EXPERT ARTICLES

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Vaccinomics and Personalized Vaccinology

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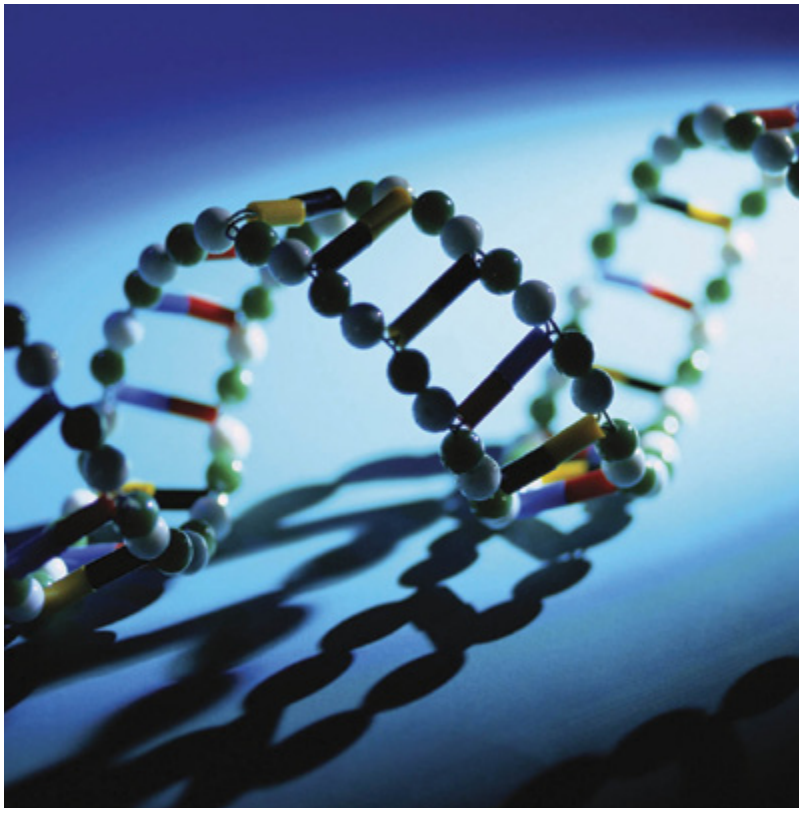
Abstract

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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FURTHER READING

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

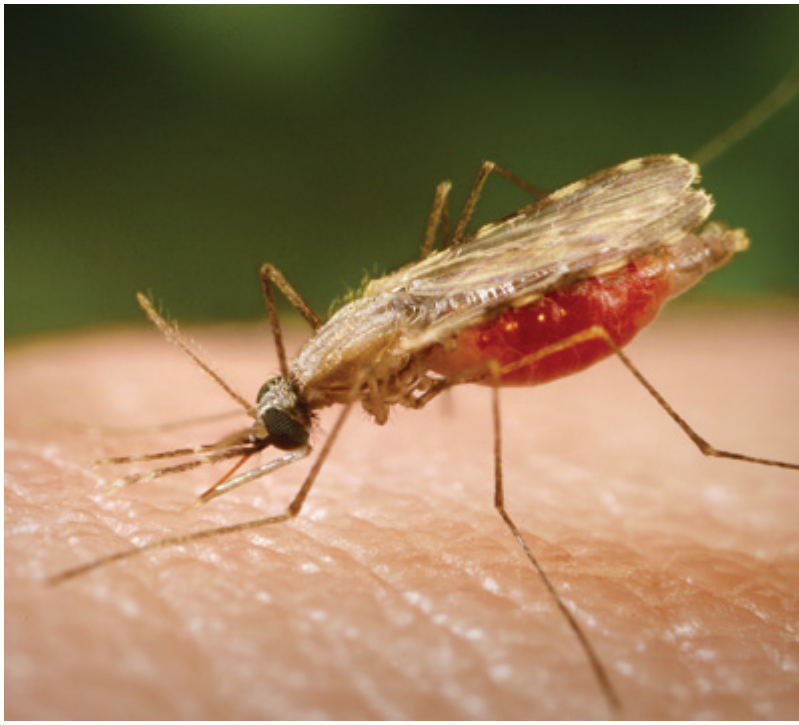
Vasee S. Moorthy, MRCP, Ph.D.

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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Dr. Moorthy serves on various independent data monitoring committees (IDMCs) and scientific advisory committees, is an editorial board member of PLoS ONE, and reviews articles for academic journals, including *The Lancet* and *Lancet Infectious Diseases.* He has a bachelor's degree (first class) in natural sciences from the University of Cambridge, a clinical medicine degree from the University of Oxford, and a Ph.D. in malaria immunology from the Institute of Molecular Medicine, Oxford.

