction in influenza among infants of mothers who received the influenza vaccine [19]. An experimental group B streptococcal vaccine is in development to prevent transmission of the bacteria from mothers to neonates. Pregnant women could be immunized against a number of other pathogens (e.g., pertussis and pneumococcus bacteria, and respiratory syncytial virus) to enable them to pass on antibodies that will protect their newborns for some months. Another increasing problem of concern to all ages is antibiotic-resistant nosocomial bacteria. Vaccines against methicillin-resistant *Staphylococcus aureus* (MRSA) are in development and could one day be offered prior to routine hospitalization.

The National Vaccine Program Office (NVPO) will play a role in guiding and coordinating activities to address these future needs. Several examples of work that will be undertaken as part of the National Vaccine Plan are addressed below.

Partnering To Develop a Vision for Future Vaccine Targets

Since 2000, new vaccines have been licensed for pneumonia, influenza, rotavirus, herpes zoster, meningitis, and cervical cancer, with many others currently under development. It is critical that we continue to be vigilant in our immunization efforts—both for recognized diseases and in anticipation of those yet to emerge.

Because vaccine development is time- and resource-intensive, understanding priorities for vaccine development and encouraging collaboration among stakeholders are essential to addressing the challenges of developing new and improved vaccines. Fostering continued investment from all sectors is critical as technological approaches and disease threats expand amid increasing costs to develop, license, and deliver vaccines. Over the next 2 years, NVPO will be working with various HHS agencies, the Bill & Melinda Gates Foundation, and the World Health Organization to develop catalogs of vaccines and vaccine technologies that are of highest need for the global and domestic communities. This effort will help inform governments and industry of future public health directions, facilitate partnerships to foster development of these tools, and identify potential policy needs and barriers to their development.

Supporting Future Vaccine Safety Studies

Because adverse events, especially serious ones, are rare, developing a robust system to enhance collection of medical histories and biological specimens from persons experiencing serious adverse events following immunization would be a significant step forward to enhance the study of biological mechanisms and individual risk factors.

NVPO is leading an effort to develop standards for a potential biospecimen repository, which could enhance the ability of scientists to carry out genetic and immunological research on vaccine safety.

In addition, in the coming years, a scientific agenda will be developed to guide future research on vaccine safety topics. Although research is being done to understand human immune responses to vaccines, opportunities still exist to better understand many factors that could relate to vaccine safety, including genetic and behavioral factors, immunological correlates for adverse events, and surveillance and regulatory issues.

Supporting Informed Vaccine Decision Making by the Public, Providers, and Policymakers

In fall 2009, NVPO conducted focus groups to gather information on beliefs, perceptions, and concerns regarding pediatric immunization. Many of the participants supported immunization, but nearly all had questions about vaccines that they thought were not being answered adequately by their healthcare providers, online resources, other media, or their peers. From these focus groups stemmed the idea for a single online resource that provides a complete portrait of vaccine issues, from development to licensure to administration.

Vaccines.gov is a new cross-departmental Web site in development that will present up-to-date vaccine and immunization information for consumers. This project is being led by NVPO with strong collaboration from key communicators across the federal government. The Web site will be a consumer

portal that draws information from across HHS and is based on the model pioneered by Flu.gov. Because women are often the primary health information seekers for their families—and may make health decisions for young children, teenagers, or aging parents—an initial primary target audience will be mothers aged 25 to 55 years. The site will present information to reflect the importance of immunization across the lifespan from children to seniors, with a particular focus on orienting consumers toward the benefits of vaccines and reestablishing social norms about immunization.

Leveraging New Opportunities in Health Information Technology

Some of the barriers to improved vaccine uptake include cost, awareness, and access problems. Community health centers, other community immunization sites (e.g., pharmacies and stores), and school-based clinics offer venues for improving vaccine uptake, in addition to traditional healthcare provider sites. There are many challenges with delivering vaccines to adolescents and adults, particularly given the lack of immunization infrastructure in these groups. The National Vaccine Advisory Committee (NVAC) and other organizations have called for vaccines to be administered to teens and adults in alternate venues outside of a doctor's office [20, 21]. For this to be done effectively and efficiently, immunization information systems (IIS) must be established and electronic health records must be available to ensure transfer of information between the alternate venue and the doctor's office. Immunization information systems (or immunization registries) are confidential, computerized databases that record all vaccine doses administered to individuals. As of December 31, 2008, 75 percent of children under the age of 6 were enrolled in an IIS [1]. According to the Task Force on Community Preventive Services, there is strong evidence that IIS could effectively increase vaccination rates [22]. HHS also has put increased emphasis on the importance of health information technology. Over the next year, NVPO will be working to understand how HHS-wide priorities in health information technology could incorporate vaccines.

Conclusion

As we look to the decade ahead, the nation's vaccine and immunization efforts will be guided by the objectives and strategies identified in the National Vaccine Plan. Scientific research will continue to present new opportunities for vaccine development and reinforce our understanding of the safety and efficacy of vaccines. These advances could be capitalized upon with the robust immunization system outlined in the National Vaccine Plan.

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As Deputy Assistant Secretary for Health and Director of the National Vaccine Program Office (NVPO), Dr. Gellin is one of our nation's top experts on vaccines and infectious diseases. NVPO was created by Congress to provide leadership and coordination among federal agencies and other immunization stakeholders, including states and municipalities, healthcare providers, and private-sector entities such as vaccine manufacturers.

Before joining NVPO in 2002, Dr. Gellin was the director of the National Network for Immunization Information, an organization he founded to be a resource of up-to-date, authoritative information about vaccines and immunizations.

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Ms. Landry has extensive experience in the vaccine policy world, having held positions at GlaxoSmithKline as Director of Vaccine Public Policy and formerly in NVPO as an Associate Director for Communications and Policy.

Ms. Landry is a graduate of the University of Maryland with a degree in zoology and Johns Hopkins University with a master's in science writing. She is the recipient of numerous awards for her vaccine- and AIDS-related work, and has authored or co-authored multiple peer-reviewed articles.

VACCINE UPDATES

Dengue

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Dengue is a mosquito-borne infection that in the 1950s affected only a few countries in Southeast Asia and Latin America [1]. The disease is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, Southeast Asia, and the Western Pacific. The World Health Organization estimates that approximately two-fifths of the world 's population is at risk of dengue infection [2]. Dengue also has started to cause outbreaks in the United States (Hawaii in 2001, Texas in 2005, and Florida in 2010) after having been absent from the country for more than 50 years. The reemergence of dengue in many parts of the world is believed to have been caused by increased urbanization and international travel and by climate changes that have affected the habitat and geographical distribution of the *Aedes* mosquitoes that spread dengue virus.

Dengue infections are caused by four different virus serotypes (DENV–1, –2, –3, and –4). The majority of dengue infections are either asymptomatic or result in a mild, self-limiting influenza-like illness called dengue fever (DF). In some cases, dengue infection results in severe disease—dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS)—which causes significant morbidity and mortality, especially in children [2].

The risk factors for developing severe dengue disease are not yet understood, but it is believed that pathogenic immune responses play an important role. Epidemiological studies have shown that the majority of DHF/DSS cases occur in secondary infections with a different serotype or in infants born to DENV-seropositive mothers. There are two main theories to explain these observations. In the first theory, antibodies produced in response to the initial infection do not neutralize the second heterotypic infection, but instead form a complex with the virus and enhance the infection by facilitating entry into Fc receptor-bearing cells. This phenomenon is called antibody-dependent enhancement (ADE) [3]. ADE has recently been demonstrated in mouse models of dengue disease [4, 5]. In the second theory, severe dengue disease is caused by pathogenic cytokines that are produced by infected T cells in response to a secondary infection with a different viral serotype. In this theory, proposed to explain severe disease in older children and adults, the secondary infection with a different serotype induces a memory T-cell response that has low affinity for the second virus and results in altered T-cell functional responses and dysfunctional cytokine production that can cause disease. Studies in human infections and animal models have provided evidence for this theory [6]. It is likely that both antibodies and T cells play a role in disease development.

Vaccines for dengue are not currently available, though research to develop a vaccine has been ongoing since the 1930s. There are several factors that have impaired the development of a dengue vaccine. First, an ideal dengue vaccine should confer strong and long-lasting neutralizing immunity against all four dengue serotypes. Partially protective or short-lasting immunity induced by dengue vaccines has the potential to cause enhanced disease if vaccine recipients are subsequently exposed to infection [7]. This potential risk has made the evaluation of dengue vaccines in endemic countries difficult. Second, there are no good animal models that recapitulate human dengue disease, and therefore it has been difficult to measure the attenuation of live vaccines and vaccine efficacy before evaluating them in humans [8]. Third, it can be difficult to achieve balanced immune responses against all serotypes in tetravalent live-attenuated vaccines, as the individual virus components of the vaccines can interfere with each other [9]. Despite these difficulties, significant progress has been made in the last few years toward developing a vaccine, and the research community is now closer than ever before to having an approved dengue vaccine on the market.

Currently, the vaccine that is most advanced in development is the ChimeriVax dengue vaccine developed first by Acambis and more recently by Sanofi Pasteur. This vaccine is a mix of four recombinant, live-attenuated yellow fever 17D vaccine viruses, each one expressing the premembrane (prM) and envelope (E) genes of one of the four dengue serotypes. This vaccine has been tested in several Phase I and Phase II clinical trials in the United States, Asia, and Latin America, in both adults and children. After three doses given 6 months apart, the vaccine confers balanced immune responses against all four serotypes and seems to be well tolerated [9]. Phase

III trials of this vaccine started in Australia in 2010 and are currently ongoing.

Three other vaccines are currently in clinical development:

1. The Laboratory of Infectious Diseases (LID) at the National Institute of Allergy and Infectious Diseases (NIAID) is developing a similar tetravalent, recombinant, live-attenuated dengue vaccine, based on an attenuated DENV-4 rather than a yellow fever 17D "backbone." LID used several novel methods to discover mutations capable of attenuating dengue virus [10]:

    - Researchers followed a reverse genetics approach to remove a stretch of 30 nucleotides shared by all serotypes in the untranslated region (UTR) of the genome. This mutation (Δ30) was attenuating and genetically stable, thus making the tetravalent vaccine safer by preventing viruses from reverting to virulent form.

    - Researchers made use of a chemical mutagenesis screen that produced an extensive collection of mutated dengue virus strains, some of which presented useful characteristics, including attenuated replication. DNA sequencing of these virus strains identified the attenuating genetic changes that would be useful for engineering a live-attenuated dengue vaccine.

    - Researchers continued improving on the original delta 30 modification by removing additional nucleotides from the UTR and by swapping UTRs bearing delta 30 between different serotypes.

    Following identification of a suitably attenuated DENV-4, LID used this strain as the background to create chimeric viruses in which the structural genes were replaced with those derived from the other three serotypes. Using a combination of these techniques, LID was able to achieve optimal levels of attenuation and immunogenicity for all four serotypes. These attenuated viruses are presently being evaluated in human trials and already have shown evidence of being safe and immunogenic. Seven LID Phase I clinical trials in the United States have evaluated different monovalent formulations to find the best candidates for use in a tetravalent formulation. In 2010, LID initiated Phase I clinical trials of four different combinations of tetravalent vaccine to determine the best formulation to induce balanced immune responses against all four serotypes. Because vaccine strains also were selected for their ability to grow well in cultured cells, the cost of manufacture should be low, thus making the vaccine attractive to developing countries in dengue-endemic areas. This vaccine technology has been licensed to industry partners in Brazil, India, and Vietnam for further development.

2. A different tetravalent, recombinant, live-attenuated vaccine is currently being developed by InViragen [11]. The backbone for this vaccine is an attenuated DENV–2 strain (PDK–53) developed by the Centers for Disease Control and Prevention that was shown to be safe and immunogenic in Phase I clinical trials. The structural genes (prM and E) of this virus have been replaced with those of the other three strains. The tetravalent vaccine is a mixture of four viruses: PDK–53 and PDK–53 expressing the structural proteins of DENV–1, DENV–3, and DENV–4. This vaccine has been shown to be safe and immunogenic in animal models. In 2010, InViragen initiated clinical evaluation of this vaccine in two Phase I trials: one in the United States (through the NIAID Vaccine and Treatment Evaluation Units) and one in Colombia. Preliminary data suggest that this vaccine is well-tolerated and immunogenic in healthy adults.

3. Another vaccine, a recombinant subunit vaccine based on the truncated form of the dengue E glycoprotein (80E), originally was developed by Hawaii Biotech and is now being developed by Merck [12]. This vaccine is produced in *Drosophila* cells and has been shown to be safe and effective in preclinical studies. In 2009, a monovalent DENV–1 vaccine formulated with alum adjuvant was evaluated in a double-blind, placebo-controlled, dose-escalation safety study in healthy people. Preliminary results show that this vaccine is well-tolerated and immunogenic. The vaccine is now being reformulated with Merck's proprietary saponin-based adjuvants. Plans for further clinical development are being discussed.

    Several additional vaccine candidates using a wide variety of approaches are currently in preclinical development. These include inactivated whole virus particles, viral expression vectors such as Venezuelan equine encephalitis replicon vectors and adenoviruses, DNA-based vaccines, epitope-based vaccines, and immunogenic fragments of recombinant E glycoprotein with a variety of adjuvants.

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VACCINE AGAINST CHIKUNGUNYA VIRUS IN DEVELOPMENT

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The National Institute of Allergy and Infectious Diseases (NIAID) soon hopes to launch a Phase I clinical trial of a candidate vaccine for chikungunya virus, a mosquito-borne pathogen that has infected millions of people, primarily in Africa and Asia, and causes debilitating pain. Researchers at NIAID's Vaccine Research Center (VRC) developed the vaccine and are making pharmaceutical-quality supplies of it in the VRC production facility for their clinical research. Phase I trial objectives include examination of vaccine safety and tolerability and early assessment of the immune response.

The vaccine uses virus-like particles (VLPs) to elicit an immune response. VLPs essentially present the outer surface of chikungunya virus, but lack DNA and therefore pose no infection risk. VRC studies in mice and nonhuman primates have shown that immunization with the candidate vaccine produces antibodies that can protect against a live virus challenge, even one nearly 4 months after immunization.

There presently is no vaccine or treatment for chikungunya virus infection. Chikungunya was isolated in Tanzania during the early 1950s. The name is derived from a tribal dialect word that means "that which bends up," reflecting the contorted posture of chikungunya patients suffering severe joint pain as a result of the disease. The joint pain can be incapacitating and long-lasting.

VLP vaccines are relatively new: The Food and Drug Administration has approved one for hepatitis B virus and one for human papillomavirus. The VRC work marks the first time scientists have used VLPs in a vaccine to protect against chikungunya virus, which is in the genus *Alphavirus*. The VRC scientists plan to determine whether VLP vaccines also will work against other alphaviruses, such as Western and Eastern equine encephalitis viruses found in the United States and o'nyong-nyong virus found in Africa.

Severe Acute Respiratory Syndrome

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Background

In the spring of 2003, the world first learned of an outbreak of a newly recognized atypical pneumonia that was subsequently 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, Vietnam, and, ultimately, to a total of 29 countries. Overall, the World Health Organization reported 8,096 probable cases of SARS and 774 fatalities in less than 1 year; 27 of those cases were in the United States [1].

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 [2]. 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 by close contact from person to person via large respiratory droplets. Initial signs of illness 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 investigations showed that SARS disproportionately affected healthcare workers and 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 develop the disease [3].

The SARS–CoV outbreak likely originated in a few exotic animals in 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 [4, 5]. Because SARS–CoV-like virus was not found in wild or farm-raised palm civets, it is thought that the horseshoe bat may serve as the natural reservoir of the virus, with the civet serving as the intermediate host. Both animals were sold in Chinese wet markets.

Months after the disease first emerged in mainland China, the clinical syndrome was characterized, the etiological agent was identified, diagnostic tests were developed, 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 successful outbreak containment. In July 2003, the World Health Organization officially declared the outbreak over. Since then 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 2003 related to restaurant exposures.

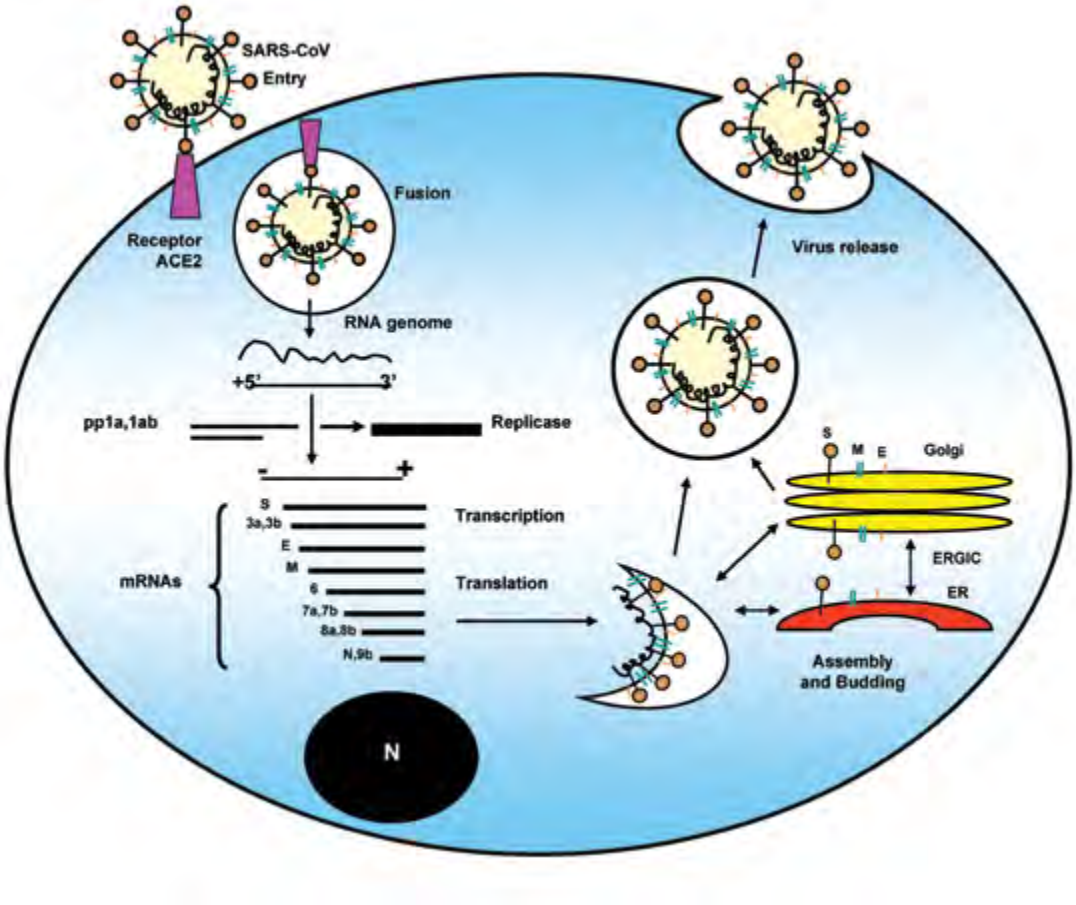
There have been no new SARS cases reported since April 29, 2004. Although the 2003 outbreak has not been repeated, the threat has not disappeared, because an animal reservoir of the precursor virus exists in nature and there is the possibility of an accidental or intentional release of the virus. The population in general, and SARS–CoV researchers specifically, remain at risk without any available prophylactic or therapeutic. Although the global health impact of the SARS 2003–2004 outbreak was tremendous, it paled in comparison to the global economic impact with respect to travel, tourism, and service industries.

SARS Research, Development, and Clinical Testing

National Institute of Allergy and Infectious Diseases (NIAID)-supported scientists have made significant advances in understanding SARS–CoV and its pathogenicity (Figure 1). For example, researchers have identified and characterized the lung receptor molecule, angiotensin converting enzyme-2 (ACE2), to which the S protein adheres [6]. Regions of interaction between the S protein and ACE2 have been mapped and characterized, and the domains of the S protein necessary for viral infection have been determined [7]. This is particularly important in designing improved candidate vaccines and therapeutics. Researchers have learned that the entry of SARS–CoV is blocked by inhibitors of the endosomal protease

FIGURE 1.

SARS–CoV life cycle



SARS–CoV binds to the target cell via interaction between S protein and the cellular receptor ACE2 (angiotensin converting enzyme-2). This complex is translocated to endosomes, S protein is cleaved by cathepsin L, membrane fusion occurs, and the viral genome is released. Viral proteins are transcribed from mRNAs, translated, nucleocapsids assembled in the cytoplasm (from genomic RNA and N protein), then processed through the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). The infected cell releases fully virulent, intact virions through exocytosis [18]. Courtesy of New York Blood Center/Dr. Shibo Jiang

cathepsin L, and a secondary receptor that augments infection, L–SIGN, also was identified and characterized.

Researchers also have discovered that the Papain-like protease (PLpro) of SARS–CoV has deubiquitinating activity, which regulates the location and stability of cellular proteins. They also determined PLpro's three-dimensional structure [8], and this work is contributing to the design of small-molecule inhibitors of this essential enzyme (Figure 2).

Researchers at the Dale and Betty Bumpers Vaccine Research Center, part of NIAID, worked in partnership with Vical, Inc., to manufacture a candidate SARS vaccine that was found to prevent the SARS–CoV from replicating in laboratory mice. The vaccine, composed of a modified piece of DNA that encodes the S protein of SARS–CoV, is expected to stimulate protective immunity in humans. A Phase I open-label clinical study to evaluate safety, tolerability, and immune response to the vaccine was completed in December 2005. The study enrolled 10 healthy volunteers, aged 18 to 50 years, who were given a three-dose vaccine regimen at 1-month intervals. The vaccine was well tolerated, with no or mild systemic or local

FIGURE 2.

PLpro active site with inhibitor

The SARS–CoV papain-like protease (PLpro) enzyme is responsible for proteolytic processing of the viral polyprotein into its functional units. The PLpro active site is depicted in ribbon, and the noncovalent, lead inhibitor in space-filling (sphere) formats [19, 20]. Courtesy of Purdue University/Dr. Andrew D. Mesecar

reactogenicity and no serious adverse events. The vaccine induced neutralizing antibodies, which are strongly associated with recovery from natural SARS infection, and produced cellular immune responses that may be an important component of SARS immunity [9].

Other efforts have been taken by private industry to advance the development of a SARS vaccine. In May 2004, 36 volunteers in Beijing, China, received an inactivated SARS virus vaccine at two dosage levels. The candidate vaccine is produced by a Beijing-based company, Sinovac Biotech Ltd. Most volunteers receiving this vaccine generated an antibody response, and no obvious adverse side effects were noted [10].

Current State of the Science

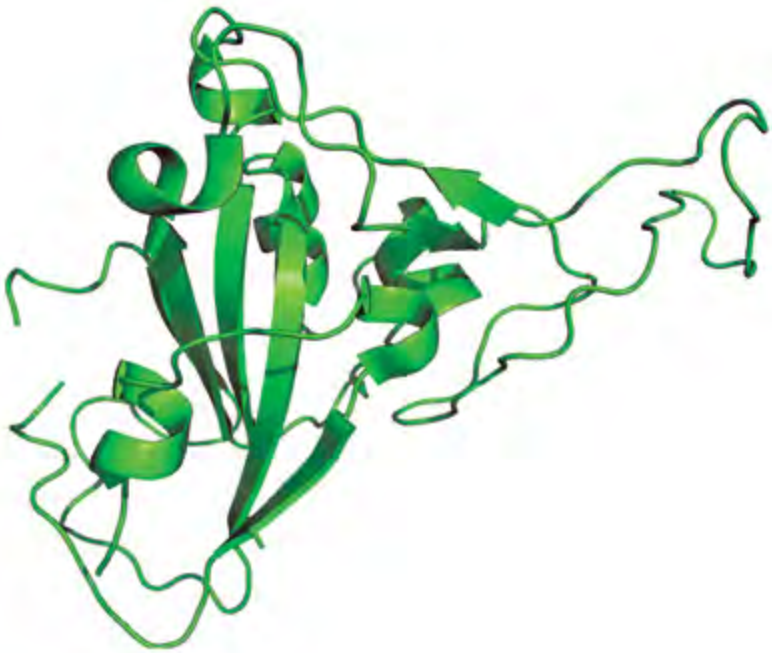
Because it is not known which type of vaccine will be most effective against SARS–CoV, NIAID supports several different approaches to vaccine development.

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 [11, 12]. S protein is used by the virus to attach to lung cells. A contract also was awarded to support the generation of a monoclonal antibody to the S protein. This monoclonal antibody demonstrated both prophylactic and therapeutic properties in animals [13]. One of the contractors, Protein Sciences Corporation, has manufactured and released clinical-grade formulations of alum-adjuvanted and unadjuvanted recombinant baculovirus-produced SARS S protein [14]. An Investigational New Drug Application was submitted in mid-2011. The NIAID Vaccine and Treatment Evaluation Units [15] are planning to conduct a Phase I dose-escalation clinical trial of the candidate vaccine in 84 subjects.

In addition, NIAID-supported investigators are pursuing several other vaccine approaches: a soluble S protein SARS vaccine expressed from mammalian cells, an alphavirus replicon vaccine against SARS, and the expression of SARS proteins in virus-like particles. Two alternate strategies being developed are a peptide-based vaccine approach and an attenuated rhabdovirus (rabies) expressing the SARS S protein. As the vaccine development process is long and difficult, it is hoped that multiple strategies will prove safe and effective in animals and, ultimately, in humans.

