

Symposium synopsis

Vaccines: New approaches and concepts

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Vaccine research has become an exciting area with notable progress on many fronts. Examples of advances include new approaches to antigen delivery, immunologic approaches to enhancing vaccine immunogenicity, and the development of vaccines that induce unresponsiveness or tolerance for the prevention and treatment of allergic and autoimmune diseases. The American Academy of Allergy and Immunology and the National Institute of Allergy and Infectious Diseases jointly sponsored the symposium, "Vaccines: New Approaches and Concepts," which was held December 9, 1994 at the National Institutes of Health. The program committee consisted of: Howard B. Dickler, MD, (Bethesda), Chairman; Philip Fireman, MD, (Pittsburgh); and Burton Zweiman, MD, (Philadelphia). Eight outstanding presentations on advances in vaccine research were provided by speakers who are leading researchers in this area of broad interest and unusually promising clinical application. This report summarizes the highlights of those presentations.

NEW MODES OF ANTIGEN DELIVERY

Many infectious agents bear antigenic epitopes that induce protective humoral or cellular immune responses. Synthetic peptides corresponding to such epitopes would have advantages as vaccines because protective immunity could be obtained while avoiding side effects and reactions to other parts of the organism. Unfortunately, this approach has been limited by the short half-life and poor immunogenicity of such synthetic peptides. These limitations can potentially be overcome by delivering the peptide on a carrier molecule. Dr. Constantin Bona (Mount Sinai School of Medi-

Abbreviations used

AIDS:	Acquired immunodeficiency syndrome
APCs:	Antigen-presenting cells
BCG:	Bacille Calmette-Guérin (vaccine)
HA:	Influenza type A virus hemagglutinin
HIV:	Human immunodeficiency virus
IFN- γ :	Interferon- γ
MBP:	Myelin basic protein
MHC:	Major histocompatibility complex
MS:	Multiple sclerosis
TB:	Tuberculosis
TGF:	Transforming growth factor

cine, New York) reviewed the work of his laboratory in which the self molecule immunoglobulin was used as the carrier.

The first approach involved the creation of a chimeric immunoglobulin molecule by using genetic engineering methods. A peptide epitope (110-120) of the influenza type A virus hemagglutinin (HA) molecule was expressed in the CDR3 region of a mouse monoclonal antibody by deleting the D segment of the V_H gene and replacing it with an oligonucleotide that encodes the peptide.¹ Exposure and availability of the viral epitope on the chimeric immunoglobulin molecule was indicated by the strong binding of a monoclonal antibody specific for HA (110-120). More importantly, the chimeric immunoglobulin molecule, in the presence of antigen-presenting cells (APCs), was capable of stimulating a T-cell hybridoma, which is specific for HA (110-120). Several aspects of this stimulation were notable: (1) it was inhibitable by anti-major histocompatibility complex (MHC) class II antibody, indicating that presentation took place on this molecule; (2) degradation and processing of the chimeric molecule were required in that both chloroquine and paraformaldehyde treatment of the APCs prevented stimulation; (3) antibody against receptors that bind immunoglobulin (Fc γ R) prevented stimulation, indicating uptake of the chimeric immunoglobulin by this receptor; (4) activation of the T-cell hybridoma by chimeric immunoglobulin was 300 to 1000 times

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more efficient than the synthetic peptide, and this was due to a higher expression of HA peptide associated with class II molecules when the APCs were pulsed with chimeric immunoglobulin as compared with synthetic peptide.

When the chimeric immunoglobulin was tested for immunogenicity in mice, it was found to be immunogenic, causing the expansion of CD4⁺ T cells, which are specific for the HA peptide. However, this response required a toxic adjuvant (complete Freund's), which cannot be used in human beings. This problem has recently been overcome by "pegylation" (treatment with monomethoxypolyethylene glycol) of the chimeric immunoglobulin, which increases the size of the molecules and causes them to persist longer in the circulation. Pegylated chimeric immunoglobulin was immunogenic in vivo when given in saline solution,² making this approach feasible for use in human beings.

More recent experiments have focused on enzymatically engineering immunoglobulins.³ Compared with genetic engineering, this approach is faster, easier, and cheaper. The synthesis begins by removing sialic acid residues from immunoglobulin with the enzyme sialidase. The terminal galactose (GAL) residues are then oxidized, creating aldehyde groups. Peptide (HA 110-120) covalently links to these groups, forming a Schiff base, which is stabilized with pyridine borane. An additional feature is that a cleavage site for cathepsins can be introduced into the peptide to facilitate release of the peptide in the lysosome. This technique has been used to attach 11 to 12 peptide groups per IgG molecule and 60 per IgM molecule. The latter stimulates the HA (110-120) specific hybridoma in an antigen-specific fashion and is 400 times more efficient than free peptide and two to three times more efficient than the genetically engineered immunoglobulin. Compared with chemically cross-linked immunoglobulin, enzymatically engineered immunoglobulin does not produce new determinants or byproducts such as heterodimers. The technique could also be used for attaching therapeutic or diagnostic moieties to targeting antibodies.