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

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Abstract

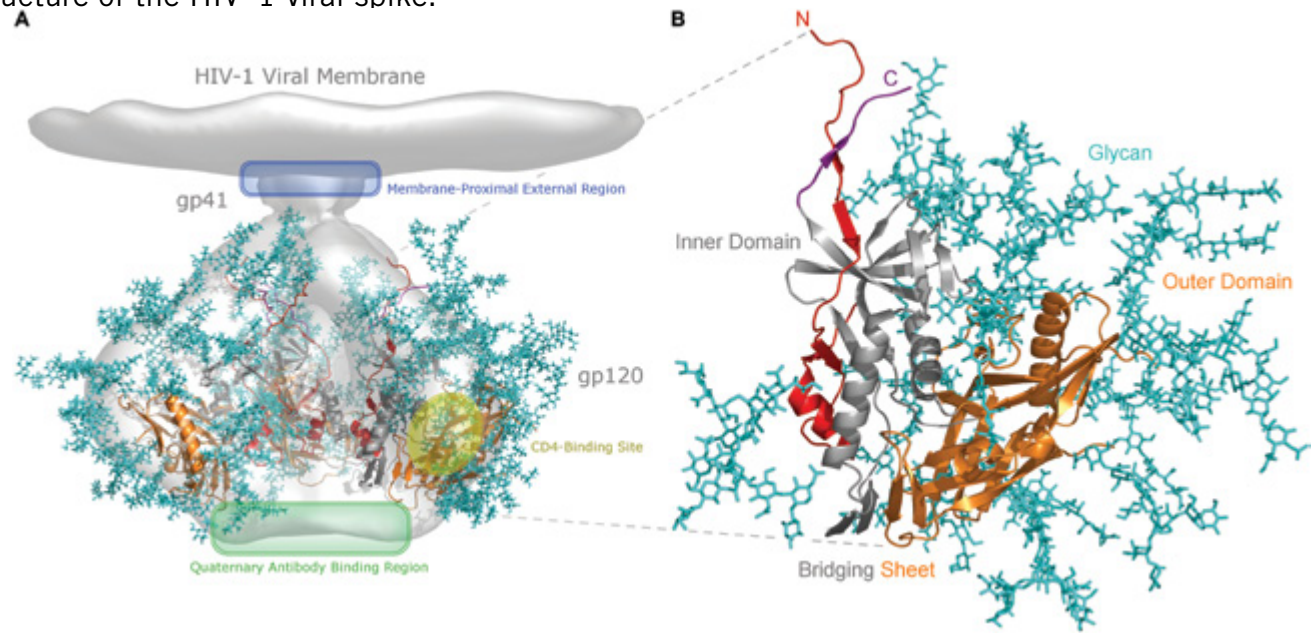
The successful development of an effective vaccine against HIV–1 will likely require novel approaches to vaccine design. At the Vaccine Research Center (VRC), part of the National Institute of Allergy and Infectious Diseases,, we have sought to harness structural biology and other informatics-related 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

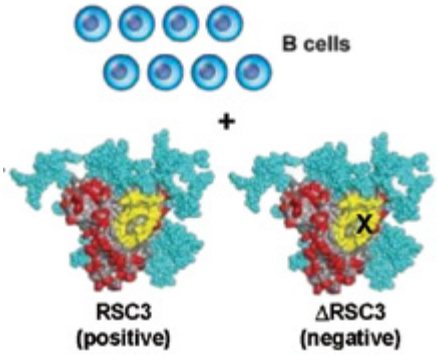
HIV–1 is an enveloped virus, with a host-derived lipid membrane that surrounds the viral core structural proteins. The only viral proteins that protrude through the protective lipid are the gp120-envelope and the gp41-transmembrane glycoproteins (Figure 1). Both are targets of neutralizing antibody, 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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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

