VACCINE RESEARCH AND DEVELOPMENT

Vaccines are a safe, effective, and efficient means of preventing morbidity and mortality from infectious diseases. NIAID is the center of vaccine research and development within the Department of Health and Human Services. The Institute's broad research programs on all classes of infectious diseases and their causative agents, together with basic research on the immune system, have nurtured comprehensive, collaborative vaccine efforts among scientists in Government, industry, and academic institutions. In setting priorities for vaccine development, NIAID weighs the severity of disease and expected health benefits, considers the scientific and programmatic gaps and opportunities, and studies the feasibility, given the status of scientific knowledge about particular diseases and their causative agents.

The Division of Acquired Immunodeficiency Syndrome (DAIDS) supports the discovery and development of safe and effective vaccines to prevent HIV infection and AIDS worldwide. Toward this end, DAIDS has a comprehensive portfolio of research grants and programs spanning basic vaccine research and preclinical testing of candidate HIV vaccines, through human clinical testing in the United States and internationally.

The Division of Microbiology and Infectious Diseases (DMID) also supports a full spectrum of vaccine research to (1) prevent infectious diseases such as tuberculosis (TB), malaria, cytomegalovirus (CMV), group B streptococcus, and chlamydial infections; (2) serve fragile populations such as infants, older people, and immunocompromised people; (3) evaluate novel vaccine approaches such as oral, transcutaneous, and combination vaccines; and (4) improve existing vaccines.

Both DAIDS and DMID support large clinical networks and have vaccine production contracts that provide opportunities to develop and advance vaccine concepts into early stages of clinical evaluation. Infrastructure for regulatory oversight, site monitoring, and data management round out the vaccine development process. In collaboration with the Fogarty International Center, both Divisions support capacity building and training in clinical research.

The Division of Allergy, Immunology, and Transplantation (DAIT) supports research designed to apply the fundamental principles of immunology to the development of improved vaccines. The Division of Intramural Research (DIR) conducts a wide-ranging vaccine program. Extensive efforts are under way to develop vaccines to prevent diseases of worldwide importance, such as malaria, AIDS, childhood respiratory infections, chlamydia, hepatitis C and E, West Nile virus, dengue fever, rabies, and genital herpes. NIAID's Dale and Betty Bumpers Vaccine Research Center (VRC) conducts research that facilitates the development of effective vaccines for human disease, with the primary focus of research being the development of vaccines for AIDS.

Division of Acquired Immunodeficiency Syndrome

The development of a safe and effective vaccine against HIV is critical to worldwide efforts to control the epidemic. Although educational and counseling efforts have had some success and remain essential, these prevention activities alone will not be sufficient to contain the worldwide spread of

disease. An HIV vaccine represents the best hope for controlling the HIV epidemic.

The NIH devotes significant resources to the development of safe and effective HIV vaccines. The AIDS Vaccine Research Working Group (AVRWG), VRC, and NIAID's comprehensive HIV vaccine research program are key to advancing HIV vaccine research. The AVRWG, chaired by Dr. Barton Haynes, stimulates HIV vaccine research and assists the NIH in developing a comprehensive research program aimed at expediting the discovery and development of a safe and effective vaccine. Serving as a focal point for intramural scientists at the NIH, VRC advances multidisciplinary research from basic and clinical immunology and virology to vaccine design and early clinical testing. This work complements the comprehensive extramural research activities of DAIDS.

DAIDS supports exploratory, high-risk, investigator-initiated HIV vaccine research at the earliest stages of concept genesis and evaluation through the Innovation Grants for AIDS Research Program. Other basic vaccine research and design efforts, including testing in animal models, mechanism-of-action studies, and studies of HIV immune correlates, are supported through the HIV Vaccine Research and Design Program. In the past year, new innovation grants were solicited and awards were made to researchers to investigate virus vector vaccine designs, T cell immunology, animal model development, and DNA vaccines. For example, HIV Vaccine Research and Design Program awards were made to support the optimization of a prime-boost vaccine approach, to evaluate genetically engineered *Listeria monocytogenes* bacteria for use as an HIV vaccine, and to examine the use of herpes simplex virus type 1 (HSV-1) amplicon vectors for HIV vaccine delivery. The Integrated Preclinical/Clinical Vaccine Development Program, which targets research at the preclinical-clinical interface of the vaccine research pipeline, made an award to examine a novel virus-like particle-based HIV vaccine intended to provide both mucosal and systemic immunity. The Novel Technologies for HIV and HIV Vaccine-Related Research Program supports the use of new technologies to detect and quantitate HIV, optimize measurement of immune responses to HIV and candidate HIV vaccines, and measure immune responses responsible for the efficacy of licensed vaccines for other infectious diseases. In the past year, awards through this program were made to assess laboratory correlates of immune protection, to evaluate a new approach to the study of T cell responses for vaccines in Uganda, and to identify minimal levels of HIV in vaccinated and HIV-exposed individuals.