Dr. Bona concluded his presentation by summarizing a number of the potential advantages of using self immunoglobulin molecules as carriers for epitopes of infectious agents for vaccination. These include lack of an immune response to the carrier itself, the ability to cross the placenta, and the fact that an immune response can be generated without adjuvant.

VACCINATION WITH GENES

Dr. Margaret Liu (Merck Research Laboratories, West Point, Pa.) presented the experience of her group with DNA vaccines comprised of non-replicating plasmids encoding viral proteins. This work began as an effort to create better vaccines for viruses that either have many different strains or alter their surface glycoproteins. One such virus is influenza, and these changes in surface antigens decrease the protection afforded by previous infection or by immunization with earlier viruses or their proteins. DNA vaccines have a number of theoretical advantages. First, because a live organism is not used, the risk of disease is completely eliminated. Second, because a vector is not used, there is no limitation caused by an immune response against that vector. Third, compared with using a limited number of peptides, DNA can encode the whole protein with all its epitopes.

The first experiments used plasmids encoding nucleoprotein, an internal protein of influenza.⁴ Spleen cells from mice immunized intramuscularly with the nucleoprotein DNA in saline solution generated nucleoprotein-specific cytotoxic T-lymphocyte responses in vitro on restimulation with either nucleoprotein peptide or cytokines. Efficacy was evaluated in mice by immunization with DNA encoding the nucleoprotein from one influenza strain and challenge with another (cross-strain challenge). Compared with unimmunized or vector-immunized controls, the nucleoprotein DNA-immunized mice had: (1) lung titers of virus that were 3 logs lower, (2) decreased morbidity indicated by lesser weight loss and faster regaining of the lost weight, and (3) markedly increased survival (100% vs 10% in controls). Because the nucleoprotein DNA-immunized mice did get sick, the role of the cytotoxic T lymphocytes appeared to be in clearing virus rather than in preventing infection. Although the nucleoprotein DNA generated good antibody responses, this antibody did not appear to play a role in the efficacy of the vaccine in that it had no effect against heterologous virus when passively transferred to new recipients.

The next group of experiments evaluated the ability of DNA encoding an influenza surface protein hemagglutinin (HA) to elicit protective antibody responses. Compared with using recombinant proteins produced in *Escherichia coli* or yeasts, immunization with DNA may provide better posttranslational modification. In some instances immunization of mice with HA DNA produced a higher neutralizing antibody titer than infection with the strain itself. Moreover, in vivo

efficacy was demonstrated by 100% survival with a 90% lethal challenge. Neutralizing and protective antibody responses were generated by HA DNA in ferrets, and neutralizing antibody responses were seen in nonhuman primates.

Impressive results were seen in ferrets that were infected with human clinical isolates of influenza.⁵ The animals were vaccinated with DNA encoding nucleoprotein and another internal protein called *matrix M1*, as well as with HA from the earlier strains A/Beijing/89 and A/Hawaii/91. When challenged with the drifted strain A/Georgia/93, they showed protection equal to immunization with DNA encoding the homologous HA from A/Georgia/93 and greater than that seen with the widely used commercial vaccine composed of killed A/Beijing/89.

Dr. Liu also summarized experiments in animal models of other viral diseases in which immune responses and protection against viral challenge were seen after immunization with DNA encoding viral proteins. These included DNA encoding human immunodeficiency virus (HIV) gp120 in monkeys and mice and DNA encoding L1, the major viral capsid protein of cottontail rabbit papilloma virus.

Dr. Liu concluded her presentation by commenting on potential concerns regarding immunization with DNA. She noted that millions of persons have been inoculated with a DNA virus (i.e., vaccinia) without safety problems. Moreover, there is no definite reason to expect greater toxicity than with current vaccines. Finally, in the preclinical models tested, neither anti-DNA antibodies nor tolerance to the encoded protein was observed. Thus DNA encoding viral proteins holds considerable promise for use as vaccines for protection against viral diseases.

ANTI-IDIOTYPES AS VACCINES

Dr. Mark Greene (University of Pennsylvania, Philadelphia, Pa.) reviewed the molecular principles that underlie the interactions between antigen and antibodies and between idiotypes and anti-idiotypes, the use of idiotypes as immunogens, and situations in which anti-idiotypes might be useful as vaccines. Idiotypes are defined as serologically detected antigenic determinants unique to an antibody or group of antibodies, and the collection of idiotypes defines an antibody's idiootype. An anti-idiootype recognizes some of these specificities.

High-resolution x-ray crystallographic studies of complex crystals between antibody and antigen or between idiootype and anti-idiootype have provided

considerable information about the basic principles of recognition.⁶ Interaction occurs over a surface area varying between 130 Å² and 900 Å². The areas of recognition on the antibody are in the hypervariable or complementarity-determining regions (CDR), which form reverse turn structures of various conformations. A limited number of such conformations exist, and therefore specificity is not due to the structure of the loop itself but rather to the residues that extend from the loop and are exposed in three-dimensional space. Of the six loops of an antibody, one or a limited number may be involved in binding. The antigen binding surface is characterized by a high density of aromatic residues, which are hydrophobic in nature. Therefore it came as a surprise that when two proteins bind, water is added rather than deleted. These hydration networks provide an extra bonding force in addition to the hydrogen and ionic bonds in some antigen-antibody interactions.