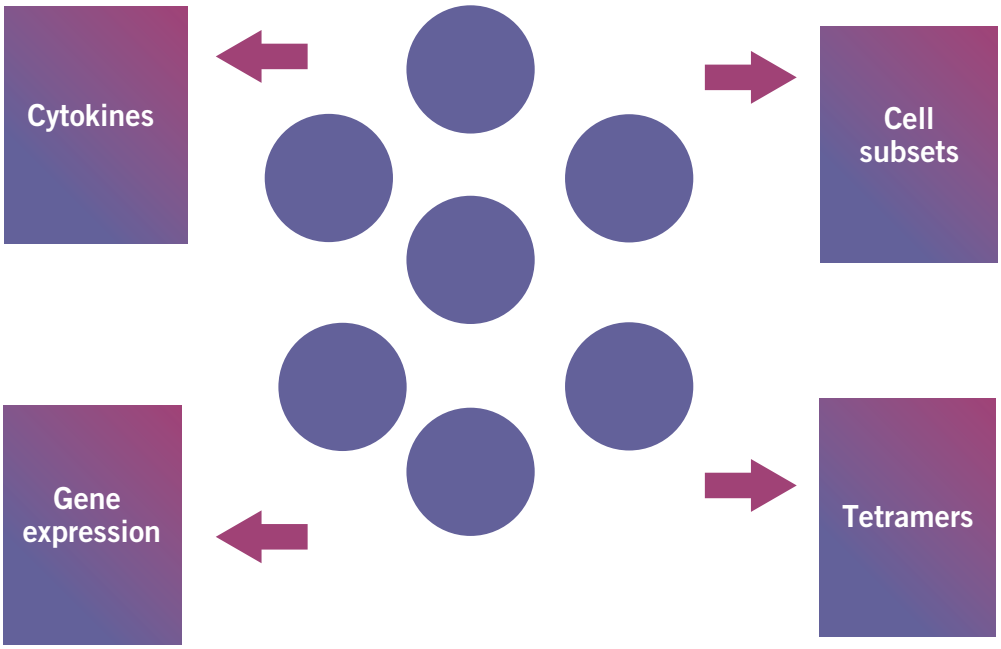
The immune system consists of a dozen or more different types of cells in the blood, lymph nodes, and spleen that respond in different ways to foreign entities and communicate with each other through a series of secreted factors or cell-surface molecules. These cells are known as white blood cells to 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

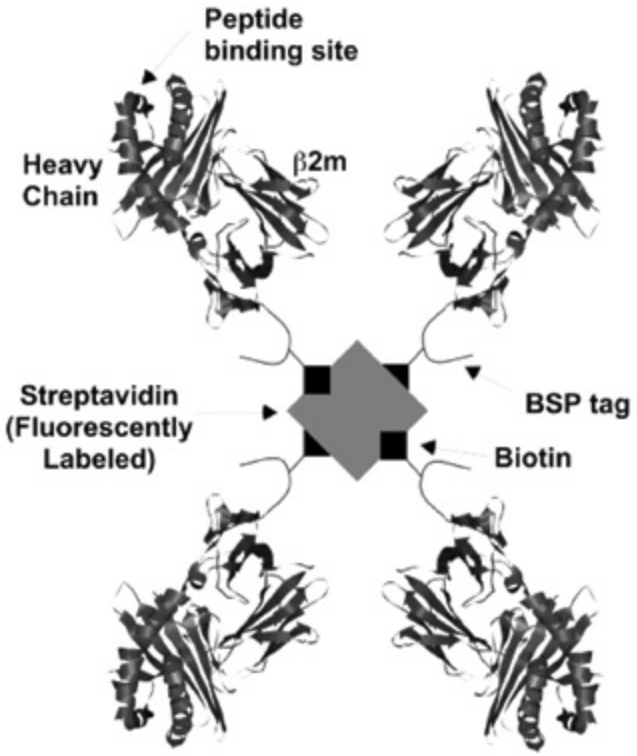
Cells of the immune system can express any of the 350 known cell-surface molecules, called CD antigens, or secrete one or more of 100+ possible cytokines. The fluorescence-based flow cytometer can catalog many of these molecules, and the new mass spectrometry–based machine, which uses lanthanide metal 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.

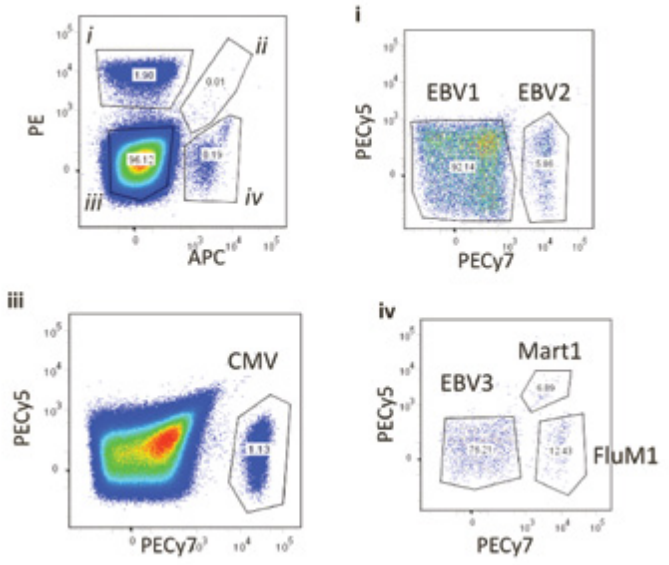
New Developments in Tetramers

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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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.