FIGURE 3.

Receptor Binding Domain crystal structure



Depiction of the X-ray crystal structure of the SARS–CoV S protein receptor binding domain (RDB), amino acids 318–510, in ribbon format. The RDB is a promising subunit vaccine candidate for SARS–CoV [17, 18]. Courtesy of New York Blood Center/Dr. Shibo Jiang

Novel subunit vaccine constructs for an S protein SARS vaccine based on the receptor binding domain (RBD) are being developed by the New York Blood Center (Figure 3). Expression of S protein RBD constructs in 293T and CHO–K1 cells has been demonstrated. All RBD proteins expressed in different expression systems have high specificity and remain in intact conformation, as demonstrated by the binding of a panel of monoclonal antibodies. Recombinant RBD (rRBD) proteins made in various expression systems induce humoral immune responses, as demonstrated by the induction of high titers of antibodies that neutralize live SARS–CoV infection in vaccinated mice [16, 17].

In addition to the vaccine work described, considerable progress has been made on the development of therapeutics for SARS–CoV. Quantitative structure-activity relationship (QSAR) and other computational analysis provided input to further chemical improvement that resulted in a current lead inhibitor with an IC50 (half maximal inhibitory concentration) of 1.6 mM (millimolars) in an enzymatic assay and an EC50 (half maximal effective concentration) of 2.5 mM against the SARS virus in cell culture assays. The development of non-covalent PLpro inhibitors with micromolar antiviral activity appears significant. The crystal structure of PLpro complexed with a lead inhibitor provides a solid foundation for further design development. Investigators demonstrated the synergy in efficacy for 3C-like protease (3CLpro) and PLpro inhibitors, and they are now pursuing parallel discovery and development of therapeutic inhibitors of both the 3CLpro and PLpro enzymatic targets that appear to be most relevant to SARS [18, 19].

Alternative SARS–CoV inhibitors have been investigated based on their ability to block viral entry. Vinyl sulfides identified as very efficient inhibitors include K777, which previously was identified as an inhibitor of *Trypanosoma cruzi*. Second-generation analogs were generated and found to be between twofold and tenfold more potent than K777 and potent against other viruses as well, including Ebola and other human CoVs. Mannose-binding lectin (MBL) can directly inhibit SARS–CoV entry. Using a panel of spike mutants, an *N*-linked glycosylation close to the receptor binding site has been identified as the primary moiety involved in MBL binding, which demonstrated that MBL can inhibit entry only if applied prior to

cathepsin L activation [20]. Unlike several other viral envelopes to which MBL can bind, both recombinant and plasma-derived human MBL directly inhibited SARS–CoV-mediated viral infection. Mutagenesis indicated that a single *N*-linked glycosylation site, N330, was critical for the specific interactions between MBL and SARS–S. Despite the proximity of N330 to the receptor-binding motif of SARS–S, MBL did not affect interactions with the ACE2 receptor or cathepsin L-mediated activation of SARS–S-driven membrane fusion. Thus, binding of MBL to SARS–S may interfere with other early pre- or post-receptor binding events necessary for efficient virus entry [21].

In addition, NIAID contractors have screened 102,000 potential antiviral drugs and other compounds for activity against SARS–CoV. Several compounds have demonstrated antiviral activity and are being further tested in animal models.

Studies also have been conducted on the molecular mechanisms regulating SARS–CoV pathogenesis in young and aged mice. The resulting data suggest that the magnitude and kinetics of a disproportionately strong host innate immune response contributed to severe respiratory distress and lethality. Although the molecular mechanisms governing acute respiratory distress syndrome (ARDS) pathophysiology remain unknown in aged animals, these studies reveal a strategy for dissecting the genetic pathways by which SARS–CoV infection induces changes in the host response, leading to death [22]. The efficacies of candidate vaccines based on a Venezuelan equine encephalitits virus (VEE) attenuated viral replicon particles (VRP) bearing either attenuated (VRP(3014)) or wild-type VEE glycoproteins (VRP(3000)) were compared in young and aged mice. Aged animals receiving VRP(3000)-based vaccines were protected from SARS–CoV disease, while animals receiving the VRP(3014)-based vaccines were not. Because the glycoproteins of VRP(3014) strain differ from those of the wild-type virus by only three amino acids, tools are likely available to elucidate the mechanism of SARS–CoV protection in aged mice [23].

Researchers in NIAID's Laboratory of Infectious Diseases (LID) studied the replication of SARS–CoV in mice, hamsters, and nonhuman 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 protect against viral replication. This work suggests that vaccines that induce neutralizing antibodies as well as strategies for immunoprophylaxis or 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 preventing and treating SARS-associated disease in hamsters [13].

The LID investigators observed no clinical illness in young mice, hamsters, or NHPs infected with SARS–CoV. However, because advanced age has been associated with poorer outcome and greater mortality in SARS patients, the NIAID 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 [24]. The role of T cells in the pathogenesis and clearance of SARS–CoV was also evaluated in aged mice. Depletion of CD8+ T cells at the time of infection did not affect viral replication or clearance, but depletion of CD4+ T cells resulted in delayed clearance of SARS–CoV from the lungs and was associated with an enhanced immune-mediated interstitial pneumonitis. CD4+ T-cell depletion resulted in reduced neutralizing antibody and cytokine production and reduced pulmonary recruitment of inflammatory cells. Viral clearance in the absence of both CD4+ and CD8+ T cells and antibodies was associated with an innate immune response. These findings provide new insights into the role of CD4+ (but not CD8+ T cells) in primary SARS–CoV infection in this model [25].

The virus-host interactions that governed development of the acute end-stage lung disease cases and deaths from SARS are unknown. LID scientists collaborated with scientists at the University of North Carolina to demonstrate that in mice, SARS–CoV pathogenesis is regulated by a STAT1-dependent but type I, II, and III interferon-independent mechanism. These scientists propose that STAT1 primarily protects mice via its role as an antagonist of unrestrained cell proliferation [26].

The LID scientists also have 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.

Challenges and Opportunities

The re-emergence of SARS is possible, and the need remains for commercial vaccine and therapeutic development. However, the cost and length of time for product development, and the uncertain future demand, result in unfavorable economic conditions to accomplish this task.

A better understanding of the abilities of and requirements for the SARS virus to infect animals without detrimental effect, and to pass from animal to animal (horseshoe bat to civet) as well as from animal to human, is needed. Findings from this research also could apply to the many other viruses that pass from animals to humans [27].

The potential exists for the exacerbation of disease on exposure to those who have been immunized, as has been seen with respiratory syncytial virus, dengue virus, and feline infectious peritonitis virus [28]. Animal studies suggest that this immunopotentiation may occur with candidate SARS–CoV vaccines that contain the N protein [29].

Improved small- and large-animal models for SARS are needed, particularly those models that better mimic human disease with respect to clinical course and symptoms. Improved animal models will help illuminate the pathophysiology of disease, including innate and adaptive immune responses and immunopotentiation, and help move vaccines and therapeutics through the regulatory and clinical phases and ultimately to licensure [30].

In the development of therapeutics and next-generation vaccines, more work is required to determine the structure/function relationships of critical enzymes and structural proteins. Once these relationships are better understood, improvements to the design of small-molecule and protein inhibitors can occur.

A long-term public health strategy should include both active and passive SARS vaccines as well as therapeutics. This strategy should focus on the impact of the disease on healthcare and service workers and on the elderly, as well as mitigation of economic impact.

As the first pandemic of the 21st century, SARS has provided a unique opportunity for research on the life cycle and components of an emerging or re-emerging disease. Although further research is needed, many recent accomplishments are leading the way toward the development of effective prevention and treatment measures.

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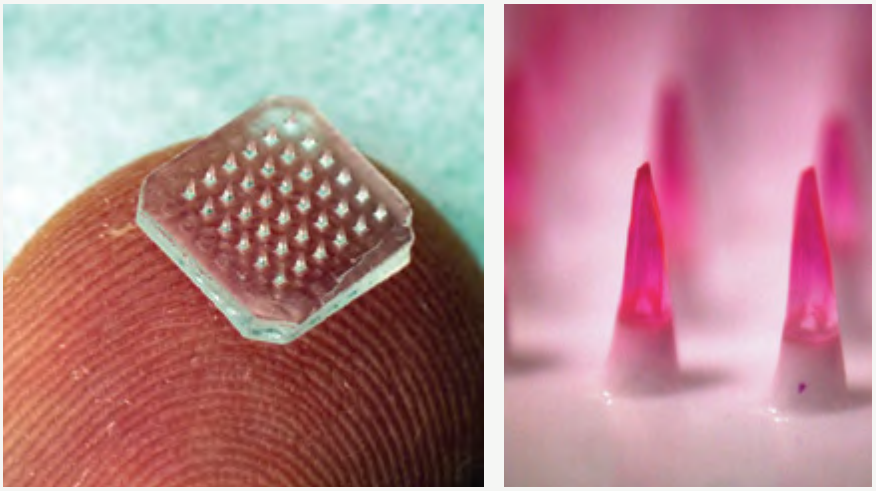
Vaccines offer the most effective method of protecting the public against infectious diseases. However, most currently licensed vaccines require multiple doses to achieve immunity, and each vaccine has unique storage requirements and different methods of administration. New and improved vaccines must be safe and easy to administer and must rapidly produce a protective immune response. Vaccines also must be safe and efficacious in populations of varying age and health status. To prepare for epidemic outbreaks of infectious diseases or the intentional release of biothreat pathogens, improved product stability and vaccination effectiveness are of great importance. Novel delivery technologies that are simple and effective could potentially help a variety of vaccines fulfill these requirements and also could have a major impact on worldwide vaccination campaigns.

New vaccine delivery technologies have evolved as we have increased our understanding of the biology of diseases and the immune response needed to confer protection. The first delivery technologies used needles (smallpox) or needles and syringes (diphtheria, pertussis, tetanus) to deliver vaccines through the surface of the skin. The next innovation was an oral vaccine (polio), and most recently, an intranasal vaccine (influenza) has been developed. Advances in biochemistry and molecular biology have enabled vaccine developers to manufacture greater amounts of vaccines with greater purity, which results in reduced costs and increased product safety. Similarly, formulation technologies have been discovered that enhance the ability of vaccines to produce protective immune responses and stabilize vaccines for storage and use in new delivery systems.



LEFT: A patch containing 36 dissolving microneedles is shown on a fingertip. Courtesy of Georgia Institute of Technology/Jeong-Woo Lee; RIGHT: Microscope image shows dissolving microneedles encapsulating a pink dye. The microneedles dissolve wihin minutes after inserstion into skin to release encapsulated drug or vaccine. Courtesy of Georgia Institute of Technology/Sean Sullivan

Recently developed vaccines, while still delivered with a needle and syringe, are quite different from vaccines of the past. Dose volumes are decreased due to increased purity, and new adjuvants are being used to help trigger the desired immune responses. Novel methods under evaluation to deliver vaccine through the skin include vaccine-coated microneedles, very small needles that contain the vaccine and are dissolved by the body's fluids just below the skin. Some DNA vaccine developers have been testing the feasibility of using electric current to carry their vaccines through the skin. Currently also in the testing stages are a group of small hand-held "needle free" devices that generate jets of high pressure air to "inject" the vaccine through the skin.

Oral delivery offers the advantage of ease of administration, while presenting unique challenges to vaccine developers. Vaccines must be able to survive the varying chemical and microbiological environments of the digestive tract and still be able to elicit the desired immune response. Orally delivered modified live bacterial (typhoid) and viral (polio, rotavirus) vaccines have been successful.

Intranasal delivery of vaccines has been investigated for a number of years with some success. This route has been tested with vaccines delivered in mists, powders, and emulsions. Unique formulations must be designed to enable vaccines to reach immune processing cells located in the nostrils. Challenges of intranasal delivery include the possibility of expelling the vaccine from the nose by an involuntary sneezing reflex, swallowing the vaccine if it is not retained in the nostrils, or inhaling the vaccine into the lungs. Any of these events can negate the vaccine's utility.

As new scientific discoveries are used to improve manufacturing, formulation, and delivery technologies of vaccines, the worldwide population will benefit from reduced time to protective immunity, increased vaccine stability, and reduced logistical requirements for storage, transportation, and delivery.

West Nile Virus

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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 spread throughout the continental United States; as of October 25, 2011, 555 cases in 42 states and the District of Columbia have been confirmed by the Centers for Disease Control and Prevention (CDC) [1]. Although infection with WNV is usually asymptomatic or causes only mild symptoms in humans, it can spread to the central nervous system and cause a variety of disease outcomes, including encephalitis, a potentially deadly brain inflammation. Other clinical presentations can be similar to those of Parkinson's disease, poliomyelitis, or Alzheimer's disease. Most cases of West Nile neurologic disease occur in elderly people and in those with impaired immune systems (people with diabetes, chemotherapy patients, etc.) [2,3]. The realization in 2002 that WNV can be transmitted by blood transfusion or organ transplantation from WNV-infected donors prompted stringent safety testing of donor blood supplies [4]. Many published studies of patients with WNV meningitis or encephalitis have confirmed that those older than 55 years are more likely to have a lengthy recovery period with long-term physical, cognitive, and functional disabilities that may last more than 2 years after acute illness [5]. Despite much effort over the last decade to develop vaccines and therapeutics, no treatment is available for WNV encephalitis, and no licensed vaccine exists to prevent disease in humans. (Although WNV vaccines have been available for prevention of disease in horses since 2002, development of vaccines for human use must adhere to Food and Drug Administration (FDA)-mandated stringent safety and efficacy testing, which extends the development timeline.) Mosquito control measures and other tactics, such as the use of mosquito repellents and the wearing of long-sleeved shirts and pants to reduce the number of mosquito bites, have thus been the only available strategies to combat the rapid spread of this emerging disease.

Faced with the continued potential for a serious WNV epidemic, researchers supported by the National Institute of Allergy and Infectious Diseases (NIAID) initiated development of candidate vaccines to protect against WNV infection. WNV vaccine development has benefited from the fact that the virus belongs to a taxonomic group known as flaviviruses, which share a number of characteristics that allow 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.

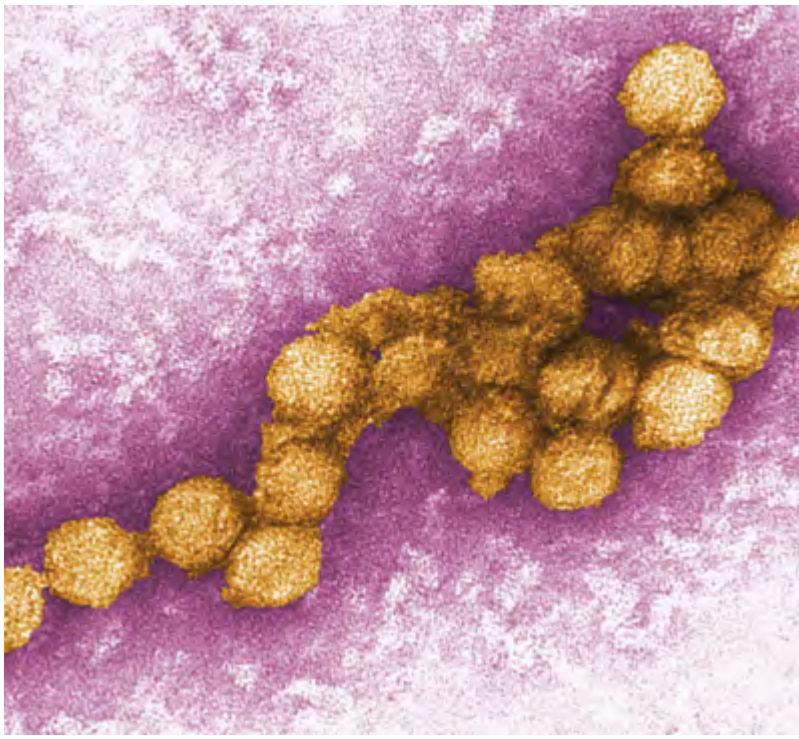
There has been great success in controlling yellow fever and Japanese encephalitis with well-organized vaccination campaigns centered on efficacious vaccines [6]. Therefore, the National Institutes of Health (NIH) has encouraged similar WNV vaccine development programs.

NIAID-supported basic research studies discovered that hamsters and mice are good models for WNV disease in humans. NIAID-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 golden hamsters were completely protected by prototype WNV vaccines and, surprisingly, were also at least partially protected against WNV infection by licensed Japanese encephalitis and yellow fever vaccines [7]. Thus, this animal model is an important resource that now is being used to test the efficacy of new vaccine candidates and antiviral medicines. Similarly, efficacious mouse models of WNV encephalitis also have been developed with NIAID support [8].

NIAID is supporting a number of WNV vaccine approaches. One of the earliest began in 1999 when NIAID funded a fast-track project by Acambis, Inc., to develop a candidate live, attenuated, "chimeric" WNV vaccine. The vaccine was constructed using the DNA/genes of the licensed yellow fever 17D vaccine virus as the backbone. For the WNV vaccine, researchers substituted certain genes (the premembrane (prM) and envelope (E) surface protein genes) of WNV for the prM and E genes of the yellow fever vaccine virus using chimeric technology that was originally developed at NIAID during the early 1990s. The "chimeric" yellow fever/West Nile DNA was then manipulated and inoculated into cell cultures

to produce the "chimeric" West Nile live, attenuated vaccine that was able to elicit anti-WNV antibodies and protect against WNV infection in vaccinated animals. This method of creating chimeric flavivirus vaccines is also being applied to developing vaccines for dengue and Japanese encephalitis viruses. The Acambis WNV vaccine (designated ChimeriVaxWN) has undergone successful preclinical evaluations in hamsters, mice, monkeys, and horses and yielded encouraging results in a Phase I clinical trial [9]. In December 2005, the vaccine was moved into Phase II clinical trial evaluation, making Acambis the first company to enter Phase II testing of a WNV vaccine. The randomized, double-blind, placebo-controlled trial was conducted in more than 200 subjects in the United States. The safety, tolerability, and immunogenicity of the vaccine at different dose levels was evaluated in a two-part study, first in healthy young adults aged 18–40 years, then in two healthy, elderly range cohorts, aged 41–64 years and age >65 years. The recently published results showed the vaccine to be highly immunogenic and well-tolerated at all dose levels and in all age groups studied. The incidence and severity of treatment-emergent adverse events (primarily fatigue, headache, and myalagia) were comparable between placebo groups and all treatment groups [10]. In 2008, Sanofi Pasteur acquired Acambis, and Acambis' West Nile, dengue, and Japanese encephalitis candidate vaccine products are now integrated within the Sanofi Pasteur vaccine development schedule.

Intramural NIAID scientists, with early assistance from collaborators from the Walter Reed Army Institute of Research (WRAIR), capitalized on advances in recombinant DNA technology and previous research on dengue viruses to produce a different candidate live, attenuated 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 (DENV–4). DENV–4 is a non-neuroinvasive virus that does not cause neurological disease in animals and humans infected peripherally. The resulting weakened, or attenuated, virus strains were safer for use in a vaccine, but still protective. The NIAID team then used this strategy to combine genes from WNV and DENV–4. This hybrid virus did not infect the brain, yet still stimulated a strong immune response with even a single dose. This WNV/DENV–4 chimeric virus was further attenuated for mice and monkeys by deleting 30 nucleotides from its 3' untranslated region (designated delta30) [11]. The WNV/DENV–4 3'delta30 candidate vaccine was evaluated for safety and immunogenicity in a Phase I clinical trial that is now completed.



Transmission electron micrograph (TEM) of the West Nile virus (WNV). Courtesy of CDC

NIAID scientists at the Dale and Betty Bumpers Vaccine Research Center (VRC) developed a DNA-based vaccine against WNV in collaboration with the CDC and 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. Two versions of the vaccine were developed, one utilizing an optimized CMV/R promoter. The VRC has completed two Phase I clinical trials to evaluate safety, tolerability, and immune responses of these recombinant DNA vaccines in human volunteers [12, 13]. As the DNA vaccine has been licensed to Vical by the CDC, any further development will be undertaken by Vical.

In addition to pursuing replicating chimeric vaccines, researchers have made advances in the development of nonreplicating subunit vaccines. Scientists at Hawaii Biotech, Inc., supported initially by an NIAID grant and then by a National Institute of Neurological Disorders and Stroke (NINDS) grant, along with other financing, are developing genetically engineered, *Drosophila*-expressed subunit vaccines containing portions of the viral E and NS1 proteins. Subunit protein vaccines cannot replicate or cause disease. Following testing of the company's WNV vaccine in the golden hamster and nonhuman primate WNV disease models [14], the WNV vaccine (designated HBV–002) completed a successful Phase

I clinical trial in 2008, which demonstrated its safety and immunogenicity in healthy adult volunteers. The company is planning future clinical trials in other populations (e.g., elderly, immunocompromised).

At L2 Diagnostics, LLC, NIAID-supported researchers have developed a recombinant *Baculovirus*-produced subunit vaccine that induces protective antiviral antibodies in a murine model of WNV infection and, importantly, prevents WNV disease in horses [15]. No Phase I clinical trials are yet planned; however, the company may pursue regulatory approvals for veterinary use of this vaccine. The company is also investigating a nanoparticle vaccine against WNV.

Other WNV vaccines in early-stage development include a mutagenized live, attenuated vaccine based on Kunjin virus (an Australian strain of WNV that is closely related to the WNV NY99 strain but rarely associated with clinical disease), a novel live attenuated vaccine (RepliVax WN) composed of WNV particles that are limited to a single cycle of replication that limits spread and renders it incapable of causing disease [16], a proprietary inactivated vaccine formulation, a dry powder WNV protein vaccine that could be administered intranasally, and a synthetic peptide-based multi-flavivirus vaccine.

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HENIPAVIRUSES (NIPAH VIRUS AND HENDRA VIRUS)

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Nipah virus and Hendra virus are closely related paramyxoviruses that emerged from bats during the 1990s to cause deadly outbreaks in humans and domesticated animals [1]. Hendra virus was first discovered in 1994 in Australia, where it caused outbreaks in racing horses and horse handlers [2]. Fourteen outbreaks have occurred in Australia from 1994 to 2010, causing 7 human infections and 4 deaths [3, 4]. Hendra outbreaks have increased in frequency; between June and October 2011 alone there were 18 spillover events in horses and 1 dog, with no confirmed human infections [5, 6]. Queensland and New South Wales have now been declared endemic for Hendra virus. Field studies following the outbreaks identified large fruit bats (*Pteropus giganteus*) as the source of infection. These bats roost on trees in horse pastures, and it is believed that horses became infected by nibbling on leftover fruit eaten by the bats or by exposure to bat secretions found in the pasture. Nipah virus was first identified in 1998 after a large outbreak in pig farms in the Malaysian peninsula caused 265 human infections and 105 deaths [7]. This epidemic is believed to have started in pig farms built on the edge of a forest where large fruit bats were roosting. Nipah virus, which is carried by bats, was passed to pigs when the pigs fed on fruit contaminated with bat saliva, which the bats dropped from their roost into the pig enclosures [8]. The infected pigs developed severe respiratory and neurological disease and are believed to have infected humans through respiratory droplets. The Nipah outbreaks in Malaysia had a devastating effect on the economy, as more than 1 million pigs had to be culled, and 800 farms had to be demolished. Several additional outbreaks have occurred in parts of Bangladesh and India, with a human case fatality rate of approximately 70 percent. Some of these outbreaks have been linked to the human consumption of fresh palm sap [9]. Field investigations have shown that palm sap, which is collected from the bark of palm trees, is often contaminated with bat saliva, as the bats like to feed from the sap-collection vessels.

No vaccine or therapeutic agents are currently available to prevent or treat Hendra and Nipah infections.

National Institute of Allergy and Infectious Diseases (NIAID)-supported investigators developed vaccines for Nipah and Hendra virus based on the soluble G-glycoproteins of the viruses formulated with adjuvants. Both vaccines have been shown to induce strong neutralizing antibodies in different laboratory animals [10, 11]. Importantly, the Hendra virus vaccine induces cross-neutralizing antibodies against Nipah virus. The Hendra virus vaccine has been shown to confer 100 percent protection against lethal viral challenges with both Nipah and Hendra viruses in cats, ferrets, and nonhuman primates [10, 11]. In May 2011, scientists at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Australia announced that this vaccine protected horses from lethal challenges with Hendra virus. In late 2011, Pfizer licensed the technology to make the vaccine for veterinary use. This vaccine has the potential to protect domesticated animals from infection and stop animal-to-human transmission of Nipah and Hendra viruses in endemic countries.

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Group B Streptococcus

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In the 1970s, group B streptococcus (GBS) emerged as the leading infectious cause of neonatal morbidity and mortality and late pregnancy-related morbidity [1, 2]. Two syndromes in neonates and young infants were recognized: early-onset disease (primarily sepsis, pneumonia, and meningitis within the first 6 days of life) and late-onset disease (primarily meningitis between 7 and 90 days of age). GBS bacteria are vertically transferred from a vaginally or rectally colonized mother to the neonate during labor and delivery, also called the intrapartum period. This typically results in colonization of the infant and less commonly in invasive early-onset disease. The mode of transmission for late-onset disease remains poorly elucidated.