Comparative studies of the binding of idiotypes to antigen or anti-idiotypes have shown that the two interactions can have similar or identical chemistry, partially similar chemistry, or completely different chemistry. The study of the binding of an idiootype to hen's egg lysozyme revealed that approximately half of the interaction sites overlapped with the interaction sites for the anti-idiootype.⁷ In contrast, the CDR3 of a particular anti-idiootype contained the structure of angiotensin II and bound to the idiootype with similar chemistry.⁸ Similarly, the CDR2 of the light chain of a particular anti-idiootype had a large region of essentially homologous amino acid sequence with the antigen reovirus type 3 hemagglutinin.⁹ Finally, two antibodies that bound the same residues in guinea fowl lysozyme did so with totally different chemistries.¹⁰ Thus in using anti-idiotypes as immunogens or vaccines, one may wish to choose one that contains the structure of the target antigen.

Even if this requirement is met, immunization with an anti-idiootype has proven to have a number of limitations. When using the anti-idiootype that contains the structure of reovirus type 3 hemagglutinin,¹¹ one needs to use adjuvant, provide a large molecular mass either by cross-linking or by coupling to a large macromolecule, and use a large mass of protein to obtain effective neutralizing antibody titers. Moreover, one needs to immunize multiple times to obtain sustained immunity. Finally, extensive attempts to generate cell-mediated immunity with the anti-idiootype failed. These included immunization with hybridoma cells expressing the anti-idiootype, which could be lysed by

cytotoxic lymphocytes specific for the antigen.¹² These limitations may be overcome in the future by linking the anti-idiotope to various cytokines, either at the protein level or through genetic immunization.

Use of anti-idiotypes as vaccines may be applicable in certain defined circumstances. One such circumstance is use of anti-idiotypes as mimics of bacterial carbohydrate antigens, which are poor immunogens. This has been accomplished for phosphatidylcholine,¹³ and protective immunity to sugar residues on meningococcus has been generated by using an anti-idiotope.¹⁴ A second example involves the case in which an idiotope causes downmodulation of an oncogene receptor with conversion of the malignant cell to a normal one. In this case the anti-idiotype can be used as a vaccine to generate the idiotope to control residual tumor (Weiner et al. Unpublished data).

On the basis of the information that he reviewed, Dr. Greene expressed reservations about whether anti-idiotypes would ever be successfully used clinically as vaccines. Nevertheless, he pointed out that the detailed molecular information derived from studies of the interactions between idiotope and anti-idiotope has provided the basis for the development of small molecular mimics, which can be used in the diagnosis and treatment of disease.

TUBERCULOSIS VACCINE

Infection with *Mycobacterium tuberculosis* (TB) is reemerging as a major health care problem. Dr. Solomon Langermann (MedImmune, Gaithersburg, Md.) reviewed the problem and the status of Calmette-Guérin bacillus (BCG) as a vaccine and then presented MedImmune's strategy for producing an improved TB vaccine.

In the United States, the incidence of TB decreased between 1953 and 1984 but increased 20% from 1984 to 1992. This is a worldwide problem, as increases in incidence in other countries demonstrate (e.g., Italy, 28% and Switzerland, 33%). The problem is exacerbated by the fact that many cases in the inner city are caused by TB that is multi-drug-resistant. Individuals most at risk are those who are immunocompromised, inner-city children, prisoners, and health care providers. A relevant public health question that arises when a TB vaccine is considered is: What groups do you target for the vaccine to inhibit the spread in the population as a whole?

BCG is a descendent of *M. bovis*, which was attenuated by multiple passages between 1908 and

1921. It is widely used as a vaccine: its use is compulsory in 64 countries and recommended in 118 countries. The major problem with BCG as a vaccine is its questionable efficacy in preventing TB in adults. A meta-analysis of more than 1200 published reports, performed by Colditz et al.,¹⁵ indicated an overall efficacy rate of 50%. A second problem is that immunization with BCG invalidates the use of the purified protein derivative skin test as an epidemiologic screening test. However, BCG has a number of useful characteristics. First, it is extraordinarily safe in that it has been administered to 2.5 billion persons with minimal problems. Second, it has immunostimulatory properties and can stimulate both systemic and secretory humoral immunity, as well as cell-mediated immunity. Third, it can be given early in life. Finally, it is both easy and cheap to produce in large amounts.

The goal of Dr. Langermann and his colleagues is therefore to improve BCG rather than start anew. Their strategy is to obtain levels of anti-TB secretory IgA in the lungs that are sufficient to prevent colonization when bacilli are inhaled and also to enhance anti-TB cell-mediated immunity such that a TB primary apical complex can be sterilized. Their approaches include improved culture conditions, hyperexpression of protective antigens, selection of an immunization route, and use of persistent strains of BCG.