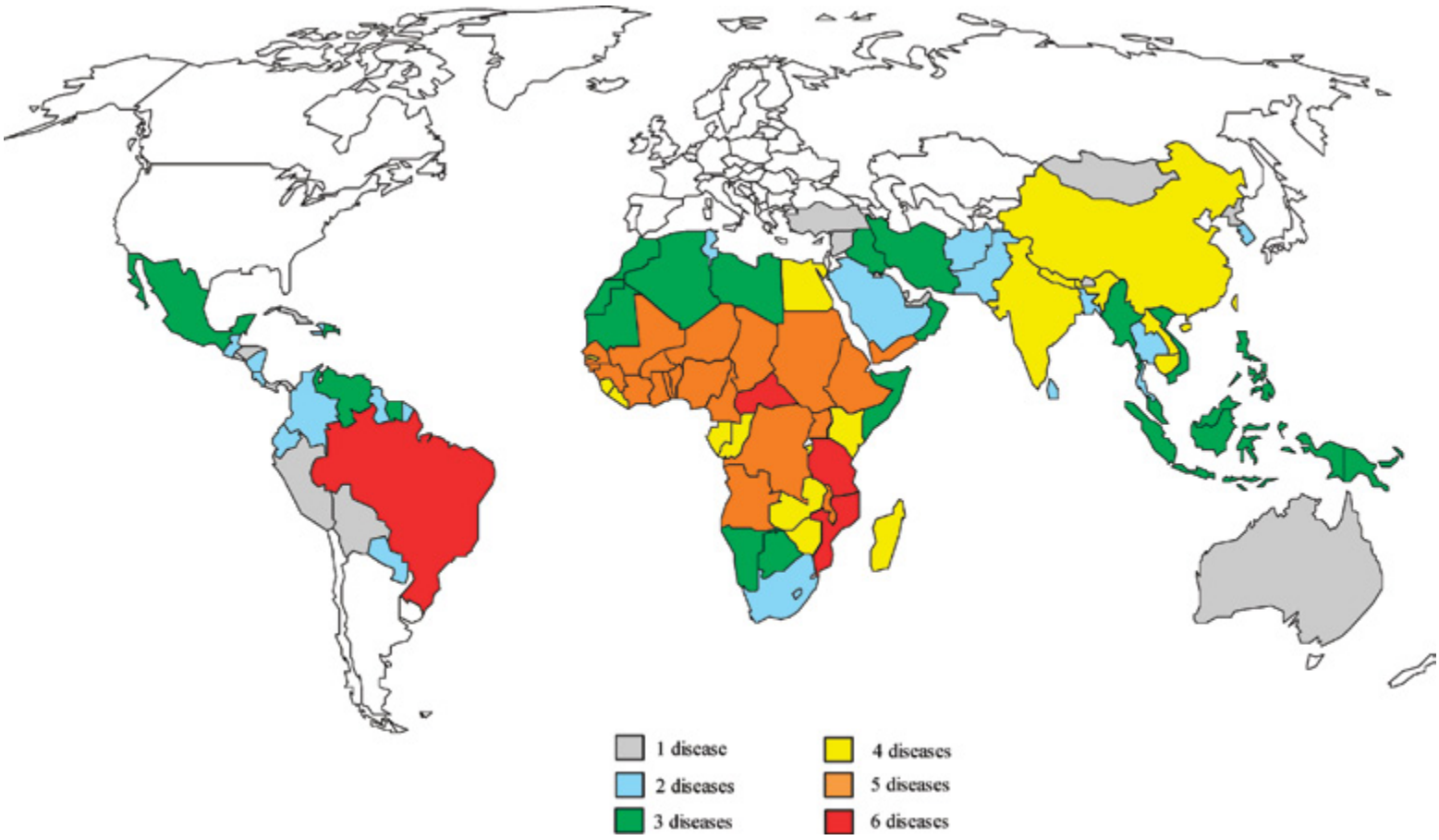


TABLE 1.

