

## BIODEFENSE

Recent world events have raised awareness of both the possibility of a bioterrorist attack and the vulnerability of the U.S. population to such an event. In fact, a terrorist attack on the United States using biological agents occurred in the fall of 2001, when *Bacillus anthracis* spores were sent through the U. S. mail, causing 18 confirmed cases of anthrax (11 inhalation, 7 cutaneous). In 2003 and 2004, ricin was found in an envelope at a postal facility in South Carolina and a Senate Office Building in Washington, D.C., and was used to contaminate several jars of baby food in California. Although the Department of Defense has developed countermeasures against biological warfare, there are additional concerns that need to be addressed to provide an adequate civilian defense from a bioterrorist attack. The number of microbial pathogens that threaten civilian populations is larger than that of classical biological warfare threats. Moreover, the populations to be protected are different because civilians include people of all ages and physical conditions.

In 2002, NIAID developed a strategic plan for biodefense research that outlines plans for addressing research needs for bioterrorism and emerging and re-emerging infectious diseases. In addition, NIAID convened a Blue Ribbon Panel of experts to provide objective scientific advice on NIAID's biodefense research agenda on so-called Category A agents. This list, which is defined by the Centers for Disease Control and Prevention (CDC), includes the most dangerous threat agents, such as smallpox and anthrax. The expert panel was asked to assess the current research, identify goals for the highest-priority areas, and make recommendations to achieve the goals. In the fall of 2002, NIAID convened a similar expert panel to assess current research and identify goals for Category B and C agents. In the areas of immunology and biodefense, NIAID has convened two more advisory bodies: an Expert Panel on Immunity and Biodefense,

to assess future immunology research most important to combat bioterrorism and emerging infectious diseases; and an Expert Panel on Atopic Dermatitis and Vaccinia Immunization, to develop a research plan to reduce the risk of eczema vaccinatum, a serious and sometimes deadly complication of smallpox immunization in atopic dermatitis patients.

In the past year, NIAID has continued to expand, intensify, and accelerate its ongoing research programs in biodefense. NIAID has launched research initiatives in areas ranging from the basic biology of microbes and their interactions with the human immune system to preclinical and clinical evaluation of new therapeutics and vaccines. These initiatives are designed to take advantage of the recent outpouring of ideas from academic and industrial scientists on ways to understand and combat potential agents of bioterrorism ([www3.niaid.nih.gov/biodefense](http://www3.niaid.nih.gov/biodefense)).

In FY 2005, NIAID made its first grant and contract awards using authorities granted by the Project BioShield Act of 2004 to expedite



**Ebola Virus.** Scanning electron micrograph of Ebola virus, which causes hemorrhagic fever and is designated a Category A agent.

research and development on critical biomedical countermeasures, including countermeasures for Category A agents. In addition, NIAID released two progress reports highlighting accomplishments in biodefense research during the 18 months subsequent to the development of the strategic plan ([www.niaid.nih.gov/biodefense/research/category\\_a\\_progress\\_report.pdf](http://www.niaid.nih.gov/biodefense/research/category_a_progress_report.pdf); [www.niaid.nih.gov/biodefense/research/category\\_bc\\_progress\\_report.pdf](http://www.niaid.nih.gov/biodefense/research/category_bc_progress_report.pdf)).

## Basic Research

One of the most important basic research tools that has evolved in recent years is the ability to rapidly sequence the entire genomes of microbial pathogens, including potential agents of bioterrorism. This capability allows scientists to identify microbial genes that play a role in disease and then design drugs that can block the activities of the proteins encoded by these genes. NIAID has made a significant investment in the DNA sequencing of the genomes of microorganisms considered agents of bioterrorism, including several Category A, B, and C agents. Organisms NIAID has helped to sequence include *Brucella suis*, *Burkholderia mallei*, *Clostridium perfringens*, *Coxiella burnetii*, *Rickettsia typhi*, *Staphylococcus aureus*, *Yersinia pestis*, *Mycobacterium tuberculosis*, *Vibrio cholerae*, *Cryptosporidium parvum*, *Giardia lamblia*, *Entamoeba histolytica*, *Toxoplasma gondii*, diarrheagenic *Escherichia coli*, *Shigella*, and *Salmonella*. In addition, NIAID has expanded its sequencing efforts of *B. anthracis* beyond the Ames strain used in the 2001 attack and has developed a comprehensive genomic analysis that includes sequencing several additional strains, clinical isolates, near neighbors, and related species. These sequences will facilitate forensic strain identification; understanding of microbial pathogenesis; discovery of new targets for drugs, vaccines, and molecular signatures; and discovery of biomarkers for diagnostics to combat bioterrorism.