To help expedite the development of promising HIV/AIDS vaccines, DAIDS also has several novel public-private partnerships under a program titled HIV Vaccine Design and Development Teams (HVDDT). These "teams" tap the different skills and talents of private industry and academic research centers, and they are given financial incentives to move strong HIV/AIDS vaccine candidates out of the laboratory and into human testing. The HVDDT program is intended to increase public-private cooperation in developing vaccines for HIV and malaria and encourages pharmaceutical companies to invest more in AIDS vaccine research by partially offsetting their financial risk. Before 2003, five contracts were awarded under this mechanism—four in 2000 and one

in 2002. In each of these cases, the contractors are moving vaccine products rapidly through production and preclinical testing. One of the original four contactors, a consortium headed by the University of New South Wales in Australia, had its two vaccines (DNA and fowlpox-vectored vaccines) enter human clinical trials early this year. The other three original awardees are on schedule to have their vaccine products ready for human clinical trials later in 2003. They include Chiron (DNA and protein-based vaccines), Wyeth Lederle Vaccine (DNA and peptide-based vaccines), and Advanced BioSciences Laboratories (DNA and protein-based vaccines). In 2003, four additional HVDDT contracts were awarded to Alphavax (alphavirus replicon-based vaccines), Epimmune (multi-epitope DNA and modified vaccinia Ankara [MVA]-vectored vaccines), Novavax (virus-like particles), and Progenics Pharmaceuticals (envelope protein vaccines).

Clinical HIV vaccine research also is carried out through the HIV Vaccine Trials Network (HVTN). This global HIV vaccine research network was established in 2000 to foster the development of HIV vaccines through testing and evaluating candidate vaccines in clinical trials. The network has the capacity to conduct all phases of clinical research, from small pilot studies that evaluate candidate vaccines for safety and the ability to stimulate immune responses to large-scale testing of vaccine efficacy. HVTN sites are located in Botswana, Brazil, China, the Dominican Republic, Haiti, India, Jamaica, Malawi, Peru, South Africa, Thailand, Trinidad and Tobago, and the United States. In the United States, sites are located in Alabama, California, Maryland, Massachusetts, Missouri, New York, Puerto Rico, Rhode Island, Tennessee, Virginia, and Washington.

Many of these sites are actively enrolling study volunteers. Internationally, enrollment is occurring in Brazil, Haiti, Peru, and Trinidad; domestically, sites enrolling patients are located in Seattle, WA; St. Louis, MO; Boston, MA; Providence, RI; New York, NY; Baltimore, MD; Birmingham, AL; Rochester, NY; San Francisco, CA; and Nashville, TN.

NIAID and the U.S. Army Medical Research and Materiel Command (USAMRMC) of the Department of Defense (DoD) continue to work cooperatively to ensure the effective integration and coordination of HIV vaccine research efforts. The formal collaboration was established through an interagency agreement in October 2002 with the transfer of the USAMRMC HIV Research and Development Program to NIAID. The merger of these two programs further ensures that U.S. Government HIV vaccine research is well coordinated, efficient, and comprehensive. The strategic and scientific strengths of the USAMRMC give NIAID greater access to the USAMRMC HIV/AIDS research program focused on vaccine product development; the DoD's presence overseas; knowledge and experience in supporting operations abroad; the extraordinary DoD medical infrastructure support, including worldwide transportation and logistics support; several well-established DoD research laboratories in developing countries; and extensive knowledge and skills in establishing and supporting operations in underdeveloped areas.

Because success in identifying a safe and effective HIV/AIDS vaccine in the shortest time possible will require unprecedented global cooperation among governments of both industrialized and resource-poor developing countries, vaccine developers in the private sector, academic researchers,

nonprofit organizations, and affected communities, NIAID recently began an innovative program called Partnership for HIV/AIDS Vaccine Evaluation (PAVE). PAVE will help ensure coordination and efficiency of U.S. Government agencies and their partners working on HIV/AIDS vaccine development, thereby accelerating progress. PAVE will accomplish this through the sharing of strategic plans and results, collaborative and comparative evaluation of candidate vaccines, and collaborations that will improve the efficiency with which clinical trials are conducted. Participation in PAVE is open to any entity or organization actively involved in HIV/AIDS vaccine research and development; all U.S. Government agencies supporting HIV/AIDS vaccine research and development are currently members. These agencies include the NIH, the Centers for Disease Control and Prevention (CDC), and the U.S. Military HIV Research Program of the DoD, as well as the NIAID/NIH-funded HIV/AIDS Vaccine Trials Network and the nonprofit Henry Jackson Foundation for the Advancement of Military Medicine.