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

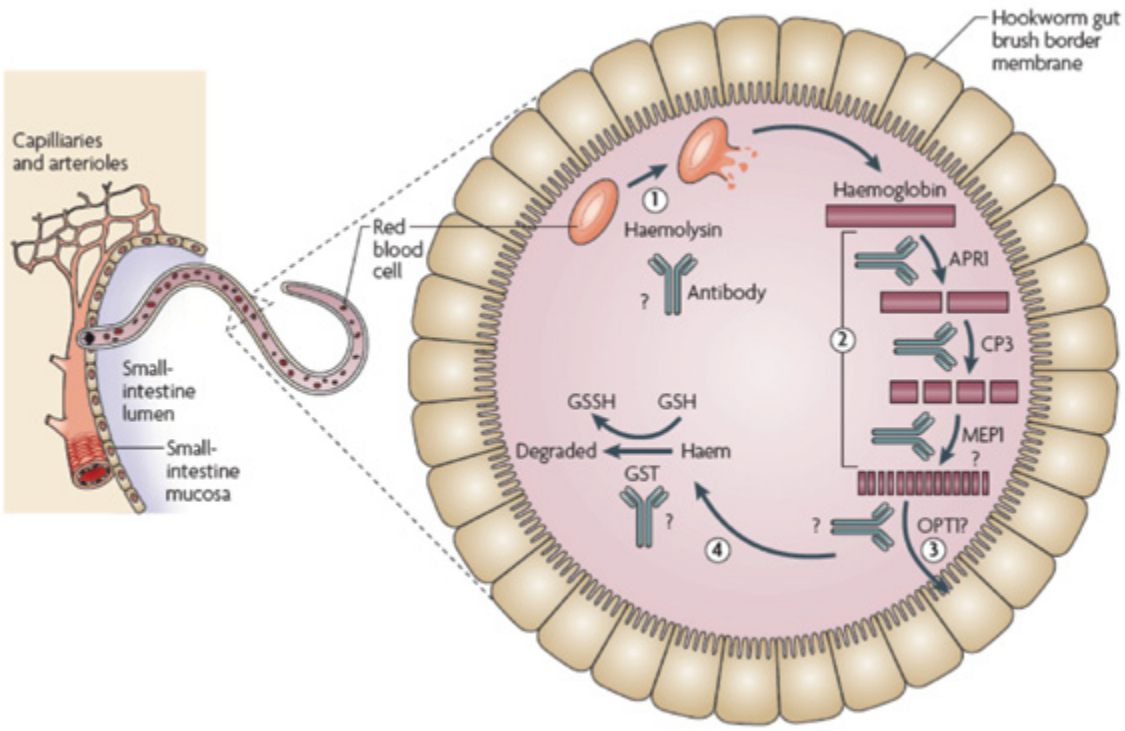
Na-GST–1 and *Na*-APR–1 are the lead hookworm vaccine antigens that have been selected for clinical development based on criteria such as efficacy in animal trials, data from epidemiological studies in individuals resident in endemic areas, and the feasibility of protein expression and manufacture using 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

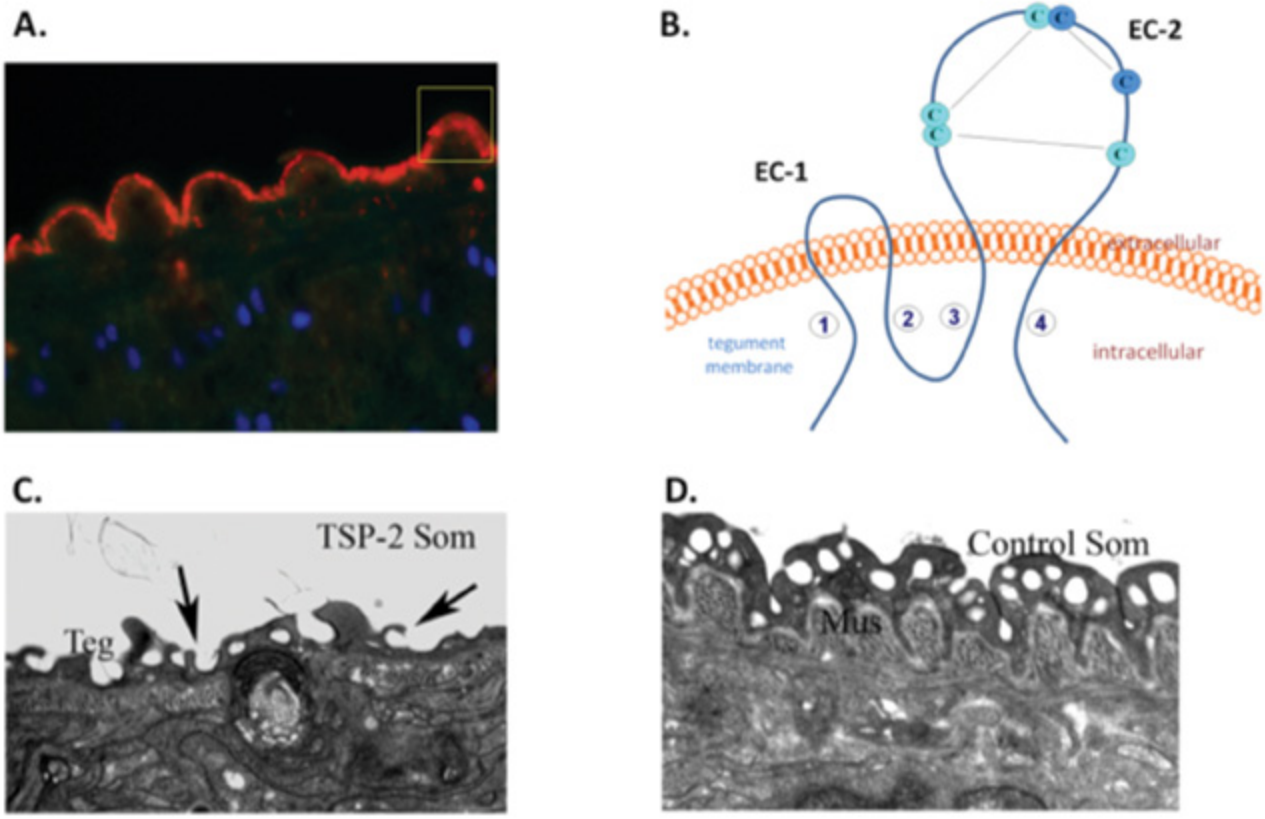
Unlike with hookworm infection, individuals residing in endemic areas can become resistant or partially immune to re-infection with schistosomiasis over time [72]. Furthermore, irradiated cercariae can elicit high levels of protective immunity in laboratory animals, and several recombinant protein vaccines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

Scott K. Fridkin, M.D. and John A. Jernigan, M.D., M.S.

