

BIODEFENSE

The intentional release of biological agents in the United States was once thought to be a remote possibility. In the fall of 2001, however, *Bacillus anthracis* spores were sent through the U.S. mail, causing 18 confirmed cases of anthrax (11 inhalation and 7 cutaneous). These and other events have raised awareness of the vulnerability of the U.S. population to a bioterrorist attack. Although defense agencies have developed research agendas to protect military personnel against biological warfare, additional factors must be considered for civilian populations. Civilian populations, unlike military populations, include people of all ages and physical conditions and could be attacked by a wider range of biological agents.

In 2002, NIAID developed a strategic plan for biodefense research that outlines plans for addressing research needs for both 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, 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 fall 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 markedly expanded, intensified, and accelerated its ongoing research programs in biodefense. NIAID has launched or expanded 35 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 (www.niaid.nih.gov/dmid/biodefense). In addition, NIAID released a progress report 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).

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*, *M. tuberculosis*, *V. cholerae*, *Cryptosporidium parvum*, *Giardia lamblia*, *Entamoeba histolytica*, and *Toxoplasma gondii*. 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 of at least 14 additional strains, clinical isolates, near neighbors, and related species. These sequences will facilitate forensic strain identification; understanding of microbial pathogenesis; the discovery of new targets for drugs, vaccines, and molecular signatures; and the 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 water-borne pathogens or toxins. The Network also will facilitate the development and evaluation of products to rapidly identify, prevent, and treat food- and water-borne diseases that threaten public health.

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, were especially productive. For example, the identification and functional characterization of the Toll-like family of receptors expressed on cells of the human innate immune system

have led to an explosion of information now being applied to the development of new vaccine adjuvants and immunostimulatory agents to boost nonspecific immune protection. Additional progress on understanding the molecular mechanisms responsible for pathogen-specific immunity mediated by antibodies and cytotoxic T cells has led to new approaches in vaccine design. For example, NIAID-sponsored scientists identified two short peptides from vaccinia—the benign virus used as a smallpox vaccine—that are recognized by the immune systems of people who have been immunized. Researchers can use these peptides to track the human immune response to the virus as they try to develop an improved vaccine. Finally, the threat of bioterrorism and the natural emergence of diseases due to microbes such as West Nile virus and severe acute respiratory syndrome (SARS) virus underscore the importance of defining the immune parameters responsible for increased susceptibility of infants, young children, the elderly, and immunocompromised individuals to infectious diseases.

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

New Diagnostic Tools

NIAID also supports research leading to the development of new and improved diagnostics. The goal of this research is to establish methods for the rapid, sensitive, and specific identification of natural and

bioengineered microbes as well as the determination of the microbes' sensitivity to drug therapy. Progress in these areas will allow healthcare workers to diagnose and treat patients more accurately and quickly.

NIAID has developed two initiatives that specifically support the development of the next generation of medical diagnostics: Biodefense Partnerships; and Cooperative Research for the Development of Vaccines, Adjuvants, Therapeutics, Immunotherapeutics, and Diagnostics for Biodefense. Under these initiatives, 67 awards were made in fiscal year (FY) 2003. In addition, the NIAID-supported Pathogen Functional Genomics Resource Center was expanded to provide the infectious disease research community with state-of-the-art research and reference reagents, which will help researchers to identify targets and proteins for use in new diagnostics.

NIAID also continued to support its Small Business Biodefense Program, which encourages the development of therapeutics, vaccines, adjuvants/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.

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 Ebola and West Nile viruses.

In FY 2003, NIAID awarded contracts to two companies to continue research and advanced development of a new anthrax vaccine, called recombinant protective antigen vaccine. The companies' challenge will be to continue to take this experimental vaccine strategy through several stages of development, including scaled manufacturing, testing for safety in laboratory and clinical trials, testing for efficacy in animals, and preparing and submitting all necessary Food and Drug Administration applications. NIAID also awarded contracts to two companies to develop the modified vaccinia Ankara (MVA) vaccine against smallpox, to assess its protection and immunogenicity in appropriate animal models, and to conduct phase I clinical trials to assess the safety and immunogenicity of MVA vaccine candidates. In addition, NIAID's Vaccine and Treatment Evaluation Units (VTEUs) have expanded by approximately 60 percent. In the past year, eight clinical trials of various smallpox vaccines have been completed or are under way at VTEU sites. In addition, clinical trials for new anthrax and West Nile vaccines are being planned for the VTEUs.