PAVE partners will initially assist each other in preparing for and conducting multiple phase III efficacy trials of candidate HIV/AIDS vaccines worldwide in an efficient, effective, and collaborative manner. Partners will work together with developing-country researchers to effectively address complex logistical and operational challenges such as training of staff and development of consistent, high-quality operating and monitoring procedures. PAVE partners will work with host countries to help ensure that the research infrastructure needed to conduct HIV/AIDS vaccine efficacy trials will be sustained and that vaccine research and development will be coordinated with the broader public health needs of the countries in which the trials take place, including prevention and care programs for those already infected with HIV. Partners will continue to individually engage host country scientists in their vaccine clinical research activities, ensuring that volunteer safety, volunteer rights, and ethical and operational standards are not compromised.

Partners have agreed to strive to coordinate trial design features so that data from different trials can be compared more readily. Initial goals include the development and sharing of central and regional laboratories that are essential for comparing different vaccines, monitoring the safety of candidate vaccines, diagnosing HIV infection rapidly and accurately, and reliably measuring other efficacy outcomes such as viral load and CD4+ cell counts. Through PAVE, the partners also will explore additional laboratory efforts that would permit a valid comparison of data across all trials.

PAVE will work with other funders and vaccine developers in the public and private sectors to ensure that sufficient capacity exists worldwide to simultaneously conduct multiple efficacy trials and to ensure that the most promising products identified through early clinical trials are targeted for accelerated development and evaluation. PAVE, which will continue to add more partners, represents a critical step forward for the coordination of public and private global HIV vaccine research efforts and will help further the field of HIV vaccine research.

To date, NIAID has supported more than 68 HIV phase I and phase II vaccine trials, involving well over 4,200 volunteers. A total of 40 candidate vaccines and 14 different

adjuvants (a substance that enhances the immune-stimulating properties of a vaccine) have been tested.

Some HIV vaccine strategies combine more than one vaccine preparation. NIAID-funded researchers have previously demonstrated that a combination vaccine approach is safe and immunogenic in volunteers at both low and high risk for HIV infection. This approach has been shown to stimulate cellular immunity, resulting in cytotoxic T lymphocytes that can kill infected cells and in the production of HIV-neutralizing antibodies that can stop HIV from infecting cells. Thus, the combination approach holds promise because it stimulates production of HIV-neutralizing antibodies and cellular immunity.

Planning is currently under way for NIAID to support the USAMRMC-sponsored phase III efficacy trial of a prime-boost vaccine candidate under development in partnership with the Thai government and Thai researchers. The study will test a combination of a canarypox virus that has been genetically altered to contain selected HIV genes, followed by a protein from the HIV outer coat called gp120. Neither the engineered canarypox virus nor the gp120 subunit protein can cause HIV infection. The phase III trial in Thailand is expected to commence in fiscal year (FY) 2004.

During the past year, NIAID's HVTN has initiated several HIV vaccine studies. Candidates under investigation include the canarypox/gp120 prime-boost approach, an alphavirus replicon vaccine containing the HIV subtype C *gag* gene, and HIV DNA vaccines with or without novel adjuvants. DNA vaccines and viral vectors both contain HIV genes that recipients' cells use to produce HIV proteins, which in turn induce an immune response.

Several other studies also completed in the past year provide more information needed to further vaccine development efforts. A study of a vaccine involving a NefTat fusion protein in combination with varying doses of gp120 found that it was well tolerated at various doses and at all points in time. Another study of the safety and immunogenicity of different doses of a canarypox vaccine (ALVAC-HIV vCP1452) found that it was less well tolerated at higher doses than at lower doses and that higher doses did not improve immunogenicity.

Future safety and immunogenicity trials will involve lipopeptides, a number of other novel DNA vaccines, a recombinant nonreplicating adenovirus vaccine, MVA-vectored vaccines, and a CTL multi-epitope peptide vaccine. HIV vaccine preparedness studies in each of the HVTN expansion sites, and a feasibility study at the HVTN sites in Brazil, also will be pursued to lay the foundation for these sites' participation in future HIV vaccine trials.

Preclinical research continues to advance knowledge and guide HIV vaccine development efforts. One recent NIAID-funded study demonstrated the importance of immune responses to the HIV proteins encoded by the *env*, *gag*, and *pol* genes and the potential role of these responses in helping to protect against death of uninfected CD4+ T cells. Findings from another recently completed NIAID-funded study suggest that chimpanzee adenoviruses that have been engineered to carry HIV genes and cannot replicate may be more suitable as vaccine carriers for use in humans than vaccines based on human serotypes.

Division of Microbiology and Infectious Diseases

Because vaccines can provide a safe, effective, and efficient means to prevent illness, disability, and death from infectious diseases, research leading to new and improved vaccines is a high priority for DMID. The goal of the DMID Program for the Accelerated Development of Vaccines, established in 1981, is to support research leading to vaccines that will improve the health of the Nation. Factors that influence priorities for vaccine research include the morbidity and mortality associated with each infectious disease, critical evaluation by the Institute of Medicine (IOM) of the National Academy of Sciences, assessment of research gaps and opportunities, and recommendations made by the National Vaccine Advisory Committee and other advisory groups.