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

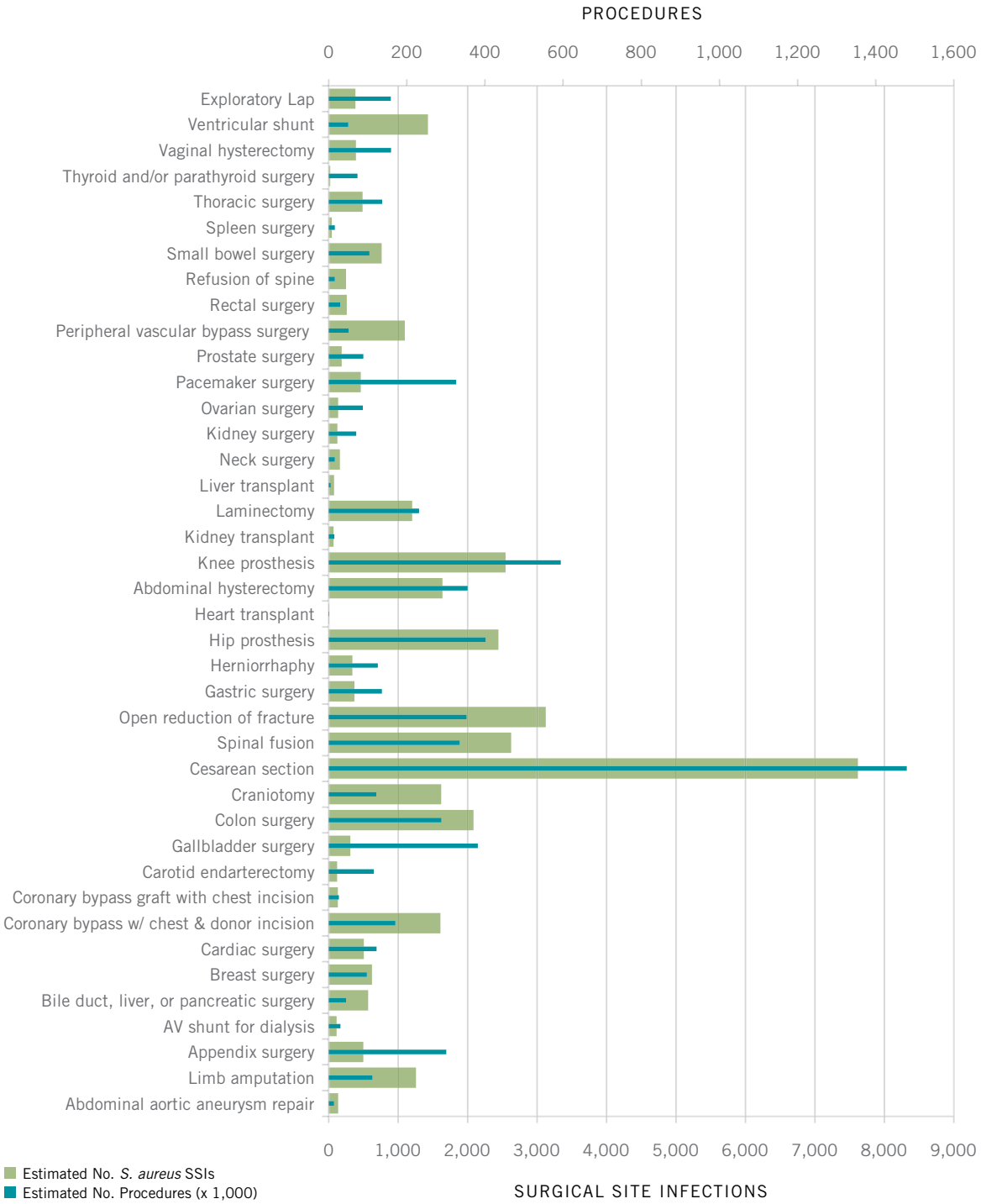
Hidden within these large population estimates are groups of people who share characteristics placing them at high risk for severe infections with *S. aureus*. Identifying these populations is critical to outlining a vaccine prevention strategy. Hemodialysis patients are known to be at highest risk of 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

