Vaccine Technologies: View to the Future

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The development of vaccines to prevent infectious diseases has been one of the most important contributions of biomedical science. Recent advances in the basic sciences are now fueling the development of a new generation of vaccines that will be based on rational design approaches. Two factors are making this possible: an improved understanding of the microbial factors required for virulence and the nature of the immune response to infection. The status of new vaccine technologies is summarized here.

The ability to control, eliminate, or even eradicate selected diseases, build sustainable immunization programs that can reach every child, and incorporate new vaccines at costs that every country can afford may not be realized with existing vaccines. In recognition of this, the Children's Vaccine Initiative (CVI) has emerged as a set of organizing principles to manage the technological transition to new vaccines. The CVI envisions the ideal vaccines of the future to be safe, heat-stable, and effective when orally administered in a few doses early in life (*1*). These objectives are now more firmly integrated in vaccine research and development, fueled by advances in the fields of recombinant DNA technology, molecular and cellular immunology, peptide synthesis, polysaccharide analytical chemistry, and microbial pathogenesis (*2*).

Here we provide a brief overview of new vaccine technologies. Successful implementation of these technologies promises to yield answers to pressing questions in vaccinology, such as: What approaches will augment a potentially protective but weak immune response? What manipulations are required to generate an adequate and sustained protective immune response even where the natural disease fails to do so? How can successful vaccines be combined without changing their safety profiles, eliciting interference, or affecting efficacy?

Combination Vaccines

Simplifying the immunization of children against multiple diseases at the same time will require the availability of safe and effective vaccines that can be delivered in combination. Such combinations may be achieved during vaccine preparation, manufacture, or administration. Combining vaccines has been recognized as the most feasible of the CVI's goals (*3*), and is of particular interest and concern to the vaccine industry because of the immediate implications for market share. Existing successful combination vaccines include oral poliovirus vaccine (OPV), diphtheria-tetanus-pertussis (DTP), and measles-mumps-rubella (MMRII) vaccines.

In the past year, two additional combination vaccines have been licensed in the United States—DTaP-Hib (diptheria–tetanus–acellular pertussis and *Haemophilus influenzae* type B) vaccines, which are combined during manufacture, and a DTP-Hib vaccine, which will be combined in the syringe just prior to injection. A number of other combination vaccines, primarily based on the building block of DTP, are being explored and have been the focus of regional assessment in both Latin America and South East Asia (*4*). The antigens proposed for combination with DTP include enhanced inactivated poliovirus (IPV), Hib, and hepatitis B virus (HBV) vaccines, which are all killed or subunit vaccines that must also be injected. The development of combinations that are based on the currently used whole-cell pertussis vaccine is limited because of the poor predictability with which combination vaccines can be made using the whole-cell pertussis antigens (*5*). It is anticipated that the availability of purified, well-characterized, acellular pertussis vaccines for use in infants, the target of six ongoing Phase III efficacy trials, will facilitate the development of these new combination vaccines.

New combination vaccines will incorporate many of the technologies reviewed below, including vaccine vectors, new glycoconjugation approaches, new delivery systems, new adjuvants, as well as nucleic acid vaccines. These technologies create a menu of options for vaccine development that will require rational evaluation. For example, the success of nucleic acid vaccines could obviate the need for adjuvants, while other technologies offer the potential for oral or mucosal delivery.

Additional research must address the gaps in our understanding of the immunologic basis for the compatibility and incompatibility of various vaccines when given in combination, as well as the need for innovative strategies to circumvent these problems, especially in infants and the elderly. Antigenic competition, selection of appropriate serotypes for global and regional needs, technical complexity of the manufacturing process, volume limitations, affordability, safety, and efficacy of varying immunization schedules, unavailability of animal models and established correlates of protective immunity, clinical trial design, and Phase IV evaluation of combinations remain important issues. Resolution of these problems will be critical for the development of vaccines for diseases in which "combinations" are required for full protection because of variation in serotypes (pneumococcus, Group B streptococcus) or strains [human immunodeficiency viruses (HIV), dengue viruses], or because the infectious agent presents multiple vaccine targets, as in malaria.

