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# MINIREVIEW

# Vaccines: All Things Considered

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This minireview is based on the diverse discussions of vaccine development presented during the GTCBIO Third Annual Conference on Vaccines: All Things Considered (3 to 4 November 2005, Arlington, Va.). As the name implies, the meeting provided an excellent overview of the concepts and concerns for vaccine developers and the vaccine industry and was relevant to individuals in academia, industry, regulatory agencies, implementation, military, government, and physicians. This minireview is divided into the categories that the keynote speaker, Michel Klein (Canadian Network for Vaccines and Immunotherapeutics, Université de Montreal), indicated to be the basis for new vaccine development: (i) biological basis for vaccine development, (ii) new technologies, (iii) new targets, (iv) bringing a vaccine to market, and (v) current issues in vaccine development.

Immunization programs have led to the elimination and/or control of several different infectious diseases, including smallpox, polio, measles, mumps, rubella, *Haemophilus influenzae* type B disease, pertussis, tetanus, and diphtheria. These vaccines were developed using technology from the 19th and 20th centuries, inactivation by heat, chemicals, and irradiation to produce a killed vaccine, vaccination with a serologically related virus à la Jenner, and attenuation by tissue culture passage to produce live vaccines with substantially reduced virulence. The vaccines of the 21st century will be developed by improvements on these basic techniques and through the use of new technologies based on the expanding understanding of the immune response. New, and still unmet, targets for vaccine development include some of the more difficult infectious agents, such as human immunodeficiency virus (HIV), cytomegalovirus, and severe acute respiratory syndrome coronavirus; bacteria, such as *Pseudomonas aeruginosa*, *Neisseria gonorrhea*, or *Mycobacterium tuberculosis*; and parasitic diseases, such as malaria or hookworm disease. In addition, vaccines will also be developed as therapies against disease for autoimmune diseases, cancer, hypertension, Alzheimer's dementia, contraception, and to promote the cessation of bad habits, such as smoking.

Bioterrorism has brought renewed interest to new and large-scale vaccine development. As Michael Moodie (Chemical and Biological Arms Control Institute) described in "Vaccines and National Security: the Need for a National Strategy," we have seen the evidence with fearsome examples that the threat of bioterrorism can be delivered by individuals or groups with religious, political, or bioterror agendas. Vaccines are an excellent, technically feasible defense against these threats with the potential to limit postattack disease spread. Vaccine readiness can even be considered a deterrent to the development and use of specific bioterror agents, because it reduces the potential for effectiveness and hence decreases its utility as a weapon.

Even though vaccination is probably the most beneficial therapy that a physician can provide a patient, there are still significant roadblocks to the development and licensing of new vaccines. These roadblocks include biological and technological issues, but to a large extent, the major roadblocks are the difficulty in preparing a 100% safe and effective product, the high cost of testing, and the almost unavoidable consequence of the occasional, no matter how infrequent and how minor, adverse event. Development of a vaccine requires more than 500 million dollars, it takes a long time to get through phase III trials, and the profit margin for such an investment is 1/10 or less of that of a successful drug that must be taken on a daily basis, such as a cholesterol-lowering statin derivative. In addition, vaccines are administered to healthy individuals, and any side effects, even if unrelated to the vaccine, make the manufacturer a target for lawsuits. Even beyond development costs, vaccine programs are expensive, vaccines are perishable, and they must be administered by a professional and may not be accepted by the populace without a defined urgent microbial threat. Clearly there is a need for a vaccine strategy that is integrated, sustainable, flexible, and consistent. The challenge will be to develop new vaccines, new mechanisms for evaluating and funding vaccines, and even ways to administer the vaccines.

## BIOLOGICAL BASIS FOR IMMUNE RESPONSE TO A VACCINE

Since the immune response evolved to provide protection against infectious diseases, the optimal development of a protective immune response by a vaccine should mimic the steps and processes elicited during the establishment of natural immunity. In the late part of the 20th century, the T cell was identified as the ultimate controller of the immune response and vaccines were just beginning to be designed to activate T cells. In the 21st century, we recognize the importance of innate responses and especially the dendritic cell (DC) in the optimal development of specific immune responses. The innate and immune responses progress through a series of stages which are orchestrated by the DC, conducted by the T cell and

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appropriate cytokines, and then delivered by activated cells and antibody. DCs are essential for initiating an immune response, presenting antigen to the T cell, and determining the nature of the immune response delivered by the T cells. A better understanding of the biology of activation of DCs, the cytokines produced by DCs, and their mechanisms for antigen presentation has fostered new developments in vaccine design and formulation. Unfortunately, DC biology is still very confusing for several reasons. First, mouse and human DCs are very different with different activation requirements and cell surface receptors. Second, there may be more than five different subsets of DCs based on their surface markers including the two major subsets which can be distinguished by expression of CD11c, the myeloid ($CD11c^+$) and plasmacytoid ($CD11c^-$) dendritic cells (9). The characteristics and development of DCs are discussed to greater extents in other reviews (3, 6, 9, 23, 32, 34, 39).