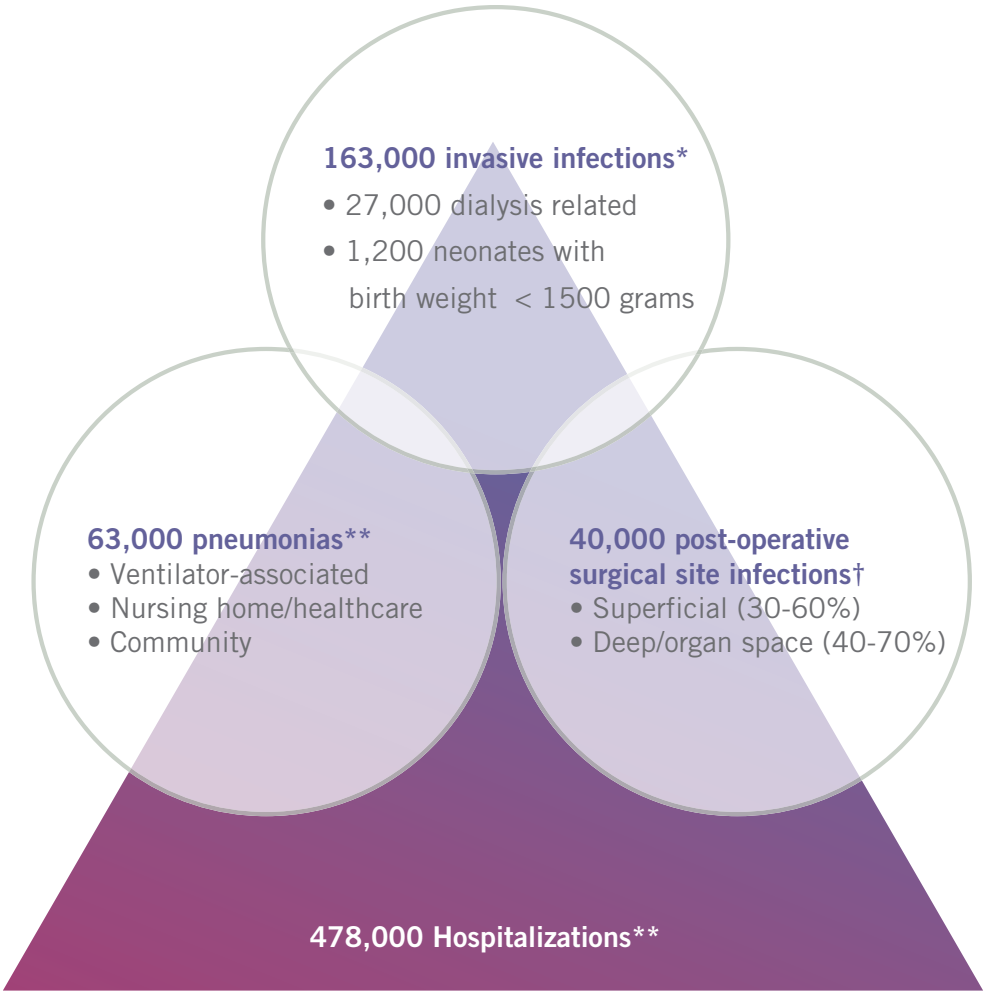
Although certain high-risk patient populations would likely benefit from an effective *S. aureus* vaccine, to have a more substantial impact on the national burden of invasive *S. aureus* infections, more comprehensive vaccination strategies are worth exploring. We recently performed an exploratory analysis 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.

Klein E, Smith DL, Laxminarayan R. Hospitalizations and deaths caused by methicillin-resistant Staphylococcus aureus, United States, 1999-2005. Emerg Infect Dis. 2007 Dec;13(12):1840-6.

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* Kallen AJ, Mu Y, Bulens S, Reingold A, Petit S, Gershman K, et al. Health care-associated invasive MRSA infections, 2005-2008. JAMA. 2010 Aug 11;304(6):641-8.

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† 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.