DMID designs and implements a comprehensive research program to develop new or improved vaccines that will prevent or reduce the incidence of disease. Advances in the fields of microbiology, immunology, and biotechnology are applied to the development of new vaccines and to the improvement of existing vaccines through research support of the following:

- New vaccines against major diseases caused by respiratory syncytial virus (RSV); malaria; group A and group B streptococci; and other bacterial, parasitic, and fungal infections of both children and adults;
- Improved vaccines against diseases such as influenza virus, viral hepatitis, and TB;
- Vaccines to prevent neonatal infections such as group B streptococcus, and congenital diseases caused by CMV infection, toxoplasmosis, syphilis, gonorrhea, and chlamydia infections;
- New vaccines to prevent and control emerging diseases, including *Helicobacter pylori*, West Nile virus, severe acute respiratory syndrome (SARS), and drug-resistant bacteria such as pneumococcus; and
- Novel technologies to enhance the effectiveness of vaccines such as adjuvants, proteosomes, and plasmid DNA approaches.

Spurred on by advances in the basic sciences, new vaccine candidates continue to be developed. Vaccines of high public health relevance, often developed in collaboration with industry, are tested for safety and efficacy in preclinical studies. If these vaccines remain promising, they may be evaluated in the DMID Vaccine Evaluation Network, which includes the Vaccine and Treatment Evaluation Units (VTEUs) and other units at universities across the United States. An integral part of NIAID vaccine research efforts, these vaccine units support carefully planned and designed clinical trials of novel bacterial, parasitic, and viral vaccines and other biologics in people of all ages and risk categories. In FY 2002, NIAID awarded new contracts for the VTEUs and several other vaccine units, expanding and reorganizing the Institute's network of university-based sites that conduct clinical trials of promising vaccine candidates and therapies for infectious diseases. As a result of this reorganization, NIAID has been able to fund more clinical trials focused on specific populations, as well as larger trials of public health importance, including those related to biodefense and vaccine safety.

DMID also supports research to develop new vaccine approaches that

- Generate long-lasting protective immunity against various infectious agents;
- Favor the development of mucosal immunity or the production of an antibody of a given isotype;
- Increase the immunogenicity of candidate vaccines or favor the expression of a cell-mediated cytotoxic immune response; and
- Simplify immunization regimens to reduce the number of immunizations required for protection and the number of visits to healthcare facilities and associated costs.

DMID is recognized as an effective participant in U.S. and global vaccine policy. In the United States, DMID collaborates with other agencies, including CDC and the Food and Drug Administration, on issues of vaccine research, vaccine safety, and national immunization strategies; this collaboration is coordinated through the National Vaccine Program Office (NVPO). Internationally, DMID participates with other national research agencies in the development and support of programs such as the Global Alliance for Vaccines and Immunization (GAVI) and the Multilateral Initiative on Malaria. GAVI has the support and participation of international agencies such as the World Health Organization, UNICEF, and the World Bank, as well as industry, nongovernment agencies, and foundations. The creation of the Global Alliance has been accompanied by significant financial commitments from the Bill and Melinda Gates Children's Vaccine Program. The mission of GAVI is to save children's lives and protect people's health through the widespread use of safe vaccines, in the belief that every child, regardless of place of birth or socioeconomic status, should be protected against vaccine-preventable diseases of public health priority.

The evaluation of vaccine safety is an integral component of the NIAID vaccine research program. Safety is evaluated in every vaccine clinical trial sponsored by NIAID. Study participants are monitored closely for any adverse effects of the vaccinations they receive. Specific safety issues such as the use of novel cell substrates for vaccine development and the evaluation of combination vaccines are explored through scientific consultation with other Government agencies and in coordination with NVPO.

NIAID, in collaboration with CDC, requested that the National Academy of Sciences Institute of Medicine (IOM) establish an independent expert committee to review hypotheses regarding the relationship between specific vaccines and adverse events. In response, IOM created the Immunization Safety Review Committee in September 2000. This committee reviews the state of knowledge regarding various specific immunization safety concerns and communicates its findings to healthcare providers and the public. In the past 3 years, the committee has met to review several important vaccine safety issues, including measles-mumps-rubella vaccine and autism, thimerosal-containing vaccines and neurodevelopmental disorders, multiple immunizations and immune dysfunction, hepatitis vaccine and neurological disorders, SV40 contamination of polio vaccine and cancer, potential role of vaccinations in sudden unexpected death in infancy, and influenza vaccine and possible neurologic complications. Within several months of each meeting, the committee publishes reports of its

findings as well as recommendations for any additional actions such as research or further surveillance that are indicated.