During a natural infection, the immature DC in the periphery is constantly taking up proteins and surveying the antigenic environment. Upon activation by interaction with structures on a microbial pathogen, the cell is activated to produce cytokines and begins to mature into an antigen-presenting cell (APC). The microbial structures, termed pathogen-associated molecular patterns (PAMPs), are generally repetitive structures including extracellular bacterial structures and nucleic acids. The PAMPs are recognized by proteins, such as Toll-like receptors (TLRs), present on the cell surface, within endocytic vesicles, and in the cytoplasm (1, 18, 25). PAMPs and their receptors, such as lipopolysaccharide (Toll-like receptor 4 [TLR4] and CD14), peptidoglycan (TLR2 and NOD2), flagellin (TLR5), single- and double-stranded RNA (TLR3, -7, and -8), and undermethylated guanosine- and cytosine-containing oligodeoxynucleotides (CpG) (TLR9) are TLR ligands and also potent adjuvants for immune responses. Imiquimod and resiquimod are examples of small-molecule, artificial ligands for TLR7 and TLR8, licensed for antiviral usage against warts and recently recognized as potential adjuvants (15, 46).

Myeloid DCs are the principal antigen-presenting cells and determinants of the nature of the T-cell response to antigen. These cells express TLR1, -2, -3, -4, -5, -6, and -7 and can respond to most PAMPs. Human plasmacytoid DCs express TLR6, -7, -8, and -9 and respond primarily to single- and double-stranded RNA, CpG, and virus infection and produce alpha interferon (20, 21, 27). Both the myeloid and plasmacytoid DCs can respond to imiquimod and resiquimod through TLR7.

The signals from the occupied TLRs initiate the maturation of the DC from a surveyor of the periphery into a cytokine-producing antigen-presenting cell. The mature DC ceases to internalize extracellular proteins, up-regulates expression of molecules involved in antigen presentation, and moves to the lymph node to present antigen to T cells. The nature of the TLR signal will determine which cytokines the DC will produce and hence the type of response the T cell will initiate, Th1 or Th2 (23). A combination of antibody and cellular immunity are produced as part of a Th1-type response (1 = first = early = local = antibody- and cell-mediated responses) which are characterized and directed by interleukin 12 (IL-12) produced by myeloid cells and gamma interferon produced by NK, NKT, and T cells. The alpha interferon produced by immature plasmacytoid dendritic cells in response to herpes simplex virus (HSV) or TLR stimulation can promote Th1 responses either directly or indirectly by stimulating NK cells to produce gamma interferon (20). The combination of antibody- and cell-mediated responses generated by Th1 responses is necessary to control intracellular (viral, bacterial, and parasitic) and fungal infections. A Th2-type response is predominantly an antibody response and will arise in the absence of IL-12 or during parasitic worm infection (3). Th2 responses are directed predominantly by interleukin 4, 5, 10, and 13. Antibody is often sufficient to control viremia, bacteremia, and other extracellular infections. Th1 and Th2 responses are antagonistic, and the cytokines produced by a Th2-type response will prevent the initiation of a Th1-type response. Immunization with a vaccine that establishes a Th2-type response will prevent the development of a Th1-type response, which may even exacerbate the disease following infection (29). This would definitely be the case for a vaccine against *M. tuberculosis*, *Mycobacterium leprae*, or leishmaniasis, for which the Th2 responses are associated with more severe disease (10). Interestingly, the antagonistic nature of the Th1 and Th2 immune responses can also be used to develop a vaccine to curtail aberrant immune responses, as will be described later for myocarditis.

The DC has the capacity to present antigenic peptides from extracellular antigens (e.g., viral or tumor proteins) to T cells on both major histocompatibility complex class I (MHC-I) and MHC-II molecules (6, 32, 34). Peptides (8 amino acids long) degraded by the proteosome and transported into the endoplasmic reticulum (ER) through the transporter associated with processing bind within the pocket at the top of the MHC-I heavy chain. Addition of the beta-2-microglobulin subunit allows the MHC-I molecule to progress through the Golgi apparatus to the cell surface. In contrast, the binding groove at the top of the MHC-II molecule is filled with the invariant chain molecule while it is within the ER to prevent acquisition of an antigenic peptide in the ER. The MHC-II molecule normally receives its peptide for presentation after transport from the ER to an endosome. Peptides from phagocytosed proteins replace the invariant chain, and the newly filled MHC-II molecule is delivered to the cell surface. More-recent findings demonstrate that exogenous peptides can also find their way onto MHC-I molecules by cross presentation pathways (38). Proteins from viral, tumor, apoptotic, and other phagocytosed cells are degraded, leave the endosomal vesicle and then find their way into the endoplasmic reticulum to decorate MHC-I molecules. Mimicking or manipulation of these pathways provides a means for enhancing the immunogenicity of a peptide for a vaccine.

## NEW TECHNOLOGIES

A combination of increased understanding of the immune response and technological advances in molecular biology and instrumentation will be the basis for many new developments in vaccine design. The use of advances in genomics, proteomics, and structural biology will also provide new candidates for peptide vaccines. From the knowledge of the genetic sequence of a bacterial species, the technology is now available to predict appropriate immunogens and make them into vaccines using reverse vaccinology. Researchers at Chiron devel-

oped a new group B *Neisseria meningitidis* vaccine in this manner, and this approach is being used to develop other vaccines (24, 30). Starting with the hypothesis that bacterial cell surface molecules will elicit protective antibody responses, potential immunogens were identified as cell surface molecules from the bacterial genetic sequence in silico (by computer). The surface location of these proteins was confirmed, and then the genes for these proteins were cloned, expressed, and then used in immunoassays of sera obtained during convalescence from infection to verify the immunogenicity of these proteins. Sufficient protein was then produced to immunize animals, and two outer membrane proteins of *N. meningitidis* were demonstrated to induce protection against challenge. Vaccines using these proteins are less type specific than capsular polysaccharide vaccines and do not require conjugation to a carrier protein to elicit complete responses.