Neonatal disease prevention strategies in the United States have focused on antenatal identification of GBS vaginal and rectal colonization in pregnant women and the use of antibiotics during labor and delivery in women who are colonized or at risk of colonization. This has led to an 80 percent decrease in the incidence of early-onset neonatal infections and a 21 percent decrease in the incidence of invasive GBS infections in pregnant women, associated with intrapartum antibiotic prophylaxis [3, 4]. Although the maternal intrapartum chemoprophylaxis strategy is effective, it is an interim solution, as the incidence of late-onset GBS disease remains unchanged. Additionally, chemoprophylaxis has resulted in use of antibiotics in 30 percent of women at delivery, raising concerns about the emergence of resistant strains [5, 6]. Recent data indicate that 20 percent of GBS isolates are resistant to clindamycin and 30–40 percent are resistant to erythromycin. Fortunately, penicillin resistance is not yet an issue [7, 8].

During the last two decades, an increase in the incidence of invasive GBS disease in nonpregnant adults has been reported [1, 9]. The majority of these cases occur in adults with underlying medical conditions, such as diabetes, neurological impairment, breast cancer, and cirrhosis, but the highest attack rates occur in those aged 65 years and older. Common clinical manifestations of GBS disease in adults include skin and soft tissue infections, bacteremia and sepsis, bone and joint infections, and pneumonia. Meningitis and endocarditis are less common, but are associated with serious morbidity and mortality. The case fatality rate is higher in adults than in neonates, and is especially high in those over the age of 65.

A safe and effective vaccine would be a major advance in the prevention of GBS disease. Active immunization of women during the third trimester of pregnancy has potential for the prevention of both maternal and infant GBS disease. Adults with underlying medical conditions also could benefit significantly from a GBS vaccine. A licensed vaccine is not yet available, but several promising vaccine candidates are in early stages of development.

Since the early 1990s, contracts funded by the National Institute of Allergy and Infectious Diseases (NIAID) have supported GBS vaccine design studies, the production of GBS glycoconjugate vaccines for serotypes Ia, Ib, II, III, and V, and more than 20 NIAID-sponsored Phase I and Phase II trials. In these studies, study participants received uncoupled capsular polysaccharides (CPSs) or CPS-protein conjugates. Each CPS was individually conjugated to tetanus toxoid (TT) or the mutant diphtheria toxoid cross-reactive material 197 (CRM197) [10–13]. In summary, results indicated that the conjugate vaccines were safe and induced functional antibody responses.

Most clinical trials involved a single injection of monovalent vaccine preparations, with the exception of a bivalent study in which type II–TT and type III–TT were administered together [14]. The immune response in bivalent vaccine recipients was comparable to that observed in the monovalent vaccine recipients. One study, in which volunteers received a type III–TT booster 21 months after the first dose, revealed that a booster response was only observed in a group that had undetectable GBS type III CPS-specific immunoglobulin G (IgG) before the first dose of type III–TT vaccine [15]. Another study showed that adsorption of a type III–TT to alum did not improve the immune response, compared with the type III CPS [15]. A randomized, double-blind, Phase I study was completed in which a GBS type III–TT was administered to 30 healthy, third-trimester pregnant women [16]. The vaccine was safe, healthy babies were delivered by all vaccine recipients, and vaccine-induced type III CPS-specific IgG was shown to be efficiently transported to the infant and functionally active through 2 months of age. These data suggest that a GBS conjugate

vaccine has the potential to prevent both early- and late-onset infant GBS disease and invasive disease in pregnant women.

More recently, additional studies have been conducted and are summarized below.

- A randomized, double-blind comparison study with GBS type V–TT and GBS type V–CRM197 vaccines tested in 35 healthy, nonpregnant women showed that both conjugate vaccines were safe and elicited specific antibody responses with opsonophagocytic killing of type V GBS [17]. Approximately 80 percent of vaccine recipients had a persistent antibody response for at least 2 years.
- A randomized, double-blind study with a GBS type V–TT vaccine tested in 32 healthy adults 65–85 years old demonstrated that the vaccine was safe and elicited specific antibody responses with opsonophagocytic killing of type V GBS and with 68 percent of recipients having a fourfold antibody increase [18]. The level of the specific antibody persisted up to 1 year, suggesting the potential for prevention of invasive type V GBS infections in healthy elderly people through vaccination.
- A Phase I, dose-escalating trial was conducted in 45 healthy adults to evaluate immunogenicity and reactogenicity of a GBS type V–TT vaccine ranging from 2.4 micrograms (mcg) to 38.5 mcg per dose [19]. The results showed that the vaccine was safe and elicited specific antibody responses with opsonophagocytic killing in all dose groups.
- Recently, a trial of a GBS vaccine in sexually active, nonpregnant women indicated that a vaccine to prevent GBS infection is possible [20]. This Phase II prospective, randomized controlled trial enrolled 668 healthy, sexually active nonpregnant women aged 18–45 years without GBS vaginal or rectal colonization at the time of their enrollment. The results showed that a GBS type III conjugate vaccine had an efficacy of 45 percent in preventing acquisition of vaginal type III colonization and an efficacy of 35 percent in preventing acquisition of rectal colonization over an 18-month period, when compared with participants who received the control tetanus and diphtheria toxoid vaccine.

In addition to the above studies, at least one pharmaceutical company has recently sponsored several studies to clinically evaluate a monovalent conjugate GBS vaccine [21].

Challenges and Future Opportunities

Although these efforts demonstrate progress in GBS vaccine development, several challenges remain:

1. Vaccine candidates that protect against multiple GBS subtypes must be developed. Serotypes Ia, Ib, II, III, and V are the predominant serotypes isolated from neonates, young infants, pregnant women, and adults with invasive GBS disease in the United States. Because antibodies against GBS CPS are serotype specific, a multivalent vaccine will be needed to provide broad protection. As a result, a number of formulation parameters, such as the number and amount of the protein carriers, will need to be optimized.
2. A correlate of immunity needs to be determined for the use of a GBS vaccine for maternal immunization. With the success of using antibiotics for prevention of neonatal sepsis, the number of cases of GBS neonatal sepsis in the United States has been reduced. Subsequently, it has been difficult to conduct the efficacy trials that are needed to reach this milestone.
3. There is a need for an established threshold for CPS type-specific antibody levels that correlate with protection. Although some data are currently available, information for all serotypes causing invasive GBS disease is required. Progress has been made in case-control comparisons of antibody levels to several GBS serotypes, including type III, in colonized mothers of infants with and without early-onset infection [22, 23]. This suggests that serotype-specific thresholds of protection can be set and will likely differ by serotype.
4. There is a need to standardize assays across laboratories for specific polysaccharide antibody levels and their biological functions.
5. Finally, additional industry commitment to GBS vaccine development is needed. Vaccine manufacturers' liability concerns have been an obstacle in the development of GBS conjugate vaccines to protect pregnant women from invasive GBS disease. The feasibility of maternal immunization has been demonstrated by the worldwide immunization of pregnant women for the prevention of neonatal tetanus, a major cause of infant mortality; however, safety data related to neonatal outcomes other than tetanus have not been collected. The risks involved in maternal immunization during the third trimester need to be better defined. The current use of inactivated influenza vaccine in pregnant women in the United States provides an opportunity to design studies to collect data to further demonstrate the safety and benefit of this approach to immunizing mother and infant.

Although NIAID's efforts in GBS vaccine development have focused on CPSs, an alternative strategy for prevention of GBS disease is to develop a vaccine based on a GBS surface protein. One advantage of this approach is that some of these proteins are immunogenic and do not need to be conjugated to other molecules. Also, recombinant DNA techniques can be used to produce large amounts of antigens for vaccine preparation.

Investigations with alpha and beta subunits of the GBS C protein, Rib protein, type V a-like and Rib proteins, and surface immunogenic protein (Sip) have demonstrated that these surface proteins are capable of eliciting antibody responses in mice and protecting against lethal bacterial challenges [24–27]. In addition to their use as immunogens, surface proteins have been used as carriers for CPS antigens. Compared with GBS CPS vaccines conjugated with TT, these conjugates have the advantage of enhancing the immunogenicity of the polysaccharide component of the vaccine and eliciting additional antibodies protective against GBS infections. Development of other formulations of GBS vaccines is another area of active research. A study with a bivalent vaccine composed of purified Rib and a proteins mixed with alum demonstrated an antibody response in mice and protected against lethal infection with GBS (serotypes Ia, Ib, II, and III) [28].

GBS C5a peptidase and beta-C protein are two surface proteins that have been conjugated to CPS antigens and are being pursued as vaccine candidates. Studies with anti-C5a peptidase antibodies demonstrated opsonic activity, suggesting that inclusion of C5a peptidase in a polysaccharide vaccine can produce another level of protection that is serotype independent [5].

A key development in the last decade includes a conserved pilus-based vaccine candidate that conferred protection against all tested GBS challenge strains in *in vitro* and *in vivo* studies [2]. In another study, a GBS CPS type III conjugated with recombinant cholera toxin B subunit administered intranasally improved the mucosal and systemic immune responses to GBS in a mouse model [29].

New strategies for GBS vaccine development include development of a universal GBS vaccine based on multiple genome screen technology. By analysis of the genome sequences of eight GBS isolates, more than 300 proteins were evaluated [30]. Four proteins that elicited protection in mice were selected, and their combination provided a high degree of protection against a large panel of strains that included all circulating serotypes.

Much progress has been made in the development of GBS vaccines during the last 30 years. Better CPS-conjugate vaccines have emerged, and the use of GBS proteins as immunogens or their conjugation to CPS offers a promising future for GBS vaccine development. However, these candidate vaccine components have yet to be studied in humans. Additional research is needed to expand serological findings to define protective levels of GBS antibodies and define immune defects in adults that result in invasive disease. There is also a need to better understand innate and adaptive responses of the immune system in relation to GBS pathogenesis in different populations. NIAID continues to fund basic research on GBS and supports both preclinical and clinical resources that may be helpful to academic and industry partners interested in collaborating on GBS vaccine development.

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CMV VACCINE SHOWS PROMISE

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Each year, approximately 8,000 infants in the United States develop severe hearing, mental, or movement impairments after becoming infected with cytomegalovirus (CMV), a common virus passed on to them while they are still in the womb. CMV is also the most common viral infection in patients who receive solid organ transplants, with up to 60 percent of transplant recipients developing symptomatic disease. Now, clinical trials supported by the National Institute of Allergy and Infectious Diseases (NIAID) have given rise to optimism that a vaccine to prevent CMV infection may be closer.

The first trial, led by pediatrician Robert Pass, M.D., of the University of Alabama at Birmingham, evaluated an experimental vaccine made from a single CMV protein, glycoprotein B, which is known to prompt an immune response. The candidate vaccine, known as CMV gB and supplied by Sanofi Pasteur, included an experimental adjuvant, MF59.

A total of 441 CMV-negative women, assigned at random to receive the candidate vaccine or a saline injection, were evaluated. Vaccinations were given to women within 1 year after they had given birth. Most women received three doses of trial vaccine or saline injection; all received at least one dose. In the final analysis, women who received the trial vaccine were 50 percent less likely to later become infected with CMV throughout the 42-month follow-up period than were women who received a saline injection.

In a second trial, led by Paul Griffiths, M.D., of the University College London Centre for Virology, the Sanofi Pasteur CMV gB vaccine was evaluated in volunteers awaiting liver or kidney transplants. A total of 67 patients received the vaccine, and 73 received a look-alike placebo. The vaccine was shown to be safe and immunogenic in all the volunteers who received it. Vaccination also reduced the posttransplant duration of viremia and the number of days of required treatment with the antiviral drug ganciclovir in patients who were seronegative at transplant but who received organs from donors who were CMV-positive.

An additional NIAID-supported Phase II trial of the experimental CMV vaccine is under way to evaluate the vaccine in healthy adolescent girls.

HIV/AIDS

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Overview

The impact of the HIV/AIDS pandemic has been profound and continues to have devastating effects worldwide. Although resources for HIV prevention and treatment have become increasingly available, the number of new infections remains at unacceptably high levels. In the United States, specific segments of the population—African Americans, Latinos, gay and bisexual men—are particularly vulnerable. And globally, in addition to the effects of the disease itself, affected populations are at higher risk for poverty, hunger, and childhood mortality. If current infection rates continue, it has been estimated that, as the need for expensive and ongoing treatment keeps pace, HIV-related costs could escalate to as high as $35 billion by 2030. The human and economic costs of HIV necessitate a preventive HIV vaccine.

The development of an HIV vaccine is complex and presents daunting scientific challenges due to HIV's unique characteristics, which include the ability to integrate into the genome of human cells without killing them and to destroy the immune system while evading the body's efforts to eliminate the virus. There are also many different genetic subtypes of HIV that circulate worldwide, and for a vaccine to be effective, it will need to induce immune responses that are broadly reactive to all or most of them.

The most rational way to design an effective vaccine is to identify the immune responses that protect against the specific infection and construct a vaccine that stimulates those responses. Because 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/or preventing or controlling infection. The two main types of immune responses are humoral immunity, which uses antibodies to defend against the virus, and cell-mediated immunity, which uses cytotoxic T lymphocytes (CTLs) to directly kill or control infected cells. The earliest vaccine research focused primarily on vaccines that elicited antibodies. Vaccine concepts involving a prime-boost combination strategy also have been tested. These vaccines stimulate a cellular immune response via CTLs (prime), as well as antibodies that bind to the virus (boost).

When a vaccine is developed, the hope is that it will be 100 percent effective in preventing infection. However, the first HIV vaccine may not be able to protect everyone from infection; it may be partially effective in preventing infection or only delay or prevent disease. Nonetheless, researchers recognize that such a vaccine could have a significant impact on the spread of new infections globally. With a decrease in the number of people susceptible to HIV infection, fewer people would be passing it on to others. If this occurs among a high percentage of people within a given population, new infections could be reduced dramatically or even eliminated. However, the benefits of a partially effective vaccine could be offset by relaxed practices of safe behaviors, education, and prevention resulting from perceived protection. Clearly, partially effective vaccines would need to be delivered in the context of a comprehensive prevention program. Thus, the National Institute of

HIV/AIDS Epidemic—Estimated Impact

Worldwide

- People living with HIV: 33.3 million
- People newly infected: 2.6 million
- Number of AIDS-related deaths: 1.8 million

United States

- People living with HIV: 1 million
- People newly infected: 56,300
- Percentage of people who don't know their HIV status: 21 percent

Sources: Joint United Nations Programme on HIV/AIDS (UNAIDS). Global report: UNAIDS report on the global AIDS epidemic 2010 [Internet]. Geneva (Switzerland): UNAIDS; 2010. Available from: www.unaids.org/globalreport/Global_report.htm

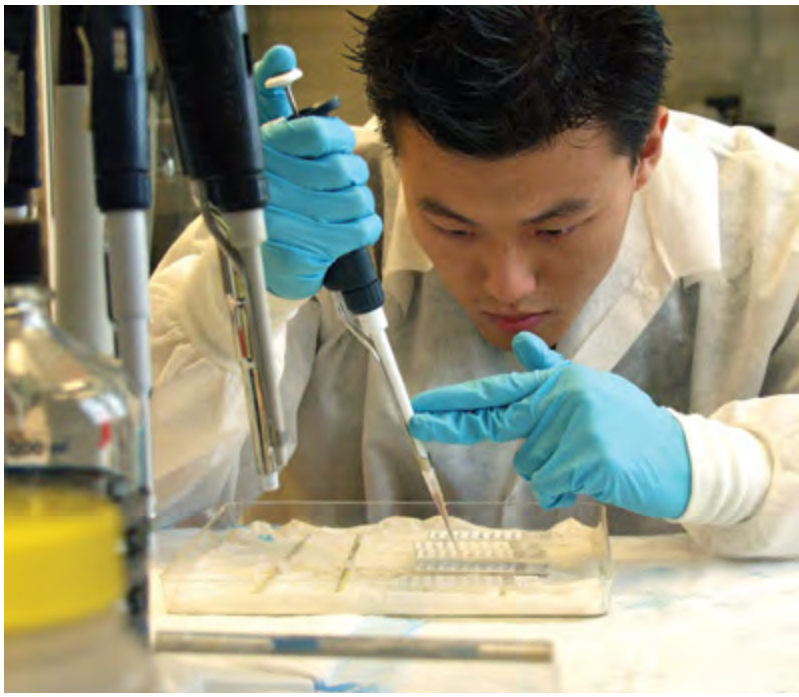
Centers for Disease Control and Prevention (CDC), Divisions of HIV/AIDS Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention. HIV in the United States [fact sheet on the Internet]. Atlanta (GA): CDC; 2010 Jul. Available from: www.cdc.gov/hiv/resources/factsheets/us.htm

Allergy and Infectious Diseases' (NIAID's) HIV prevention research encompasses a variety of methods, such as topical microbicides, antiretroviral therapy (ART) to reduce the ability of HIV-infected persons to infect others, and pre-exposure prophylaxis (PrEP) to reduce the risk of HIV infection.

The Need for Partnership

Not only will multiple strategies be needed to fully prevent HIV, but also multiple organizational partners will be critical to identifying those strategies as quickly as possible. NIAID supports and oversees the vast majority of HIV vaccine research through collaborative partnerships with other government agencies, academic institutions, industry, private organizations and foundations, and the community. These partnerships greatly extend NIAID's scientific capacity, leverage resources (financial and otherwise), and encourage a coordinated approach that will potentially accelerate the development of an HIV vaccine. Among NIAID's partners and collaborators are the following:

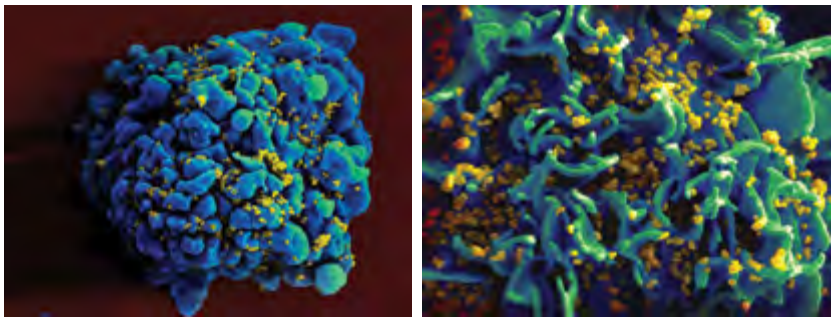
- **Global HIV Vaccine Enterprise (GHAVE).** GHAVE, also known as "the Enterprise," is a consortium of independent organizations, including NIAID, committed to accelerating the development of a preventive HIV vaccine. With its 2010 Scientific Strategic Plan, the Enterprise seeks to speed the development, execution, and analysis of HIV vaccine trials; better integrate preclinical and clinical research; capitalize on progress from recent HIV vaccine and other non-HIV research; and bring in new researchers from outside the field of HIV, as well as new funders. (See www.hivvaccineenterprise.org/scientific-strategic-plan.)
- **HIV Vaccine Trials Network (HVTN).** Funded by a cooperative agreement from NIAID, the HVTN is a clinical trials network of international scientists and researchers. The HVTN's mission is to evaluate candidate preventive HIV vaccines in all phases of clinical research, from evaluating experimental vaccines for safety and the ability to stimulate immune responses to testing vaccine efficacy, while at the same time generating information that will guide the design of improved vaccine concepts. (See www.hvtn.org.)
- **International AIDS Vaccine Initiative (IAVI).** IAVI was founded in 1996 to speed the discovery of an HIV vaccine; its partners include private companies, academic institutions, and government agencies, including the National Institutes of Health (NIH). (See www.iavi.org.)



Scientist at work. Courtesy of the U.S. Military HIV Research Program

- **NIAID HIV Vaccine Research Education Initiative (NHVREI).** NHVREI was established in 2006 as NIAID's primary mechanism for educating and fostering partnerships with key influencers within community-based, nonscientific organizations. The purpose of these partnerships is to promote understanding of and garner support for HIV vaccine research, especially among the more vulnerable and hard-to-reach populations. NHVREI is implemented through a contract with the Academy for Educational Development and Getting Your Message Right public relations. (See www.bethegeneration.nih.gov.)

  When the NHVREI contract expires in fall 2011, the scope of the project will be expanded to encompass all biomedical preventive research, including microbicides and PrEP. This effort, known as the Biomedical Prevention of HIV Research Education Initiative, will disseminate information on NIAID's prevention and vaccine clinical research activities and cultivate ongoing dialogue and relationships with key opinion leaders and organizations that reach highly affected populations.
- **South African AIDS Vaccine Initiative (SAAVI).** SAAVI was formed in 1999 to coordinate the research on and development and testing of HIV vaccines in South Africa. SAAVI is based at the Medical Research Council of South Africa and works with key national and international partners to identify an affordable, effective, and locally relevant AIDS vaccine. NIAID works collaboratively with SAAVI in



Scanning electron micrograph of HIV particles infecting a human T cell. LEFT: Image of an HIV infected H9 T cell, colorized by Anita Mora at RML. Image taken by Beth Schmidt in the Research Technologies Branch, Courtesy of NIAID. RIGHT: Close up view of an HIV infected H9 T cell, colorized by Anita Mora at RML. Image taken by Beth Schmidt in the Research Technologies Branch. Courtesy of NIAID

conducting HIV vaccine trials in South Africa. (See www.saavi.org.za.)

- **Community Advisory Boards (CABs).** NIAID highly values and actively seeks community input in all aspects of the research process. A key partner is the Global Community Advisory Board (GCAB), a group of community representatives who work with the leadership of the HVTN and site-specific CABs. CAB members help develop research plans, set research priorities, and participate as full members of protocol teams. CAB members also relay community needs and concerns, provide input on planned and ongoing research, and help assess the feasibility of a given trial in their community. (See www.niaid.nih.gov/topics/HIVAIDS/Research/Pages/outreach.aspx.)
- **U.S. Military HIV Research Program (MHRP).** MHRP was established in 1985 by the U.S. Army Medical Research and Materiel Command to protect U.S. troops entering areas with a high prevalence of HIV. Bringing together scientists from the U.S. Army, Navy, and Air Force, MHRP is dedicated to HIV vaccine development, HIV prevention, disease surveillance, and HIV care and treatment. NIAID jointly plans and executes HIV vaccine research projects and clinical trials with MHRP through an interagency agreement, which helps ensure that U.S. government-funded HIV vaccine research is well coordinated, efficient, and comprehensive. (See www.hivresearch.org.)

The Role of Basic Vaccine Research

The identification of new, improved candidate vaccines is urgently needed. Basic research in the fields of HIV natural history, pathogenesis, immunology, virology, viral and host genetics, and animal model development can lead to novel discoveries and increase our understanding of the earliest events in HIV infection and early immune responses. Scientific advances that define how the human immune system attempts to protect itself against HIV continue to unfold; provide a better understanding of the earliest events in natural infection, particularly in those who show an immune capacity to resist the virus; and are beginning to shape new vaccine approaches.

NIAID conducts basic research through the Dale and Betty Bumpers Vaccine Research Center (VRC) and the NIAID-funded Center for HIV/AIDS Vaccine Immunology (CHAVI), as well as individual grantees at academic centers throughout the United States. The 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 on envelope structure and potential targets for broadly neutralizing antibodies, new methodologies for enumerating protective T lymphocytes, and fundamental studies on adjuvants and potential vaccine vectors.

CHAVI is a virtual center designed to support intensive and highly collaborative projects that address key immunological roadblocks to the discovery and development of a safe and effective HIV vaccine. Established in 2005, this center currently focuses on elucidating early viral and immunological events and host genetic factors associated with HIV transmission, establishment of productive infection, and (partial) containment of virus replication; determining correlates of the simian form of HIV, simian immunodeficiency virus (SIV), immune protection in primates; designing, developing, and testing novel immunogens and adjuvants that elicit persistent mucosal and/or systemic immune responses in humans and primates; and advancing HIV vaccine candidates into early phase clinical trials.

In March 2008, following disappointing results from an NIAID-funded HIV vaccine trial (see page 126), NIAID held a summit on HIV vaccine research and development. In garnering input on how best to reinvigorate and advance HIV vaccine research, a scientific consensus emerged: Enormous advances in fundamental research are needed to design a safe and effective HIV vaccine. As a result, NIAID expanded and strengthened its portfolio of basic vaccine discovery research.

Two of NIAID's recent basic vaccine research programs are the Basic HIV Vaccine Discovery Research Initiative and the B Cell Immunology Partnerships for HIV Vaccine Discovery.

The Basic HIV Vaccine Discovery Research Initiative funds a broad range of basic research in areas such as immunology, virology, cellular and structural biology, and host genetics. The B Cell Immunology Partnerships for HIV Vaccine Discovery fosters cross-fertilization between B cell immunologists and HIV vaccinologists, seeking to facilitate discovery of novel vaccine design and immunization strategies for eliciting protective anti-HIV antibodies. Both programs have the potential of leading to new discoveries, expanded knowledge, and novel concepts and approaches applicable to HIV vaccine design.

Other important NIAID-funded basic research initiatives include the Phased Innovation Awards in AIDS Vaccine Research, which supports early stage AIDS vaccine research, and the Highly Innovative Tactics to Interrupt Transmission of HIV (HIT–IT). HIT–IT funds risky but rational approaches that could potentially provide long-term protection from acquiring HIV infection and that are based on newly gained knowledge of HIV pathogenesis, biology of HIV transmission, and human genetics. HIT–IT was specifically designed to attract investigators from outside the HIV research field, as well as those applying for their first grant.