BCG viruses produced in roller-bottle-dispersed cultures have proven to have less reactogenicity and to induce more uniform responses than BCG viruses grown on surfaces.¹⁶ Moreover, vectors that allow the production of recombinant BCG and hyperexpress foreign antigens have been developed.¹⁷ One such antigen is the outer surface protein A (Osp A) of *Borrelia burgdorferi* (the agent in Lyme disease). Use of this antigen allows evaluation of BCG as a vaccine vehicle and serves as a model for hyperexpression of TB antigen.

The intranasal route has proven to be very effective for producing the types of responses that are needed for an efficacious TB vaccine. When a single droplet of Osp A-BCG was applied to the nares of mice, long-lasting specific systemic IgG and IgA responses developed, whereas intraperitoneal administration evoked only IgG.¹⁸ Intranasal immunization also produced focal immune responses throughout the mucosal immune system, and specific secretory IgA responses were demonstrated in the lungs.

Intranasal immunization with BCG also induces TB-specific T_{H1} responses (required for cell-medi-

ated immunity) from lung lymphocytes. When stimulated *in vitro* with mycobacterial antigens, lung lymphocytes specifically secrete the cytokine interferon- γ (IFN- γ) (Langermann et al. Unpublished data). Evidence for protection has also been seen after intranasal immunization with BCG in animal models. Mice were protected from disseminated TB after aerosol challenge, and guinea pigs showed a 4 log reduction of systemic organisms and a 1/2 log reduction of organisms in the lungs (Langermann et al. Unpublished data). After intranasal immunization with BCG, organisms could be cultured from the lungs after 9 weeks. This indicates that there is relatively good persistence. If greater persistence is required to generate high efficacy, mutant BCG organisms will be evaluated. Although the means exist to hyperexpress TB antigens in BCG, to date no antigen has been identified that is not also expressed by BCG. Efforts are ongoing to identify a TB-specific protective antigen.

In conclusion, some of the elements needed to produce a highly efficacious TB vaccine are in place. The prospects for continued improvements seem bright.

SELF ANTIGENS AS VACCINES FOR AUTOIMMUNE DISEASE

Autoimmune diseases are those in which the immune system attacks the body's own tissues with resulting tissue destruction and inflammation. The minimal requirement for inducing an autoimmune disease is the presence of autoreactive T cells. In multiple sclerosis (MS), it is postulated that myelin basic protein (MBP)-specific autoreactive T cells become activated in the periphery by a superantigen, a viral antigen that cross-reacts with a self antigen, or some other signal. This signal in the presence of a costimulatory signal, such as that provided by B-7 molecules, results in activated T cells. Once activated, these T cells traverse the blood-brain barrier into the central nervous system, where they recognize the MBP antigen in the context of the MHC, presumably on microglia cells. This recognition results in the secretion of T_{H1}-type cytokines, such as IL-2, IFN- γ , and tumor necrosis factor- α , from the autoreactive T cells. Dr. David Hafler (Harvard Medical School, Boston) suggested that because autoimmune disease is postulated to be caused by the activation of autoreactive T_{H1} cells, immunotherapy or vaccines for autoimmune diseases should induce the activation of autoreactive T_{H2} regulatory T cells. These autoreactive regulatory T cells would migrate into the

involved organ or tissue, secrete downregulatory cytokines, such as transforming growth factor (TGF)- β , IL-4, and IL-10, and turn off the immune response induced by the T_{H1} autoreactive T cells. Dr. Hafler proposed three methods for altering the cytokine profile of cells: (1) alteration of costimulatory signals, (2) use of altered peptide ligands, and (3) mucosal stimulation.

In patients with MS, the frequency of autoreactive T cells specific for MBP is the same as that in normal subjects.¹⁹ However, the frequency of activated autoreactive T cells specific for MBP is higher in patients with MS than in normal subjects, with no difference in activated T cells specific for tetanus toxoid.²⁰ Patients with MS also have a higher frequency of activated T cells in peripheral blood and spinal fluid than subjects with other inflammatory neurologic diseases, in which T cells reactive to MBP would be expected.²⁰ The two immunodominant epitopes of MBP, regions 84-102 and 143-168, are the same in both central nervous system and peripheral blood T cells.^{19, 20} In binding of MBP (84-104) to DR β 1*1501, Val (89) and Phe (92) are critically important, whereas in binding to DR β 5*0101, Phe (92), Lys (93), and Ile (95) are critical MHC contact points. Thus one peptide, which can bind to two different MHC molecules, can use different residues in binding to each MHC.

The residues important for T-cell receptor binding are Phe (91), His (90), and Lys (93). The Phe residue at position 91 is critical for every T-cell clone, suggesting that it is a primary T-cell receptor contact site, whereas the His and Lys residues are important for different clones.²¹

Dr. Hafler and colleagues examined the stimulation abilities of peptides altered at various MHC and T-cell receptor contact residues to determine whether they could alter T-cell function. Changes at Val (89) resulted in decreased binding of the peptide to the MHC and decreased thymidine uptake. Peptides with substitutions for Phe (91), a critical T-cell receptor-binding residue, did not induce proliferation but bound to the MHC reasonably well. A peptide with substitution of Ala for His (90) was able to inhibit thymidine incorporation by the native peptide but unable to stimulate thymidine incorporation itself. This peptide resulted in no activation of p56 *lck*, whereas a peptide substituted at position 93, which induced proliferation, also induced autophosphorylation of *lck*. Calcium flux followed the same pattern. The peptide with Ala at position 90 induced new messenger RNA synthesis and protein secretion of

TGF- β 1, but no other cytokines. Induction of even low levels of TGF- β 1 was postulated to be important in regulating and limiting the immune response to self antigens.²²

Dr. Hafler asked, "Why are there autoreactive T cells in normal individuals?" He suggested that the autoreactive T cells represent the price we pay for a very broad T-cell receptor repertoire. Another possibility is that autoreactive T cells play an important role in regulating tissue destruction associated with immune responses. The pattern of cytokines secreted by T cells after ligand engagement of their T-cell receptor determines the T-cell function and thus the nature of the immune response.