Approaches to Enhancing Immunogenicity

The advent of recombinant DNA technology has stimulated the production and testing of new subunit vaccines designed to be safer and more efficient. Unfortunately, the limited immunogenicity of many of these candidates has hindered their development as potential vaccines. Strategies to enhance the immunogenicity of these candidate vaccines are therefore critical. Several types of immunoenhancers are under investigation. They work in a variety of ways: (i) by changing the conformation of the antigen, thereby enhancing antigen presentation; (ii) by preventing proteolytic destruction of the antigen in the stomach, thus allowing it to pass into the intestines intact for presentation to the gut-associated lymphoid system; (iii) by targeting antigen directly to M cells of the gut to induce mucosal immune responses; (iv) by targeting macrophages to activate their capacity to destroy bacteria; and (v) by inducing the production of various immunomodulatory cytokines that act directly on thymus-derived helper T lymphocytes (T_H cells) to stimulate specific types of immune responses.

Conventional adjuvants. The immune response after vaccination is often far weaker than that occurring after natural disease, and protection against subsequent exposure to pathogen can be variable. Conventional adjuvants are substances that, when mixed with antigen, can amplify cell-mediated and humoral immune responses to that antigen.

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The only adjuvant approved for human vaccines in the United States is aluminum salt (aluminum hydroxide or aluminum phosphate), which is used in diphtheria, tetanus, and hepatitis B vaccines. Like other depot-type adjuvants, aluminum salt was originally thought to work by increasing antigen stability, but recent work suggests that it may also mediate cytokine release (*6*). Aluminum salt adjuvants are limited in that they preclude lyophilization or freezing of the vaccine, they are not effective with all antigens, and they do not stimulate cell-mediated immunity.

The development of alternative conventional vaccine adjuvants is approached empirically by mixing an antigen with the potential adjuvant, and then testing the mixture in animals or humans to determine its effectiveness and safety. Research in this area has focused on oil-based emulsions that contain biodegradable materials. Candidates include the Syntex formulation SAF-1 [containing squalene oil, an amino acid derivative of muramyl dipeptide (threonyl-MDP), and nonionic block polymers], the Ciba-Geigy formulations [containing squalene, surfactants, and a fatty acid derivative of muramyl tripeptide (MTPPE)], the Ribi formulation (containing monophosphoryl lipid A and mycobacterial cell walls), and the saponin derivatives, especially the Cambridge Biotech QS21. The Ribi formulation has been clinically tested with a candidate malaria vaccine (*7*). QS21 formulations, which are stable to both freezing and thawing, have been shown to be a useful adjuvant in a subunit vaccine for feline leukemia virus (*8*).

Nonionic block copolymer surfactants act as adhesive molecules, binding to antigen and complement components with their hydrophilic component, and binding to oil with their hydrophobic portion. This conformation improves the presentation of antigen to the immune system. The block copolymers produce an intense immune response with an increased proportion of the immunoglobulin G_2 (IgG_2) isotype (*9*).

The development of new adjuvants has been dominated by concerns about safety (*10*). Some of the empirically developed adjuvants have been too toxic for use in humans, causing tissue damage at the site of injection and later granulomatous reactions, pyrogenicity, arthritis, and anterior uveitis. Careful collection of safety data and evaluation of each candidate will be required.

Epitope-based strategies. Strategies for immunization with molecules representing preselected epitopes of immunogens have developed as a result of an enhanced understanding of the mechanisms for antigen recognition by B and T cells. In principle, these strategies result in an immune response directed only to the relevant epitope on the pathogen and therefore may avoid any toxicity associated with an immune response to other epitopes. The simplest approach is to link B cell and T cell [T_H cell and cytotoxic T lymphocyte (CTL)] epitopes and use these linear polyepitopes as vaccines. In practice, a good humoral immune response may be elicited, but genetic restrictions may limit the vaccinee's ability to mount an appropriate cell-mediated response to these immunogens. How to optimize the arrangement of epitopes and how to present antigens to the immune system in a manner that maintains conformational and functional integrity (as synthetic peptides versus peptides expressed in vectors such as vaccinia virus) have not yet been determined, but are questions under investigation in many laboratories.

Although epitope-based approaches stimulate good antibody responses, they do not stimulate potent cellular immunity, especially CTL responses. Therefore, variations of this approach are being pursued. One interesting example is the use of Multiple-Antigen Peptide Systems (MAPS), which consist of selected T and B cell epitopes that are conjugated to a polylysine core without a carrier protein (*11*, *12*). MAPS are structurally defined, contain a quantifiable amount of well-characterized, pure antigen, can be administered intraperitoneally, and generate antibodies with high specificity. This approach has been applied to the development of totally synthetic vaccines for HBV, malaria, and HIV (*13*).