Recent Progress

In 2010, scientists in NIAID's VRC discovered two potent human antibodies that can stop more than 90 percent of known global HIV strains from infecting human cells in the laboratory. It is hoped that these antibodies can be used to design improved HIV vaccines or can be further developed to prevent or treat HIV infection. The antibodies, known as VRC01 and VRC02, are naturally occurring and were found using a novel molecular approach that honed in on the specific cells that make antibodies against HIV. Both VRC01 and VRC02 were found to neutralize more HIV strains with greater overall strength than previously known antibodies to the virus. The atomic-level structure of VRC01 when attached to HIV also was determined, helping define precisely where and how the antibody attaches to the virus. With this knowledge, scientists have begun to design components of a candidate vaccine that could teach the human immune system to make antibodies similar to VRC01 and that might prevent infection by the vast majority of HIV strains worldwide [1, 2].

Basic research has led to a more thorough understanding of the earliest stages of HIV infection, including the "eclipse" phase, when HIV infection is becoming established but the virus is not yet detectable in the blood. CHAVI scientists also have examined "transmitter/founder" viruses by sequencing the genomes of viral particles in the plasma of 12 individuals prior to the emergence of HIV-specific immune responses. In 80 percent of heterosexual cases, they found that HIV infection stemmed from a single founder virus (range 1–6). In contrast, injection drug users were infected with a median of three viruses (range 1–16). Direct analysis of those viruses actually responsible for clinical infection may lead to important clues as to whether these viruses possess common features that could be effective targets for vaccine-induced immune responses [3, 4].

In another CHAVI study, uterine epithelial cells were identified as possible targets of HIV infection and transmission. Previously, the mechanisms of HIV transmission in the female reproductive tract were poorly understood. However, the likely exposure of these tissues to HIV is relevant to development of intervention strategies and may create a "window of vulnerability" that has not yet been systematically explored [5]. CHAVI scientists also characterized the critical role of the T-cell immune response in early virus control. Through analysis of host-immune responses to HIV infection, they showed that the first CD8+ T cells, despite limited breadth and very rapid virus escape, suppressed HIV as the amount of HIV in the blood was declining from a peak level. This implies that vaccine-induced HIV-specific T cells could contribute to the control of acute viremia (amount of HIV in the blood) if they are present before or early in HIV infection [6].

Preclinical Research

Discovery Strategies

Preclinical and clinical studies build on basic research findings and shed light on new and improved vaccine approaches. In addition to the B cell partnership program, which crosses from basic into discovery research, NIAID supports other preclinical initiatives, such as the HIV Vaccine Research and Design (HIVRAD) Program and Integrated Preclinical/Clinical AIDS Vaccine Development (IPCAVD) Program. These initiatives fund multidisciplinary research, including animal model development, immunogen structure, mechanism of vaccine action and vector development, and advanced-stage vaccine product development for investigators transitioning vaccines into human clinical studies. The HIVRAD and IPCAVD programs also foster and support public-private partnerships of scientists from industry and/or academia, to help advance promising vaccine concepts.

Through multiple contracts, NIAID also provides substantial resources for all phases of preclinical development and evaluation of candidate HIV vaccines, including *in vitro*

laboratory studies and *in vivo* testing in nonhuman primates. The Reagent Resource Support Program for AIDS Vaccine Development produces or purchases reagents needed for use in AIDS vaccine research, while the HIV Database and Analysis Unit compiles and analyzes data in several areas relevant to AIDS vaccine research. The unit encompasses the HIV Genetic Sequence Database, the HIV Molecular Immunology Database, and the Nonhuman Primate Vaccine Trials Database. Another important resource is the Preclinical Master Contract, which provides a complete spectrum of support for investigator-initiated vaccine development.

Recent Progress

The value of a T-cell-based HIV vaccine was brought into question after unexpected results from the Step Study (a Phase IIb proof of concept). The study's findings were announced in September 2007, when the trial was halted prematurely. This clinical trial enrolled individuals at high risk for HIV infection and evaluated a vector-based vaccine using recombinant adenovirus serotype 5 (rAd5), which is related to the virus that causes some forms of the common cold. The vector-based vaccine did not prevent HIV or significantly reduce set-point viral loads, or levels of infection, among study participants. However, research funded through the IPCAVD program recently demonstrated that an improved T-cell-based vaccine regimen using two distinct adenoviruses (rAd26 and rAd5) was able to substantially increase the protective efficacy, compared with an Ad5-based regimen in nonhuman primates. This improved regimen reduced viral set point and decreased AIDS-related mortality. The vaccine only expressed a single SIV antigen (Gag), suggesting that the partial immune control was mediated by a vaccine-elicited T-cell response (Gag-specific cellular immune response) rather than an antibody-based effect, since the vaccine lacked the SIV envelope protein [7].

Another important study found that a new HIV vaccination strategy using a "mosaic" design could expand the breadth and depth of immune responses in rhesus monkeys. The mosaic vaccine was designed through computational methods that created small sets of highly variable artificial viral proteins. When combined, these proteins theoretically could provide nearly optimal coverage of the diverse forms of HIV circulating in the world. In one NIAID-funded study, mosaic vaccines were embedded in specialized vectors designed to elicit strong T-cell responses. In rhesus monkeys, this vaccine resulted in a fourfold improvement in the monkeys' immune response, compared with previously tested vaccines, demonstrating that mosaic vaccines may improve the immune response against genetically diverse HIV–1 viruses [8–10].

VRC researchers also have developed a new "scaffold" strategy, which would teach the immune system to recognize certain protein structures on the viral surface and produce antibodies that bind to those structures and neutralize HIV. The technique involves extracting an epitope (an antibody-recognizable portion of the surface of a viral envelope protein) and placing the surface fragment into a different scaffold protein, which is intended to scaffold-lock the epitope in the shape recognized by the immune system. In theory, when a fixed epitope is introduced into an animal model (or eventually, a person), the immune system would recognize the envelope epitope and make antibodies against it. To test this scaffolding technique, VRC scientists applied it to an epitope on the surface of HIV that changes shape and is recognized by an HIV-neutralizing antibody known as 2F5. The epitope adopts a helical or spiral shape when removed from the surface of HIV, but the 2F5 antibody-recognizable version of it has an irregular, kinked shape. The scientists placed copies of the kinked epitope into scaffolds that locked it in that kinked form. Then the researchers injected these scaffold-bound epitopes into guinea pigs. In response, the animals' immune systems made antibodies very similar to 2F5 that bound tightly to the epitope. This study demonstrates that the engineering of protein scaffolds is a potentially useful approach in vaccine design. VRC researchers are continuing to refine this technique and apply it to the design of HIV vaccines, as well as vaccines for other infectious diseases [11].

Role of Nonhuman Primate Research

HIV vaccine testing in animal models continues to be an important step in evaluating the potential of vaccines. Nonhuman primate studies provide critical information regarding safety and potential efficacy, and help scientists understand how the body responds to infection. The hope is that, by examining the earliest events after mucosal infection (0–4 days) and the effects of vaccine interventions on those events, we will be able to learn more about how to prevent virus expansion beyond local mucosal tissue. Observing differences in these early interactions between animals that are successfully protected by vaccination and those that are not, and among different vaccine modalities, will provide valuable information for rational HIV vaccine design.

Although not ideal, nonhuman primates represent the best available surrogate model for research on AIDS pathogenesis

and vaccine development. Because HIV does not infect monkeys naturally, researchers conduct experiments with the closely related SIV. Combining parts of the HIV envelope and the inner core of SIV, researchers also have engineered chimeric simian-human immunodeficiency viruses (SHIVs) that mimic HIV infection and cause AIDS-like illness in macaque monkeys. Pathogenic chimeric SHIVs allow researchers to study the immune responses to the envelope-based HIV vaccines and the ability of these responses to stop or control the virus in a live model.

In addition to the HIVRAD and IPCAVD programs, NIAID carries out AIDS vaccine-related studies in the nonhuman primate model through the Simian Vaccine Evaluation Units (SVEUs). The SVEUs provide nonhuman 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. NIAID also supports three Non-human Primate Core Immunology and Virology Laboratories contracts to carry out immunological and virological assessment of animals under study.

Recent Progress

Using a novel strategy previously developed by NIAID-funded researchers to identify transmitted HIV genomes in acutely infected people, researchers have been able to determine the molecular features of SIV transmission in experimentally infected macaques. They demonstrated that repeated intrarectal exposure of rhesus macaques to low doses of SIV replicates many of the features of human HIV mucosal transmission, at both the biological and molecular levels. Because an HIV vaccine will need to stop HIV at or near the moment it is transmitted across a mucosal membrane or in the early period before infection, this gives researchers a more reliable model to use in testing new vaccines and other preventive modalities [12].

Other NIAID-funded SIV research has shown that challenging monkeys with a cytomegalovirus (CMV)-based SIV vaccine results in containment of virus. Typically, virus replication and dissemination occurs within days after infection, whereas vaccine-induced T cell activation and recruitment to sites of viral replication takes weeks. Researchers hypothesized that vaccines designed to maintain activated effector memory T cells might impair viral replication at its earliest stage. They developed an SIV gene-containing vector based on rhesus CMV (RhCMV), because natural RhCMV infection in monkeys induces lifelong effector memory T-cell responses. In fact, when this vaccine was used in monkeys, it stimulated robust and persistent T-cell responses against all five proteins (Gag, Rev, Tat, Nef, and Env) encoded by the SIV genes inserted into the vector. Furthermore, these responses were generated regardless of preexisting immunity to RhCMV. When a low-dose challenge with a pathogenic SIV was

HERPEVAC TRIAL FOR WOMEN CONCLUDES

Amanda Schleif, M.P.H., National Institute of Allergy and Infectious Diseases, National Institutes of Health

In September 2010, a large-scale genital herpes vaccine trial called the Herpevac Trial for Women drew to a close. Supported by GlaxoSmithKline (GSK) Biologicals and the National Institute of Allergy and Infectious Diseases (NIAID), the Phase III clinical trial enrolled more than 8,000 women aged 18 to 30 years at 50 sites across the United States and Canada. Ultimately, results showed that the experimental vaccine, while safe and generally well tolerated, did not prevent genital herpes.

Genital herpes is estimated to affect 1 in 4 women in the United States, causing painful lesions or sores in the genital area. The disease has no cure; the causative herpes simplex virus (HSV) stays in the body permanently, where it can reactivate and cause periodic outbreaks. Herpes can lead to an increased risk of contracting HIV/AIDS and also can cause other health complications. For example, a woman with herpes can pass the disease on to her newborn, putting the baby at risk of serious brain, skin, or eye problems.

In earlier studies, the experimental herpes vaccine was found to prevent genital herpes infection in more than 70 percent of the female study volunteers who had no history of prior herpes virus infection, but it had no clear effect in the men. These studies formed the basis for the Herpevac Trial.

Although initial analysis of the Herpevac Trial results showed that the primary endpoint, prevention of herpes disease, was not accomplished, the trial was successful in many respects. Over 8 years of research, significant enrollment numbers and successful participant follow-up resulted in a firm conclusion. Data continue to be evaluated at this time, but one outcome is already clear: the results from the Herpevac Trial for Women will be an invaluable source of information to guide future research toward a new, improved vaccine to prevent genital herpes.

repeated, the vaccinated rhesus macaques showed increased resistance to acquisition of progressive SIV [13].

Scientists also have used a new approach to demonstrate that long-lasting neutralizing antibodies can be delivered by gene transfer *in vivo* and can provide continuous protection against SIV challenge. With this approach, the genes for SIV-specific antibodies are packaged into an adeno-associated virus (AAV) and then delivered by intramuscular injection. After AAV enters cells, those genes are expressed and result in production of the neutralizing antibodies. Intramuscular injection of this vaccine resulted in sufficient antibody production to protect against SIV infection in some animals and could provide a long-term method of producing antibodies without relying on the adaptive immune system of the host [14].

NIAID HIV Vaccine Trials as of June 2011

121 Cumulative Trials Conducted
- 112 Phase I
- 6 Phase II
- 2 Phase IIb
- 1 Phase III

19 Ongoing Trials
- 16 Phase I
- 2 Phase II
- 1 Phase IIb

Clinical Research

Background and Vaccine Concepts

At present, NIAID-supported HIV vaccine clinical trials are conducted primarily through the HVTN, a global network of international scientists and researchers whose mission is to evaluate preventive vaccines against HIV/AIDS. The HVTN conducts all phases of clinical research and, with sites in the United States, Africa, Asia, South America, and the Caribbean, spans four continents. An operations center, statistical and data management center, and central laboratory complete the network.

To date (June 2011), NIAID has supported a total of 121 HIV vaccine trials involving 79 products, 19 adjuvants, and approximately 29,500 trial participants. These trials have involved a number of different strategies, including component or subunit vaccines (made with a structural piece of HIV, such as an envelope or a core protein), live vector vaccines (a live bacterium or virus that transports genes that make HIV proteins), peptide (small pieces of HIV proteins) or fusion protein vaccines (two proteins merged together), DNA vaccines (direct injection of HIV genes), and vaccine combinations, such as a 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 of a gp160 subunit candidate vaccine opened in 1987 at the NIH Clinical Center. The vaccine was tested in healthy, uninfected volunteers at low risk for HIV infection and 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 at high risk for infection due to injection drug use, multiple sex partners, or sexually transmitted infections. Although these early vaccine candidates, as well as many others designed against the HIV envelope proteins, stimulated production of antibodies, antibody levels decreased within a relatively short period of time and rarely elicited CTLs.

Early studies also demonstrated that protection against HIV may require cell-mediated immune response, which involves the activation of specific CD8+ T cells that target HIV-infected cells. To elicit CD8+ T-cell 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 vectors only carry a small part of HIV genetic material, they cannot cause HIV infection. Different types of viral vector vaccines have been evaluated or are being evaluated, including poxviruses (e.g., canarypox and modified vaccinia Ankara (MVA), which is a weakened vaccinia virus), alphavirus, and Ad5. The canarypox vaccine was the first candidate HIV vaccine shown to induce a CTL response against diverse HIV genetic subtypes.

Researchers also have been exploring other possible vaccines, including DNA vaccines (containing one or more HIV genes or potential adjuvants). Vaccination, usually intramuscularly, will cause 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. Subsequently, new technologies, such as codon-optimization and higher doses, were shown to enhance the performance of DNA vaccines.

In 1992, researchers turned their attention to a combination, or prime-boost, approach to improve the immunogenicity of HIV vaccines. Since then, prime-boost approaches have used combinations of DNA vaccines, viral vector vaccines, and subunit or peptide vaccines. Studies have shown the combination vaccine approach to be safe and immunogenic in volunteers at low and high risk for HIV infection, and that this approach can stimulate cellular immunity and the production of HIV-neutralizing antibodies.

Recent Progress

In late 2007, the HIV vaccine research field had disappointing news. The vaccine used in HVTN 502, also known as the Step Study, failed to prevent HIV infection and did not affect the level of viral load in those participants who were vaccinated but still became infected. More disturbingly, study participants—especially a subset of men who were uncircumcised and had naturally occurring neutralizing antibodies to Ad5 (the virus used to make the vaccine vector that delivered the HIV vaccine) at the time of enrollment—appeared to be at increased risk for infection. This study was terminated early as a result [15]. A related study, known as Phambili (HVTN 503), was evaluating the same adenovirus-based vaccine, and was suspended, as well.

The Step Study was testing Merck's vaccine candidate, the MRK Ad5 HIV–1 gag/pol/nef trivalent vaccine, based on a weakened adenovirus that had been altered to be rendered unable to replicate and infect humans. The study, involving 3,000 volunteers at high risk for acquiring HIV in regions with a high prevalence of HIV clade B, was designed to determine whether the vaccine either reduced HIV acquisition or lowered the viral set point in those volunteers who became infected.

Hoping to gain insight into the lack of efficacy, the HVTN laboratory program began evaluating HIV immune responses of Step Study volunteers who became infected during the study. Extensive analysis suggested that the immune responses induced by the vaccine put some early pressure on the virus, but did not have a significant impact on virus levels. A long-term follow-up study of participants, HVTN 504, was immediately launched to help researchers better understand the results; it evaluated the rate and risk of HIV infection among Step Study participants in the United States. Although there was an overall increased

CHLAMYDIA VACCINE BEING TESTED IN NONHUMAN PRIMATES

Harlan D. Caldwell, Ph.D. and Ken Pekoc
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The World Health Organization (WHO) estimates that more than 140 million people, mostly women and children in developing countries, are infected with the bacterium *Chlamydia trachomatis*, making chlamydia the most common bacterial disease in the world.

In the United States, chlamydia is perceived primarily as a "silent" disease that, despite no apparent symptoms in more than half of the infected population, can damage reproductive organs and cause infertility. Chlamydia is the leading reported sexually transmitted infection in the United States; in 2009, the Centers for Disease Control and Prevention reported approximately 1.2 million cases.

But chlamydia has an entirely different meaning in more than 50 developing countries, where infection is associated with the disease trachoma, which can cause blindness. WHO estimates that trachoma has left approximately 6 million people blind in Africa, the Middle East, Central and Southeast Asia, and Latin America. Trachoma causes the eyelid to fold inward and rub on the eyeball, abrading the corneal surface and resulting in impaired vision and blindness. Trachoma has been identified as one of the world's most neglected infectious diseases.

WHO hopes to eliminate blinding trachoma by 2020 through its SAFE strategy—Surgery, Antibiotics, Facial cleanliness, and Environmental change. Scientists at the National Institute of Allergy and Infectious Diseases (NIAID) are doing their part to complement this public health strategy by developing a vaccine to prevent trachoma.

The NIAID vaccine in development is designed to prevent infection from all 15 varieties of *C. trachomatis.* Researchers are testing the vaccine in nonhuman primates, following successful tests in cell culture and mouse models.

The focus of the vaccine is a protein antigen known as PmpD, or polymorphic membrane protein D, which was identified by NIAID's Harlan Caldwell, Ph.D. PmpD helps the bacteria infect host cells and suppress host immunity. Researchers are trying to learn whether a PmpD-based vaccine can generate multifunctional neutralizing antibodies capable of interfering with *C. trachomatis* infection and blocking the immunosuppressive effect of PmpD. One of the greatest challenges to fighting chlamydial infection, which the PmpD vaccine might solve, is that people do not develop a sustained protective immune response to the infection.

RV144 tested the "prime-boost" combination of two vaccines: ALVAC® HIV vaccine (the prime) and AIDSVAX® B/E vaccine (the boost). Courtesy of the U.S. Military HIV Research Program

risk of HIV among uncircumcised men, the higher rate of HIV acquisition was seen primarily during the initial vaccination phase of the trial, during the vaccination phase or the year thereafter (first 18 months), and then waned over time [16]. In another study, human leukocyte antigen (HLA) allele expression, which is known to influence progression of HIV disease and/or viral load set point, was significantly linked to viral load, although the effect did not appear to be mediated through increased breadth or magnitude of vaccine-induced responses; broader Gag responses may be associated with increased control of viral replication in Step Study vaccinees [17].

Following the early termination of the Step Study, plans to implement several other studies involving Ad5-based vaccines were put on hold or modified. One such vaccine being developed by the VRC consists of a multiclade recombinant Ad5-based component administered to boost immune responses induced by the prime DNA vaccine. A trial of this VRC vaccine regimen, HVTN 505, began as a small focused study with the primary goal of determining if the vaccine decreases viral load in study participants who later become infected with HIV. However, the trial was expanded in August 2011 so that it could also determine if the vaccine regimen prevents HIV infection. The results of RV144, discussed below, and a series of studies in nonhuman primates that showed that the VRC vaccine regimen prevented SIV infection 50 percent of the time in two-thirds of the monkeys tested, supported the expansion of HVTN 505. The study will now enroll a total of 2,200 participants and will evaluate if the VRC vaccine regimen is at least 50 percent effective in preventing HIV acquisition during the 18 months following immunization. As a safety precaution, participants must be circumcised and without Ad5 antibodies at the time they are enrolling. Although rAd5 is not likely to advance to licensure, this trial will generate useful information on the impact of the induced immune response on the virus and perhaps correlates for HIV vaccine protection.

NIAID is also supporting a number of other studies involving alternative adenovirus vectors, including a study of the VRC rAd5 combined with NYVAC (poxvirus vector) vaccine (HVTN 078), alternative lower seroprevalence rAd vectors (e.g., HVTN/IAVI study of an Ad26/Ad35 vaccine), and the VRC Ad5 vaccine with extensive mucosal assessment (HVTN 076). Several Phase I trials also are underway with the VRC Ad5 vaccine in collaboration with the HVTN to evaluate how delivery, timing, combinations, and host genetics influence the breadth and location of T-cell responses (HVTN 082, HVTN 083, HVTN 084, and HVTN 085).

Almost 2 years after the disappointing results of the Step Study, the field was infused with new optimism. Announced in September 2009, the Thai HIV Vaccine Trial, also known as RV144, showed that a candidate vaccine (based on a canarypox vector and gp120 protein) was 31 percent effective at preventing HIV infection. While the effect was modest, it was statistically significant. This was the first time an HIV vaccine had demonstrated an ability to prevent infection in people, and the trial thereby reinvigorated the field and gave us all a glimpse of what was possible [18]. (See page 126.)

The RV144 trial also provided the first opportunity to investigate immune correlates of vaccine efficacy in humans. Initial studies indicated that the antibody and T-cell responses were similar to those previously observed in studies using this regimen. Several RV144 working groups, which were established in the fall of 2009 and comprise various HIV vaccine stakeholder organizations and experts in the field, are working toward identifying the potential immune correlates of protection. NIAID also has established an HIV Mucosal

Immunology Group (MIG) program, which will share protocols for mucosal sample collection and assays to characterize and standardize the measurement of mucosal immune response across the field.

Future Directions

Discovery and Nonhuman Primate Research

While additional analysis of RV144 is expected to yield new information to increase scientists' understanding of how a highly effective HIV vaccine might work, it also will generate new questions. NIAID is positioned to answer the key questions with the efforts already underway and several new programs. One such program is the Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery, which will seek to identify an immunogen that induces durable, highly effective, broadly protective immune responses. The program will support a multidisciplinary team of researchers focused on a number of critical scientific questions that require a "big science" approach.

The Innovation for HIV Vaccine Discovery initiative, which is designed to address gaps in HIV vaccine discovery, will fund basic research on new target molecules or pathways needed for designing an effective HIV vaccine. The HIVRAD program will continue to be an important component of NIAID's discovery effort, supporting projects for research that have advanced beyond the exploratory stage and that further address hypotheses crucial to vaccine design.

If additional analysis and follow-up studies from RV144 identify correlates of immunity, researchers will be able to optimize candidates that are already in the pipeline. However, in the absence of known correlates of protection, researchers will continue to seek other candidate vaccines and stimulate potentially protective immune responses.

Research studies also are being planned to explore the use of other vectors with greater immunogenicity, better adjuvants, and the use of additional protein boosts. Some vectors that have provided interesting results will be further investigated; these include replicating vectors (CMV, in particular), as well as vectored antibodies, which insert broadly neutralizing antibodies into a vector. In addition, mosaic inserts, described earlier, have already been studied in animals and have shown some success in enhancing the breadth and depth of immune responses. CHAVI researchers are currently designing the first human trial of a mosaic HIV vaccine candidate.

In 2011–2012, NIAID will establish the Consortia for AIDS Vaccine in Nonhuman Primates, to better understand the viral and host events that occur at the earliest stages of mucosal infection and the ways these events can be blocked or modulated by immunization. In addition, this program could help increase our understanding of the viral and host factors responsible for the nonpathogenic nature of SIV infection in natural host species.

Clinical Research

In future Phase IIb efficacy trials, NIAID will consider using an adaptive trial design so that a clear signal of efficacy can be identified early on. This would allow for changes to the trial design before the trial's natural conclusion. Specific milestones and points of analysis would be defined prior to trial initiation, and changes in trial design, based on what is learned at given time points, would be prescribed in advance.

In the wake of RV144, additional studies are being planned that could help identify potential correlates of protection and ultimately improvements to this or subsequent vaccine regimens. Because data from RV144 indicated that protection against HIV was highest at 6 to 12 months after vaccination, two smaller studies are being planned (RV305 and RV306) that will add a secondary boost to try to extend and increase early immune responses. In addition, Phase IIb trials are being planned to determine whether the results of RV144 can be extended to other populations (e.g., higher risk individuals) and regions (e.g., with higher incidence, with different clades, and in which different routes of transmission are predominant). These Phase IIb trials will seek to improve on the initial design with additional boosts and a different pox virus and/or different adjuvants.

NIAID will continue to pursue these and other clinical trials in collaboration with its many partners, including funded researchers and research organizations, government agencies, foundations, industry, and the community. By combining scientific resources, we hope to build on exciting new advances, continue to deepen the understanding of HIV vaccine design, and accelerate the development of an effective and safe HIV vaccine that can be used worldwide.

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PROMISING HIV VACCINE TRIAL RESULTS: RV144, THE THAI HIV VACCINE TRIAL

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The field of HIV vaccine research was greatly encouraged when promising results of a preventive HIV vaccine trial in Thailand were announced in September 2009. This was the first time an investigational vaccine was shown to prevent HIV infection among some vaccinated individuals, giving the world great hope that a safe and effective HIV vaccine will one day become a reality.