**Enhancing with adjuvants.** Better understanding of dendritic cell and T-cell activation and regulation will foster the development and use of new adjuvants, cytokines, chemokines, and costimulatory molecules in vaccine formulations to enhance the immunogenicity and development of memory and direct the type of response elicited by the vaccine. Adjuvants, by definition, enhance the immunogenicity of a vaccine by promoting uptake of the immunogen and activating DCs to initiate the immune response (7, 33, 35). Natural adjuvants include TLR ligands (31) and the cytokines or chemokines produced in response to natural stimulation. Artificial adjuvants enhance the immunogenicity of antigens by activating cytokine responses similar to TLR activation from DCs or promoting uptake of the immunogen. The ideal adjuvant promotes a more natural immune response with less immunogen.

The classical adjuvant for vaccines, and until recently, the only FDA-approved adjuvant, is alum (4). Alum provides a particle upon which the vaccine is precipitated. Although precipitation onto alum promotes uptake of the immunogen, alum is a poor activator of DCs and does not induce the production of IL-12. As a result, alum-based vaccines initiate Th2-type antibody responses. Complete Freund's adjuvant (CFA) is a powerful adjuvant consisting of inactivated bacillus Calmette-Guérin (BCG) (a strain of *Mycobacterium bovis*), and contains a mixture of different TLR ligands in a mineral oil solution. Emulsification of the immunogen in the mineral oil provides a depot for slow antigen release to promote phagocytosis, while BCG is a strong activator of DCs and Th1 responses. CFA is not approved for human usage. Newer adjuvants approved for use in human vaccines include monophosphoryl lipid A (MPL), which includes the endotoxin-like component of bacterial lipopolysaccharide; Montanide ISA51 (Seppic), which consists of a well-characterized mixture of the oil-like components of complete Freund's adjuvant without BCG; and MF59, which consists of squalene microfluidized in an oil and water emulsion. QS21 and other saponin derivatives have also been used extensively as Th1-promoting adjuvants. These adjuvants have some of the activities represented in CFA and with greater safety. Combinations of alum and other adjuvants, such as MF59, QS21, or MPL, can enhance the immunogenicity of a vaccine. The combination of alum and MPL (AS04) was used for the human papillomavirus (HPV) virus-like particle (VLP) vaccine (17) and herpes simplex virus glycoprotein D subunit vaccines developed by GlaxoSmithKline (43).

Jeffrey Ulmer (Chiron) described several different approaches that Chiron has taken to develop new types of adjuvants, in addition to their MF59 adjuvant. On the basis of the discovery that small molecules, such as imiquimod, can bind and activate TLRs, they initiated large-scale in vitro screening procedures to discover small-molecule immune potentiators as possible adjuvants. Their screening procedures are based on the ability of the molecule to promote tumor necrosis factor alpha production from spleen cell preparations. This procedure has already identified several candidate adjuvants.

**Taking advantage of antigen presentation.** Although peptides can be antigenic, their small size often limits their ability to initiate an immune response. Classically, peptides would be attached to larger protein carriers, such as keyhole limpet hemocyanin, to increase their visibility to DCs. However, this incorporates additional epitopes from the carrier into the immunogen, and the response is usually a Th2-directed antibody response to the peptide of interest. Several approaches were discussed at the meeting that promote the immunogenicity of peptides by manipulating their interactions with MHC molecules, T-cell receptors, and DCs.

Dan Zimmerman (CEL-SCI Corp.) and Ken S. Rosenthal (Northeastern Ohio Universities College of Medicine) described the L.E.A.P.S. (ligand epitope antigen presentation system) technology for converting small peptides into immunogens (13, 14, 36, 37, 47). This technology converts epitope-containing peptides that are too small to elicit an immune response into immunogens. In addition, the technology can also determine the type of immune response, Th1 or Th2, that will be initiated by the LEAPS vaccine. The LEAPS approach utilizes small peptides (approximately 18 to 20 amino acids) obtained from MHC-I, MHC-II, or other molecules that are immune cell binding ligands (ICBL) to facilitate immunogen interaction with MHC molecules and the T-cell receptor. The ICBL is covalently attached through a triglycine linker to the epitope-containing peptide. Vaccines using this technology have been prepared with peptides from *M. tuberculosis*, *Plasmodium* species, and herpes simplex virus. The G ICBL is a 15-amino-acid peptide from the beta chain of MHC-II which will promote Th2-type responses to the attached peptide. The J ICBL is a 13-amino-acid peptide obtained from beta-2-microglobulin and upon covalent attachment, will promote Th1-type responses to an epitope. Unlike large protein carriers, no detectable immune response to the J or G ICBL peptide can be detected. Protective immunity was elicited in mice by attachment of the J ICBL to epitopes as small as 8 amino acids from the HSV proteins ICP27, glycoprotein B, and glycoprotein D. These vaccines elicited T-cell responses that were sufficient for protection. The J-ICBL-based vaccines appear to activate T cells, and production of antibody to the epitope is observed only upon antigenic or infectious challenge in a prime-boost type manner. Some of the G-ICBL-based LEAPS vaccines elicit antibody responses without the need for a boost but were not protective against HSV.