There is some evidence that genetic fusion of immunogenic peptides with the nontoxic B subunit of cholera toxin functions as an adjuvant for inducing mucosal immune responses in mice (*14*). This combination targets the Peyer's patches in the intestine and results in a brisk, sustained immune response to the attached peptide sequence. Nontoxic derivatives of cholera toxin (and the related *Escherichia coli* heat-labile toxin) are also being evaluated.

Particulate antigens. Liposomes and microspheres can protect antigens from proteolytic destruction in the stomach, allowing antigen to pass into the intestines intact for presentation to gut-associated lymphoid tissue. Different types of liposomes have been tested over the past 20 years. Recently, immunostimulating reconstituted influenza virosomes (IRIVs)—spherical, unilamellar vesicles that combine the hemagglutinin membrane glycoprotein of influenza virus with antigen—have been tested in a hepatitis A virus vaccine in humans. High antibody titers were obtained after one dose of vaccine (*15*), and efficacy trials are in progress.

Microcapsules consist of an inner reservoir of antigen surrounded by an outer, biodegradable polymer wall through which the antigen is slowly released in the lymphoid tissue. The technology has been available for 30 years, but has been explored with vaccines only recently (*16*). Microcapsules can vary in composition and size, and have been shown to produce strong, sustained immune responses in the case of toxoids and viral antigens. Although the microcapsules are made of the same material used to make resorbable sutures, the possibility of adverse reactions to a slow-release allergen remains a safety concern, albeit a theoretical one, at this point. Microcapsules between 5 and 10 μm in diameter are taken up by the Peyer's patches of the gastrointestinal tract, and oral administration of these capsules has been shown to effectively prime and boost IgG and IgA responses in mice (*17*). Microcapsules maintain the peptide in the dry state, avoiding the need for refrigeration of the vaccine. However, the microencapsulation process does expose antigens to organic solvents, which can reduce their immunogenicity (*18*).

Another approach has been to incorporate antigens into solid particles called ISCOMs (immunostimulatory complexes). These structures are generated by mixing antigen with a biocompatible detergent and the complex adjuvant Quil A. The ISCOM self-assembles into stable 35-nm cage-like structures held together by the hydrophobic interactions between the matrix (Quil A), added lipids, and the antigen. ISCOMs containing viral membrane proteins have been tested in animals and found to induce antibody titers that were 10 times higher than those seen in controls. When complexed with glycoprotein, ISCOMs may also induce CTL responses, perhaps through the delivery of antigen directly to the cytosol for presentation with major histocompatibility complex (MHC) class I molecules (*19*). Cytosolic antigen delivery by membrane-active adjuvants mimics the antigen presentation that occurs during viral infection or after immunization with live attenuated vaccines. Concerns about the safety of Quil A must be resolved before ISCOMs can be administered to humans.

Protein cochleates, which are stable precipitates of protein, phospholipid, and calcium, are new formulations used to enhance the immunogenicity of antigens. The name derives from their unique structure, a rolled-up lipid bilayer maintained by calcium bridges. Membrane proteins or peptides with lipid anchors can be integrated into this lipid bilayer, which protects them from intestinal acid and allows them to be slowly taken up by the Peyer's patches. Protein cochleates can thus be used for presentation of multiple antigens. In experiments with mice, they have been found to stimulate

circulating and mucosal antibodies and CTLs that protect against subsequent challenge with pathogen. This approach is currently being tested in animals with influenza, parainfluenza, and HIV vaccines (*20*).

Glycoconjugation Technologies

The protection elicited by antibodies to the polysaccharides of encapsulated bacteria form a rational basis for their development into vaccines against Hib, meningococcus A and C, and pneumococci. However, simple polysaccharide vaccines do not protect young infants (a high-risk group for these infections) unless the polysaccharide is conjugated to a suitable carrier protein. Conjugation changes the polysaccharide from a T cell–independent to a T cell–dependent immunogen (*21*). Protein-polysaccharide immunogens show enhanced immunogenicity, elicit predominantly IgG class antibodies, and induce a secondary response upon revaccination. Production of a successful glycoconjugate vaccine depends on the physical nature and chain length of the carbohydrate, the selection of carrier protein, the ratio of carbohydrate to protein, the coupling methodology (linker molecule versus reductive amination), and the conformation of the antigenic determinants (*22*).