To expand its current enteric pathogens research network, NIAID established the Food and Waterborne Diseases Integrated Research Network to include multidisciplinary research on all food- and waterborne pathogens or toxins. The network facilitates the development and evaluation of products to rapidly identify, prevent, and treat food- and waterborne diseases that threaten public health.

NIAID funds the Centers for Medical Countermeasures against Radiation, which will focus on basic and applied research to develop new products for measuring radiation exposure, protect against exposure, and minimize and treat the effects of exposure to a wide range of radioactive compounds.

## Immunity and Biodefense

Considerable knowledge about the mechanisms of host immune responses to microbial pathogens has been gained in recent years. Studies of innate immune mechanisms, which serve as a nonspecific first line of defense against pathogenic infection, have been especially productive. In FY 2004 and 2005 several of the contracts awarded under the Immune Epitope Discovery Program made significant progress in the identification of antibody and T cell epitopes to such pathogens as influenza, vaccinia virus, and *Clostridium botulinum* neurotoxins. Finally, the threat of bioterrorism and the natural emergence of diseases due to microbes such as West Nile virus (WNV) and severe acute respiratory syndrome (SARS) virus underscore the importance of defining the immune parameters responsible for increased susceptibility to infectious diseases of infants, young children, the elderly, and immunocompromised individuals.

To gain a better understanding of the human immune response to potential agents of bioterror, NIAID funded eight Cooperative Centers for Translational Research on Human Immunology and Biodefense. These centers, located throughout the country, focus on the rapid development of

bioterrorism countermeasures, such as vaccines and therapies.

Also contributing to the biodefense vaccine effort are a number of recent contracts awarded to identify immune epitopes for Category A, B, and C pathogens; define human genetic variance that contributes to infection susceptibility or vaccine efficacy; identify new candidates for vaccine adjuvants; develop reagents for nonhuman primate studies of new drug or vaccine candidates; and address the problem of eczema vaccinatum as a serious adverse consequence of the current smallpox vaccine.

### New Diagnostic Tools

NIAID also supports research leading to the development of new and improved diagnostics. The goals of this research are to establish methods for the rapid, sensitive, and specific identification of natural and bioengineered microbes, as well as to determine the microbes' sensitivity to drug therapy. Progress in these areas will allow healthcare workers to diagnose and treat patients more accurately and quickly.

NIAID continues to support initiatives specifically for the development of the next generation of medical diagnostics, and also continues to support its Small Business Biodefense Program, which encourages the development of therapeutics, vaccines, adjuvants and other immunostimulants, diagnostics, and selected resources for biodefense by the small business research community. This program expands the duration and dollar limits for small business grants to develop specified products that are considered high priority for biodefense.

NIAID supports a range of biodefense genomics research projects that provide comprehensive genomic, bioinformatics, functional genomics, and proteomic research resources to the scientific community to help researchers identify targets and proteins for use in new diagnostics. Through these projects, NIAID awarded contracts in

FY 2004 for eight Bioinformatics Resource Centers to develop and maintain comprehensive, relational databases for genomic and related data for microorganisms responsible for emerging and re-emerging infectious diseases and for those considered agents of bioterrorism ([www.niaid.nih.gov/dmid/genomes/brc/default.htm](http://www.niaid.nih.gov/dmid/genomes/brc/default.htm)).