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.

NIAID made four awards in FY 2003 under a new initiative called *In Vitro* and Animal Models for Emerging Infectious Diseases and Biodefense, which will provide a range of resources for preclinical testing of new therapies and vaccines, including nonhuman primate models. NIAID also made two awards under the *In Vitro* Antiviral Screening Program to facilitate the identification of antiviral agents with the potential for treatment of viral infections of public health importance, including those for newly emerging infections and those that are not a high priority for the pharmaceutical industry.

NIAID also has expanded the Collaborative Antiviral Study Group (CASG) by approximately 20 percent. In the past year, CASG developed a clinical protocol for the treatment of smallpox with cidofovir, in the event of an outbreak or release. A phase I clinical study is planned in FY 2004 to assess

initial safety, tolerability, and pharmacokinetics of a promising new oral derivative of cidofovir in normal volunteers.

Research Resources

Over the past year, NIAID has devoted considerable resources to 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 will assist the research community in conducting studies of biodefense pathogens. Several significant accomplishments NIAID has made during this past year to expand biodefense research capacity and infrastructure are featured in the following text box “Expanding Research Capacity in Biodefense and Emerging Infectious Diseases.”

Focus on

Expanding Research Capacity in Biodefense and Emerging Infectious Diseases

Our ability to detect and counter a bioterrorist attack requires basic research aimed at understanding the organisms that might be used as agents of bioterrorism as well as deciphering how the human immune system responds to these organisms. The research needed to obtain this knowledge, which will be used to develop new and improved diagnostic tests, vaccines, and therapies, must be carried out in specialized, high-containment labs. To that end, the Division of Microbiology and Infectious Diseases (DMID) has funded several new programs to expand high-containment laboratory capacity. Three of the most prominent programs are the Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases, the National Biocontainment Laboratories, and the Regional Biocontainment Laboratories.

Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases (RCE)

The RCE program will establish eight centers that will provide a coordinated, comprehensive mechanism to support interdisciplinary biodefense research. RCEs will support investigator-

Expanding Research Capacity in Biodefense and Emerging Infectious Diseases, *Continued*

directed research; train researchers and other personnel for biodefense research activities; create and maintain supporting resources, including scientific equipment; emphasize research focused on the development and testing of vaccine, therapeutic, and diagnostic concepts; make core facilities available to approved investigators from academia, Government, biotech companies, and the pharmaceutical industry; and provide facilities and scientific support to first responders in the event of a national biodefense emergency.

Each center comprises a lead institution and affiliated institutions located primarily in the same geographic region. The eight institutions that received an RCE grant are as follows:

- Duke University
- Harvard Medical School
- New York State Department of Health
- University of Chicago
- University of Maryland, Baltimore
- University of Texas Medical Branch (Galveston)
- University of Washington
- Washington University in St. Louis

National Biocontainment Laboratories (NBLs) and Regional Biocontainment Laboratories (RBLs)

In 2002, a Blue Ribbon Panel, convened to advise NIAID on its biodefense research agenda, determined that the amount of biosafety level 4 (BSL-4) and BSL-3 lab space is inadequate. To address this critical national need, DMID is funding the construction of two NBLs and nine RBLs, to be located throughout the country. The new laboratories will give scientists space to conduct critical biodefense research safely.

The objective of the NBL program is to provide funding to design, construct, and commission state-of-the-art BSL-4, BSL-3, and BSL-2 laboratories, as well as associated research and administrative support space; the RBL construction program will provide funding for similar facilities containing BSL-3 and BSL-2 labs. All BSL labs will be designed and built using the strictest Federal standards, incorporating special engineering and design features to prevent microorganisms from being released into the environment. Numerous safety and decontamination features provide multiple layers of protection for lab workers and the surrounding community.