Known as RV144 or the Thai HIV Vaccine Trial, this Phase III trial tested a prime-boost combination of two vaccine candidates (ALVAC–HIV and AIDSVAX B/E), which were based on the strains of HIV that commonly circulate in Thailand. The 6-year study, which began in 2003, was designed to test the vaccine regimen's safety and ability to prevent HIV infection, as well as its ability to reduce the amount of HIV circulating in the blood (the viral load) of those who became infected during the time they were participating in the study.

RV144 demonstrated that the vaccine was safe and that individuals who received the vaccine regimen were 31 percent less likely to contract HIV than those who received a placebo injection. Despite these encouraging results in preventing HIV infection, the vaccine regimen did not have an impact on viral load in those who became infected.

Scientists continue to examine the trial data to understand how the vaccine prevented HIV infections and determine whether the vaccine can be improved. The data are providing scientists with valuable insights that will guide the design and testing of future HIV vaccines.

RV144 was sponsored by the U.S. Military HIV Research Program and conducted jointly by the Thai Ministry of Public Health and U.S. Army. Specifically, the U.S. Army Medical Component of the Armed Forces Research Institute of Medical Sciences assisted with the conduct of the trial in Thailand.

Laboratories at the Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok, Thailand. Courtesy of the U.S. Military HIV Research Program

Major funding and other support were provided by the National Institute of Allergy and Infectious Diseases (NIAID), a component of the National Institutes of Health. The two vaccine products used in the trial were provided by Sanofi Pasteur (ALVAC–HIV) and Global Solutions for Infectious Disease (AIDSVAX B/E).

More than 16,000 non-infected men and women were enrolled in the study. Because the study was designed as a community-based trial, the volunteers were not selected based on HIV risk factors; they were mostly between 18 and 30 years of age and included individuals at both high and low risk of HIV infection. Approximately 40 percent of study participants were women.

Since the completion of RV144, trial collaborators and other experts in the field have been investigating what made this specific vaccine regimen work in some study participants. Ongoing studies hope to determine the specific types of immune responses responsible for protecting individuals from HIV infection. Identifying these "correlates of protection" would provide a critical measurement against which other vaccine products and approaches could be evaluated and optimized before taking them into large efficacy trials.

The knowledge gained from RV144 stands to benefit HIV prevention research efforts worldwide. NIAID will continue to work with its partners to develop and test potentially improved HIV vaccines.

For more information about RV144, please see:

MHRP: U.S. Military HIV Research Program [Internet]. Rockville (MD): The Program; c2011. RV144 Trial: Thai Phase III HIV Vaccine Trial; [cited 2011 Apr 28]; [about 3 screens]. Available from: www.hivresearch.org/research.php?ServiceID=13

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Influenza

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Introduction

Influenza remains among the leading causes of vaccine preventable morbidity and mortality worldwide, with annual epidemics occurring in all age groups. In the United States, pneumonia and influenza together are among the top 10 causes of mortality, and between 1976 and 2007, the number of reported deaths associated with seasonal influenza ranged from 3,349 to 48,614 [1]. The World Health Organization (WHO) uses available country-specific data to estimate that each year seasonal influenza epidemics cause 3 to 5 million cases of severe illness and 250,000 to 500,000 deaths globally [2].

Despite prior vaccination or infection, susceptibility to influenza infection persists. As the virus replicates, mutations arise in its two main surface proteins: the hemagglutinin (HA) and neuraminidase (NA). Over time, new "versions" of the viruses emerge because they have accumulated enough mutations to antigenically alter these proteins (referred to as "antigenic drift"), rendering the population susceptible to reinfection and prior year influenza vaccines ineffective. As a result, the virus strains that will be used to produce influenza vaccines must be reviewed annually to see how closely they match the evolving strains that are circulating around the world and whether needed vaccine strains are updated to match those expected to cause the next epidemic.

The type of antigenic variation that results in a pandemic ("antigenic shift") occurs when a new type A influenza virus is introduced into the human population and that virus is able to transmit efficiently from person to person. Wild aquatic birds, such as ducks and shore birds, are the natural hosts of influenza A viruses, and strains containing one of the 16 known types of HA and one of the 9 known NA types have been isolated from birds. Co-infection of animals or humans with different influenza viruses can result in an exchange of their genetic material known as "reassortment," creating new forms of the virus. Influenza viruses that infect animals can also directly infect humans. Antigenic shifts resulting in pandemics occurred in the 1950s and 1960s, when reassortment resulted in the introduction of genes from, respectively, influenza HA-type 2 (H2) and HA-type 3 (H3) avian influenza viruses into human influenza viruses, and in 2009, when an influenza virus containing a mixture of genes that tracked back to swine, birds, and human sources was circulating in swine and directly infected humans, causing the first influenza pandemic of the 21st century [3].

Flu Vaccines: First Steps to Today

Influenza vaccines are the primary means of preventing influenza disease and its related health complications. The first influenza vaccines were whole-virus vaccines produced by growing viruses in embryonated chicken eggs and inactivating them by chemical treatment. Clinical trials sponsored by the U.S. military conducted in the 1940s demonstrated that intramuscular administration of a dose of the inactivated virus was highly effective in preventing influenza illness in healthy young adults, provided there was a good match between the HA and NA proteins of the virus in the vaccine and those on the epidemic strain(s) [4]. Licenses were issued in 1945 to several companies in the United States for commercial production. Since the availability of eggs needed to manufacture influenza vaccines could be susceptible to an outbreak of avian influenza, there has been an investment by public and private sectors over the last decade to move to a cell culture-based manufacturing technology. Several companies have received regulatory approval in Europe using this approach, and in the United States, influenza vaccines produced in cell cultures are in late-stage clinical testing [5, 6].

In the United States, two types of influenza vaccines are Food and Drug Administration (FDA) approved to prevent seasonal influenza: trivalent inactivated vaccines (TIV) that are further purified into either split or subunit forms and administered via an intramuscular injection, and the live-attenuated influenza vaccine (LAIV), which is minimally purified and administered as a weakened form of the virus given as a nasal spray.

Over the last decade, several approved influenza vaccine manufacturers have left the U.S. market, and one of the National Institute of Allergy and Infectious Diseases' (NIAID's) efforts was to establish partnerships with the private sector to increase the availability of influenza vaccines. Through its clinical network of Vaccine and Treatment Evaluation Units (VTEUs),

NIAID has collaborated with the private sector to conduct clinical studies that helped support the approval of two new inactivated influenza vaccines in the United States [7, 8]. Additionally, much of the early stage research to support proof-of-concept studies on the intranasal LAIV (FluMist) was conducted by NIAID laboratories and in clinical trials supported by NIAID and the private sector. FluMist is currently approved to prevent influenza illness in healthy children and adolescents, aged 2 to 17 years, and healthy adults, aged 18 to 49 years.

3D graphical representation of a generic influenza virion's ultrastructure. A portion of the virion's outer protein coat has been cut away, which reveals the virus' contents. Courtesy of CDC

Research Aimed at Expanding Vaccine Options for Those at Greater Risk

For many years, the elderly were considered to be the population at greatest risk for health complications due to influenza, and pregnant women were identified to be at an increased risk during influenza pandemics. More recently, the substantial morbidity and mortality associated with influenza also has been recognized for very young children, individuals with underlying health conditions, and obese populations. As a result, in early 2010, the Centers for Disease Control and Prevention's (CDC's) Advisory Committee on Immunization Practices (ACIP) recommended annual influenza vaccination for all people 6 months of age and older for the upcoming influenza season unless the vaccine was contraindicated [9]. The ACIP noted that individuals may be unaware of whether they fall within a higher risk group and a "universal" recommendation sent a clear, more practical message [10].

A long-standing focus of the NIAID Influenza Program has been to better understand the breadth and duration of the immune response following influenza vaccination of "at-risk" populations and to identify strategies to improve vaccine effectiveness. Since early 2009, two studies with seasonal inactivated influenza vaccine and two studies with one or two doses of 2009 pandemic influenza vaccines have been initiated in pregnant women. To evaluate whether increasing the dosage of the vaccine also will increase the immune responses in the vulnerable age group of very young children, NIAID's VTEUs are currently conducting a study in which influenza vaccine-naive and fully primed 6- to 35-month-old children are being immunized with two doses of seasonal vaccine either at the currently recommended level (7.5 mcg of HA protein per strain) or at the standard adult dose (15 mcg of HA protein per strain). Results will compare data on safety and immunogenicity of the vaccines, and if the higher dosage results in higher antibody responses (which are thought to provide greater protection), they could support a recommendation that it be given routinely.

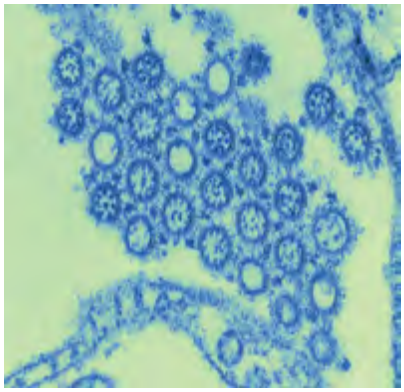
Collaboration With Industry on Developing a High-Dose Influenza Vaccine

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 has been reported to be as low as 30 to 40 percent. NIAID-supported clinical investigators have conducted several studies to assess the safety and immunogenicity of high-dose vaccines in elderly and immunocompromised populations [11]. These data helped support an FDA approval of a high-dose influenza vaccine for individuals 65 years of age and older [12]. With the availability of this approved higher dose seasonal influenza vaccine, additional studies are being planned to look for possible benefits in other at-risk populations, including immunocompromised individuals.

Pandemic Influenza

The Vaccine Response to the 2009 H1N1 Pandemic

By May 2009, a few weeks after the 2009 H1N1 virus had first been reported, the virus was identified in more than 30 countries [13]. An urgent public health priority was the production of sample lots of vaccine that could be evaluated for safety and immunogenicity in U.S. government- and industry-supported clinical trials. Because the 2009 H1N1 virus contained a novel HA protein, the dosage of the vaccine and the number of doses needed to elicit a robust immune response was unknown. Under contract to the U.S. Department of Health and Human Services' (HHS') Biomedical Advanced Research and Development Authority (BARDA), approved manufacturers rapidly produced vaccine for their own and for NIAID clinical trials. Through its VTEUs, NIAID initiated three clinical trials in adults on August 7, 2009, to evaluate the safety of the inactivated 2009 H1N1 vaccine given alone or in combination



A highly-magnified, digitally-colorized transmission electron micrograph (TEM) depicting virions from an H1N1 influenza isolate. Courtesy of CDC

with the 2009–2010 inactivated seasonal influenza vaccine and the ability of these vaccines to induce protective levels of antibodies. Following a review of the safety data from the ongoing adult study, NIAID initiated similar studies in children aged 6 months to 17 years less than 2 weeks later. Within several weeks, preliminary results from NIAID's studies and independent studies conducted by vaccine manufacturers confirmed that a single 15 mcg dose of the vaccine elicited a robust immune response in healthy adults and older children. The NIAID studies also showed that while one dose of the vaccine generated significant antibody responses in pregnant women, children 9 years old and younger would need two doses of the vaccine [14]. These data were used to help inform vaccination recommendations for the 2009 H1N1 vaccines, which were approved by the FDA in September and distributed the first week of October. In collaboration with BARDA and influenza vaccine manufacturers, NIAID's VTEUs also completed a clinical study evaluating an inactivated 2009 H1N1 vaccine made by one company mixed with an oil-in-water emulsion adjuvant produced by a different company. In addition to assessing the safety and immunogenicity of combining these two products just prior to administration, the feasibility and logistics of this "mix-and-match" approach may serve as a guide for future pandemic preparedness and response efforts.

H5N1 Influenza Vaccines

In 1997, the highly pathogenic avian influenza (HPAI) H5N1 strain infected humans in Hong Kong directly from infected poultry. During this outbreak, 18 people became infected, 6 of whom died. The virus was successfully controlled with the culling of approximately 1.5 million chickens. In 2003, H5N1 viruses reappeared with two cases in family members from Hong Kong who had recently traveled to China.

Since 2003, H5N1 influenza viruses have caused outbreaks in 51 countries and have become endemic in avian populations in several countries (e.g., Indonesia and Egypt), resulting in 566 known human cases and 332 fatalities, primarily among poultry workers or others in close contact with domestic birds. Deaths occurred due to pneumonia, severe acute respiratory distress, or organ failure [15]. These ongoing outbreaks continue to raise concerns of an increase in human exposure to H5N1 viruses. Clustering of H5N1 cases suggests that limited human-to-human transmission has occurred among persons with intense, close contact; however, it is not yet known whether sustained human-to-human transmission of these viruses could be acquired through mutation alone or would require reassortment with currently circulating epidemic strains.

The public health community is concerned that H5N1 viruses may emerge as the next pandemic strain because of the number of human infections that have occurred. Recent pandemic preparedness efforts by NIAID have focused in large part on the clinical evaluation of influenza vaccines made using different forms of the H5N1 virus that have infected people in Asia. WHO 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 (clade 1), A/Vietnam/1203/2004 (clade 1), A/Indonesia/5/2005 (clade 2.1), A/whooper swan/Mongolia/244/2005 (clade 2.2), A/bar-headed goose/Qinghai Lake/1A/2005 (clade 2.2), A/turkey/Turkey/1/2005 (clade 2.2.1), A/Anhui/1/2005 (clade 2.3.4), A/Egypt/1394-NAMRU4/2007-like (clade 2.2.1), A/goose/Guiyang/337/2006 (clade 4), and A/chicken/Vietnam/NCVD-016/2008 (clade 7).

In 1998, NIAID awarded a contract to Protein Sciences for the production of the first H5N1 vaccine, which was evaluated for safety and immunogenicity in a clinical trial conducted by the NIAID VTEUs [16]. In 2004, NIAID awarded contracts to Sanofi Pasteur and Chiron Corporation to support the production of vaccines against more recent forms of the virus for evaluation in adults, the elderly, and children. Over the last 6 years, NIAID has sponsored and/or supported, in collaboration with BARDA, more than 20 clinical trials to evaluate different dosage levels, routes of administration (intramuscular vs. intradermal), and studies with and without adjuvants. A series of studies also has been done showing that immunization with one H5N1 vaccine can prime for a more robust and broader cross-reactive antibody response following receipt with a second vaccine made from an antigenically distinct strain [17], as well as inactivated and live-attenuated H5N1 vaccines in a variety of populations, including healthy adults, the elderly, and children. One of the studies, a multicenter, double-blind

two-stage Phase I/II study using vaccine obtained under NIAID contract to Sanofi Pasteur, was conducted in healthy adults aged 18 to 64 years. The results from this trial were the basis of an FDA approval of the first H5N1 vaccine (two doses at 90 mcg vaccine for healthy adults) in 2007.

In 2005, NIAID announced a cooperative research and development agreement with MedImmune to produce and test LAIV for influenza A viruses with pandemic potential, beginning with vaccines for the highest priority HA subtypes, including H5. These vaccines are 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 of HPAI viruses will be modified to alter virulence determinants.

Both NIAID and MedImmune 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. Clinical trials were initially conducted at Johns Hopkins Bloomberg School of Public Health's Center for Immunization Research in Baltimore, and are now being conducted at the University of Rochester in Rochester, NY, to assess vaccine safety, infectivity, and immunogenicity. Clinical trials of H5N1, H6N1, H7N3, and H9N2 vaccines have been completed. The vaccines were safe and well tolerated but were variably immunogenic.

New Vaccine Strategies

Over the last decade, a variety of new technologies have facilitated the development of innovative approaches to influenza vaccine development. NIAID and HHS through BARDA continue to encourage and supported multiple efforts to develop "next-generation" influenza vaccines.

Innovative vaccine strategies that do not require replication of the influenza virus are also being developed. This includes purified protein vaccines produced by recombinant DNA technology. These vaccines comprise 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 formulations 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 is included in a plasmid or viral vector, which, once injected in a person, enters the cells of the host, where it produces limited amounts of the viral proteins that elicit a specific 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 a possible target. 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. Although it is still in early stages of investigation, M2e may be an additional immune stimulus to augment the immune response and increase protection. A different type of common epitope vaccine focuses on the stalk region of the HA molecule, which is highly conserved, though immunorecessive. On removal of the immunodominant globular head region of HA, NIAID-supported investigators have generated a "headless HA" vaccine candidate that was shown to generate antisera with broader reactivity than those obtained from mice immunized with full-length HA. The headless HA provided full protection against death and partial protection against disease following lethal challenge in mice [18].

Innovative vaccine technologies provide new options to develop vaccines rapidly in response to a newly emergent strain. If successful, such advances could further increase vaccine production capacity and enhance preparedness against seasonal influenza and potential pandemic influenza strains [19].

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NIAID CENTERS OF EXCELLENCE FOR INFLUENZA RESEARCH AND SURVEILLANCE

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The *National Institute of Allergy and Infectious Diseases* (NIAID) has a long history of supporting research activities to provide more effective approaches to controlling influenza virus infections. These activities include both basic and applied research on influenza basic biology and replication, pathogenesis, epidemiology, and clinical research to develop new and improved diagnostics, antiviral drugs, and vaccines. Due to the ever-present threat of an influenza pandemic, in 2007 NIAID established the Centers of Excellence for Influenza Research and Surveillance (CEIRS) to expand its worldwide influenza surveillance program and bolster influenza research in key areas, including understanding how the virus causes disease and how the immune system responds to infection with the virus. The goal of the CEIRS program is to provide essential information for the development of public health strategies crucial to both lessening the impact of seasonal influenza and responding to a pandemic.

Following the 2009 novel H1N1 influenza outbreak, the CEIRS sites quickly began work with the virus. The scientists used their infrastructure to provide essential information regarding the newly circulating virus. Some highlights of the CEIRS 2009 H1N1 research results include:

- First description of the origins and evolutionary genomics of the 2009 H1N1 virus [1].
- First description of the pathogenesis and transmission of the 2009 H1N1 virus in the ferret model [2].
- Detailed characterization of the 2009 H1N1 virus *in vitro* and *in vivo* and antiviral drug treatment after animal model infection with the virus [3].
- Description of the fitness of the 2009 H1N1 virus and the prediction that it would be the dominant influenza virus circulating for the upcoming influenza season [4].

From 2007 through 2011, CEIRS scientists published more than 450 peer-reviewed scientific journal articles and collected more than 475,000 influenza virus samples from multiple species—including wild birds, domestic poultry, swine, marine mammals, and humans. More than 17,000 influenza positive samples have been identified. In addition, more than 1,000 of these viral genomes have been fully sequenced and deposited in public databases. For more information, see www.niaid.nih.gov/research/resources/ceirs/.

Current activities of the CEIRS sites seek to expand the NIAID influenza virus surveillance program, both internationally and domestically, and to conduct research on such topics as the prevalence of avian influenza; how influenza viruses evolve, adapt, and are transmitted; and the immunological factors that determine whether an influenza virus causes only mild illness, severe illness, or death. Some sites will continually monitor international and domestic cases of animal and human influenza to rapidly detect and characterize viruses that may have pandemic potential and to generate pandemic vaccine candidates. The centers are laying the groundwork for new and improved control measures for emerging and re-emerging influenza viruses.

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Malaria

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Malaria, caused by the parasite *Plasmodium falciparum* and related species, remains a major public health threat, especially among children and pregnant women in Africa. More than 500 million cases of malaria occur annually among the world's poorest populations [1], and this disease claims the lives of nearly 1 million children each year in Africa alone [2]. An effective malaria vaccine would be a valuable tool to reduce the disease burden and could contribute to eliminating malaria from some regions of the world. Current malaria vaccine candidates are directed against human and mosquito stages of the parasite's life cycle. RTS,S is the most advanced vaccine candidate because it has consistently demonstrated partial protection against malaria in Phase II clinical trials and in an ongoing Phase III trial in Africa [3]. New vaccine targets are being identified to improve the chances of developing a highly effective malaria vaccine.

The *P. falciparum* life cycle in humans is classified by three stages: the pre-erythrocytic stage (liver stage) that initiates the infection, the asexual erythrocytic stage (blood stage) that causes disease, and the gametocyte stage that infects the mosquitoes that transmit the parasite. Optimism that a safe and effective malaria vaccine can be developed is based on the fact that natural *P. falciparum* infection induces clinical immunity. In areas of intense *P. falciparum* transmission, where individuals are infected by hundreds of mosquito bites each year, immunity to severe, life-threatening disease is usually acquired early in childhood, whereas immunity to mild disease is not typically acquired until late adolescence. However, even in adults who have had decades of exposure to *P. falciparum*, sterile immunity to infection rarely develops and an occasional episode of fever can occur [4]. Thus, the immunity ultimately acquired by adults confers protection against the disease caused by the blood stages of *P. falciparum*, the stage in the life cycle of the parasite that causes symptoms in humans, and not protection from infection per se. The hope is that knowledge of the immune mechanisms and their *P. falciparum* targets that ultimately provide protection from disease in adults can be used to develop a vaccine that would induce in a child a facsimile of adult immunity. Alternatively, by understanding the clinically silent stages that precede the blood stage infection (i.e., sporozoite and hepatocyte stages), vaccination might be possible to evoke protective immune responses that do not normally develop in natural infection—namely, responses that prevent the blood stage infection from occurring at all. Both broad approaches to vaccine development are being taken [5, 6]. Compounding the difficulty of the vaccine effort are the large gaps in understanding *P. falciparum* infection biology, including how *P. falciparum* invades its target cells and causes disease.

Biologist checks culture volume in a fermenter growing *Pichia pastoris* yeast. This culture medium expresses a malaria antigen that the lab is evaluating for possible vaccine development. Courtesy of NIAID

Pre-Erythrocytic Stage Vaccines

The most advanced vaccine in development, RTS,S, consists of a recombinant protein expressed at the pre-erythrocytic stage that covers the parasite's surface—the circumsporozoite (CS) protein [7]. The idea of a pre-erythrocytic vaccine took shape with the seminal observation by Ruth Nussenzweig that vaccination of mice with irradiated sporozoites resulted in protection [8] and that protection could be achieved by immunization with the CS protein alone [9]. Development of pre-erythrocytic vaccines began with cloning of the *P. falciparum* CS protein [10] and collaboration in 1985 between the Walter Reed Army Institute of Research and industry partners. This research led to the development of the RTS,S vaccine. RTS,S consists of hepatitis B surface antigen (HBsAg) particles fused to the CS protein and formulated with the adjuvant AS01 [7, 11]. In a series of Phase II clinical trials, 30–50 percent of malaria-naive adults immunized with RTS,S were protected against challenge by mosquitoes that were infected with the

homologous *P. falciparum* clone [11–16]. For this vaccine, protection correlated with CS-specific antibody and CD4+ T-cell responses [16], but re-analysis of the data suggests that the contribution of T-cell immunity to protection may be minimal [17]. In Phase II field trials in The Gambia [18] and Kenya [19], RTS,S conferred short-lived protection against malaria infection in approximately 35 percent of adults, but the results from the trial in Kenya did not reach statistical significance. Among children and infants who were immunized with RTS,S in Phase II trials conducted in Mozambique, Tanzania, and Kenya, approximately 30–50 percent were protected from clinical malaria [20–24], but protection was generally short-lived. In field trials, immunization with RTS,S induced antibodies that correlated with protection from *P. falciparum* infection [25, 26] but not clinical disease [20, 24, 25, 27].

The RTS,S vaccine entered Phase III clinical trials in 2009. Based on results from Phase II trials, RTS,S is likely to provide only partial protection. However, precluding any unpredictable adverse effects, the vaccine could benefit millions of children by substantially reducing malaria morbidity and mortality. Initial results of the Phase III trial indicate that the RTS,S vaccine reduces episodes of clinical malaria by half in children aged 5–17 months over the first year of follow-up. Efficacy and safety results in 6- to 12-week-old infants, and longer term protectivie effects of the vaccine, are expected by the end of 2014 [3]. Efforts to improve the efficacy of CS protein–based vaccines with alternative adjuvants [28] or viral vectors [29, 30] have been unsuccessful to date, but several studies are still ongoing. Preclinical research efforts are focusing on inducing higher levels of CS protein–specific antibody [31]. In one study, the CS repeat peptide conjugated to the mosquito stage ookinete surface protein Pfs25 induced high levels of uncommonly long-lasting antibodies to both vaccine components in mice [31]. In principle, this vaccine strategy could confer protection against liver infection and block transmission by the mosquito vector.

THE INTERNATIONAL CENTERS OF EXCELLENCE FOR MALARIA RESEARCH

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A major resurgence of interest in and funding for malaria research, control efforts, and new product development has occurred during the last decade. Several successes have emerged from these investments, ranging from sequencing of the genomes of *Plasmodium falciparum, Plasmodium vivax,* and *Anopheles gambiae*, to more applied areas such as improved drugs, diagnostics, and insecticides, as well as to public health interventions such as widespread use of long-lasting insecticide-treated bed-nets and highly effective artemisinin combination therapies. According to the World Health Organization's *World Malaria Report 2010,* many malaria-endemic countries are presently experiencing a decrease in the incidence of malaria after years of increase or stagnation. Despite these recent gains, basic epidemiological information about the "malaria reality" on the ground in several endemic countries is still lacking.