DMID will continue to apply the latest advances in the fields of immunology, microbiology, and biotechnology to the development of new or improved vaccines against infectious diseases. These applications include the following:

- Use of recombinant DNA technology for the production of defined immunogens as well as the preparation of plasmid DNA vaccines;
- Development and use of various immunomodulators to augment the immune response to poorly immunogenic candidate vaccines;
- Development of novel vaccine delivery systems to promote long-lasting immunity or to generate immune response in selected host tissues; and
- Research on novel approaches to the development of multicomponent vaccines and simpler vaccination regimens to reduce healthcare costs and the number of visits to healthcare facilities.

Division of Allergy, Immunology, and Transplantation

DAIT supports research on immunologic mechanisms and novel technologies applicable to vaccine design and development. The Division funds vaccine-related research projects on innate and adaptive immunity that aim to increase our ability to rationally manipulate immune responses through better understanding of the underlying molecular, cellular, and systemic aspects of natural host defenses and antigen-specific immunity. Projects include basic studies of innate immune receptors for pathogen molecules, antigen processing and presentation, the development of antibody and cellular responses, and the elaboration of immunologic memory. Topics more immediate to vaccine applications include the development of new adjuvants to enhance immunity, the design of approaches that can induce protection in mucosal tissues, and the discovery of novel methods for more effective delivery of immunizing agents. Projects laying the groundwork for improved vaccine approaches include discovering and cataloging new pathogen epitopes, which are the molecular regions of bacteria and viruses that stimulate immunity, and analyses of the impact of variability in the human genome on immune responses.

DAIT continues to fund four Vaccine Immunology Basic Research Centers that focus on the fundamental aspects of human protective immune mechanisms in infectious diseases. Through the Human Immunology Centers of Excellence Program, DAIT supports numerous mechanistic studies that will contribute to our basic understanding of human immunity and vaccine responses.

In FY 2002, the Hyperaccelerated Award/Mechanisms in Immunomodulation Trials research program was expanded to support indepth study of immunologic mechanisms during clinical trials of vaccines including analyses of the underlying mechanisms of protective immunity, specificity and kinetics of immune responses, and immune memory. Studies proposed under this program must make use of clinical samples from a parent clinical trial that is supported by other funding. For example, NIAID recently funded research to analyze indepth the cell-mediated immune responses

of participants in a smallpox vaccine clinical trial.

DAIT has established a program called Application of Data on Human Leukocyte Antigen (HLA) to the Improvement of Vaccines, which supports several projects on hepatitis C, TB, malaria, and HIV. Investigators funded under this program recently developed a novel way to identify malaria vaccine antigens. They used a mathematical algorithm to predict which segments of individual malaria proteins would bind the majority of the immune system receptor molecules of the major histocompatibility complex (MHC); binding of these malaria protein segments, called supertype antigenic epitopes, to MHC molecules is required for activation of immune cells called T lymphocytes. Concurrently, the investigators used proteomic and genomic techniques to select 27 genes expressed in different life stages of the parasite for further analyses. They found that 16 of the 27 candidate genes contained supertype epitopes that were recognized by T cells from individuals vaccinated against malaria but not by T cells from control individuals. Using these strategies, the investigators identified three times more malaria T cell antigens than have been previously described. Furthermore, some of these novel antigens stimulated the immune system more vigorously than any of the antigens that are the basis of current vaccine trials. This approach has the potential to identify many new malaria antigens and will be invaluable in the discovery of vaccine candidates in other diseases as well. Also under this program, DAIT supports the HLA Ligand/Motif Online Database, a Web-based, searchable database of human MHC molecules and peptide ligands. The database specifies amino acid sequences of peptides derived from viral, bacterial, parasite, and human proteins in association with human MHC molecules. This resource enables users to search for specific human MHC/peptide combinations or to determine ligand amino acid motifs. Support is provided under a NIAID contract with the University of Oklahoma. The Web address is *http://hlaligand.ouhsc.edu.*

Grants funded under the Cooperative Centers for Translational Research on Human Immunology and Biodefense Program will facilitate the translation of research in animal models such as the mouse, into studies in the human. New technologies will be developed to allow more definitive studies of human immune responses and regulation, and research on human immunity to NIAID Category A to C priority pathogens will be conducted to support improved vaccine designs and the development of novel immunotherapeutic agents.

Contracts awarded under the Innate Immune Receptors and Adjuvant Discovery Program will support research from the discovery/molecular response evaluation stage through preclinical testing of new adjuvants that can stimulate the human innate immune system. The adjuvant products developed under this program might be used both as vaccine adjuvants—to elicit T and B cell responses when co-administered with an immunogen—and as stand-alone immunomodulators—to stimulate short-term protective responses against a broad range of infectious agents. Research must be directed toward vaccine adjuvants and immune stimulation strategies to defend against NIAID Category A, B, or C agents. Multiple awards will be made in FY 2004.