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

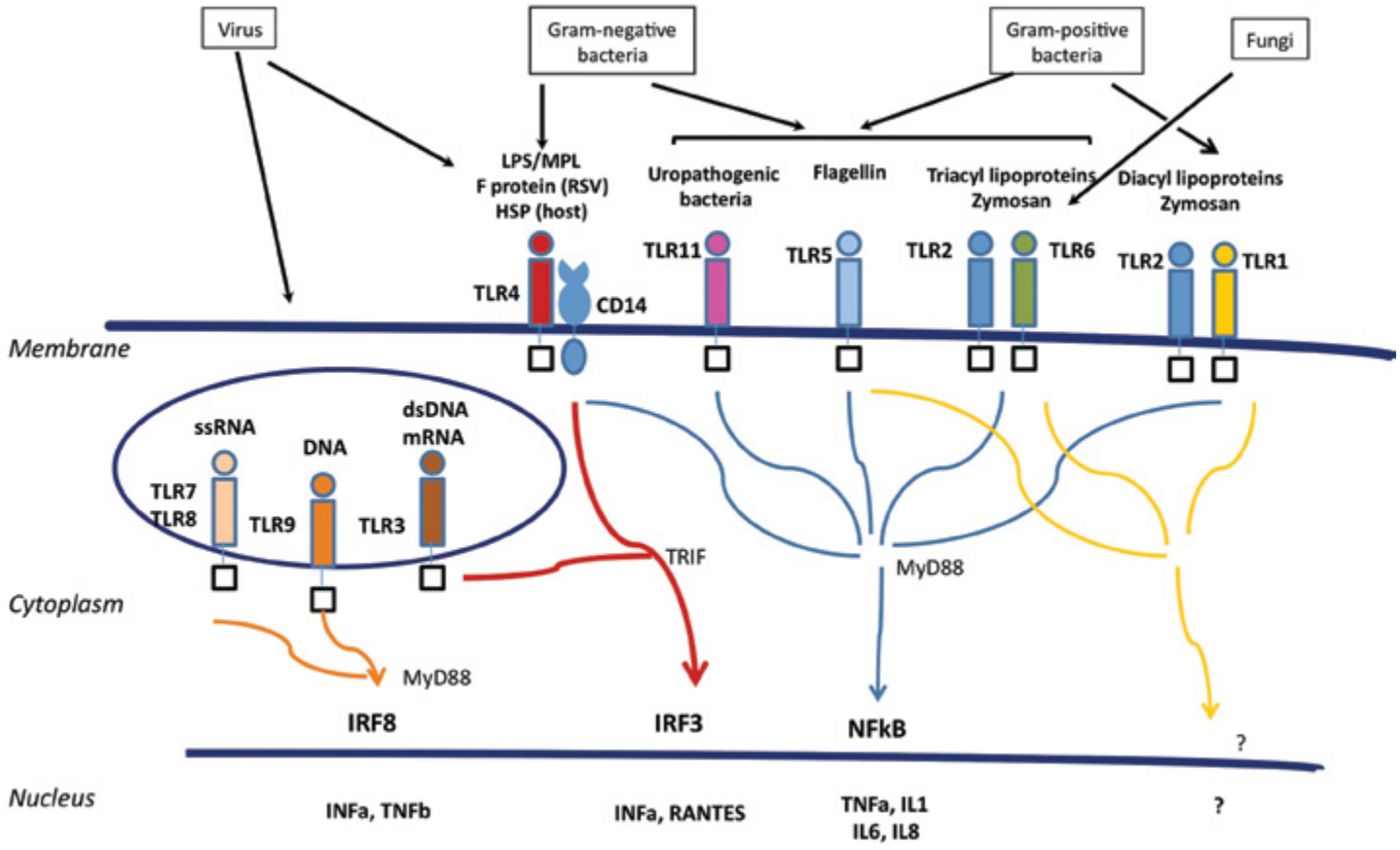
Virosomes are reconstituted viral envelopes that display desired vaccine antigens but lack the viral genome. Their mode of action has been described as being through endosomal fusogenic properties that enable them to present antigens in the cytosol in the context of the MHC class I antigen presentation 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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She then moved to the United States, where she spent 4 years at the Baylor College of Medicine in Houston, first as a postdoctoral research fellow, then as an assistant professor, working on vaccine delivery systems and immunopotentiators.

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.

Dr. Gellin has had broad experience in public health aspects of infectious diseases and has held positions at the National Institute of Allergy and Infectious Diseases (part of the National Institutes of Health), the Centers for Disease Control and Prevention, the Rockefeller Foundation, and the Johns Hopkins Bloomberg School of Public Health. In addition, he has been a regular consultant to the World Health Organization. He is board certified in internal medicine and infectious diseases and is currently on the faculty at George Washington University School of Medicine and Vanderbilt University Schools of Medicine and Nursing.

Dr. Gellin is a graduate of the University of North Carolina (Morehead Scholar), Cornell University Medical College, and the Columbia University School of Public Health. He is an infectious disease expert with training in epidemiology. He has written extensively about public health aspects of infectious diseases in medical and nonmedical texts and the peer-reviewed medical literature and also has served as a medical advisor to Encyclopedia Britannica.

Sarah R. Landry, M.A.

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Prior to joining NVPO in May 2010, Ms. Landry was Director of the Office of Program Planning, Operations, and Scientific Information at the Division of Allergy, Immunology, and Transplantation of the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health. She was responsible for oversight and management of her division's strategic planning, including budgeting, communications, and advocacy efforts, as well as a variety of programmatic planning and grants management activities. Ms. Landry worked extensively in infectious diseases, including HIV/AIDS, during her 16-year career at NIAID.

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.