Several Hib conjugate vaccines are licensed at this time; these consist of polyribosylribitolphosphate capsule or derivative oligosaccharides linked to protein carriers (including tetanus or diphtheria toxoids) or to an outer membrane protein complex of *Neisseria meningitidis* group B (*22*). Conjugate vaccines are relatively expensive to produce, so an increase in the efficiency of the manufacturing process may be a prerequisite for their accessibility to the developing world (*23*).

Cytokines

An emerging area of immunologic enhancement involves the use of cytokines to direct and boost immune responses. $CD4^+$ T_H lymphocytes have been subdivided into two classes (T_H1 and T_H2), which differ in the pattern of cytokines they produce. T_H1 cells are prominently involved in cell-mediated immunity and produce cytokines such as interleukin-2 (IL-2) and interferon-γ (IFN-γ), whereas T_H2 cells assist in antibody production and produce cytokines such as IL-4 and IL-10. In certain chronic infections, such as leishmaniasis and schistosomiasis, the severity of the disease is determined by whether the predominant immune response is T_H1-like or T_H2-like. Thus, in principle, the ability to manipulate the immune response toward a T_H1- or T_H2-like response may lead to enhanced immunologic protection and reduced immunopathology (*24*).

IL-12 is a recently characterized cytokine that may play a pivotal role in immunomodulation. The adjuvant activity of IL-12, when administered with antigen, has been demonstrated in a mouse model (*25*). Immunization of BALB/c mice with *Leishmania major* antigens and IL-12 induced leishmania-specific $CD4^+$ T_H1 cells that conferred protection against *L. major*, whereas immunization of control animals with antigen alone elicited T_H2-type immune responses that were not protective.

Nucleic Acid Vaccines

Injection into muscle of plasmid DNA encoding an antigen of interest has been shown to result in sustained expression of the antigen and generation of an immune response (*26*). This approach, termed "nucleic acid vaccines," is receiving much attention for several reasons. First, in animal models these vaccines appear to stimulate persistent humoral and cell-mediated immune responses, without integration of plasmid into chromosomal DNA (*27*). The animals are protected from lethal virus challenge. This is potentially useful in protecting against viral infections in which the antibody response alone is not protective, or where there is antigenic diversity of surface proteins among strains. Second, several routes of vaccine administration are possible—parenteral, mucosal, or via a gene "gun" that delivers tiny amounts of DNA-coated gold beads (*28*). Finally, this strategy results in relevant antigen production in primates without the use of infectious agents (*29*). Thus, this approach to vaccine development may be pertinent to several diseases, including AIDS, and will undoubtedly continue to receive intense scrutiny. Hypothetical safety concerns, including the potential integration of plasmid DNA into the host genome or the generation of anti-DNA antibodies, will need to be addressed.

Vaccine Vectors

For many diseases, the ideal vaccine is a live attenuated derivative of the pathogen, which induces strong, long-lasting protective immune responses to a variety of antigens on the pathogen without causing illness. Potential barriers to the development of such vaccines include difficulties in propagating the pathogen in the laboratory, difficulties in attenuating the pathogen without reducing its immunogenicity, and difficulties in ensuring that the attenuated pathogen does not revert to virulence.

One strategy for overcoming these obstacles is to insert one or more of the pathogen's genes into a nonpathogenic organism that can be easily administered, ideally by the oral route. This avirulent organism thus serves as a vector for expression of the genes coding for important antigens of the pathogen within the host (*30*). Several such vectors have been tested and are in various stages of development as vaccines, as summarized below.

Vaccinia virus. Several features of vaccinia virus, including its ability to tolerate insertions of large segments of foreign DNA as well as its replication within the cytoplasm of host cells, make this virus an ideal candidate vector for expression of antigens from a large range of pathogens (*31*). Vaccinia virus is also being used to express cytokines that modulate the immune response and to produce large quantities of proteins for testing as subunit vaccines. Recently, a vaccinia strain attenuated by genetic engineering (NYVAC) has been developed and is being investigated for its potential as a vector for foreign antigens (*32*). Concerns that must be addressed before vaccinia recombinants can be widely used in humans include the relatively low immunogenicity of the expressed antigens, the potential reactogenicity of the vaccine, the difficulty in using the vectored vaccine twice, and the possibility of disseminated disease in immunocompromised persons.