Daniela Cihakova (Johns Hopkins School of Medicine) reported that a LEAPS vaccine can also be used to manipulate the immune response to prevent and treat experimental autoimmune myocarditis, a Th2 immune response-mediated disease. Immunization with a myosin-derived peptide attached to the J ICBL elicited a Th1 response and significantly reduced

the incidence and severity of myocarditis. The LEAPS approach may be useful to modulate other immune diseases.

Robert Humphreys (Generex) described two approaches to enhancing immunogenicity by manipulating the interaction of an antigenic peptide with MHC-II molecules (22). Through extensive analysis of the interaction of the invariant chain with the MHC-II molecule, a four-amino-acid peptide, named IiKey, which binds and opens the groove of the MHC-II molecule to accept the peptide, was identified. Attachment of an epitope to IiKey through a three-amino-acid spacer creates a peptide that will open the groove on MHC-II molecules that are on the surface of an APC and promotes the binding of the peptide epitope within the groove. The APC can then present the tethered antigen to T cells. The immunization can be performed using peptides or as a DNA vaccine that expresses the peptide sequence for an IiKey epitope vaccine. Vaccines to influenza virus epitopes, including epitopes from H5N1 viruses, were developed using this technology. In another approach, the expression of the invariant chain in DCs was suppressed with antisense RNA technology (small interfering RNA) to allow MHC-II molecules to acquire an antigenic peptide in the endoplasmic reticulum, and like MHC-I molecules, display them at the cell surface (17, 40). When given with a DNA vaccine for a viral or tumor peptide, the small interfering RNA for the invariant chain can enhance the T-cell-mediated response by allowing the APC to present the same antigenic peptides to both CD8 T cells (through MHC-I molecules) and also to CD4 T cells (through MHC-II molecules) (19, 45).

Recognizing that the DC is the ultimate APC and that appropriate stimulation of the DC determines the nature of subsequent immune responses, Brian Czerniecki (University of Pennsylvania) described a very exciting approach that he and colleagues have developed for a DC-based anti-breast tumor vaccine. Their vaccine utilizes DCs that were generated rapidly and efficiently from autologous monocytes. Depending upon the stimuli, the monocytes can be converted into Th1-promoting DCs (DC1) or Th2-promoting DCs (DC2). DC1 cells can be generated by maturation of monocytes to DCs in the presence of gamma interferon or a ligand of TLR8, such as resiquimod. They demonstrated that optimal stimulation of antitumor T cells requires DC1 cells. In early clinical trials, immunization with in vitro-generated DC1 cells incubated with HER-2/neu promoted T-cell responses to the tumor cells and clinical evidence of antitumor responses in the breast (2, 26).

**Size matters.** Particles of the size of microbes are preferentially taken up by DCs and macrophages. Going beyond the alum concept, Chiron developed microparticles of chemically modified poly(lactide-*co*-glycolide) (PLG) microparticles with either a negative or positive surface charge to carry proteins or DNA, respectively (42). These particles are 1 μm in diameter, the approximate size of a bacterium. The potency of the protein-decorated particles was enhanced further with the coadministration of CpG oligodeoxynucleotide, a potent TLR7 activator. For example, the immunogenicity of the outer membrane protein of *Neisseria meningitidis* was enhanced by adhesion to the positively charged particle, but coadministration of CpG oligodeoxynucleotide significantly boosted the response. The antibody production to the PLG-meningitis B protein (287) plus CpG vaccine was 2 times greater than that for 287 in Freund's adjuvant, 30 times more than that for PLG-287 alone, 100 times more than that for CpG plus 287, and 50 times more than that for 287 adsorbed to alum (41).

The positively charged PLG particles were used to enhance the activity of DNA vaccines. Immunization with PLG-hepatitis C virus DNA and PLG-HIV DNA vaccines generated thousand-fold-higher antibody titers with fewer immunizations than DNA alone did (40).

Using the particle approach, Ronald Ellis (ID Biomedical) described the development of a noninfectious particle-based influenza vaccine that can be administered as an aerosol. The intranasal proteosome influenza vaccine (FluINsure) incorporates influenza virus hemagglutinin and neuraminidase proteins into particles containing *Neisseria meningitidis* outer membrane protein preparations. The outer membrane protein acts as an adjuvant, and the particulate form of the vaccine enhances its uptake and immunogenicity. Single doses of these vaccines were effective at eliciting mucosal secretory immunoglobulin A and protection from influenza virus challenge in human volunteers. This aerosol-administered influenza vaccine offers a straightforward approach to customizing the synthesis of the annual influenza vaccine.

Anne Schuind (GlaxoSmithKline) described the development and clinical trials of their VLP-based divalent vaccine against the human cervical carcinoma-associated papillomavirus strains, HPV16 and HPV18. They took advantage of Mother Nature by letting the genetically engineered and in vitro-produced L1 large capsid protein of both HPV types self-assemble into VLPs of ~30 nm. The VLP is readily taken up by DCs and macrophages, and this enhances the immunogenicity of the viral proteins. Women ($n$ = 1,113) between 15 and 25 years of age and receiving three doses of a bivalent vaccine consisting of VLPs from HPV16 and HPV18 in their AS04 proprietary adjuvant were protected from acquisition of HPV disease (91%) or persistent HPV disease (100%) (17). Although it was not presented at this meeting, Merck has developed a similar vaccine, which received FDA approval in June 2006. Prevention of infection by HPV16 and HPV18 should prevent most cervical cancers.