In FY 2005, NIAID continued to support contracts for the Biodefense Proteomics Research Centers to develop and enhance innovative proteomic technologies and apply them to the understanding of the pathogen and/or host cell proteome for the discovery and identification of novel targets for the next generation of drugs, vaccines, diagnostics, and immunotherapeutics against microorganisms considered agents of bioterrorism. NIAID funds seven centers that are working on a range of proteomics studies including agents from Categories A, B, and C.

### Vaccines

NIH-supported researchers are developing vaccines against many infectious agents, including those considered to be bioterrorism threats, for use in civilian populations of varying ages and health status. Vaccines are being developed using both traditional and novel technologies. Significant progress has been made in the development of next-generation vaccines for anthrax and smallpox, and in the development of new vaccines for other diseases such as Venezuelan equine encephalitis (VEE), West Nile virus, plague, and cholera. Ongoing modified vaccinia Ankara (MVA) and recombinant protective antigen contracts for smallpox and anthrax vaccines, respectively, continue to support scaled manufacturing of the vaccines, as well as further safety testing in humans and safety and efficacy studies in animals. In 2005, NIAID awarded three contracts to fund development of new vaccines against tularemia and botulinum. The tularemia and botulinum contract awards will fund early-stage product development of the respective vaccines, including dosage formulation,

pilot batch production, and initial clinical assessment.

## Therapeutics

NIH therapeutics research focuses on the development of new antimicrobials and antitoxins, as well as the screening of existing antimicrobial agents to determine whether they have activity against organisms that might be used by bioterrorists. Knowledge gained from basic and applied research is helping to identify additional targets for medications and immune-based therapies against agents of bioterrorism.

Through the *In Vitro* and Animal Models for Emerging Infectious Diseases and Biodefense Program, NIAID continues to provide a range of resources for preclinical testing of new therapies and vaccines, including nonhuman primate models. Under these contracts, small animal and nonhuman primate models will be developed and validated for licensure of vaccines and therapeutics by the U.S. Food and Drug Administration (FDA).

Also, through the *In vitro* and Animal Models for Emerging Infectious Diseases and Biodefense Program, NIAID is screening existing FDA-approved antimicrobials for efficacy against inhalational anthrax. Through its *in vitro* antiviral screening contracts, NIAID has supported the evaluation of compounds for *in vitro* activity against models for NIAID biodefense Category A, B, and C viruses, such as influenza, yellow fever, dengue, West Nile virus, VEE, Pichinde virus (a surrogate for arenaviruses), and Punta Toro virus (a surrogate for Rift Valley fever, sandfly fever, and hantavirus). Approximately 1,600 compounds were screened in FY 2005.

The NIAID *in vivo* antiviral screening contracts support the evaluation of many compounds against NIAID Biodefense Category A, B, and C viruses or their surrogates such as influenza, orthopoxviruses (including surrogates for smallpox), Punta Toro virus, Pichinde, SARS

coronavirus (CoV), Banzi (a surrogate for yellow fever), Venezuelan equine encephalitis, and West Nile virus.

Grants awarded for Accelerated Product Development for Radiation Countermeasures will support projects focused on protecting the immune system from radiation or restoring the immune system following radiation exposure.

Contracts for the Development of Radiation Countermeasures were awarded to evaluate promising compounds to prevent, reduce, or treat symptoms of radiation exposure. Additionally, three contracts were awarded for Development of Improved DTPA (diethylenetriaminepentaacetate) for Radionuclide Chelation. DTPA can be used to remove certain radioactive compounds from the body. If an individual is exposed to one of these compounds, DTPA can be given intravenously to help eliminate the contamination. For use following a terrorist attack, however, DTPA would be practical only in an easier-to-administer form. These contracts will seek to develop alternate ways to effectively administer DTPA, either by inhalation, oral liquid, or pill.