The Large-Scale Antibody and T cell Epitope Discovery Program supports the rapid identification and verification of the particular molecular structures on pathogens, called epitopes, that are recognized by specific antibodies or T cells during the immune response. A related effort will establish a comprehensive centralized database to provide a Web-based, searchable source of information on pathogen epitopes for researchers. Included in the database is an analysis resource to facilitate data analysis and prediction of novel pathogen epitopes.

The NIAID Tetramer Facility produces MHC/peptide reagents for T cell detection and has provided more than 1,500 tetramers to investigators worldwide. Reagents are provided for the study of T cell responses relevant to vaccine research and development for many diseases including intracellular bacterial, viral, and parasite infections and autoimmune diseases. Information on the NIAID Tetramer Facility can be found at *www.niaid.nih.gov/reposit/tetramer/index.html.* The National Cancer Institute also provides funding for the Tetramer Facility.

Division of Intramural Research

DIR is working to develop vaccines against many infectious agents of public health importance including respiratory and gastrointestinal viruses, hepatitis viruses, West Nile virus, and infectious agents that cause common tropical diseases such as malaria and dengue. This work often involves collaborative research and development efforts that span years—or decades—before coming to fruition. For example, an intranasal influenza vaccine licensed in 2003 is the result of more than 20 years of collaborative research involving Dr. John Maassab of the University of Michigan School of Public Health and DIR scientists, with support from DMID. A hepatitis E vaccine developed by DIR researchers currently is undergoing a clinical trial in collaboration with scientists from the military and industry. Candidate vaccines for pandemic influenza, parainfluenza, RSV, and the newly discovered RSV-like human metapneumoviruses are tested for safety, immunogenicity, genetic stability, and efficacy at the DIR-supported VTEUs. Each year about 10 candidate vaccines are evaluated at the Johns Hopkins University VTEU.

DIR scientists have furthered the development of candidate vaccines that target the two most important viral agents of pediatric respiratory tract disease worldwide, RSV and human parainfluenza type 3 (HPIV3). Clinical trials of a vaccine candidate called HPIV3 cp45 have demonstrated its safety in very young infants and both its safety and immunogenicity in older infants and children, suggesting further development is warranted.[52] In addition, DIR researchers found that a candidate vaccine for HPIV3 could be made more effective in primates by inserting the gene for granulocyte-macrophage colony-stimulating factor (GM-CSF) into the vaccine virus. The three- to sixfold increase in virus-neutralizing antibodies the scientists observed is a very large increase in practical terms. Provided the vaccine passes appropriate safety tests and has a similar effect in humans, this result might lead to more effective live attenuated vaccines for HPIV3, RSV, and other viruses in this family.[53]

DIR researchers also are working to improve the current licensed hepatitis A vaccine made from killed virus. This vaccine requires multiple booster shots given

intramuscularly—an expense and inconvenience that inhibits its use in less developed countries. Scientists are attempting to develop a live hepatitis A vaccine made from a deliberately weakened form of the virus that could be given orally in a single dose. Their studies have revealed the genes responsible for hepatitis A virulence, but in experiments in which these genes were mutated to weaken the virus, the virus often reverted to its more virulent form. Studies to determine the feasibility of this live oral vaccine approach are currently under way.[54]

DIR scientists made a major advance in malaria vaccine development by the production, preclinical testing, and commencement of human phase I clinical trials of a candidate vaccine based on the *Plasmodium falciparum* antigen AMA1. Following immunization with AMA1, Aotus monkeys were protected from challenge with malaria parasites. Unfortunately, different parasites carry slightly different forms of AMA1, and, although antibodies raised against one form are able to bind to other forms of AMA1, they bind best to the form used to raise the antibody. However, combining two different types of AMA1 generated an immune response that was better at recognizing the AMA1 from many different parasites. This combination of two forms of AMA1 is the basis of the malaria vaccine now in a human trial. The scientists do not yet know if two types of AMA1 will be sufficient to cover all malaria parasites, but they have established a proof of principle that will guide further vaccine development.[55]

Dengue is a mosquito-borne viral infection that causes an estimated 50- to 100-million cases of dengue fever and several hundred thousand cases of potentially fatal dengue hemorrhagic fever each year. Four subtypes of dengue virus exist; infection with one subtype does not provide immunity to the others, so persons living in dengue-endemic areas can be infected by each subtype during their lifetimes. DIR scientists are developing a recombinant live attenuated dengue vaccine that would provide protection against all four dengue subtypes. Preclinical testing of this candidate vaccine is under way.[56] Vaccines also are being developed for other mosquito-borne and tick-borne viruses. These include West Nile and St. Louis encephalitis viruses, both of which are widely disseminated throughout the United States, and tick-borne encephalitis virus, which is widely disseminated throughout the northern hemisphere.