The VLP can also be modified to generate protective antibodies to other diseases. Martin Bachman (Cytos Biotechnology) discussed the use of chemically modified VLPs to make vaccine-induced therapies for smoking and hypertension. By chemically affixing nicotine onto the surface of bacteriophage Qβ, an immunogen that is very stable and is easy and inexpensive to make was developed. The nicotine-modified Qβ elicits a neutralizing antibody that inhibits the uptake of nicotine by the brain, which limits the reward from smoking. The results of a phase 2 study indicate a successful reduction in smoking for individuals who have developed high titers of antinicotine antibodies in their blood in response to the vaccine. The same technology can be applied to develop antibody-mediated therapies to other diseases. Initial work has begun with a vaccine to prevent hypertension using a Qβ modified with angiotensin-II.

## NEW TARGETS (AND RENEWED OLD TARGETS)

Although it sometimes seems that all of the easy vaccines have already been developed, new understanding of the microbiology and immunology of pathogens and new technology

are providing opportunities to develop vaccines against pathogens that have eluded vaccine control. In addition to HIV, there is opportunity for developing new vaccines and immunization programs for diseases of the developed world and even the more challenging targets that are prevalent in underdeveloped countries, such as Ebola fever, dengue, and hookworm disease. Careful analysis of the disease patterns of the populace can also point out the need for new immunization programs using modifications of established vaccines.

Respiratory syncytial virus (RSV) has long been a prospective target for vaccine development. Developing a vaccine against RSV has been a challenge, because antibody is insufficient for protection and inactivated vaccines that generate a predominantly antibody response (Th2) can promote exaggerated disease. An early formalin-inactivated alum-precipitated vaccine enhanced disease, and temperature-sensitive live-attenuated intranasal vaccines were ineffective. Jonathan Klein-Evans (MedImmune Inc.) very effectively described several different approaches that have been taken towards an RSV vaccine. These include subunit vaccines with purified viral glycoproteins, a polypeptide vaccine, DNA vaccines expressing the F and G glycoproteins of the virus, and live virus vectors including vaccinia virus, bovine parainfluenza virus, and adenovirus which express the F and G glycoproteins (11). MedImmune Inc. developed a cold-passaged temperature-sensitive attenuated viral vaccine by passage of RSV at temperatures less than 32°C. This virus can establish upper respiratory infections but cannot replicate in the warmer environment of the lungs. Phase I/II trials of this vaccine have been promising. He stressed the importance of keeping the patent lawyers, like himself, involved in the vaccine development process to secure the company's investment in new technologies (12).

Although the use of adenovirus as a platform for developing vaccines against different viruses was initially developed as a way to provide an antigenic boost to the priming of immune responses elicited by a DNA vaccine, John Dong (GenPhar, Inc.) described the use of adenovirus as a platform for developing vaccines against different viruses, including hepatitis B, HIV, Marburg, Ebola, and dengue viruses. They have developed an adenovirus strain 5 vector that can be genetically modified to include genes from other viruses or immunogens. Injection or aerosol administration of high doses of a mixture of adenoviruses expressing one or more viral antigenic proteins can be administered alone or supplemented with adenoviruses expressing cytokines, such as IL-2, gamma interferon, or granulocyte-macrophage colony-stimulating factor. Sufficiently high doses of these vaccines can develop appropriate antibody- and cell-mediated responses consistent with protection. Administration of high titers of the adenovirus-based vaccine is the key to generating the protective responses. Rhesus monkeys injected with an HIV vaccine elicit high titers of antibody to the envelope protein. Similarly, vaccines for Marburg, Ebola, or dengue virus promoted antibody- and cell-mediated responses (44).

Although vaccine development is motivated by its benefit to mankind, the primary drive remains profit, since vaccines are usually made by pharmaceutical companies. Maria Elena Bottazzi described the work of the Human Hookworm Vaccine Initiative (HHVI) which is working on developing and delivering a recombinant vaccine for treating and preventing hookworm-induced malnutrition and anemia. Hookworm disease is one of three major soil-transmitted helminth infections with a prevalence of 740 million people and 65,000 deaths per year. Currently, the infection is treated with mebendazole or albendazole, drugs that affect the adult worm but not the larva and do not prevent the very high rate of reinfection. Despite the great benefit that such a vaccine would provide, the lack of a commercial market for such a vaccine required that charitable or governmental funds and the facilities of a nonprofit, government, or academic institution be utilized for its development. HHVI is a public-private partnership centered at George Washington University with The Oswaldo Cruz Foundation in Brazil and the London School of Hygiene and Tropical Medicine, sponsored by the Sabin Vaccine Institute with major funding from the Bill & Melinda Gates Foundation. HHVI had to overcome many challenges in the development of the first antiparasite vaccine, including identifying an appropriate antigen, cloning and expressing the antigen, demonstrating efficacy for the vaccine, and developing good manufacturing practice methods for vaccine production (16). The ancylostoma-secreted protein-2 (ASP-2) of the larva was chosen as the target for vaccine development, since antibodies to the protein inhibit larval invasion in in vitro studies, which will prevent or reduce the potential for future infection. The gene for ASP-2 from *Necator americanus* was cloned, expressed, and secreted by *Pichia pastoris*, large-scale production methods were developed, and the vaccine protein was purified by ion-exchange chromatography and adsorbed to Alhydrogel. Vaccination is administered within 3 weeks of drug-induced deworming. Human trials have already begun. The pathway for development, clinical evaluation, regulatory approval pathway, licensure, and distribution of the hookworm vaccine will serve as a model and example for the development of other important but potentially unprofitable vaccines. Most importantly, HHVI has demonstrated that development of such a vaccine can be "pulled off" within an academic setting. As Nelson Mandela has said, "Life or death for a young child too often depends on whether he is born in a country where the vaccines are available or not. The issue is of fundamental fairness."