The RCEs, NBLs, and RBLs, will be part of the NIAID RCE Biodefense Network. The labs will serve as national and regional resources, will offer preferential support for the research activities of the RCEs and other NIAID-funded biodefense research, and will be available to assist national, state, and local public health efforts in the event of a biodefense emergency.



transmission. The mouse model has been used by the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) to evaluate a new plague vaccine candidate. Both models will be used to investigate the role of specific *Y. pestis* virulence factors at the molecular level, to characterize the host response to naturally acquired infection, and to evaluate new vaccines. This work is important to NIAID's biodefense efforts as well as to efforts to control naturally occurring plague epidemics. In light of recent plague outbreaks in human populations in India and Africa and the emergence of multiple antibiotic-resistant strains of *Y. pestis*, plague remains an international public health concern.

DIR researchers also are conducting studies of the mechanisms of pathogenesis of the Ebola virus. By inserting different combinations of Ebola viral genes into human tissue culture cells, scientists at NIAID's Vaccine Research Center (VRC) demonstrated that only three viral proteins are needed to form new Ebola virus particles in infected cells. A thorough understanding of how the Ebola virus assembles may lead to a better understanding of the virus' lifecycle and how it causes disease.

To better understand the innate immune response, NIAID DIR scientists are studying infection-fighting white blood cells called neutrophils, which are an essential part of human innate immunity. Although much is understood about the innate immune response to infection, the molecular basis for termination of inflammation and resolution of infection in humans is not clearly understood. To that end, NIAID researchers have produced a comprehensive new picture of the interaction between many kinds of disease-causing bacteria and neutrophils. 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.³³

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

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 poxviruses scientists are collaborating with USAMRIID researchers and others in nonhuman primate studies of MVA's efficacy as a smallpox vaccine. They also have recently collaborated in the development of a rapid test for measuring vaccinia-neutralizing antibodies, which will speed assessments of the efficacy of MVA and other candidate smallpox vaccines.³⁴

In addition, researchers at VRC are working with other DIR scientists to evaluate MVA in two phase I clinical trials in both vaccinia-naïve (never vaccinated) and vaccinia-immune

(previously vaccinated against smallpox) populations.

Another longstanding NIAID intramural research program, focused on studies of the tick-borne flavivirus complex, has ramped up efforts to develop a vaccine against tick-borne encephalitis virus and other highly virulent members of this group, which includes the hemorrhagic fever viruses that cause Kyasanur Forest disease and Omsk hemorrhagic fever. These viruses, along with a nonvirulent member called Langat virus, share components that can induce cross-resistance among other members of the group. In a collaborative effort with USAMRIID, NIAID scientists are refining an approach they devised nearly 10 years ago that uses a chimeric live virus—composed of parts of different flaviviruses—as a candidate vaccine. This approach allows the scientists to significantly weaken the vaccine candidate while maintaining the advantages of a live vaccine in inducing a strong protective immune response. This strategy also has been applied to construct a vaccine against West Nile virus.

Hemorrhagic fevers such as Ebola 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 between NIAID and USAMRIID currently in place allows for collaboration in animal studies, assay performance, and data analysis.

A potentially effective adenoviral vector-based (ADV) vaccine for Ebola virus infection in nonhuman primates has been developed under

an interagency agreement between NIAID and USAMRIID. An ADV-only vaccine that elicited protective immunity in monkeys after a 4-week postvaccination challenge, in contrast to previous 10-week or 6-month vaccine regimens, could be especially useful in an acute Ebola outbreak. A second-generation product also will be evaluated that would provide coverage for Marburg and possibly Lassa viruses. In addition, VRC began a phase I trial of a DNA-based vaccine for Ebola in October 2003.

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 may help to explain certain side effects associated with the smallpox vaccine and suggest new ways to modify some of these side effects. Investigators also are evaluating different methods of detecting the smallpox vaccine virus in clinical specimens, including sensitive cell culture methods and polymerase chain reaction.

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 fever, and botulism. For example, DIR researchers are developing preparations of monoclonal antibodies from chimpanzees—which are virtually identical to human antibodies—that can bind specific antigens on the vaccinia virus and might therefore be used in treatment of complications arising from the use of this virus as a smallpox vaccine.