Traditionally, identification of potential new vaccine candidates has been a slow and laborious process conducted one gene or protein at a time. However, genome sequencing and other high-throughput analytic techniques now provide far more rapid and efficient methods to identify parts of an infectious agent that might form the basis of human vaccines. DIR scientists are using these modern tools to identify potential vaccine components for group A streptococcus, *Mycobacterium tuberculosis*, and other agents that cause significant morbidity and mortality worldwide.

Vaccine Research Center

The role of VRC is to stimulate multidisciplinary vaccine research and to translate basic research into candidate vaccines ready for clinical trials. After September 11, 2001, the biodefense role of VRC expanded to include development of preventive and therapeutic vaccines against potential agents of bioterrorism.

In November 2002, VRC initiated the first trial of a preventive HIV vaccine that has the potential for global reach in the war against HIV. The initial phase of this trial, involving the first multiclade, multigene HIV vaccine candidate to enter human trials, is designed to determine the vaccine's safety at three dose levels. A total of 50 healthy, HIV-negative volunteers have been enrolled. During the yearlong trial, VRC scientists are assessing the vaccine's safety and evaluating the immune response to it. If this trial is successful, a larger trial to further evaluate safety, immune response, and dosing schedule will be conducted through NIAID, DAIDS, and HVTN at several domestic sites. A phase I clinical trial with 30 healthy volunteers also will be done in Uganda as a collaboration among the Makerere University-Walter Reed Project, DAIDS, and VRC. DAIDS' Adult AIDS Clinical Trials Group also will conduct a phase I clinical trial in HIV-infected volunteers.

The first phase I clinical trial of an HIV vaccine invented at VRC was launched in May 2001. This study of a DNA vaccine containing a modified *gag-pol* gene is following 21 healthy HIV-negative men and women to determine whether the candidate vaccine is safe and whether the body makes an immune response to these proteins.

VRC is currently testing MVA, an attenuated poxvirus that has the potential to protect against both vaccinia, the virus used to vaccinate against smallpox, and variola, the virus that causes smallpox. The vaccine was provided by Therion Biologics Corporation as part of a collaboration with VRC. Two phase I clinical trials are now under way testing MVA as a component of a safer smallpox vaccine in both vaccinia-naive and vaccinia-immune populations. Preclinical studies also have shown promise for the use of recombinant vaccinia as a vector. Administration of an MVA vector to macaques demonstrates production of both humoral and cellular immune responses to both vaccinia antigen and recombinant.

The development of an effective vaccinia vaccine with a lower rate of complications, particularly for vaccine-naive and immunocompromised recipients, and an easier method of administration than traditional scarification, is of great interest. Such a vaccine could be more widely used in the event of smallpox bioterrorism or as an agent that can be administered to vaccine-naive researchers working with vaccinia strains.

Additional plans for FY 2004 include clinical testing of a novel vaccine for the Ebola virus. The rapid progression of disease after Ebola infection allows little opportunity to develop natural immunity, and there is currently no effective antiviral therapy. Therefore, vaccination offers a promising intervention to prevent infection and limit spread as well as an important public health benefit for healthcare workers involved in care of patients and containment of outbreaks.

The development of a contractor-leased and contractor-operated Vaccine Pilot Plant (VPP), which will manage production of multiple vaccine candidates originating from VRC, is a high priority. To achieve this objective, VPP will function in concert with the Vaccine Production Laboratory located at the Bethesda campus to transfer new vaccine technology for pilot-scale production of vaccine material for use in clinical trials.

Focus on

Story of Discovery: Developing a Recombinant Vaccine for Hepatitis E

Hepatitis E virus (HEV) is one of the most important causes of acute hepatitis (inflammation of the liver) among adults in Southeast and Central Asia, the Middle East, and North Africa. HEV also poses a risk to others, including military personnel, who travel to these areas. Although children become infected with HEV, the virus is more likely to cause acute hepatitis in people aged 15 to 40 years, for whom the mortality rate is 0.4 to 4.0 percent. The disease is most serious in pregnant women, in whom the mortality rate can reach 20 percent. HEV infects as many as one-third of the world's population, and there is no effective treatment. Therefore, the development of an effective HEV vaccine is a high priority. To meet that goal, NIAID intramural researchers and their colleagues have developed a recombinant HEV vaccine based on the viral capsid protein of a Pakistani (genotype 1) strain of HEV. The development of the recombinant HEV vaccine has been an international effort, with contributions from U.S. Government scientists, academic scientists, the U.S. military, the pharmaceutical industry, and foreign governments.