Oral tolerance can be viewed as a way of switching T-cell function from a T_{H1} to a T_{H2} phenotype. T-cell clones were isolated from the mesenteric lymph nodes of mice that had been orally tolerized to MBP.²³ Although the CD4⁺ clones had T-cell receptor α and β chains identical to encephalitogenic clones, these cells secreted TGF- β 1 in addition to various amounts of IL-4 and IL-10. Each T-cell clone that secreted TGF- β did not secrete IFN- γ . When adoptively transferred to naive animals, these cells could suppress the development of experimental allergic encephalomyelitis induced by MBP or a different antigen, proteolipid. Anti-TGF- β 1 monoclonal antibodies abrogated the protective effect of the TGF- β 1 clones. Thus low-dose feeding of antigen induces T_{H2}-type cells, which secrete TGF- β 1.²³ These T cells can migrate to the area of the immune response to the fed antigen and suppress T_{H1} cell activation and/or function. In addition to this mechanism of active suppression, feeding of high doses of antigen can induce apoptosis or inactivation of both T_{H1} and T_{H2} type cells, leading to clonal anergy or clonal deletion. In animals transgenic for the ovalbumin-specific T-cell receptor, feeding increasing concentrations of ovalbumin leads to an increasing percentage of apoptotic T cells.²⁴ Thus oral feeding of antigen can induce tolerance by active suppression, clonal anergy, or clonal deletion.

Small trials of feeding type II collagen to treat rheumatoid arthritis or feeding myelin to treat MS have been completed, and larger trials are in progress.^{25, 26} The early results suggest a beneficial clinical effect in both trials. At the National Eye Institute, clinical trials of feeding S-antigen to treat uveitis are in progress. With evidence that feeding insulin will prevent diabetes in nonobese diabetic mice, there are plans for a large human trial with oral insulin. Trials of feeding MHC peptides to

block transplant rejection are also in progress. In patients enrolled in the original trial of feeding of MBP, Dr. Hafler found that the frequency of T cells secreting TGF- β 1 was significantly higher in the patients who were fed antigen than in control subjects.²⁷

In summary, autoimmune disease is caused by activated autoreactive T cells secreting T_{H1} cytokines. Vaccination, with altered peptides or oral feeding of antigen, should lead to the production of specific regulatory clones that secrete TGF- β 1 and T_{H2} cytokines. These clones would downregulate the action of the destructive autoreactive T cells found in this disease, preventing or ameliorating the destructive process.

CYTOKINES AS ADJUVANTS

IL-12, a cytokine produced by macrophages and B cells, has been shown in a variety of experimental systems to have potent immune regulatory effects. It stimulates the production of IFN- γ and selectively induces T_{H1} differentiation. Because of its ability to stimulate cell-mediated immunity, it has been suggested that IL-12 could be used as an adjuvant for vaccines for diseases known to be controlled by cell-mediated immunity, including *Leishmania major*.^{28, 29} Dr. Alan Sher (National Institute of Allergy and Infectious Diseases, Bethesda) discussed experiments testing this possibility.

Schistosoma mansoni, a helminth found worldwide, causes significant morbidity largely because of the fibrosis induced by the granulomatous response to the deposition of parasite eggs in portal areas of the liver. Vaccines to prevent or reduce infection by *S. mansoni* have largely focused on immunization against the invading larval stages with only limited success. Egg granuloma formation has been shown to be a CD4⁺ T-cell-dependent process dominated by a T_{H2} cytokine response. Because IFN- γ stimulates T_{H1} differentiation and downregulates T_{H2} responses, Dr. Sher and colleagues attempted to determine whether this cytokine plays a role in the inflammation resulting from the deposition of parasite eggs. Because IL-12 is a regulator of the production of IFN- γ by natural killer cells, they also investigated its role in the development of egg granulomas.³⁰