Avipoxviruses. A variety of avian viruses closely related to vaccinia virus are also being evaluated as potential vaccine vectors. The great advantage of these viruses is that they produce an abortive infection in humans (that is, they infect cells, but the infection does not spread because avipox is strongly host restricted to avian species), and therefore do not cause disease. If these viruses can be engineered to express high levels of an immunogen, they could be useful for delivery of antigens. Because avipoxviruses are not strongly immunogenic, they presumably could be used repeatedly to deliver a variety of immunogens. A rabies vaccine that is based on an attenuated canarypox vector has been tested in animals, and canarypox vectors expressing measles glycoproteins are also being evaluated for their vaccine potential (*32*).

Adenoviruses. Despite their small genome size, adenoviruses are also being considered as potential vaccine vectors. Vaccine strains currently used in the military have been engineered to express genes from respiratory syncytial virus, HBV, and HIV. Although studies in rodents looked promising, studies in primates—and recent human clinical studies with HBV surface antigen expressed in adenovirus type 7—suggest that antigen expression or presentation may be insufficient to elicit efficacious protective immune responses.

Polioviruses. Attenuated polioviruses (Sabin vaccine) are widely used as vaccines for polio. Recent work has shown that it is

possible to genetically engineer polioviruses to express foreign antigens, thereby giving them added utility as potential vaccine vectors. Three basic strategies are being used. The first involves inserting large pieces (500 to 800 base pairs) of foreign DNA into the poliovirus VP1 structural protein gene. The resulting virus requires a helper virus to replicate, but infected cells produce large quantities of the foreign protein, which is released when infected cells lyse. The second strategy involves inserting small gene segments (up to 60 base pairs) into the "loop" region of the poliovirus VP1 structural protein. Poliovirus recombinants expressing these epitopes replicate normally, but they express these new epitopes on a highly immunogenic domain of the native poliovirus virion. In a more recent approach (*33*), foreign antigens (up to 400 amino acids) are expressed as part of the poliovirus polyprotein precursor and are then released when the precursor is cleaved during infection by viral proteases.

Salmonella. A variety of different *Salmonella* species have been extensively studied for their potential as vaccine vectors. The rationale for this approach stems from the extensive literature on the value of attenuated *Salmonellae* as vaccines. A currently licensed typhoid fever vaccine, Ty21a, is an attenuated strain of *Salmonella typhi*. Because these strains are administered orally and interact for long periods of time with the gut-associated lymphoid tissues, where they stimulate high levels of IgA production as well as cellular immune responses, they have been most actively studied for their use in diseases requiring a strong mucosal immune response for protection. *Salmonella* recombinants expressing purified *Bordetella pertussis* components, HBV surface antigen, *Plasmodium* antigenic repeat sequences, and *E. coli* fimbriae and toxin subunits have all been tested in animals. *Salmonella* Ty21a recombinants expressing *Vibrio cholerae* lipopolysaccharide antigens and *Shigella* invasion determinants have been tested in human clinical trials. These studies have shown that *Salmonella* is an acceptable vector, and most research is now focused on deriving better-characterized *Salmonella* strains and developing techniques to increase expression of the foreign antigens.

Other vector systems being explored include the Herpesviruses (herpes simplex virus, cytomegalovirus, and varicella virus) and the mycobacterium bacillus Calmette-Guérin (BCG), all of which have large genomes. Research on most of these vector systems is still largely in the early developmental stages, but a recombinant BCG-OspA vaccine against *Borrelia burgdorferi* (the causative agent of Lyme disease) will soon enter clinical trials.

Conclusion

The future promises many new vaccines to prevent, control, and possibly eradicate disease. The CVI has forged a new synthesis of basic science and public health by emphasizing the need for links between public health strategies and new vaccine technologies. Its success will rest on the development and application of the technologies such as those briefly described here, as well as those that will surely arise in the future as a result of rapid advances in this field. Important changes in the development, manufacture, quality assurance, and control of the new vaccines, and in the immunization programs that deliver them, will also be required (*34*).

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