Discovery of Hepatitis E Virus

In the late 1970s, NIAID researchers began to suspect that a previously unrecognized virus was the cause of some hepatitis epidemics. Other scientists had previously isolated hepatitis A virus (HAV) as the cause of many hepatitis cases. Like HAV, the unknown virus was transmitted through the oral-fecal route, in which the virus shed in an infected person's feces pollutes water consumed by someone else. The symptoms it caused—jaundice, abdominal pain, malaise, vomiting, enlarged liver, and fever—were identical to those of HAV. And like HAV, the unknown virus was eliminated from the body after the illness had run its course, leaving the patient immune to further infection. Despite these similarities, the unknown virus was not identical to HAV. NIAID intramural researchers, in collaboration with Indian scientists, found that many people infected during hepatitis epidemics in India did not generate antibodies against HAV. However, HEV was not isolated until 1983, when a Russian scientist deliberately drank a solution containing fluid from hepatitis patients, took blood and stool samples during the course of his illness, and then analyzed the samples after he recovered. The same researcher also infected cynomolgus macaques with the virus, thereby creating an animal model of the disease. But like other scientists since then, he was unable to grow the virus in cultured cells, a barrier that has made it impossible to develop a traditional vaccine based on weakened, live virus.

Steps Toward a Recombinant HEV Vaccine

The next major step toward an HEV vaccine occurred in 1991, when researchers at Genelabs, Inc., a California biotechnology

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Story of Discovery: Developing a Recombinant Vaccine for Hepatitis E, *Continued*

company, determined the entire 7,200-nucleotide sequence of the RNA genome of HEV. Using the Genelabs sequence information, NIAID researchers began to generate HEV proteins in pure form to improve antibody-based diagnostic techniques for the virus. The NIAID scientists showed that people who had recovered from HEV infection had a high concentration of antibodies against the capsid protein that forms the viral coat and that people who could make these antibodies were unlikely to become infected with HEV. To develop an HEV vaccine based on the viral capsid protein, the NIAID researchers and their colleagues engineered an insect virus to contain the gene for the HEV capsid protein, allowed the recombinant virus to infect cultured insect cells, and extracted the target protein from the culture fluid. After showing that this approach would generate the needed large quantities of HEV capsid protein, the NIAID team enlisted the aid of Novavax, another biotechnology company, to scale up production and make an initial supply of vaccine-grade material.

With a nonhuman primate model of hepatitis and the recombinant HEV capsid protein at hand, initial testing of the recombinant vaccine proceeded. In experiments in the mid-1990s, NIAID researchers and their collaborators injected macaques with the recombinant capsid protein. Four weeks later, the researchers injected the monkeys with a challenge dose of live HEV. Many of the vaccinated monkeys became mildly infected, particularly after receiving higher doses of the virus, but the candidate vaccine prevented liver inflammation and none of the monkeys became ill. In a more recent study, NIAID intramural researchers and their colleagues demonstrated that a two-dose, recombinant HEV vaccine based on the virus capsid protein protects rhesus monkeys against multiple strains of HEV that occur worldwide. As little as 1 µg of the recombinant HEV vaccine, administered to the monkeys in two doses, protected against the genotype 1 (Pakistani strain) of HEV used to make the vaccine as well as genotype 2 (Mexican strain) and genotype 3 (U.S. strain) of HEV.*

NIAID sought out corporate partners to help develop the vaccine further, and GlaxoSmithKline agreed to license the technology and bring its expertise in vaccine development. Because the U.S. Army also was interested in an HEV vaccine to protect troops deployed overseas, researchers at the Walter Reed Army Institute of Research began human safety testing of the candidate vaccine. Initial clinical trials in Nepal, where HEV is endemic, and in the United States indicated that the recombinant vaccine is safe and that it also stimulates the generation of antibodies against the HEV capsid protein. Next, NIAID, GlaxoSmithKline, and the U.S. Army worked with authorities in Kathmandu, Nepal, to initiate a much larger field trial of the vaccine, which began in 2001 and is still under way. The results of this trial are expected early in 2004. This trial, with more than 2,000 participants, is designed to reveal whether the candidate HEV vaccine can prevent disease in an area where the virus is a serious public health problem and to determine the optimal vaccine dose. Only genotype 1 of HEV occurs in Nepal, and it will be important to show in

Story of Discovery: Developing a Recombinant Vaccine for Hepatitis E, *Continued*

future studies that the recombinant vaccine also is effective against the other HEV genotypes that occur in different parts of the world.

Using New Knowledge for Public Health

Possession of an HEV vaccine would, for the first time, provide public health officials in developing nations with an effective tool to control HEV epidemics. Given the inadequate sewage treatment in many of these nations, authorities at present can do little more than urge people in affected areas to boil their water. The development of the new, recombinant HEV vaccine is based on nearly two decades of research by NIAID scientists who, with the assistance of many collaborators, ultimately translated their Government laboratory investigations into a potentially useful and greatly needed product. ❖

*Purcell RH et al. Pre-clinical immunogenicity and efficacy trial of a recombinant hepatitis E vaccine. *Vaccine* 2003;21:2607-2615.