Depletion of IFN- γ or natural killer cells by antibodies to these molecules resulted in an increase in granuloma size around intravenously injected eggs and was associated with increased expression of T_{H2} cytokines and a significant decrease in T_{H1} cytokine expression. IL-12 is increased in animals after the injection of eggs; this

increase was abrogated by the depletion of natural killer cells or IFN- γ . Depletion of IL-12 with specific neutralizing monoclonal antibodies in mice injected with eggs resulted in increased granuloma size, decreased T_{H1} cytokine expression, and increased T_{H2} cytokine expression. The pattern of cytokine expression was similar to that seen with IFN- γ depletion.³⁰ In contrast, treatment with exogenous recombinant (r)IL-12 inhibited primary granuloma formation while increasing IFN- γ , IL-2, IL-10, and pulmonary IL-12 mRNA levels and suppressing IL-4, IL-5, IL-6, and IL-13 mRNA expression. Because IL-12 increased both IFN- γ and IL-10, studies in which each of these cytokines were individually depleted after IL-12 administration indicated that the effects of rIL-12 could be attributed primarily to increased IFN- γ . IL-12 also inhibited the formation of secondary granuloma in mice previously sensitized with eggs, demonstrating that IL-12 could reverse an established T_{H2} cell response. Mice that were given IL-12 at the time of egg sensitization to precommit them to a T_{H1} response developed only minimal granulomas on subsequent egg challenge.³⁰ When mice sensitized with eggs plus IL-12 were challenged percutaneously with *S. mansoni* larvae, the number of worms or eggs recovered from these animals was not reduced compared with unsensitized animals.³¹ However, the granulomas formed were significantly smaller and contained fewer eosinophils. Interestingly, there was a dramatic inhibition of egg-induced fibrosis. The levels of hepatic hydroxyproline and mRNA for type I and type III collagen were decreased in the mice sensitized with IL-12 and eggs compared with unsensitized mice and those sensitized with eggs alone. There was no increase in necrosis within the granulomas in the treated group. Because TGF- β can directly stimulate fibroblasts to produce type I and type II collagen, the levels of TGF- β were examined. The expression of this cytokine was decreased but was equally decreased in animals sensitized with eggs without IL-12. Thus TGF- β cannot wholly explain the decrease in fibrosis.³¹ These findings demonstrate that IL-12 used as an adjuvant during immunization can decrease the disease found in response to a pathogen without necessarily preventing infection. Because the major manifestation of disease in schistosomiasis is the host response to the eggs and not the worms themselves, Dr. Sher suggested that this new anti-pathology vaccine may prove useful in preventing the human morbidity associated with this disease and that other T_{H2}-mediated diseases

may also benefit from such a vaccination strategy.

ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS) VACCINES

Since the virus responsible for AIDS was discovered in 1983, there has been interest and activity in the development of a vaccine to prevent infection. Dr. Mary Lou Clements (Johns Hopkins University, Baltimore) presented an overview of the development of AIDS vaccines.

Several obstacles to development of an effective vaccine for HIV have been perceived.³² First, the disease can be transmitted by infected cells, as well as by the free virus in body fluids. This is not uncommon and is seen with several other viruses, including measles, for which there are effective vaccines. Second, antigenic variation of the viral proteins has been perceived as an obstacle but has not hindered the development of influenza vaccines. Third, antibodies to surface viral proteins can enhance or facilitate infection and result in increased viral replication in *in vitro* assays. Although there has been concern about the *in vitro* phenomenon of antibody enhancement of infection, it has not turned out to be a significant problem for the development of vaccines against other viruses that elicit this phenomena; for example, there are safe, effective vaccines for yellow fever and tickborne encephalitis. An additional problem with the development of live attenuated or inactivated HIV vaccines is the potential for integration of HIV DNA into the host cell genome.

Recently, studies that have shown dose-dependent effects on transmission of the virus and on disease development have raised the question of whether total protection against infection is necessary. Several studies have shown a dose-dependent effect of the amount of measurable virus in the mother's blood on the rate of transmission to an infant. The lower the maternal viral load, the lower is the rate of transmission to the infant.³³ In addition, long-term survivors may have reduced virus load, particularly at the onset of the infection.³⁴ Thus there may be a role for vaccine-induced immunity to decrease the HIV viral load. This may reduce the rate of transmission of HIV infection from infected vaccinated persons to unvaccinated contacts and from infected vaccinated women to their infants, as well as prevent the development of or ameliorate the disease.

Ideally, two types of immune responses induced by vaccination would be desirable. The develop-

ment of high levels of neutralizing antibodies in body fluids might limit virus infection and reduce the load of free virus in body fluids, whereas the induction of HIV-specific cytotoxic T-cell responses of the CD8⁺ type might eliminate virus-infected cells. To prevent a persistent infection and to reduce HIV viral load in chronic infection, both neutralizing antibodies and cell-mediated immunity are probably needed. For preventive vaccines, initial strategies have focused on the envelope (gp120 or gp160) proteins of HIV. Envelope proteins have been used because they contain at least five epitopes that are responsible for neutralizing antibodies, as well as epitopes that are responsible for cytotoxic T-cell activity. Vaccinia virus and, more recently, canarypox virus vectors have been used to express the genes that code for envelope proteins, V3 loop peptides, core proteins, and cytotoxic T-lymphocyte epitopes of polymerase and nef gene products. The canarypox virus vector has several safety features, including the fact that it does not replicate in mammalian cells. Live recombinant viruses can be administered as vaccines; or recombinant proteins expressed in poxviruses can be extracted after growth in tissue culture, purified, mixed with an adjuvant, and administered as a subunit vaccine. There may be an advantage to combining the two approaches (i.e., priming the immune system by administering a live recombinant vaccine first and then boosting with a subunit vaccine). An advantage of a live recombinant vaccine is that it may be able to induce cytotoxic T-cell activity and prime for cell-mediated and humoral immunity, but it does not elicit a high titer of neutralizing antibody by itself. However, subunit vaccines have been more effective in inducing neutralizing antibodies. Thus the combination may be more effective than a single vaccine.