New vaccine programs for varicella-zoster virus (VZV) and pertussis resulted from reevaluation of the immune status and disease prevalence in older individuals. As will be discussed at the 2006 meeting, a modified formulation of the children's varicella-zoster virus vaccine has been developed for administration to adults to boost immunity and prevent zoster (shingles). Martin Wasserman (GlaxoSmithKline) described how careful review of CDC statistics indicated that the immune response to pertussis elicited by the inactivated diphtheria-pertussis-tetanus (DPT) vaccine administered to infants may dissipate over time, putting teens at risk to infection and disease. Adolescents are also the "at-risk" group for fatalities from meningococcal disease. By recommending the newly developed booster Tdap (combination tetanus, diphtheria, and acellular pertussis vaccine) (5) and the new meningococcal conjugate vaccine for teens, the risk for both of these diseases is minimized. The current recommendations of the Advisory Committee on Immunization Practices for vaccines for teenagers includes boosters for tetanus and diphtheria and "catch-up" vaccines for hepatitis B, measles-mumps-rubella (MMR), and varicella. In 2000, 35 million teenagers were missing at

least one of the recommended vaccines. Reformulation and development of combinations of vaccines can streamline vaccine administration and increase utilization. To quote the Advisory Committee on Immunization Practices, an added bonus to a "strong adolescent immunization platform is that it can serve as a driver to enhance prevention and improve adolescent health care" by promoting a visit to the physician.

**Bringing a vaccine to market.** The challenge for the 21st century will be to develop approaches to vaccine production that retain the safety, reliability, and reproducibility of current methods but add adaptability, ease of scale-up, speed, and lower cost. New advances in technology can provide new approaches to increase the efficiency of development, manufacturing, and testing of vaccines, and time equals money. All aspects of vaccine production and evaluation must meet with the highest standards. The FDA provides guidelines and oversight towards maintaining these standards.

Development of a new vaccine or even a change in procedures requires validation and FDA review. George Robertson (DOR BioPharma, Inc.), drawing from his experience in quality control labs in the Army and at Merck and Wyeth, stressed the importance of established and dependable manufacturing and laboratory practices. Vaccine development procedures should follow GDP (good development practices), a combination of GLP (good laboratory practices), GMP (good manufacturing practices), and GCSP (good common sense practices). Manufacture of vaccines requires established, validated equipment, validated procedures, and even validated individuals (to carry out the procedures) at all stages of the development process to ensure that the vaccine product remains the same and retains high quality throughout. Validation of laboratory procedures is an important part of this process. Each assay should be validated with respect to its qualifications. As George Robertson said "accuracy, precision and specificity, as well as its limits of detection, quantitation, linearity, range and robustness (reproducibility over time)." Once developed and validated, any change in a raw material, procedure, system, or personnel requires revalidation and represents a major expenditure. These concerns are valid for production procedures and analytical procedures. Development of systems and standard operating procedures for "change control" becomes necessary to promote stability, reduce costs, and ensure quality.

The seasonal and pandemic influenza vaccines are special cases for the vaccine industry due to the need for new vaccines. Norman Baylor (Director of the Office of Vaccines Research and Review of the Center for Biologics Evaluation and Research) discussed FDA's approach to meeting the challenge of an influenza pandemic. The FDA is responsible for ensuring the safety of vaccines and assuring the public confidence. They have defined a path for demonstrating vaccine safety, efficacy, quality, and reproducible manufacture. The FDA has started working with industrial partners at earlier stages to facilitate the proper path towards the development and testing process of the vaccine and have developed accelerated mechanisms for approval. In recognition of the special situation for influenza vaccines, especially pandemic flu, the FDA has carefully looked at its legal requirements and developed accelerated pathways to facilitate rapid evaluation and licensure. For example, "clinical data are not required for approval of a change in the virus representation to accommodate the annual change in endemic influenza for licensed manufacturers of inactivated flu vaccine," and a surrogate marker for efficacy can be used to test the annual vaccine, e.g., the hemagglutinin inhibition activity of antihemagglutinin antibody rather than protection in human trials. Use of the surrogate markers shortens the approval time of the vaccine. In addition, FDA views pandemic vaccines made using licensed processes as supplements rather than new licenses—this can speed and reduce the burden and costs of a response to a pandemic influenza outbreak. In summary, the FDA is working with the vaccine industry to ensure the availability of quality influenza vaccine.