In 2010, the National Institute of Allergy and Infectious Diseases, a component of the National Institutes of Health, established 10 International Centers of Excellence for Malaria Research (ICEMRs) to address some of the malaria research gaps that currently exist in global endemic settings, including parts of Africa, the Pacific Islands, and Latin America.

Renewed involvement and commitment by research institutions, control programs, governments, and funding agencies has resulted in a rapid scale-up of access to malaria control measures, which in turn are changing the landscape of malaria. With centers located in every malaria-endemic region of the world, the ICEMRs are uniquely positioned to capture this shifting epidemiology in real time across the globe, and these data will inform future malaria control and elimination programs.

Several features of the ICEMRs distinguish them from other initiatives. Most observational studies in malaria are restricted to a single field site with a relatively homogeneous population. In contrast, each ICEMR has multiple field areas, which are thought to be distinct with respect to disease transmission and burden. It is anticipated that data gathered from these heterogeneous sites, using a common study design, may provide an opportunity to generalize the findings beyond the study areas. All centers are adopting a multidisciplinary approach to study the complex interactions between the human host, the malaria parasite, the vector, and the ecology at the molecular, cellular, organismic, population, and field levels. It is expected that such studies will provide the knowledge base necessary for improved clinical and field management of malaria, as well as guide the development of new tools and interventions.

Efforts also are ongoing to develop vaccines that induce T-cell immunity to the pre-erythrocytic stage through either irradiated [32] or genetically attenuated [33] sporozoites, or through expression of *P. falciparum* liver stage proteins in viral vectors [34]. The irradiated sporozoite strategy is based on an observation that the bites of irradiated infected mosquitoes protected humans from challenge with infected mosquitoes that were not irradiated [35], suggesting that irradiated sporozoites in humans could be an effective vaccine—just as effective as they were first shown in mice [8]. This approach is not practical because protection required the bites of more than 1,000 infected, irradiated mosquitoes [36]. As an alternative, manufacturing processes have been developed to purify and cryopreserve irradiated sporozoites from aseptic mosquitoes in the quantities necessary for vaccination [32]. In the first clinical trial, the irradiated, purified, and cryopreserved sporozoite vaccine was safe and well-tolerated but only modestly immunogenic and protected only a few individuals. The next clinical trial will attempt to improve efficacy by optimizing the route of administration [37]. Studies are also in progress to determine whether sporozoites can be attenuated for use as vaccines by methods other than irradiation [33, 38]. A Phase II trial is underway to test this strategy in humans.

In mouse models of malaria, immunization with irradiated sporozoites induces CD8+ T cells that kill parasite-infected hepatocytes. The known targets of CD8+ T-cell killing, in addition to CS protein, include thrombospondin-related anonymous protein (TRAP) and liver stage antigen (LSA). In *P. falciparum*–naive adults, immunization with viral vectors containing TRAP peptides led to partial protection from challenge by infected mosquitoes through mechanisms that involved the induction of large numbers of TRAP-specific interferon gamma (IFNγ)-producing T cells [39]. Disappointingly, this vaccine did not induce protection in children in Africa [40]. For unknown reasons, the level of TRAP-specific INFγ-producing T cells was considerably lower in vaccinated African children compared with that in *P. falciparum*–naive adults [39, 40]. Efforts are ongoing to improve the T-cell immunogenicity of TRAP with simian adenovirus vectors [34].

Asexual Erythrocytic Stage Vaccines

The asexual blood stage of the parasite's life cycle begins with the release of merozoites into the bloodstream from ruptured infected hepatocytes. The blood stage is the only stage in the parasite's life cycle that causes disease [41]. Because immunity to disease develops with repeated *P. falciparum* infections, the acquisition of naturally acquired immunity by a vaccine may be able to be mimicked and accelerated. One key component of blood stage immunity is antibodies. This was demonstrated by experiments in which the transfer of immunoglobulin G from immune, adult Africans to partially immune African [42] or Thai [43] children rapidly reduced parasitemia and fever. These experiments suggest that a vaccine could theoretically be developed that would elicit in children the antibodies that protect against disease in adults. At present, the specificity of antibodies that confer protection against malaria is not fully characterized, and the precise mechanisms of antibody-mediated protection are unknown.

Several blood stage antigens are in clinical development as vaccines:

- Apical membrane antigen 1 (AMA1) [44]
- Erythrocyte binding antigen-175 (EBA–175) [45]
- Glutamate-rich protein (GLURP) [46, 47]
- Merozoite surface protein 1 (MSP1) [48]
- Merozoite surface protein 2 (MSP2) [49]
- Merozoite surface protein 3 (MSP3) [46, 50–52]
- Serine-rich antigen 5 (SERA5) [52]

All of these antigens are highly expressed on the surface of the merozoite. Unfortunately, recent Phase II trials of the most advanced blood-stage candidates, AMA1 and MSP1, did not demonstrate efficacy in African children [44, 48]. Efforts are ongoing to enhance the vaccine efficacy of AMA1 and MSP1 with novel adjuvants [54, 55] or viral-vectored prime-boost strategies [34] or by combining AMA1 and MSP1 [56]. However, extensive parasite genetic diversity, due to the selective pressure exerted by the human immune response, presents a major hurdle for the development of blood stage vaccines [57, 58]. For example, the AMA1 antigen is highly polymorphic, with hundreds of haplotypes that affect the ability of antibodies specific for one haplotype to block invasion by other haplotypes [59]. Unless strategies are developed to overcome such genetic diversity, highly polymorphic *P. falciparum* antigens, such as AMA1, are unlikely to be useful [57, 59].

Combining Pre-Erythrocytic and Erythrocytic Stage Vaccines

The World Health Organization's guidelines for measuring the efficacy of malaria vaccines in Phase III clinical trials recommend defining the primary endpoint to the time of the

first clinical malaria episode [60]. By these criteria, the RTS,S vaccine has demonstrated 30–50 percent efficacy in Phase II trials [27]. Preliminary data from an ongoing Phase III trial are consistent with these results [3]. However, an important unanswered question remains: How does partial pre-erythrocytic immunity influence the time to onset of clinical malaria, which occurs during the erythrocytic stage? One possibility is that a partially effective pre-erythrocytic vaccine reduces the number of infected hepatocytes, thus decreasing the number of merozoites that are released into the bloodstream and allowing more time for blood stage immunity to develop before the fever threshold is reached. If so, combining *P. falciparum* antigens that target the pre-erythrocytic and blood stages may further decrease the probability of reaching the disease threshold. This eventuality provides the rationale for several multistage vaccine candidates that are currently being evaluated in clinical trials.

Transmission-Blocking Vaccines

Transmission-blocking malaria vaccine candidates target antigens on gametes, zygotes, or ookinetes in the mosquito midgut. Antibodies induced in the human blood by these vaccine candidates and ingested with the parasite can block the parasite's life cycle development in the mosquito [61]. These vaccines could be important tools to eliminate malaria and protect against epidemics if *P. falciparum* parasites are reintroduced after a period of elimination. A transmission-blocking malaria vaccine would not confer protection to the vaccinated individual unless it is combined with an effective pre-erythrocytic [31] or erythrocytic vaccine.

P. falciparum proteins, such as Pfs25, that are expressed only in the mosquito are not polymorphic because they are not under adaptive immune pressure in the human host [62]. Gamete proteins, such as Pfs48/45 and Pfs230, which are expressed in the human host, are more polymorphic than Pfs25, but still have conserved domains that are present in all parasite clones studied to date [63]. Pfs230 has the additional advantage of being the target of antibody-dependent complement lysis [64]. In a mouse model, antibodies to HAP2, a *Plasmodium* protein thought to be involved in the fusion of male and female gametes in the mosquito midgut [65], also have transmission-blocking activity *in vivo* and *in vitro* [66].

Current evidence suggests that the levels of antibodies in blood that would be required to significantly affect parasite development in the mosquito may need to be extremely high [67]. Conjugation of Pfs25 to a carrier, such as outer membrane protein complex (OMPC) of *Neisseria meningitidis* serogroup B, may overcome this problem, because the conjugate induces high titer antibodies in rhesus monkeys that persist for at least 2 years [68]. Preclinical and clinical development of transmission-blocking vaccines is underway because of their promise for malaria elimination.

Conclusion

Malaria is a complex parasitic disease that imposes an enormous disease burden, and for which a vaccine is not currently available. Optimism that a vaccine can be developed comes from observations that malaria immunity can be acquired through natural and experimental infection. However, many *P. falciparum* proteins are highly polymorphic and their biological functions are redundant, resulting in significant challenges to vaccine design. Nevertheless, by recruiting experts in all aspects of *P. falciparum* infection biology and immunity to work on this problem, the development of a highly effective malaria vaccine may be possible.

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Respiratory Syncytial Virus

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Respiratory syncytial virus (RSV) is the single most important cause of severe lower respiratory tract infection in infants and young children. RSV disease also affects the elderly and the immunocompromised. It is a frequent cause of winter outbreaks of acute respiratory disease. RSV infects repeatedly and causes disease throughout life, including a wide array of respiratory symptoms, from rhinitis and otitis media to pneumonia and bronchiolitis—of which the latter two have significant morbidity and mortality. In the United States, 3.5–4 million children younger than 4 years of age acquire RSV infection annually. Among infants less than 1 year of age, RSV accounts for an estimated 75,000–125,000 hospitalizations annually. RSV infects nearly all children by 2 years of age, and re-infections occur later during childhood and adulthood that are generally associated with milder disease. Recent evidence points to a link between RSV infection and the development of wheezing and asthma [1].

Recently, RSV has been recognized as a significant cause of severe respiratory infections in older populations. Among the elderly in the United States, RSV accounts for an estimated 14,000–62,000 hospitalizations annually. Outbreaks of RSV are complicated with pneumonia among elderly patients in nursing homes and hospitals. Each year, RSV affects 5–10 percent of nursing home populations. Two to 8 percent of these cases are fatal, amounting to approximately 10,000 deaths per year

IMPACT OF REGULATORY SCIENCE ON INFLUENZA VACCINE DEVELOPMENT

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The Food and Drug Administration (FDA) pursues and promotes advances in regulatory science—the science of developing new tools, standards, and approaches to assess the safety, efficacy, quality, and performance of FDA-regulated products. The agency's Center for Biologics Evaluation and Research (CBER) regulates complex and diverse products, including vaccines intended to protect against both seasonal and pandemic influenza.

As part of its efforts to advance regulatory science, CBER plays a pivotal role in the development of tests that ensure the potency of seasonal and pandemic strain–specific influenza vaccines. Antibodies against the hemagglutinin (HA) protein from the influenza virus strain(s) that will be included in the vaccine are essential to testing the potency of the vaccine. CBER scientists typically remove the HA protein from influenza viruses using a standard chemical technique; these proteins are injected into sheep, whose immune systems make anti-HA antibodies. CBER collects the sheep sera containing these antibodies and supplies the sera for use in potency tests for influenza vaccines.

Although this approach to developing anti-HA antibodies is typically effective, there have been instances in which the peculiar characteristics of some strains of influenza virus make it difficult to obtain sufficient amounts of HA protein. Therefore, CBER developed an alternative approach that does not require the presence or purification of influenza virus or removal of HA protein. Instead, the center uses recombinant DNA techniques to produce plasmid DNA coding for HA protein and injects the plasmid into sheep. The HA protein expressed *in vivo* from this DNA triggers development of antibodies against the specific HA protein. CBER scientists then inject into the sheep genetically engineered viral vectors that produce HA protein to boost antibody production. These sheep anti-HA antibodies have worked effectively in tests designed to evaluate commercially produced H1N1 and H5N1 vaccines.

This work demonstrates the feasibility of an alternative approach to producing potency reagents [1] and provides an effective backup technique for anti-HA antibody production when the standard technique does not work well or fast enough to produce potency antibodies for a novel influenza virus. It is an example of the critical role CBER research plays in ensuring the safety, purity, potency, and effectiveness of biological products through regulatory science.

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from RSV among persons older than 64 years of age. Among elderly persons followed for three consecutive winters, RSV infection accounted for 11.4 percent of hospitalizations for obstructive pulmonary disease, 10.6 percent of hospitalizations for pneumonia, 7.2 percent of hospitalizations for asthma, and 5.4 percent of hospitalizations for congestive heart failure [2]. Severe RSV infections are also a problem in immunocompromised persons of any age, especially transplant recipients.

An effective vaccine to prevent RSV could be useful in reducing morbidity, the frequency of hospitalizations, and the death rate from this infection. Although the development of a vaccine has been a priority of NIAID, a licensed vaccine is not yet available because of several challenges. The most significant obstacle is the unexpected enhancement of disease post-vaccination (i.e., increased severity of infection when vaccinated children were exposed to natural RSV infection). In a study conducted in the 1960s, immunized children who were seronegative for RSV before vaccination and were subsequently exposed naturally to RSV experienced enhanced disease. This included a significant increase in the frequency and severity of RSV lower respiratory tract diseases (bronchoconstriction and pneumonia) and greater incidence of hospitalization, compared with children in the control group who were not vaccinated [3]. Scientists are studying possible mechanisms responsible for this enhanced disease following vaccination.

To develop an effective vaccine, a more complete understanding of the protective and disease-enhancing immune responses to RSV is imperative. Research efforts have focused on the individual components of these responses, including cell-mediated events and production of serum and secretory antibodies. Vaccine candidates under development are evaluated in a stepwise progression: first in animal models, next in adults, then in children—those who have already been exposed to infections (seropositive individuals), older nonimmune or seronegative children, and younger seronegative and highly susceptible infants.

RSV includes two subgroups: A and B. A successful vaccine would induce resistance to both of these subgroups. The major protective antigens of RSV are the fusion (F) and attachment (G) glycoproteins found on the surface of RSV. These proteins induce neutralizing antibodies that protect against wild-type RSV infection. The F surface protein is highly conserved among the RSV subgroups and functions to promote fusion of the virus and host-cell membranes. The structure of the G surface protein is the major difference between RSV subgroups A and B. The G protein is responsible for attaching RSV to a susceptible cell. Despite 47 percent amino acid sequence diversity between the G proteins in RSV subgroups A and B, the G protein contains a central conserved domain that is flanked by two hypervariable regions.

Subunit RSV Vaccine Candidates

Several potential vaccine candidates contain purified F protein (PFP). PFP–1 and PFP–2 are subunit vaccines that were tested in various populations in Phases I and II human clinical trials. In studies with 12- to 48-month-old RSV seropositive children, PFP–1 and PFP–2 have been shown to be safe and immunogenic. These studies were not designed to evaluate the efficacy of the vaccine (i.e., whether recipients are actually protected from RSV infection) [4].

Subunit vaccines may be particularly useful in specific groups of high-risk children and adults. In a pilot study of children with cystic fibrosis, the PFP–2 vaccine induced a significant antibody response and a significant reduction in the number of lower respiratory tract illnesses [5]. Other studies have demonstrated that the PFP–2 vaccine is safe and immunogenic in ambulatory adults older than 60 years of age and in seropositive children who have bronchopulmonary dysplasia [6, 7].

A Phase II, double-blind, controlled, multicenter study of the safety, immunogenicity, and effectiveness of the PFP–3 subunit vaccine was conducted in RSV seropositive children with cystic fibrosis. The study found that the PFP–3 subunit vaccine is safe and immunogenic; however, the study did not demonstrate a reduction in the incidence of lower respiratory tract illnesses [8].

Maternal immunization with a PFP subunit vaccine is a potential strategy being evaluated to protect infants younger than 6 months old from RSV disease. The rationale is based on (1) reports of the efficient transfer of specific neutralizing antibodies from mothers to infants during pregnancy and (2) demonstration of the possible prophylactic value of high-titer anti-RSV polyclonal antiserum or humanized monoclonal antibody (MAb) that is administered to high-risk children to protect against lower respiratory tract RSV disease and hospitalization [9]. Infants younger than 6 months old are most at risk for RSV infection, but usually least responsive to vaccines. Thus, maternal immunization may be beneficial because pregnant women respond well immunologically to vaccines and placental transfer of maternal antibodies occurs naturally during the third trimester. A Phase I, double-blind, placebo-controlled study was conducted with 35 healthy women who were in their third trimester of pregnancy. The

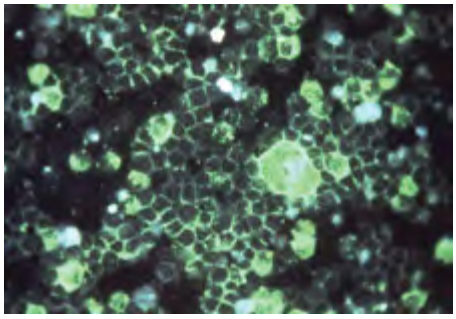
PFP–2 vaccine was found to be safe and immunogenic. Transplacental transfer of maternal neutralizing antibodies to RSV was efficient. Infants born to vaccine recipients were healthy and did not experience adverse events related to maternal immunization [9].

The G protein fragment of RSV is the basis of another subunit vaccine currently being developed. A novel recombinant vaccine candidate, BBG2Na, has been constructed by fusing the conserved central domain of the G protein (G2Na) of RSV Long strain to BB (the albumin-binding region of streptococcal G protein). A clinical trial was conducted in 108 healthy adults. The BBG2Na vaccine was found to be safe, well-tolerated, and immunogenic [10].

A subunit RSV vaccine consisting of F, G, and M proteins also is being developed. Little is known about the function of the M protein (Matrix protein), but some data suggest that the M protein is associated with RSV nucleocapsids and, like the matrix proteins of other negative-strand RNA viruses, can inhibit virus transcription. The primary target of this vaccine is to prevent significant respiratory disease in study populations primed by previous natural RSV infection. Two Phase I clinical trials have been conducted in healthy adults. These trials support the safety and immunogenicity of this product. The first trial compared an aluminum phosphate formulation of the vaccine (n=30) with aluminum phosphate control (n=10). The second trial compared the aluminum phosphate formulation (n=10) with a formulation containing a new adjuvant—poly[di(carboxylatophenoxy)phosphazene]—in a different sample of young, healthy adults (n=30). Both vaccines were found to be well-tolerated and immunogenic [11]. Additional studies of the F/G/M protein vaccine are being conducted.

Several other subunit vaccines are in preclinical development:

- Recombinant chimeric RSV FG glycoprotein vaccines adsorbed onto aluminum hydroxide gel with or without the addition of 3-deacylated monophosphoryl lipid A
- PFP formulated with alum with or without G protein (from subgroups A and B)
- Synthetic peptide of the conserved region of the G protein with or without cholera toxin as a mucosal adjuvant
- Recombinant fragment (BBG2Na) of the G protein formulated with dimethyldioctadecylammonium bromide, a nasal adjuvant
- Recombinant fragment of the G protein in a liposome encapsulated formulation, prepared by including a variety of different lipids



Photomicrographic detection of respiratory syncytial virus (RSV) using indirect immunofluorescence technique. Courtesy of CDC

- Mimotope (peptide that mimics antigenicity) of a conserved and conformationally determined epitope of the F protein recognized by an anti-RSV MAb (MAb19) that neutralizes RSV
- Recombinant RSV F virus-like particles
- Recombinant RSV F and G proteins using Newcastle disease virus-like particles
- Recombinant F and G proteins using Sendai virus as a vector

Live Attenuated RSV Vaccine Candidates

NIAID laboratories are actively pursuing the development of a live attenuated RSV vaccine that is administered intranasally. Live attenuated vaccines appear to offer several advantages over nonreplicating or subunit vaccines, especially for RSV-naive infants and young children. Intranasal immunization with a live attenuated vaccine induces both systemic and local immunity and therefore may protect against upper as well as lower respiratory disease. Also, the immune response to a live vaccine more closely resembles the response to natural infection and therefore is less likely to produce enhanced disease on exposure to natural infection. In addition, like other live attenuated intranasal respiratory virus vaccine candidates, live intranasal RSV vaccine candidates have been shown to replicate in young infants in the presence of maternally acquired antibodies.

Early attempts at developing live attenuated RSV strains included conventional methods of attenuation by cold passage (cp), cold adaptation, chemical mutagenesis, temperature-sensitive (ts) selection, and combinations of these methods. These efforts resulted in several vaccine candidates that appeared to be substantially attenuated in experimental animals. These candidates were then evaluated in Phase I clinical studies, which involve a stepwise progression from adults to seropositive children to seronegative children to RSV-naive infants. These viruses proved to be insufficiently attenuated. The most promising candidate was a cold-passaged,

temperature-sensitive mutant called cpts248/404, which was well-tolerated and immunogenic in seronegative children older than 6 months of age. However, cpts248/404 was associated with mild-to-moderate upper respiratory congestion when administered to 1- to 2-month-old infants, indicating that more attenuation was needed [12].

To construct more-attenuated vaccine candidates, the technology of reverse genetics was employed, whereby complete infectious RSV is recovered from cDNA. This provides the means to insert predetermined mutations into infectious viruses via the cDNA intermediate. This technique was coupled with sequence analysis to determine the basis of attenuation in the incompletely attenuated, biologically derived viruses noted above. This resulted in identification of the mutations involved in the attenuated cp phenotype and of six independent ts-attenuating mutations. In addition, four accessory viral genes were identified (SH, NS1, NS2, and M2–2) that are nonessential in cell culture but are attenuating *in vivo;* thus, deleting these genes provides another means of attenuation. With this information, a series of further-attenuated, cDNA-derived viruses were constructed. In particular, a recombinant version of cpts248/404 (the mutant described above) was further attenuated by deleting the SH gene and including yet another attenuating mutation, yielding a virus called cp248/404/1030ΔSH. When evaluated in 4- to 12-week-old infants, this virus was well-tolerated and immunogenic [13]. Additional studies are needed to determine whether cp248/404/1030ΔSH can induce protective immunity against wild-type RSV.

Other candidates are being prepared for clinical studies. Deleting the M2–2 coding sequence resulted in a virus that is reduced one-thousandfold for replication in experimental animals and has the unusual phenotype of decreased RNA replication and increased gene transcription and antigen expression. Another candidate that is presently being prepared for clinical evaluation involves deleting the NS1 gene, which was shown to strongly suppress the induction of type I interferon. Both the delM2–2 and delNS1 viruses may have increased immunogenicity due to, respectively, increased antigen expression and the adjuvant effect of increased interferon expression. Additional candidates involving combinations of gene deletions and point mutations designed to increase genetic stability also are being developed. The vaccine candidates to date represent RSV antigenic subgroup A; a subgroup B component also will likely be included in an RSV vaccine, which can be readily achieved using the same attenuating mutations that have been identified for subgroup A.

Another strategy is to express the RSV F and G protective antigens from genes added to a live human parainfluenza virus type 3 (HPIV3) vaccine as vector. HPIV3 is a particularly apt choice, because immunization against both RSV and HPIV3 ideally should begin early in infancy. Presently, lead constructs have been developed based on an attenuated PIV3 consisting of bovine PIV3 in which the F and HN genes have been replaced by those of HPIV3, thus combining the host-range attenuation of bovine PIV3 with the major protective antigen genes from HPIV3. A construct in which the RSV F protein is expressed from an added gene between the N and P genes of the PIV3 vector is currently in Phase I clinical trials. On one hand, this approach combines two necessary vaccines into a single recombinant virus and, being based on PIV3, avoids the poor growth and physical instability of RSV. But on the other hand, the construct lacks most of the RSV antigens. Combining a PIV-vectored RSV vaccine with an attenuated RSV strain may be the best way to increase the potency of immunization against RSV while including a PIV3 component.

The live attenuated approach was evaluated in healthy young adults, showing that these viruses are highly restricted and over-attenuated in RSV-experienced individuals [14]. The live attenuated approach will likely not be useful in adults because a virus that replicates well in RSV-experienced individuals likely will retain residual virulence for RSV-naive contacts. However, RSV subunit vaccines have been shown to be well-tolerated and safe in RSV-experienced individuals, which is consistent with the observation that, to date, disease enhancement has been observed in only RSV-naive individuals [3]. The immunogenicity of previous formulations of RSV subunit vaccines was disappointing, but several commercial companies are developing improved versions. An RSV subunit vaccine could be combined with the inactivated influenza vaccine for yearly immunization. Maternal immunization with an inactivated vaccine represents another possible approach to increasing the resistance of young infants to severe RSV disease.

Future Directions

Ideally, immunization for RSV should begin during the first 2 months of life. However, developing a vaccine for RSV is challenging because this is a time when immune responses are reduced due to immunologic immaturity and the presence of maternal antibodies. Safety concerns also are paramount during this time. In addition, RSV infects and causes disease

at the lung mucosal surface, where immune protection is less complete. However, the recent success of the live attenuated, topically administered rotavirus vaccine indicates that substantial reduction in severe disease from a mucosal pathogen can be achieved in infancy [15]. While still elusive, live attenuated RSV vaccine candidates with promising characteristics are now moving into expanded clinical trials. The development of improved subunit vaccines has great potential for use in healthy adults, the elderly, and specific groups of high-risk older children, as well as for maternal immunization.

In addition, substantial progress has been made in developing new adjuvants for human use. These adjuvants may augment the immunogenicity of subunit vaccines and possibly live vaccines. With appropriate adjuvants, RSV subunit vaccines might be made safe for RSV-naive individuals. New RSV vaccine platforms, including virus-like particles and replication-defective vectors such as alphaviruses and adenoviruses, have yielded promising results in preclinical testing. Thus, the prospects for developing RSV vaccines are encouraging.