NIAID also has expanded the Collaborative Antiviral Study Group (CASG) by approximately 20 percent since it was established in 1986. In 2003, CASG developed a clinical protocol for the treatment of smallpox with cidofovir, in the event of an outbreak or release. NIAID will soon be supporting a phase I clinical study by Chimerix, Inc., to assess initial safety, tolerability, and pharmacokinetics of a promising new oral derivative of cidofovir in normal volunteers. The CASG will conduct future phase I/II studies with the drug after the initial phase I study is complete. The CASG is also conducting a phase I/II study on the safety and tolerability of an immune globulin treatment for West Nile virus neuroinvasive disease and is conducting a natural history study of the clinical outcomes of WNV neuroinvasive disease. In addition, the CASG

is initiating a chart review study of the use of oseltamivir in young children less than 2 years of age with confirmed or suspected influenza. The FDA recently cleared ST-246, a smallpox antiviral developed by SIGA Technologies, Inc., for a phase I clinical trial that will be supported by NIAID and conducted at the NIH Clinical Center. This trial is awaiting Institute review board approval and is slated to begin March 2006.

NIAID also funds research focused on the discovery and development of botulism therapeutics that would be effective in a post-exposure scenario.

## Research Resources

NIAID continues to support the expansion of centralized laboratory resources, including regional biosafety laboratories, *in vivo* and animal model resources, drug-screening contracts, the production of standardized and validated reagents and tests, and genomic and bioinformatics resources. The availability of such resources assists the research community in conducting studies of biodefense pathogens.

In FY 2005, NIAID completed a national network of 10 Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases (RCEs), to support research focused on countering threats from bioterror agents and emerging infectious diseases. Each center is comprised of a consortium of universities and complementary research institutions serving a specific geographical region. The primary objective of the RCE program is to support the NIAID biodefense and emerging infectious diseases research agenda. The RCEs, located throughout the United States, will build and maintain a strong scientific infrastructure supporting multifaceted research and development activities that promote scientific discovery and translational research capacity required to create the next generation of therapeutics, vaccines, and diagnostics for the NIAID Category A, B, and C agents. The

research being conducted within the RCEs spans a broad range of biodefense and emerging infectious disease topics including:

- Basic research on bacterial and viral disease processes;
- New approaches to blocking the action of anthrax, botulinum, and cholera toxins;
- Developing new vaccines against anthrax, plague, tularemia, smallpox, and hemorrhagic fevers;
- Creating new immunization strategies and delivery systems;
- Generating new antibiotics and other therapeutics;
- Designing new advanced diagnostic methods and devices;
- Conducting immunological studies of host-pathogen interactions; and
- Developing computational and genomic approaches for studying infectious diseases.

In 2005, NIAID enlarged its network of National Biocontainment Laboratories (NBLs) and Regional Biocontainment Laboratories (RBLs) by awarding grants to fund the construction of 4 more RBLs, for a total of 2 NBLs and 13 RBLs. The NBLs will serve as national and regional resources for research on biodefense and emerging infectious disease agents that require biosafety level (BSL) -4/3/2 biocontainment, and the RBLs will serve as regional resources for research requiring BSL-3/2 biocontainment. These laboratory facilities will be designed and built using the strictest federal standards, and will incorporate multiple layers of safety and security to protect laboratory workers and the surrounding environment.

The NBLs and RBLs will complement and support the research activities of NIAID's RCEs.

The biosafety labs also will be available and prepared to assist national, State, and local public health efforts in the event of a bioterrorism or infectious disease emergency.

NIAID established the Biodefense and Emerging Infections Research Resources Repository in September 2003 to provide unique and quality-assured biodefense-related reagents and resources to the scientific community for basic research and product development. This program facilitates the understanding of the pathogenesis of NIAID Category A, B, and C priority pathogens and emerging infectious diseases organisms and toxins and aids in the development and evaluation of vaccines, therapeutics, and diagnostics for these organisms and agents. The repository also assists with access to reagents not held in the program.

In order to facilitate research and product development for biodefense and emerging infectious diseases, the repository collects information about biodefense-related reagents and standards and disseminates this information through print, electronic media, and workshops; enhances technology transfer through development and publication of methods; and facilitates commercial development of reagents through proactive communication with biotechnology and pharmaceutical companies. In addition to securing acquisition, storage, and the distribution of biological agents and toxins, the repository generates new reagents as scientific advances are made.