Production of the influenza vaccine poses some of the greatest challenges for both production and licensure because it changes annually. Every year many millions of fertilized chicken eggs are utilized in the production of the annual influenza vaccines. Unfortunately, our dependency on egg-based vaccines puts the vaccine supply at risk for problems with the supply of eggs (e.g., adequate advanced planning, a sufficient number of hens, and susceptibility of the hens to disease) and the number of facilities capable of production. In addition, the egg-based manufacturing procedures are labor-intensive and take up large amounts of space and cannot be performed under stringent biosafety conditions, such as for biosafety level 3. The avian H5N1 flu outbreak creates an additional dilemma, because it puts the laying hens at risk for infection and hence jeopardizes the supply of eggs for vaccine production. Peter Khoury (Baxter International) described their efforts for developing tissue culture-based vaccines for influenza and other vaccines. Choice of the cell line and growth methods is determined by the growth characteristics, availability, lack of adventitious agents, and yield of virus and the engineering aspects related to suspension growth or growth on microcarriers. Baxter International chose to develop procedures for growing large quantities of Vero cells, African green monkey kidney cells, on microcarriers in serum-free medium in bioreactors for influenza vaccine production. Interestingly, Vin Singh and Brian Douglass (ATCC) described their efforts at making available earlier passages of Vero cells for vaccine production. In comparison to egg-based vaccines, the Vero cell vaccine can be brought online quicker, with less planning, and with less biosafety concerns for working with virulent seed viruses, since cell culture is performed under closed conditions. Vero cells are already being used to grow inactivated rabies and polio vaccines and can also be used to grow viruses for other vaccines, including West Nile encephalitis and severe acute respiratory syndrome coronaviruses. Baxter International and Chiron have both been awarded contracts for cell culture-based vaccine production from the National Health Service in the United Kingdom. On 4 May 2006, the U.S. Congress divided a $1 billion award among GlaxoSmithKline, MedImmune Inc., Novartis Vaccines and Diagnostics, DynPort Vaccine Co., and Solvay Pharmaceuticals Inc. to develop cell culture-based influenza vaccines. Baxter International has also developed an automated robotic procedure to harvest cells from fertilized eggs and make chicken embryo cell aggregates. This alternative can be handled under tissue culture conditions, like the Vero cells, for producing vaccines to significant pathogens. This system is being used to produce vaccinia virus and a vaccine against tick-borne encephalitis virus. Switching to tissue culture cell-based vaccines requires considerable valida-

tion, testing, and approvals from the FDA before a tissue culture cell-based vaccine can be utilized to make a vaccine for the populace, but its many advantages over the fertilized egg method may provide the impetus to make the switch.

## CURRENT ISSUES IN VACCINE DEVELOPMENT

**Money, money, and legal issues.** The challenge of the 21st century will be to find the money to fund the development of new vaccines and their distribution to the populations in need. It takes a lot of money and time, and time is money to develop, produce and test a vaccine before it can be brought to the market. The classical route towards funding the development of a new company progresses through a series of steps starting with "maxing" out the credit cards, approaching the FFF group (friends, family, and fools), and finding "angels" to invest in the idea until venture capital can be attracted. Once the business is established, then the usual sources of funding for vaccine development are investment companies, the stock market, corporate partners in "big pharma," and Uncle Sam. At each stage of investment, the inventor's share and control in the endeavor become diluted into the investor pool. Michael Salgaller (Toucan Capital Corp.) noted that investors see the vaccine industry as plagued by particularly slow, risky, and expensive development stages towards licensure and marketing. As a result, much of the funds for vaccine development is from the government.

Jill Hackel (Wyeth Vaccines) described the issues that must be considered in the development of a vaccine beyond manufacturing, and again, they boil down to time and money. The amount of time required for each stage of vaccine development is increasing, and the probability of success at each phase of development is decreasing, resulting in higher risk and higher cost to a pharmaceutical company with an increasingly limited potential for profit. The cost for developing a vaccine has gone from $231 million in 1987 to $802 million in 2000 and continues to increase. The industry faces an increasing regulatory burden due to evaluation of new technology and increased product complexity and the necessary zero tolerance for risk. In addition, differing international requirements for licensure require redundancy in testing. Jill Hackel suggested that one way to ease the burden involved in producing the seasonal influenza vaccine is the use of appropriate biomarkers as indicators of vaccine efficacy in early stages of vaccine development in lieu of protection from challenge; this would speed up the research and phase I trials. Careful coordination of trials and use of electronic data management would also increase the efficiency of the trial. Most importantly, careful and early consultation with the FDA facilitates the approval process. The FDA is developing "a critical path initiative to develop new, publicly available scientific and technical tools—including assays, standards, computer modeling techniques, biomarkers, and clinical trial endpoints—to make the development process itself more efficient and effective and more likely to result in safe products that benefit patients."

Recently, government funding of vaccine development has been driven by fear: fear of bioterrorism, fear of being blamed for inaction, and concern over lost revenue due to absence due to illness. Fear of the potential threat of a pandemic influenza outbreak has garnered the allocation of considerable funds and research from several different governmental agencies. Fear of the threat of bioterrorism elevates infectious diseases to the status of a military weapon and changes the definition of a vaccine program into a military deterrent. Project Bioshield was the answer that the U.S. government came up with to create, fund, and activate vaccine development to control bioterror agents. Frank Rapoport (a lawyer with McKenna, Long and Aldridge LLP) and Monique K. Mansoura (a scientist with the Office of Research and Development Coordination) of the U.S. government described the goals of Project Bioshield, which are to accelerate the research, development, purchase, and availability of priority medical countermeasures to protect the U.S. population from the effects of chemical, biological, radiological, and nuclear threat agents. Basically, this program will create vaccines for the strategic national stockpile that can be distributed upon need. Since 2004, contracts for over 80 million doses of anthrax vaccine have been awarded, and contracts for botulinum antitoxin and a next-generation smallpox vaccine are in progress. The estimated cost of smallpox vaccines for 2004 to 2012 is $1.9 billion. Project Bioshield will spend over $5.6 billion to stockpile vaccines for anthrax, smallpox, botulinum, Ebola fever, and plague.