Neutralizing antibody responses in serum have usually been evaluated by using assays that measure a reduction in growth of laboratory HIV strains. Newer assays, in which patient isolates of HIV grown in peripheral blood mononuclear cells are used, have been developed. However, it is not clear that results of either type of assay correlate with protective immunity. The gp120 vaccines have elicited the highest titers of neutralizing antibodies against laboratory strains, but not patient isolates, of HIV; and these vaccines have seldom induced CD8⁺ cytotoxic T-lymphocyte activity. Some HIV vaccines have been tested for immunogenicity in primate systems. Because the chimpanzee can be challenged and infected with HIV, one can assess the ability of a vaccine to confer protection against

HIV infection in this animal model. However, the chimpanzee does not develop disease, so one can use this system only as a test to evaluate efficacy of a vaccine to provide sterilizing immunity. Indeed, the chimpanzee model can be considered an *in vivo* neutralization assay.

In 1988, the National Institutes of Health established an AIDS Vaccine Evaluation Group for testing HIV vaccines. Many of the risks of HIV vaccines are the same as those of other vaccines, such as local and systemic side effects. One effect of the vaccines that has caused concern is the development of antibodies in sera of vaccine recipients that react positively in the screening tests for diagnosing HIV infection. Unlike gp160 and multiple protein vaccines, the gp120 vaccines have not caused false-positive screening results except transiently.

Between 1988 and 1994, over 13 vaccines with a total of 10 different adjuvants had been tested in 18 trials involving over 1240 volunteers.³⁵ These have included phase I trials, in which the safety, immunogenicity, optimal dose and route of administration, and dose response of various vaccines have been shown. Also, one phase II trial of two gp120 vaccines, one perinatal phase I trial of envelope subunit vaccines in infected pregnant women, and two phase I trials of envelope subunit vaccines in nonpregnant infected adults have been conducted. The vaccines tested have been derived mainly from clade B strains of HIV. Various adjuvants have been used in different trials.

Several of the new adjuvants that caused significant local or systemic side effects were not shown to be clearly superior to other less reactogenic adjuvants in terms of immunogenicity. V3 loop peptide vaccines have been administered by parenteral and oral routes. Overall, all candidate vaccines tested have been quite safe, with no clinically serious adverse effects. As with hepatitis B vaccine, multiple doses of vaccine seem to be required for production of maximum immune responses, because little immune response is seen after two immunizations and anamnestic antibody responses are detected after the third and fourth doses. Dr. Clements stated that completion of the full series of immunizations will be needed to achieve maximum immune responses induced by the vaccines. Between 1988 and 1994, 12 HIV infections were detected in vaccinated persons and placebo recipients in the AIDS vaccine trials. Three of these occurred in placebo groups; among the other nine, some occurred in those who had received fewer than three injections.³⁶

The decision process leading to conducting phase III trials is complicated.³⁷ The risk-versus-benefits ratio of an efficacy trial will depend to a large extent on the urgency of the need for a vaccine to reduce HIV transmission. In this country a decision was made by the National Institute of Allergy and Infectious Diseases in June 1994 to wait until more promising vaccine candidates were available or until more compelling information about the effectiveness of gp120 vaccines was available. In October 1994, however, a committee of international experts convened by the World Health Organization recommended that trials to assess the efficacy of gp120 or other suitable vaccines be conducted, if the authorities in the country where the trial would be conducted gave their approval.

Dr. Clements concluded by commenting that much could be learned from well-designed, properly conducted efficacy studies. Randomized, placebo-controlled, double-blind, large-scale studies of candidate vaccines are needed to determine the effect of vaccine-induced immunity on virus load and the course of disease in infected individuals and to elucidate the correlates of protective immunity. If the viral load could be reduced in vaccine recipients, immunization with even a partially effective vaccine might result in reduction of both clinical disease and vertical or horizontal transmission of the virus.

ALLERGEN PEPTIDES AS VACCINES TO PREVENT AND TREAT ALLERGIC DISEASE

The normal immune response to a protein allergen involves uptake by an APC and presentation of peptides to T cells. In the presence of costimulatory molecules expressed on the surface of the APC, T cells are induced to proliferate and produce cytokines. One of the key mediators in the allergic response is IL-4 produced by T cells, which is necessary for production of IgE by B cells. Classically, clinical interventions have focused on IgE, the production of which occurs late in the process. In his talk Dr. Geoffrey Sunshine (ImmuLogic Pharmaceuticals, Waltham) addressed strategies that could modify the immune response by altering earlier steps in its development. Ongoing responses to allergens can be modified if the allergen is presented to T cells in the absence of costimulation. In this case T cells are turned off (anergized) rather than turned on. Other mechanisms of nonresponsiveness may involve deletion of T cells or active suppression.