It is anticipated that in the long-term the Biodefense and Emerging Infections Research Resources Repository will become a national resource and clearinghouse for biological organisms and toxins, reagents, and information on these organisms. By centralizing this function, access to and use of these materials can be monitored and quality control of the reagents assured. Information about this resource is now available on the Web site at [www.beiresources.org](http://www.beiresources.org).

For more information on the numerous NIAID resources available to biodefense researchers, visit [www2.niaid.nih.gov/biodefense/research/resources.htm](http://www2.niaid.nih.gov/biodefense/research/resources.htm).

## NIAID Intramural Research Programs

### *Basic Research Discoveries*

The NIAID Division of Intramural Research (DIR) studies of *B. anthracis*, the bacterium that causes anthrax, are focused on the identification, genetic regulation, and analysis of anthrax lethal toxin and other virulence factors, as well as the development of improved vaccines and therapeutics. The anthrax toxin is the primary cause of damage to animal tissues during an anthrax infection. DIR scientists are studying the action of the toxin in appropriate small animal models to identify molecular targets of anthrax toxin and opportunities for specific therapy of anthrax infections. Recent studies to determine why mice strains differ greatly in susceptibility to anthrax lethal toxin revealed that steroid therapy increased their sensitivity to the toxin.<sup>24</sup> This result suggests that steroid therapy for anthrax could have potentially detrimental consequences that should be considered in treatment protocols for this disease.

In addition, NIAID intramural anthrax research has promising nonbiodefense applications. For example, new knowledge about anthrax toxin structure and function is also being used to create toxin-based therapeutic agents targeted specifically to cancer cells. These toxins have shown efficacy in mouse tumor models and are being developed by a licensee for possible clinical application.

DIR investigations of *Yersinia pestis*, the bacterium that causes plague, have resulted in the development of both mouse and rat models of bubonic plague that incorporate the natural flea-borne route of transmission. The mouse model has been used to test two plague vaccine

candidates. The first, developed by the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) was recently found to be 100 percent effective in mice. The second, a new live-attenuated vaccine for plague, remains under investigation.

The plague investigators also characterized the Brown Norway rat as a small animal model in which to study *Y. pestis* transmission and pathogenesis and the host response to plague. The development of the gross pathology and histopathology in the bubo, or infected lymph node, was characterized and shown to closely resemble human bubonic plague. Characterization of the disease and host response in this small animal model enables discoveries that could help scientists develop improved therapies and vaccines.<sup>25</sup> This work is important to NIAID's biodefense efforts as well as to efforts to control naturally occurring plague epidemics.

To better understand the innate immune response, DIR scientists are studying infection-fighting white blood cells called neutrophils, which are an essential part of human innate immunity. In 2005, NIAID researchers completed microarray studies of human neutrophil polymorphonuclear leukocyte function and are pursuing research to elucidate the role of selected genes and proteins identified in these studies. By describing changes in neutrophil gene expression in response to bacterial invasion, the investigators have identified dozens of possible targets for drug therapies. These findings are likely to be broadly applicable to many types of microorganisms that cause disease in humans and could lead to new treatments that augment the immune response against multiple high-priority pathogens.<sup>26</sup>

Additional investigations underway in NIAID laboratories include studies of the pathogenesis of *C. burnetii*, the agent of Q fever; studies of multidrug-resistant tuberculosis; studies of relapsing fever agents with a focus on improving diagnostic tests; and a new program to identify

and characterize antigens suitable for use in a vaccine against *Burkholderia mallei* and *Burkholderia pseudomallei*, the causative agents of glanders and melioidosis, respectively; and a new tularemia research program. The tularemia-causing agent, *Francisella tularensis*, is a highly infectious bacterium whose virulence is enhanced by its ability to survive and replicate within host cells called macrophages—normally strong defenders against bacterial infection. The new program focuses on understanding how the bacterium survives inside the macrophages of the host in order to uncover novel targets for the design of vaccines and therapeutics against tularemia. This research is supported by enhanced genomics and proteomics capabilities on the Bethesda campus and at the Rocky Mountain labs.