Redefinition of vaccine development as a deterrent to biowarfare with the establishment of Project Bioshield has enticed the military defense industry to get involved in vaccines. For example, Lockheed, an aerospace, military defense company, bought Dyneport, a vaccine company. The extensive experience in lobbying Congress for funding by the military defense complex may change the nature of future vaccine development.

The ogre for the vaccine industry of the 21st century will continue to be liability and lawsuits. Vaccines are a unique clinical intervention because they are administered to healthy individuals to elicit immune responses that in some individuals are unpredictable, and there is zero tolerance for side effects. Side effects and unknown outcomes are the basis for lawsuits. Fear and the cost of lawsuits have reduced the numbers of companies that make vaccines and caused the price of vaccines to rise enormously. For example, the cost of the DPT shot rose from $0.11 to $11.00 from 1981 to 1986 due to costs of litigation. James Wood (Reed Smith), a lawyer who has been defending the vaccine and drug industry against litigation, described vaccines and the vaccine industry as a natural target for litigators with a defined pathway into the courts. There are ongoing lawsuits based on the use of thimerosal in vaccines and side effects of the DPT and measles vaccines. He mentioned an interesting approach to legal action that is being pursued, an approach based on the potential consequences of the possible acquisition of simian virus 40 (SV40) in recipients of the early polio vaccines (8). SV40 can cause tumors in rodents but has no known association with human cancer (28). Wood suggested that a solution could be for Congress to enact a federal vaccine act that would create an efficient method of providing adequate compensation for vaccine-injured persons but also protect vaccine producers from punitive damages as long as the company showed compliance with FDA and Public Health Service rules. Such an action would reduce the legal costs for the development and marketing of vaccines and encourage new vaccine programs.

## SUMMARY

Vaccines remain the primary means for preventing disease and reducing health care costs of treatment and lost work time due to sickness. Despite our greater understanding of immunology and the technological advances that have been made, we are in jeopardy of losing the acceleration of vaccine development that has occurred since Jenner's discovery in 1775 due to the incredible expense, small profit margin, great risk against success, and even greater risk for litigation. The presentations at this meeting presented the warning with optimistic suggestions for improvement. New technology, new approaches to production, and greater cooperation between the FDA and vaccine developers will enhance vaccine development. The vaccine industry can help themselves by careful analysis of potential markets prior to development, by working closely with the legal and production people early in the development process, by carefully designing vaccine trials to ensure applicability to the regulations of multiple countries, by dialogue with the FDA early in the development process, and by having a plan for dealing with change. The federal government can help in many ways: initiate tort reform to help remove/lessen the manufacturer's liability; develop new incentives for early stage investment in vaccine development by providing tax advantages, such as lower capital gains rates, or by allowing the sale/transfer of research and development tax credits; reduce the regulatory burden on vaccine development without compromising safety by categorizing vaccines within orphan drug status for clinical trials and by greater utilization of surrogate indicators of efficacy, especially in phase I trials; change patent rules to promote mandatory patent pooling and extend the life of a patent for time lost in the regulatory process to increase the incentives and the return on the investment.

Government can facilitate vaccine development by enriching the funding of vaccine research and development. Although Project Bioshield has been given a considerable budget, its funds are distributed to a very limited number of companies and for very limited and defined projects. As a result, the benefit to the advancement of the vaccine field is also limited. NIH remains the primary funding source for most vaccine projects, both academic and industrial. Review of the CRISP (Computer Retrieval of Information on Scientific Projects) database of NIH projects for the past 5 years indicated that vaccine projects represent approximately 3% of NIH projects compared to the approximately 17.5% for drug or pharmaceutical projects. Interestingly, the number of NIH intramural vaccine projects suggests that they have a higher priority within the NIH campus than is applied to extramural projects. In addition to the many approaches that NIH is currently taking to enhance vaccine development, more development could be reached by increasing the number (including Small Business Innovation Research [SBIR]/Small Business Technology Transfer [STTR]) of study sections that review immunology/vaccine projects, increasing the representation of reviewers on the study sections who come from vaccine segments of industry, and by increasing and expanding the number of requests for applications specific for vaccines. In addition, greater recognition can be paid by funding sources and the FDA towards vaccines that elicit protections other than antibody and therapies for noninfectious diseases, such as autoimmunity, allergy, and cancer, and therapies for diseases, such as Alzheimer's dementia, obesity, smoking, drug abuse, and hypertension.

Many developments have been made within the vaccine industry, but interestingly, there are relatively few new technologies that have entered the vaccine market. There are many other diseases that can be targeted for vaccine prevention or treatment, but funding, risk, and limited profit/return reduce their initiation. The development of vaccines in the 21st and even the 22nd century is going to require a team effort from all the different constituencies that were represented at the Vaccines: All Things Considered meeting and presented in this minireview. The fourth annual meeting will be held in November 2006 in Washington, D.C. (http://gtcbio.com/confpage.asp?cid=28).

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