In the past, immune responses to allergens have

been modified by using multiple injections of the allergen over months to years (i.e., immunotherapy). There is little agreement on the exact mechanism of this therapy. Recent evidence suggests that IL-4 production may be decreased in human beings who have undergone allergen immunotherapy, and IFN- γ production may be induced in these patients. This shift in cytokine production could modulate the immune response by pushing the immune response away from the production of IgE.

Another strategy is to use peptides derived from specific allergens to modulate the immune response. After proteins that react with IgE are isolated from patients, these antigens can be purified, characterized, cloned, and sequenced. With the sequence, a set of overlapping peptides can be synthesized, and these peptides can be used to refine the peptide candidates by testing them in patient-derived cell lines to define the minimal size and maximal T-cell reactivity. The safety of the peptides can be enhanced by selecting those that have minimal effect on B cells and histamine release.

Dr. Sunshine presented data obtained by using this strategy for modulating cat allergy. The major cat allergen is Fel d 1; persons allergic to cats have positive skin test responses to purified Fel d 1 and IgE that specifically reacts with Fel d 1. Fel d 1 is a two-chain molecule, but with the use of cell lines from patients allergic to cats, it was shown that the major area of T-cell reactivity is in a region of chain 1. Two peptides from this area, IPC-1 and IPC-2, were synthesized.

To determine whether these T-cell epitopes could modulate immune responses, an animal model was set up. IPC-1 and IPC-2 were injected into mice either intravenously or subcutaneously without adjuvant, and the mice were later challenged with peptide or the entire recombinant chain 1 molecule in adjuvant. Cells isolated from these animals were tested for antigen-specific T-cell proliferation and cytokine production. Preinjection of IPC-2 led to decreased production of IL-2 in comparison with mice that did not receive the peptide. Preinjecting a combination of peptides IPC-1 and IPC-2 decreased responses to the same degree as preinjecting chain 1.³⁸ The results were the same whether the peptide was given intravenously or subcutaneously and over a wide dose range. The phenomenon was long-lasting because at 100 days, there was still a decrease in cytokine production. Proliferation and production of IL-3, IL-4, and IL-5 were all decreased in the

group that received the peptide injections. These results were seen in spleen, lymph node, and peripheral blood T cells, suggesting that peptides produce a systemic effect. The effect was seen in isolated CD4⁺ cells. Interestingly, the same effect of peptide-preinjection decreasing T-cell responses was seen in both naive and primed mice. Mice primed with an injection of protein in adjuvant and then given injections of peptide without adjuvant showed the same decrease in cytokine production after challenge.

To determine whether deletion, anergy, or suppression was the mechanism of this decreased T-cell response after peptide injection, Dr. Sunshine and coworkers used mice that carried a transgene for the T-cell receptor specific for pigeon cytochrome C peptide 88-103. In these mice more than 60% of the T cells respond to a single peptide. Peptide injection into these animals resulted in decreased T-cell IL-2 and IL-4 synthesis. Transgenic mice were also thymectomized so that their existing T-cell population was fixed and could not be renewed by thymic differentiation. In the thymectomized animals the same decrease in T-cell response was seen after treatment with peptide. In addition, even after 35 days, the number of cells in the CD4⁺ subset specific for the transgene was the same. Thus these cells were not deleted.³⁹ Dr. Sunshine suggested that the decrease in T-cell function after peptide injection is most likely due to anergy, but other mechanisms may apply in different peptide-mediated T-cell tolerance systems.

After establishing that injection of allergen epitopes can downregulate the immune response to those peptides and the protein from which they are derived, this strategy of immunomodulation by peptide was tested in an animal model in which the clinical impact could be evaluated. Mice develop experimental allergic encephalomyelitis, a paralyzing demyelinating disease similar to MS after the injection of myelin basic protein (MBP) in adjuvant. Preinjection of a dominant peptide T-cell epitope of MBP just before the onset of the disease prevented a rise in the clinical scores of disease observed compared with scores in saline-injected controls. In addition, if the injections of peptide were given at the onset of symptoms, the clinical scores were better than those of animals that did not receive peptide. Furthermore, when animals that had undergone their first episode were given peptide injections, they did not relapse as did control, untreated mice. Thus peptide injection can prevent the development of experimental al-

lergic encephalomyelitis and can also treat existing disease.⁴⁰

Multicenter clinical phase I and phase II trials in which peptide immunotherapy was used to treat cat allergy have been completed. Patients allergic to cats received injections of a combination of IPC-1 and IPC-2 or control injections. Patients were evaluated 1 and 6 weeks later by systematic scoring of signs and symptoms during cat room exposure. At 6 weeks, those who had received the highest dose of peptide showed the most improvement in nasal and lung scores. As seen in most allergy studies, there was also a significant effect of placebo. However, over 85% of those receiving the highest dose improved.⁴¹ Approximately a third of the patients did have delayed allergy-like symptoms after the injection of peptide, which were self-limited or controlled with antihistamines. Thus this is a safe and apparently efficacious therapy for allergic diseases, which needs evaluation in phase III trials. It may also have application to autoimmune diseases.

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