The molecular events that underlie Ebola virus cytopathicity are poorly understood. Scientists at the NIAID Vaccine Research Center (VRC) have identified a cellular mechanism responsible for Ebola glycoprotein (GP) cytotoxicity. Through its effects on specific cell-surface molecules, Ebola virus disrupts several processes essential for immune activation and recognition, such as cell trafficking and antigen presentation. By altering the trafficking of select cellular proteins, Ebola GP inflicts cell damage and can facilitate immune escape by the virus. This mechanism is likely responsible for the inflammatory dysregulation, immune suppression, and vascular dysfunction that are hallmarks of lethal Ebola virus infection. These findings are important for developing countermeasures against the pathogenic effects of the virus.

### Vaccines

NIAID has a longstanding intramural research program aimed at shedding light on the molecular biology and gene expression mechanisms used by vaccinia—the virus used in the current smallpox vaccine—and other poxviruses. A primary aim of this program is the development of MVA as a carrier for the delivery of vaccine components and gene therapies to

target cells. Intramural poxvirus researchers, who have decades of experience with MVA, and other poxvirus scientists are collaborating with USAMRIID researchers and others in nonhuman primate studies of MVA's efficacy as a smallpox vaccine. In a study comparing MVA and Dryvax (the traditional licensed smallpox vaccine) in a monkey model, scientists found that after two doses of MVA or one MVA dose followed by Dryvax, the immune response was equivalent or higher than that induced by Dryvax alone. After challenge with monkeypox virus, unimmunized animals developed hundreds of skin lesions and became gravely ill or died, whereas vaccinated animals were healthy and asymptomatic, except for a small number of transient skin lesions in animals immunized only with MVA. Their findings were important steps in the evaluation of MVA as a replacement vaccine or pre-vaccine for those with increased risk of severe side effects from Dryvax.<sup>27</sup>

In addition, the VRC completed two clinical trials to evaluate the safety and immunogenicity of MVA. One study involved young adults who had never been vaccinated against smallpox; the other was designed for older adults who had been vaccinated more than 10 years ago. The vaccine was provided by Therion Biologics Corporation as part of a collaboration with the VRC. MVA is an attenuated strain of vaccinia virus that is replication-defective, and therefore does not cause a lesion at the site of inoculation typical of Dryvax. MVA had fewer side effects than Dryvax and was found to be well tolerated and immunogenic. MVA given prior to Dryvax reduced Dryvax-related side effects and improved immunogenicity.

NIAID DIR scientists also continued basic investigations to determine if pieces, or subunits, of the vaccinia virus could be used to make a protective vaccine against smallpox. Such a vaccine would likely have fewer side effects than the current vaccine. Toward this goal, they found that a vaccine made from three recombinant

proteins of the outer membranes of intracellular and extracellular virus protected mice from 10 times the usual lethal dose of the Walter Reed vaccinia strain. The results of these studies suggest that an effective smallpox vaccine could be made from subunits of vaccinia.

Hemorrhagic fevers such as those caused by Ebola virus are associated with a high mortality rate, particularly for the Ebola Zaire subtype. Traditional public health measures to prevent future outbreaks are limited, thus increasing the urgency for development of an effective vaccine. An interagency agreement currently in place between NIAID and USAMRIID allows for collaboration in animal studies, assay performance, and data analysis.

Investigators at the VRC, with scientific collaborators at USAMRIID and the CDC, have developed a potentially effective vaccine strategy for Ebola virus infection in nonhuman primates. In November 2003, the VRC initiated the first human clinical trial of a DNA vaccine designed to prevent Ebola infection. The vaccine was well tolerated and there is evidence of both humoral and cellular immune responses at all dose levels. The VRC also has plans to evaluate a fast-acting, recombinant adenoviral vector Ebola vaccine. Such a vaccine would be especially useful in an acute outbreak setting. If this vaccine proves to be effective in humans, it could one day be used to quickly contain Ebola outbreaks with the same ring vaccination strategy used in the past against smallpox. This product is currently in the preclinical testing phase and a phase I study is projected to begin in summer 2006.