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Tuberculosis

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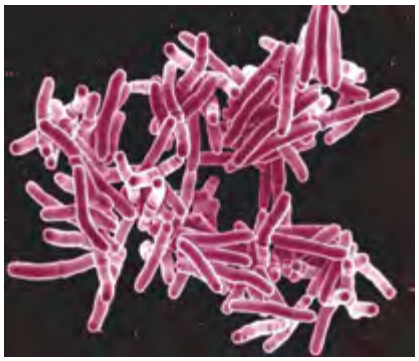
Background

Despite significant advances in tuberculosis (TB) research and improvement in treatment strategies, TB remains one of the leading infectious killers worldwide. Although curable, TB claims an estimated 1.7 million lives each year [1]. Failure to contain this disease can be attributed to a number of factors, including insufficient TB treatment and care infrastructure in endemic, resource-limited countries; the lack of integration of TB and HIV/AIDS healthcare services in areas where the spread of TB is closely linked to the HIV co-epidemic; the lack of rapid and sensitive diagnostics; the lack of treatment options to shorten therapy from the current 6–9 months; the spread of drug-resistant disease; and the lack of a highly effective vaccine.



Scanning electron micrograph of *Mycobacterium tuberculosis*. Courtesy of NIAID

In most cases, infection with *Mycobacterium tuberculosis (Mtb)* is controlled by the immune system and leads to a spectrum of manifestations ranging from asymptomatic colonization (often referred to as latent or persistent infection) to subclinical disease. Weakening of the immune system, as is the case in persons also infected with HIV or with diabetes, can result in progression from subclinical infection to active, symptomatic TB disease. While TB can manifest itself in a multitude of forms, pulmonary disease is of greatest public health importance since it is responsible for the transmission of the pathogen in communities. Patients with active TB are generally treated with combination chemotherapy under direct observation (DOT, directly observed treatment) for 6–9 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 TB. According to modeling studies, a combination of prevention strategies using more effective vaccines and/or more efficient treatment of latent disease, combined with proactive identification and treatment of TB patients, are needed to eliminate this disease as a global public health burden [2].

The currently available TB vaccine, *M. bovis* Bacille Calmette-Guérin (BCG), was developed almost 100 years ago. Worldwide, a variety of BCG strains are available and are widely administered to newborn children under the World Health Organization's (WHO's) Expanded Programme on Immunization. One BCG vaccine strain (Tice) is licensed in the United States against TB but is not recommended for general use. Despite its lack of consistent, reproducible efficacy in clinical trials to prevent adult pulmonary TB, BCG provides reasonable protection against childhood complications of and death from TB.

Development of more effective vaccines either to prevent infection with *Mtb* or to block 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' (HHS') Advisory Council for Elimination of Tuberculosis, 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 become available, many of which are now being evaluated in humans in clinical trials.

State of the Science in Tuberculosis Vaccine Development

Until the early 1980s, the incidence of TB in the United States had been steadily declining. A sudden spike in new cases was reported between 1986 and 1992. This resurgence of TB was attributable largely to a deteriorating public health infrastructure and also was coincident with the HIV epidemic. In 1993, TB was declared a global health emergency by WHO [3]. Following these events, awareness of the global impact of TB increased and led to the realization that improving our understanding of the natural history of TB and the interaction of host and pathogen is a prerequisite for identifying better ways to diagnose, prevent, and treat this disease. Research funding

has steadily increased since 1992, with NIAID developing a comprehensive research program to stimulate and support all aspects of TB biomedical research and product development. Significant gains in knowledge were made through the sequencing of the genome of laboratory and clinical strains of *Mtb* and other mycobacterial species, and the development of microbiologic and genetic tools that helped dissect the interaction of the pathogen and the host immune response. These efforts have been aided by the development of research resources for TB, including 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)-wide initiatives. These investments in biomedical research have resulted in the first-ever portfolio of TB vaccine candidates, many of which have entered clinical trials, with others completing preclinical evaluations. These candidates are representatives of a diverse set of vaccine classes and include recombinant BCG and live-attenuated *Mtb* strains; various other live vectors (bacterial and viral); and DNA, protein, and peptide subunit vaccines.

Significant effort has been expended to develop relevant animal models of TB that approximate distinct stages of human disease, to aid in the characterization and selection of preclinical and clinical vaccine candidates. Since the pathogenesis of TB varies among different animal species, with dynamic immunological factors modulating disease outcome after infection with *Mtb*, several different animal species are currently employed in preclinical vaccine testing, to assemble comprehensive datasets about vaccine candidates. Through the increasingly detailed characterization and refinement of these models, which now extend from rodents (mice and guinea pigs) to rabbits to nonhuman primates, researchers continue to gain insight into immunological and microbiologic factors that are involved in the development of TB in these animals and thus create scientific hypotheses for how human TB may develop. Although it is recognized that BCG provides critical protection against pediatric TB, this live vaccine can lead to significant adverse events and even death in children also infected with HIV, and thus, safer and more effective versions of BCG are being developed. Clinical development strategies for new TB vaccines include boosting of neonatal BCG with novel vaccines at a later stage in life, as well as replacement of BCG with safer and more effective recombinant strains that will improve boosting later in life. Both strategies to prevent primary infection and/or reactivation of latent TB are being pursued, as are strategies to use vaccines and immune stimulants to improve and shorten chemotherapy. Because about one-third of the world's population is thought to harbor asymptomatic infection with *Mtb*, and HIV co-infection increases the chance of developing active disease from 1 in 10 over the course of a person's life to 1 in 10 per year, prevention of reactivation disease is considered critical to curbing the spread of TB [4].

Several candidates that demonstrated protection against infection with *Mtb* in small animal models equally well or better than BCG have entered human clinical trials. These are the first studies of new, engineered TB vaccine candidates since the introduction of BCG in 1921. This new generation of clinical candidates includes recombinant BCG vaccines expressing various immunodominant *Mtb* antigens intended to replace BCG as a primary vaccine and fusion proteins composed of immunogenic *Mtb* peptides and virally vectored constructs intended to boost either current or potential recombinant BCG. In addition, various non-TB mycobacteria, such as *M. vaccae* and *M. w,* are being evaluated for their ability to stimulate immune responses against TB. Also, clinical studies are being conducted to better define the immune protection elicited by BCG in pediatric populations and to aid in the development of immune assays for the characterization of immune responses in human clinical trials. Overall, the research community is developing a comprehensive approach to designing improved vaccination strategies for TB. Currently, it is estimated that combination approaches of improved priming and boosting vaccines 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 TB vaccinology is available that can be used as guidance for developing or improving new TB vaccines. Although TB vaccine research has made tremendous advances over the last 10 to 15 years, a number of critical questions remain to be answered. The answers will likely provide the keys to faster TB vaccine development.

- Why are some individuals able to contain infection with *Mtb* as a latent, asymptomatic infection while others develop subclinical disease and still others progress to fulminate active disease? To answer this question, longitudinal human studies of *Mtb* infection are needed to define approaches and solutions to preventing progression to active disease.

- What markers can serve as correlates of immunoprotection in humans to allow assessment of immunogenicity in clinical trials? Since BCG is not able to protect against adult TB, these correlates of immune protection will likely not be identified until vaccines that provide more effective protection are evaluated in advanced clinical trials. Research in immunology of TB has provided suggestions as to what markers may be of relevance in protection, and these markers are progressively being integrated into clinical immune assays and also in animal studies of TB vaccines. Only with the aid of data from human vaccine trials will researchers be able to benchmark animal models to help identify those candidate vaccines with the highest chance of improving protection against TB in humans. For these reasons, it is critical that vaccine candidates be 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 comorbidities in patients at high risk for *Mtb* infection and progression to active disease? Do such co-infections or comorbidities have an impact on potential efficacy of vaccines?
- What are the most relevant animal models to predict efficacy of human vaccines against infection, disease, and/or transmission?
- How will persons already infected with *Mtb* respond to vaccination?
- What is the impact of vaccination on disease pathogenesis, and does natural and induced immunity affect the evolution of *Mtb* strain phenotypes? How do clinical trials have to be designed to study these complex interactions?
- What role will diagnostics play in the development of TB vaccines? Rapid and accurate identification of patients with *Mtb* infection, as well as ruling out active TB in adults and pediatric populations, will be critical for enrollment into clinical trials that evaluate post-exposure vaccines. Diagnostics that accurately and rapidly indentify infected persons are likely going to rely on a combination of host immune and bacterial markers. Diagnostic development therefore should be closely coupled with immunology and vaccinology research in TB to leverage scientific findings in these areas.
- How does BCG work in children? This is a currently understudied but important aspect of vaccine development. Little is known about general or TB-specific differences in immune response and vaccine efficacy among infants, children, and adults. It is recognized that the clinical presentation of TB in young children is different from that in adults and that BCG efficacy differs significantly in these populations.
- How can studies be designed to minimize the sample size and study duration? The current global capacity for registration-quality clinical trials for TB vaccines is insufficient to support Phase III trials. Furthermore, these trials are expected to require substantial numbers of trial volunteers and financial support, and it is unclear how development of clinical sites and funding for the clinical trials will be supported.

NIAID-Supported Tuberculosis Vaccine Research

Many challenges exist that will influence the design of efficacy trials in humans. To answer the above questions, NIAID is funding not only investigator-initiated research but also solicited research on TB immunology, pathology, pathogenesis, vaccine development, target antigen identification, diagnostics, development of improved tools for epidemiological studies, and development of markers of immunoprotection. All research in TB is included under Category C of NIAID's Biodefense Research Program. In addition, NIAID provides resources through its genomics and bioinformatics programs that are available to the TB research community.

NIAID's preclinical contract research resources include critical research materials from pathogenic and nonpathogenic mycobacteria, as well as vaccine-testing services in small animal models. Other contracts bridge 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. Support services also are available to help advance promising preclinical candidates to clinical testing. NIAID's Tuberculosis Research Unit and Vaccine and Treatment Evaluation Units provide clinical trials infrastructure for TB projects to evaluate vaccine candidates and conduct studies on establishing surrogate markers of protection (see www.niaid.nih.gov/labsandresources/resources/Pages/default.aspx).

Knowledge gained from research over the last 14 years has led to a diverse pipeline of vaccine candidates, with several products being evaluated in various stages of clinical trials. The advancement of the current global TB vaccine pipeline, as well as an updated Blueprint for Tuberculosis Vaccine Development, is being discussed by members of the Stop TB Partnership's working group for vaccines. Its most recent publication, "The Global Plan to Stop TB 2011–2015," not only summarizes the ongoing efforts in the field of TB vaccine development but also,

for the first time in the history of TB control, acknowledges the need to include fundamental research in human TB as an integral part of a global strategy to eliminate this disease [5]. This publication attests to the continued need for new vaccines against TB and 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 TB 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 pharmaceutical quality preclinical and also clinical development of vaccine candidates.

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

HEPATITIS C VIRUS: PROSPECTS FOR VACCINE DEVELOPMENT

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In the United States, there are approximately 20,000 new hepatitis C virus (HCV) infections every year. Acute HCV infections become chronic in the majority of infected individuals. Chronic HCV infection is associated with a high risk of progressive severe liver disease, including cirrhosis, liver cancer, and end-stage liver disease. There are an estimated 3–4 million individuals with chronic HCV infection in the United States and more than 170 million worldwide.

Multiple challenges exist with regard to developing an HCV vaccine. HCV mutates at an unusually high rate in an infected patient; immune responses such as virus-neutralizing antibodies and T-cell responses are compromised by the emergence of variant viruses. HCV proteins directly target and inhibit both innate and adaptive host immune responses. Also, a convenient small animal infection model for HCV is lacking. Currently, the only animal that can be infected with HCV is the chimpanzee.

The National Institute of Allergy and Infectious Diseases (NIAID) supports basic and clinical research on HCV replication and pathogenesis, virus-host interactions involved in pathogenesis, and immune responses; development of cell culture and small animal model systems for virus replication; development of vaccines and therapeutics, including programs to develop and test vaccines against HCV; and support of preclinical and clinical development resources.

Notably, in addition to individual investigator-initiated awards, NIAID has established five cooperative research centers for studying HCV, each engaged in studies on the host immunological response to infection.

Specific HCV vaccine candidates currently in clinical development include:

- A prime-boost approach with recombinant adenovirus and modified vaccinia Ankara (MVA) vectored vaccines preparing to enter Phase II trials
- Yeast vector vaccine in Phase IIb trial for therapeutic use
- Synthetic peptide vaccines—Phase II trials for therapeutic use completed
- MVA vector vaccine in Phase II trial for therapeutic use

The long-term, progressive clinical manifestations of chronic HCV infection provide opportunities, post-infection, to intervene with so-called "therapeutic" immunization approaches. Studies in chimpanzees suggest that it may be possible to develop both a prophylactic vaccine to prevent chronic HCV infection as well as therapeutic vaccines that may lower virus levels and ameliorate chronic liver disease. Given the large number of individuals with chronic HCV, safe and effective therapeutic vaccines that may potentially be used in conjunction with drugs would have great impact on the public health burden of HCV.

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Rotavirus Vaccines

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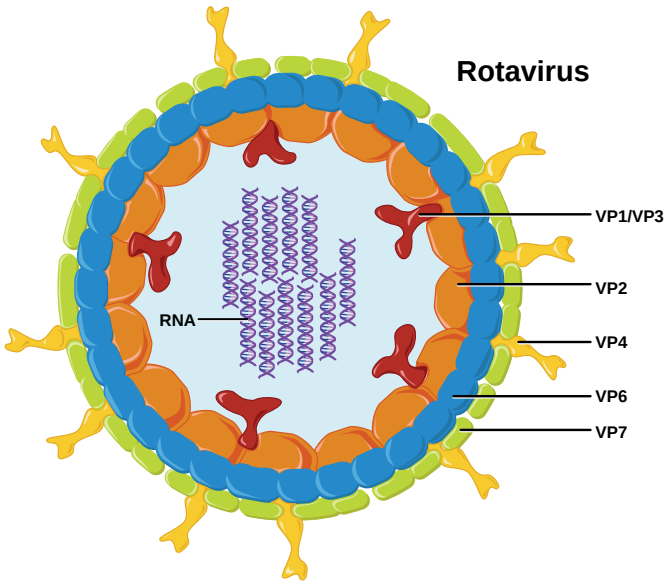
Rotaviruses are the leading cause of severe acute gastroenteritis among children around the world [1]. Before rotavirus vaccines were made available, nearly all children in the United States had rotavirus gastroenteritis by the age of 5, according to the Centers for Disease Control and Prevention (CDC). In the pre-vaccine era, rotavirus infections were responsible for 400,000 doctor visits, more than 200,000 emergency room visits, 55,000 hospitalizations, and 20 to 60 deaths annually among children under 5 years of age in the United States [2, 3].

Following the availability of rotavirus vaccines, reductions in severe and fatal diarrheal disease have been observed in low-middle, middle, and high-income countries [4]. It is estimated that in the United States rotavirus vaccines prevented approximately 650,000 diarrheal-associated hospitalizations between 2007 and 2009, and saved $278 million in treatment costs [5].

Vaccine-preventable deaths still continue, however. The World Health Organization estimates that more than 520,000 children under the age of 5 die from vaccine-preventable rotavirus infections each year, primarily in poor countries due to the lack of health care and adequate resources [1].

FIGURE 1.

Depiction of rotavirus



A rotavirus is a wheel-shaped virus consisting of 11 double-stranded RNA segments that generate six structural proteins (VP1, VP2, VP3, VP4, VP6, and VP7) and six nonstructural proteins (NSP1–6). Each virus particle is surrounded by a triple layer coat composed of the different structural proteins. Courtesy of NIAID

History of Rotavirus Vaccines

Credit for the discovery of human rotaviruses goes to Dr. Ruth Bishop in Melbourne, Australia, who first identified rotavirus as an agent of children's diarrhea in 1973. She recognized that naturally attenuated strains of rotavirus infecting neonates could protect them against severe gastroenteritis for multiple years.

Researchers later determined that rotaviruses consist of 11 segments of double-stranded RNA housed within concentric shells composed of three structural protein layers (Figure 1). There are seven rotavirus serogroups, A to G, with A being the most common. Proteins that form the outer shell include VP7 and the VP4 spike proteins. They stimulate the production of neutralizing antibodies and are, thus, targets for host protection by vaccines. VP6, which forms the next shell layer, has important antigenic determinants specific to each serogroup. One of the nonstructural proteins, NSP4, is now identified as an enterotoxin. When intestinal cells are infected with different strains of rotavirus—human or animal—genetic material from each strain may combine to produce a reassortant virus.

The National Institute of Allergy and Infectious Diseases (NIAID) has a long history of supporting rotavirus candidate vaccine development during the 1970s and 1980s, leading eventually to formation of a rhesus rotavirus quadrivalent vaccine expressing the most common human rotavirus serotypes: G1, G2, and G4, along with a rhesus G3. This vaccine advanced into clinical trials and was found to be safe and welltolerated. Upon its licensure in 1998 as RotaShield, it became the first rotavirus vaccine licensed in the United States. RotaShield was later voluntarily withdrawn from the market when data collected through postlicensure surveillance suggested an increase in a rare associated adverse event called intussusception. Currently, there are two licensed rotavirus vaccines.

RotaTeq (RV5) was initially developed by NIAID grantees and was licensed in the United States in 2006. It is a live oral human-bovine pentavalent reassortant rotavirus vaccine. RotaTeq is given to infants in three doses as an oral liquid at 2, 4, and 6 months. Large clinical trials showed no increase in intussusception with RotaTeq when compared to the placebo group. A threefold increase in serum immunoglobulin A (IgA) antibodies was seen in a subgroup of infants receiving RotaTeq, compared with those receiving placebo [6]. Efficacy against any rotavirus gastroenteritis matching the vaccine serotypes in the first year was 74 percent and rose to 98 percent against any severe rotavirus gastroenteritis.

Rotarix (RV1) is a live attenuated oral human vaccine containing only the most common human genotype, G1, yet proved in trials to protect against severe diarrhea for G1, G2, G3, G4, and G9 rotavirus strains. Given to infants in two doses between 6 and 24 weeks old, Rotarix was originally approved for use in more than 90 countries; it was licensed for use in the United States in 2008. No increase in intussusception was seen during clinical trials when comparing Rotarix to placebo.

Looking Forward

Current rotavirus vaccines have improved the health of children around the world. However, new vaccines could continue to reduce the global impact of rotaviruses. Together Rotarix and RotaTeq are licensed in more than 100 countries but remain cost-prohibitive for many developing countries. Considerations for next-generation vaccines include: affordability, ease of delivery, ambient storage, and use in higher-risk populations, such as infants with compromised immune systems or poor nutrition.

Isolates of human rotaviruses taken from asymptomatic infants are still considered a promising source of new vaccines. An example of government and private sector collaboration exists in the development of a vaccine that is now taking place in India. A naturally occurring rotavirus strain was isolated in a neonatal unit in India, adapted to Primary African Green Monkey Kidney (PAGMK) cells by CDC, and later transferred to NIAID for production of clinical lots. The resulting vaccine was tested in the United States by NIAID in adults and children. The vaccine was then transferred to a biotechnology company in India where it was adapted to Vero cells and tested in Phase I and II studies. The newly formulated vaccine is currently in Phase III studies in India under support from the Bill & Melinda Gates Foundation.

Other rotavirus vaccine candidates moving forward in clinical trials include an oral vaccine based on a neonatal strain of rotavirus and vaccines made from recombinant virus-like particles that are incapable of replication yet have proven effective against animal rotavirus. Another option being advanced uses killed rotavirus strains delivered by injection, in hopes that such vaccines may be more protective in higher risk populations, where oral vaccines are typically less effective.

In order to increase accessibility to rotavirus vaccines, NIAID has negotiated agreements with pharmaceutical companies in Brazil, China, and India for the transfer of human-bovine rotavirus vaccine technology and biological starting materials that have been developed by NIAID scientists. The goal is to have local companies make affordable vaccine, raising the hope that the vaccine will be incorporated into local programs and the disease burden will be reduced. The success of current rotavirus programs demonstrates that research on new prevention strategies, including vaccines, can make a significant impact on improving health and decreasing costs [7].

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APPENDIXES

APPENDIX A: Status of Vaccine Research and Development, 2012

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Ancyclostoma duodenale	Recombinant protein	+	+	+		
Bacillus anthracis	Recombinant protein (eg rPA, rPA + other anthrax proteins)	+	+	+	+	
	Viral vectored	+	+			
	Bacterial vectored	+	+			
	Conjugate (eg PGA-PA)	+	+	+		
	Spore vectored	+				
	DNA	+	+	+		
	AVA + CpG	+	+	+		
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 (polyvalent)	+	+	+	+	
	DbpA	+				
	DbpB	+				
	AcGal	+				

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
	RevA	+				
	BBK32,BB0323	+				
Brugia malayi	Purified parasite antigens (paramyosin, etc.)	+	+			
Campylobacter jejuni	Whole cell (intact)	+	+	+	+	
	E. coli recombinant flagellin, intranasal delivery	+	+	+		
	Protein subunit vaccine	+	+	+	+	
	Whole cell	+	+	+		
	Polysaccharide capsules	+	+			
	Flagella secreted protein A1 (FspA1)	+				
	ACE393 recombinant protein, systemic delivery with alum	+	+	+	+	
	Monovalent capsule conjugate (CRM197)	+	+			
Candida albicans	Cell surface oligomannosyl epitope	+	+			
	Recombinant Als1p surface protein	+	+			
	Recombinant Als3p surface protein	+	+			
Chikungunya virus	Live attenuated (conventional)	+	+	+	+	
	Adenovirus-vectored (various)	+				
	Adeno-associated virus-vectored	+				
	Newcastle disease virus-vectored	+				
	Alphavirus-based chimeras	+				
	Virus-like particle vaccine	+				
	Cationic-liposome-DNA complex	+				
Chlamydia pneumoniae	Purified, major outer membrane protein, heat shock protein	+				
	Outer membrane protein-based DWA vaccine	+				
Chlamydia trachomatis	Major outer membrane protein (MOMP)	+	+			

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
	Polymorphic membrane protein D	+				
	Chlamydia-secreted protease factor (CPAF)	+				
Clostridium botulinum	Toxoids	+	+	+	+	
	Recombinant AB vaccine	+	+	+	+	
	Recombinant heavy chain	+	+			
	Recombinant light chain LHN	+				
	Viral vectored	+				
	Replicon based	+				
	DNA	+				
	Nonneurotoxic peptides	+				
Clostridium difficile	Toxin mutants	+	+	+	+	
	Toxin mutants expressed in a bacterial system (endotoxin-free *Bacillus megaterium*)	+				
	Surface layer protein	+				
Clostridium tetani	Recombinant toxin	+	+			
	Salmonella vector	+	+	+		
	Microencapsulation	+	+			
	Transcutaneous immunization	+	+			
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	+	+			

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 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	+				
	Replication-defective	+				
Dengue virus	Purified rDNA-expressed viral proteins	+	+			
	Yellow fever/dengue chimeric virus	+	+	+	+	
	Inactivated whole virus particle	+	+	+		
	VEE replicon vector	+	+			
	Naked DNA	+	+			
	Vaccinia vector (live)	+	+			
	Vaccinia subunit	+	+			
	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
	Yeast subunit	+	+			
	Drosophila-expressed recombinant subunit	+	+	+		
	Baculovirus-expressed recombinant subunit	+	+			
	Live attenuated dengue virus (monovalent)	+	+	+	+	
	Live attenuated dengue virus (combined quadrivalent)	+	+	+	+	
	Adenovirus vector	+	+			
Eastern equine encephalitis virus	Inactivated whole virus particles	+	+	+	+	
	VEE virus replicon particle	+	+			
	DNA vaccine	+				
	Alphavirus-based chimeras	+				
	Cationic-liposome-DNA complex	+				
Ebola virus	Recombinant protein subunit (various virus and eucaryotic expression and delivery systems)	+	+			
	VEE virus replicon particle	+	+			
	Kunjin virus replicon particle	+				
	Plasmid DNA prime/adenovirus-expressed protein boost	+	+			
	Plasmid DNA	+	+			
	Virus-like particle (VLP)	+				
	Various adenovirus-vectored	+	+			
	rVSV-vectored	+	+			
	Paramyxovirus-vectored	+				
	CMV-vectored	+				
	Combination DNA/VLP	+				
	Multi-agent DNA	+				

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
	rVSV-vectored multi-agent vaccine (Lassa, Ebola, Marburg)	+				
	Prophylactic monoclonal antibodies	+				
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 *Salmonell*a	+	+			
Epstein-Barr virus (EBV)	Glycoprotein subunit (gp350)	+	+	+	+	
	Vaccinia recombinant virus expressing gp350	+	+	+		
	Peptide induction of CTL	+	+	+		
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	+	+			
	Attenuated EHEC vector based vaccine (attaching/effacing determinants attenuated)	+	+			
Enterotoxigenic *E. coli* (ETEC)	Killed cells and beta-subunit of cholera toxin	+	+	+	+	
	Nontoxigenic ETEC derivative, live attenuated	+	+	+	+	
	Formalin killed over expressed colonization factors CFAI, CS2, CS4, CS5 and CS6	+	+	+		
	Killed whole cells vaccine containing CTB	+	+	+	+	+
	ACE527 polyvalent live attenuated vaccine	+	+	+	+	