The VRC also is developing vaccines against West Nile virus and SARS. For West Nile virus, the VRC's candidate vaccine is based on an existing codon-modified, gene-based DNA plasmid vaccine platform designed to express WNV proteins. In April 2005, following preclinical safety studies and viral challenge studies, the VRC initiated a phase I clinical trial to evaluate the safety, tolerability, and immune responses of this

recombinant DNA vaccine in human volunteers. Also in collaboration with Vical, Inc., the VRC is currently developing a second-generation DNA vaccine using an improved expression vector expressing the same WNV proteins. A phase I clinical trial is planned for spring 2006.

In response to the recent global outbreak of SARS, VRC investigators began work immediately on the development of a potential vaccine. The VRC contracted with Vical, Inc., to manufacture a single closed, circular DNA plasmid-based vaccine encoding the S protein of SARS-CoV. VRC studies using mice as an experimental model demonstrated that this vaccine induces T cell and neutralizing antibody responses, as well as protective immunity. A phase I open-label clinical study to evaluate safety, tolerability, and immune response was initiated in FY 2005, fully enrolled volunteers, and is now closed to accrual.

### **Therapeutics**

NIAID clinical investigators have an approved protocol in place that will allow them to evaluate and treat persons exposed to or infected with anthrax, and to conduct immunologic evaluations of recipients of anthrax vaccines. In addition, DIR investigators and their colleagues in the NIH Clinical Center are collecting serial blood samples and throat swabs from healthy persons who receive the smallpox vaccine in order to measure serum cytokines and look for the smallpox vaccine virus. Identification of specific cytokines induced after vaccination could help to explain certain side effects associated with the smallpox vaccine and suggest new ways to modify some of these side effects. The investigators also evaluated different methods of detecting the smallpox vaccine virus in clinical specimens, including sensitive cell culture methods, polymerase chain reaction studies, and a direct fluorescent antibody test that detects viral proteins. These studies indicated which test detects the virus most reliably, which is the fastest to perform, and which tests give false-positive results for other viruses.

DIR poxvirus researchers also identified three poxvirus proteins that are required for virus entry and fusion with the cell. Remarkably, these proteins are conserved in all poxviruses analyzed to date, indicating that the mechanism of poxvirus entry into cells is also conserved. The identification of the entry proteins is crucial for understanding how poxviruses spread and cause disease. In addition, these proteins are potential targets for poxvirus therapeutics.

Protective antibodies are produced by the host in response to infection or immunization. Administration of sera containing protective antibodies to people exposed to a pathogen is called passive immunoprophylaxis and has long been used to prevent disease in exposed populations. However, monoclonal immunoglobulin preparations tailored to act specifically on the most vulnerable parts of an invading pathogen could be of higher and more consistent potency.

DIR researchers are pursuing several prophylaxis and treatment strategies based on monoclonal antibodies, including the development of preparations that can be used to prevent or treat complications of smallpox vaccination, smallpox, anthrax, SARS, West Nile virus, botulism, rabies virus, Japanese encephalitis virus, and the tick-borne encephalitis virus complex. For example, DIR researchers derived monoclonal antibodies from chimpanzees—which are virtually identical to human antibodies—that can neutralize the protective antigen toxin of *Bacillus anthracis*, and tested them in both cell culture and in a rat model. These monoclonal antibodies attached to the anthrax toxin with greater strength than any previously developed anthrax antibodies, and they protected rats from death following infusion of anthrax toxin. The results of these studies are very encouraging for the development of a monoclonal-based immunotherapy to neutralize the effects of anthrax toxin in infected or exposed humans.

**Resources**

In FY 2005, the Vaccine Research Center established the Biodefense Research Section, a new laboratory within the Center focusing on three major areas: (1) development of vaccines and antivirals against hemorrhagic fever viruses

such as Ebola, Marburg, and Lassa; (2) studies of the mechanism of vaccine-induced immune protection; and (3) basic research to understand the mechanism of virus replication (entry) and neutralization.