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
	Anti-CFAI and CfaE bovine IgG (passive immunization)	+	+	+	+	
	Peru-15 (pCTB plus ETEC)	+	+	+	+	
	Double mutant heat labile enterotoxin	+	+	+		
	Heat labile enterotoxin transcutaneous delivery (patch) for traveler's diarrhea (Iomai)	+	+	+	+	+
	Attenuated EHEC vector based vaccine for delivery ETEC adhesin and toxin targets (CFAI & LT)	+	+			
	Adhesin-toxoid chimera CFAI/CfaE-CTA2 candidate	+	+			
	Heat labile and heat stable toxin A (LT-STa)-chimera toxoid	+				
	Conjugated (BSA) heat stable toxin	+				
	Molecular targets on *E. coli* surface, early stages of discovery	+				
	E. coli veriome including all major pathotypes (diarrheal and extraintestinal)	+				
	Attenuated ETEC strains PTL002 and PTL003 expressing CFA/II	+	+	+	+ PTL003	
	Shigella strains expressing ETEC adhesions antigens (CVD1208, 1233, 1252)	+	+	+		
	S. flexneri 2a (SC602) expressing CfaB and LTB	+	+			
Escherichia coli (urinary tract)	Anti-FimH adhesin	+	+			
Francisella tularensis	Live attenuated	+	+	+	+	
	Detoxified *F. tul* endotoxin	+				
	Deletion mutants, live vaccines	+				
	O antigen capsular polysaccharide	+				
	Bacterial vectored	+				
	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.*

Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
	Isolated outer membrane proteins	+				
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)	+	+	+		

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
	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	Recombinant subunit	+	+			
	Nonreplicating adenovirus vector	+	+			
	Naked DNA	+	+	+		
	VSV vector	+				
Helicobacter pylori	Recombinant *H. pylori* urease and cholera toxin-oral vaccine	+	+	+		
	Recombinant *H. pylori* vacuolating cytotoxin A (VacA), cytotoxin associated antigen A (CagA) neutrophil activating protein (NAP) with aluminum hydroxide	+	+			
	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	+	+			

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Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
	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 and woodchuck antigens expressed by rDNA	+	+			
	HBV proteins expressed in yeast cells by rDNA	+	+	+	+	+
	Salmonella and *Listeria* monocytogenes vectors	+	+			
	Variants	+	+			
	Generation of cytotoxic T lymphocytes	+	+	+	+	
	DNA vaccines	+	+			
	rDNA, plants	+	+	+		
	Intranasal vaccines	+	+			
	HBV recombinant vaccine with MPL adjuvant (Fendrix)	+	+	+	+	
	HBV vaccine with novel adjuvants—chitosan, oil emulsions, hydrogels delivering GM-CSF, AS02v, etc.	+	+	+		
Combined HAV/HBV vaccine	Combined inactivated components	+	+	+	+	+
Hepatitis C virus (HCV)	Recombinant pro-apoptotoci BCG (rpaBCG) vaccines that express HCV antigens	+				
	MVA-based rVac w/3 NS protein genes	+	+			
	Recombinant viruses carrying HCV non structural genes: adenovirus	+	+			
	Bacterial recombinants with HCV proteins: *Listeria monocytogenes*	+				

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
	Cell-based vaccines; yeast	+				
	Plants systems for HCV protein expression	+				
	Human dendritic cells (matured *in vitro* with HCV peptides), for autologous transfer	+				
Hepatitis D virus (HDV)	Synthetic peptides	+	+			
	Recombinant pro-apoptotic BCG (rpaBCG) vaccines that express HCV antigens	+				
	Lentivirus derived HCV-like particles	+				
Hepatitis E virus (HEV)	Expressed proteins	+	+	+	+	
	Recombinant protein (*E. coli* expressed)	+	+	+	+	+
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)	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)	+	+	+	+	+
	Live attenuated (interpandemic)	+	+	+	+	+
	Live attenuated (pandemic)	+	+	+	+	+
	Liposome containing viral HA	+	+	+	+	+
	Recombinant viral proteins	+	+	+	+	+
	Inactivated with novel adjuvants	+	+	+	+	+

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
	Cell culture derived influenza vaccine	+	+	+	+	+
	M2e vaccines	+	+	+	+	
	Recombinant viral vector vaccines	+	+	+	+	
	Plant-based NECVLP vaccine	+	+	+	+	
	DNA vaccines	+	+	+		
	Peptide vaccines	+	+	+		
Japanese encephalitis virus	Whole, inactivated virus particles (JE-VAX, mouse brain-derived)	+	+	+	+	+
	Whole, inactivated virus particles, (IXIARO, Vero cell culture-derived, licensed in the United States in 2009)	+	+	+	+	+
	Infectious clone	+	+			
	Purified DNA expressed protein	+	+			
	Live attenuated virus (SA-14-14-2)	+	+	+	+	+
	Vaccinia vector (live)	+	+	+		
	Live attenuated YF17D-vectored JE chimera (ChimeriVax-JE)	+	+	+	+	
	Fusion loop peptide	+				
Junin virus (Argentine hemorrhagic fever)	Live attenuated (Candid #1)	+	+	+	+	
	Live attenuated YF17D-vectored multi-valent chimera (YF, Junin and other arenaviruses)	+				
Lassa virus	Chimeric live reassortant Mopeia/Lassa virus	+				
	DNA vaccine	+				
	Viral-like particles	+				
	Live attenuated YF17D-vectored bivalent chimera (YF, Lassa)	+				
	rVSV-vectored multi-agent vaccine (Lassa, Ebola, Marburg)	+				

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
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	+				
	Epicutaneous strategies with cholera toxin adjuvant	+				
Marburg virus	DNA	+	+	+		
	DNA/adenovirus boost	+	+	+		
	VEE virus replicon particle	+				
	Various adenovirus-vectored vaccines	+	+			
	Virus-like particle (VLP)	+				
	rVSV-vectored multi-agent vaccine (Lassa, Ebola, Marburg)	+				
	Prophylactic monoclonal antibodies	+				
Measles virus	rDNA HA and fusion proteins	+	+	+		
	Live attenuated	+	+	+	+	+
	High-titer live (multiple strains)	+	+	+	+	+
	VEE virus replicon particle	+	+			
	Dry powder	+	+			
	DNA + Vaxfectin	+	+			
Moraxella catarrhalis	High molecular weight, outer membrane proteins CD, E, B1, and LBP for use in conjugate vaccines	+	+			

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
	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)	+	+	+	+	+
	Mycobacterium indicus pranii (MIP)	+	+	+	+	+
	BCG + killed *Mycobacterium leprae*	+	+	+	+	+
	ICRC bacilli, heat killed (Indian Cancer Research Center strain)	+	+	+	+	+
	Mycobacterium w (M. welchii), live and killed	+	+			
	BCG-70M (secreting a fusion protein of BCG Hsp70 and *M. leprae* major membrane protein II)	+				
	BCG-SM (secreting *M. leprae* major membrane protein II)	+				
	Adjuvanted *Mycobacterium leprae* antigen ML0276	+				
	BCG homologous and heterologous boosting	+	+	+	+	+
	BCG delivered orally	+	+	+		
	Mycobacterium vaccae, heat killed	+	+	+	+	+
	Recombinant BCG with endosome escape, overexpressing several key antigens	+	+			
	Recombinant BCG with endosome escape (rBCGΔUre:CHly+)	+	+	+		
	Superoxide dismutase (SOD) diminished BCG	+	+			
Mycobacterium tuberculosis	Live attenuated *Mycobacterium tuberculosis* strains	+	+			
	Modified vaccinia virus expressing *Mycobacterium tuberculosis* Ag85A (MVA-85A)	+	+	+	+	
	Ag85B + ESAT6 (Hybrid-1) subunit vaccine in IC3 adjuvant	+	+	+		

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
	Ag85B + TB10.4 (HyVac4) subunit vaccine in IC3 adjuvant	+	+	+		
	M72f (Mtb39 + Mtb32) subunit vaccine in adjuvant AS01 adjuvant	+	+	+	+	
	Hsp65 DNA vaccine	+	+			
	AERAS-402/Crucell Ad35 (replication deficient Adenovirus 35 expressing Mtb antigens, Ag85 A, Ag85 and B, and TB10.4	+	+	+	+	
	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	+	+	+		
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)	+				

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
	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 *Neisseria*l antigen (Universal)	+				
	De-N-acetyl sialic acid polysaccharide derivative-TT	+	+			
	Recombinant Protein B (TspB)	+				
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 G glycoproteins	+	+			
	Soluble G glycoproteins	+	+			
Norwalk virus (Human Noroviruses including Norwalk (GI.1))	Norwalk VLPs (GI.1)	+	+	+		

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
	Norwalk VLPs in transgenic potatoes	+	+	+		
	Norwalk VLPs in transgenic tomatoes (lyophilized)	+	+			
	Intranasally–delivered Norwalk VLPs w/MPL and Chitosan	+	+	+		
	Intranasal Norwalk VLPs: challenged with characterized Norwalk virus pool	+	+	+		
	Intramuscular Norwalk VLPs and Norovirus (GII.4) VLPs plus MPL, Chitosan	+	+	+		
Onchocerca volvulus	Recombinant proteins	+	+			
Paracoccidioides brasiliensis	Purified yeast cell proteins	+	+			
	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	+	+	+		
	VAR2CSA, pregnancy associated antigens	+	+			

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
	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	Codon-deoptimized poliovirus for new IPV seed stock	+				
	miRNA target inserted poliovirus in high fidelity polymerase mutant backbone for a new IPV stock and OPV	+	+			
	Chimeric vaccines such as wild type capsid protein expressing from Sabin strain backbone or type 3 capsid in type 1 backbone	+	+			
	Mutant poliovirus carrying mutations in 5'non-coding region for a new IPV seed stock	+				
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	+	+	+		
	Live attenuated *Pseudomonas* vaccine (aroA mutant)	+	+			

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	+	+	+	+	
	RSV live attenuated strains	+	+	+	+	
	Nanoparticle vaccine carrying G polypeptide against CXC3 motif of RSV G protein	+	+			
	Recombinant Sendai virus expressing RSV F protein	+	+	+		
	Recombinant attenuated parainfluenza virus type 3 expressing RSV F protein	+	+	+		
	Recombinant Newcastle Disease virus expressing RSV F protein	+	+			
	Recombinant RSV virus F protein particle vaccine (VLP)	+	+	+		
Ricin toxin	Recombinant inactivated toxin	+	+	+		
	Ricin A (RiVax) plus adjuvant vaccine—formulation optimization	+	+			
	Vaccine formulation and lyophilization for intradermal/intranasal delivery	+	+			
Rickettsia rickettsii	Subunit vaccine containing major surface proteins (155 and 120 kD)	+	+			

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Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
Rift Valley fever virus	Inactivated	+	+	+	+	
	Live attenuated virus (MP-12)	+	+	+	+	
	VEE virus replicon particle	+				
	Sindbis virus replicon particle	+				
	Virus-like particle (VLP)	+				
	Live attenuated recombinant virus	+				
Rotavirus	Attenuated human rotavirus strain 89-12 P1A[8] ,G1	+	+	+	+	+
	Salmonella expressing VP4, VP7, or both	+	+			
	Attenuated bovine/human virus reassortants (G1-WC3; G2-WC3; G3-WC3; G4-WC3; P1A[8]-WC3)	+	+	+	+	+
	Human nursery strains—116E (India)	+	+	+	+	+
	Human nursery strains—RV3 (Australia)					
	Purified rotavirus proteins rDNA-derived virus-like particles (VLPs)	+	+			
	Vaccina virus recombinant expressing		+			
	VP4, VP7, or both	+				
	DNA vaccines	+	+			
	VP6 vaccines with maltose binding protein (MBP)	+	+			
	Inactivated rotavirus vaccine (G1p[8]) with Alum	+	+			
	Intranasal attenuated rotavirus vaccine or IN rotavirus VLPs	+	+			
Rubella virus	Live attenuated	+	+	+	+	+
Salmonella typhi	Vi carbohydrate	+	+	+	+	+
	Live attenuated Ty21a vaccine	+	+	+	+	+
	Live attenuated auxotrophic mutants	+	+	+	+	
	Vi conjugate vaccine	+	+	+	+	+

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Target Agent	Vaccine	Basic R&D	Preclinical	Phase I	Phase II	Phase III
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 expresssing S1 or N	+	+			
	B- and T-epitope peptide-based vaccine	+				
Shigella dystenteriae	Live auxotrophic, attenuated mutants	+	+	+		
	Polysaccharide-protein conjugate	+	+	+	+	
Shigella flexneri	*E. coli* hybrids	+	+	+	+	
	Polysaccharide-protein conjugate	+	+	+	+	

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
	Live attenuated oral vaccines	+	+	+	+	
	LPS proteosome (intranasal)	+	+			
	LPS-invasin proteins (IpaB/C) complex	+	+	+	+	
Shigella sonnei	Live attenuated (WRSS1) oral vaccine	+	+	+	+	
	LPS proteosome (intranasal)	+	+			
	Polysaccharide-protein conjugate	+	+	+	+	
	Nucleoprotein	+	+			
Shigella flexneri/sonnei	Polysaccharide-protein conjugate	+	+	+	+	+
Staphylococcus aureus	Clumping factor B	+				
	rAls3p-N	+				
	Polymeric N-acetylglucosamine	+	+	+		
	S. aureus protein/polypeptide antigen expressed in yeast					
	Surface proteins IsdA, IsdB, SdrD, SdrE	+				
	Pentavalent vaccine candidate	+	+	+		
	Tetravalent bioconjugate vaccine CP5-EPA/ CP8-EPA and clumping factor A (ClfA) and-alpha toxoid)	+				
Staphylococcal entertoxin B	Recombinant toxin	+	+			
Streptococcus pneumoniae	Glycoconjugate vaccine (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 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	+	+			

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
	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	+	+	+	+	
	Drosophila-expressed recombinant subunit vaccine	+				
	Live attenuated dengue/TBE chimera	+				
	Recombinant vaccinia virus	+				
Toxoplasma gondii	Recombinant parasite surface protein (p30)	+	+			
	Live attenuated parasites	+	+			
	Parasite surface protein expressed in viral vector	+	+			
	Polyepitope DNA	+				
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
Variola	DNA subunit	+	+			
	Non-replicating attenuated strain	+	+	+	+	
	Repicating attenuated strain	+	+			
	Recombinant subunit	+	+			
Venezuelan equine encephalitis	Inactivated, whole virus particles	+	+	+	+	
	Live attenuated virus strain (TC-83)	+	+	+	+	
	Live attenuated mutagenized virus (V3526)	+	+	+		
	Inactivated V3526	+				
	Infectious clones	+	+			
	VEE virus replicon particle	+	+			
	DNA	+				
	Adenovirus-vectored	+				
	Multi-epitope peptide	+				
	Cationic liposome-DNA complex	+				
	Alphavirus-based chimeras	+				
	Prophylactic monoclonal antibodies	+				
Vibrio cholerae	Killed bacteria plus toxin B subunit	+	+	+	+	+
	Live recombinant O1	+	+	+	+	+
	Live recombinant O139	+	+	+	+	
	Conjugate lipopolysaccharide (LPS)	+	+			
	Killed bivalent (O1/O139)	+	+	+	+	+
	Live attenuated oral O1	+	+	+	+	
Yellow fever virus	Live attenuated (YF17D, Licensed in the United States)	+	+	+	+	+
	Infectious clone	+	+			
	Inactivated whole virus particles	+				
	Recombinant, bivalent YF17D/Lassa	+				

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
	Fusion loop peptide	+				
	Prophylactic monoclonal antibodies	+				
Western equine encephalitis virus	Inactivated, whole virus particles	+	+	+	+	
	VEE virus replicon particle	+	+			
	DNA	+				
	Baculovirus-expressed subunit protein	+				
	Cationic-liposome-DNA complex	+				
	Alphavirus-based chimeras	+				
West Nile virus	YF17D/ WNV chimera	+	+	+	+	
	Dengue4/WNV chimeras	+	+	+		
	DNA plasmid vaccines	+	+	+		
	Drosophila-expressed recombinant subunit vaccine (HBV-002)	+	+	+		
	Engineered, attenuated, single-cycle virus vaccine	+				
	Mutated, live attenuated vaccine	+				
	Kunjin-based WNV DNA vaccine	+				
	Fusion loop peptide	+				
Yersinia pestis	F1-V fusion protein	+	+	+	+	
	F1 + V protein	+	+	+		
	Nanodelivered F1-V	+	+	+		
	Bacterial vectored	+				
	Viral vectored	+				
	LcrV subunit	+				
	rV10 subunit	+	+			
	Flagellin adjuvanted F1-V	+	+			
	MVA-V combination	+				

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
	T-4 display	+				
	Outer membrane proteins	+				
	PNAG conjugated vaccine	+				
	Oral bacterial vectored	+				

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: NIAID-Supported HIV Vaccine Candidates in Preclinical Development, July 2011

Vaccine*	HIV Subtype	Preclinical Partners**	Manufacture
Multi-gene DNA (+ GM-CSF) + MVA	B	NIAID (Moss lab), GeoVax (Robinson)	VGXI/BioReliance
Multi-gene DNAs + cytokines DNA + Electroporation devices	A, B, C	U. Penn (D. Weiner), Inovio	Althea and VGXI/Inovio
VEE replicons	C	Alphavax	Alphavax
Novel serotype Adenoviral Vectors (Ad25, Ad35) + protein	A, mosaics	Harvard (Barouch)	Crucell
Multi-gene DNAs + cytokines DNA + Electroporation devices + VSV Gag	B	Profectus/Ichor	Boeringer Ingelheim/DSM Biologics/Henogen/Ichor
AAV-based vectors	A	Children's Hospital of Philadelphia	Targeted Genetics
Mosaic DNA + NYVAC HIV Env	mosaics	CHAVI (Haynes), Sanofi, EuroVacc	Althea/IDT and Sanofi
NYVAC + Env protein + adjuvant (Poly IC/LC)	C	FHCRC (J. McElrath), Oncovir, Sanofi	Sanofi/TBD
Multigene DNA + NYVAC	C	IPPOX, Eurovacc	Vical/IDT and Sanofi
Replication Competent Adenovirus, Type 4	mosaics	NIAID (Connors), NCI (Guroff)	PaxVax
Envelope Proteins	A,C	Novartis	TBD
Chimp-serotype based Adenovirus vectors	B	Wistar (Ertl)	TBD
Multi-clade DNA and proteins	A, B, C, D, E	U. Mass (Lu)	TBD

APPENDIX C: Ongoing Clinical Trials of HIV Vaccine Candidates in HIV-Uninfected Adults, July 2011

Protocol	Type of Vaccine – Prime – Boost	Vaccines – Prime – Boost	HIV Antigens [Clade] – expressed by Prime – expressed by Boost	Adjuvants – with Prime – with Boost	Developer/ Manufacturer – Prime – Boost	Phase	Location of Clinical Sites
DNA (alone)							
HVTN 080	DNA plasmids	*PENNVAX(TM)-B [PV-B]* via INOVIO® Electroporation Device	Gag [B], Pol [B], Env [B]	IL-12 DNA plasmid	U Penn. School of Medicine	1	USA
DNA plus Live Vector: Adenovirus 5 (Ad5)							
HVTN 505	DNA plasmids Live Vector	*VRC-HIVDNA016-00-VP* *VRC-HIVADV014-00-VP*	Gag [B]; Pol [B]; Nef [B]; Env [A]; Env [B]; Env [C] Gag-Pol [B]; Env [A]; Env [B]; Env [C]	— —	VRC VRC	2	USA
HVTN 077	DNA plasmids Live Vector	*VRC-HIVDNA044-00-VP* *VRC-HIVADV038-00-VP*	Env [A] Env [A]	— —	VRC VRC	1-B	USA
HVTN 082	DNA plasmids Live Vector	*VRC-HIVDNA016-00-VP* *VRC-HIVADV014-00-VP*	Gag [B]; Pol [B]; Nef [B]; Env [A]; Env [B]; Env [C] Gag-Pol [B]; Env [A]; Env [B]; Env [C].	— —	VRC VRC	1-B	USA
DNA plus Live Vector: Adenovirus 35 (Ad35)							
HVTN 077	DNA plasmids Live Vector	*VRC-HIVDNA044-00-VP* *VRC-HIVADV027-00-VP*	Env [A] Env [A]	— —	VRC	1-B	USA
Live Vector: Adenovirus 5 (Ad5)							
Merck 018/ HVTN 050	Live Vector	*MRKAd5 HIV-1 gag [homologous boost]*	Gag [B]	—	Merck	1	USA, Puerto Rico, Peru, Haiti, S. Africa, Malawi
HVTN 071	Live Vector	*MRKAd5 HIV-1 gag/pol/ nef [homologous boost]*	Gag [B]; Pol [B]; Nef [B]	—	Merck	1-B	USA
HVTN 083	Live Vector	*VRC-HIVADV038-00-VP [homologous boost]*	Env [A]	—	VRC	1	USA
HVTN 083	Live Vector Live Vector	*VRC-HIVADV038-00-VP* *VRC-HIVADV052-00-VP*	Env [A] Env [B]	— —	VRC VRC	1	USA
HVTN 503 'Phambili'	Live Vector	*MRKAd5 HIV-1 gag/pol/ nef [homologous boost]*	Gag [B]; Pol [B]; Nef [B]	—	Merck	2-B	South Africa
VRC 015	Live Vector	VRC-HIVADV014-00-VP [given by Biojector versus needle]	Gag-Pol [B]; Env [A]; Env [B]; Env [C]	—	VRC	1	USA

Protocol	Type of Vaccine – Prime – Boost	Vaccines – Prime – Boost	HIV Antigens [Clade] – expressed by Prime – expressed by Boost	Adjuvants – with Prime – with Boost	Developer/ Manufacturer – Prime – Boost	Phase	Location of Clinical Sites
Live Vector: Adenovirus 35 (Ad35)							
VRC 012 Part A	Live Vector	*VRC-HIVADV027-00* *[dose escalation for Part B]*	Env [A]	—	VRC	1	USA
HVTN 083	Live Vector	*VRC-HIVADV027-00*	Env [A]	—	VRC	1	USA
Live Vectors: Adenovirus 5 (Ad5) and Adenovirus 35 (Ad35) or Ad35 and Ad5							
VRC 012 Part B	Live Vector Live Vector	*VRC-HIVADV027-00-VP* *VRC-HIVADV038-00-VP*	Env [A] Env [A]	— —	VRC VRC	1	USA
HVTN 077	Live Vector Live Vector	*VRC-HIVADV027-00-VP* *VRC-HIVADV038-00-VP*	Env [A] Env [A]	— —	VRC VRC	1-B	USA
HVTN 083	Live Vector Live Vector	*VRC-HIVADV027-00-VP* *VRC-HIVADV052-00-VP*	Env [A] Env [B]	— —	VRC VRC	1	USA
HVTN 083	Live Vector Live Vector	*VRC-HIVADV027-00-VP* *VRC-HIVADV038-00-VP*	Env [A] Env [A]	— —	VRC	1	USA
DNA plus Live Vector: Modified Vaccinia Ankara (MVA)							
HVTN 205	DNA Plasmid Live Vector	*pGA2/JS7 DNA [GeoVax]* *MVA/HIV62*	Gag-PR-RT- Env-Tat-Rev-Vpu [B] (as single transcript) Gag-Pol-Env [B] (same gene sequences as in DNA)	— —	GeoVax GeoVax	2-A	USA, Peru
HVTN 073/ SAAVI 102	DNA Plasmid Live Vector	*SAAVI DNA-C2 (multigene)* *SAAVI MVA-C (multigene)*	Gag-RT-Tat-Nef [C]; and gp150 [C] Gag-RT-Tat-Nef [C];	— —	SAAVI SAAVI	1	South Africa
RV 262	DNA Plasmids Live Vector	*PENNVAX-G DNA (env & gag)* *MVA-CMDR*	Gag [multisubtype consensus]; Env [A]; Env [C]; Env [D] HIV-1 CM235 Env gp150 [E] plus CM240 Gag/Pol [A]	— —	U Penn. School of Medicine WRAIR & NIAID/LVD	1	USA, Kenya, Uganda, Tanzania, Thailand
Live Vectors: Adenovirus 26 (Ad26)							
IPCAVD 001	Live Vector	*Ad26.ENVA.01* *[homologous boost]*	Env [A]	—	D Barouch/ Crucell	1	USA
IPCAVD 003	Live Vector	*Ad26.ENVA.01* *[homologous boost]* [Innate & Mucosal immunity]	Env [A]	—	D Barouch/ Crucell	1	USA
Live Vectors: Adenovirus 26 (Ad26) combined with Adenovirus 48 Highly Variable Region (HVR48)							
IPCAVD 002	Live Vector	*Ad5HVR48.ENVA.01* *[homologous boost]*	Env [A]	—	D Barouch/ Crucell	1	USA, Kenya, Rwanda, S. Africa
Live Vectors: Adenovirus 5 (Ad5) and NYVAC or NYVAC and Ad5							
HVTN 078	Live Vector Live Vector	*VRC-HIVADV014-00-VP* *NYVAC-HIV-B*	Gag-Pol [B]; Env [A]; Env [B]; Env [C] Gag-Pol-Nef [B] and gp120 [B]	— —	VRC EuroVacc	1	Switzerland

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