Working with dangerous bugs

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As part of the national effort in the US to protect civilians from bioterrorist attacks, the US National Institute of Allergy and Infectious Diseases (NIAID) was charged with the development of diverse research resources. The NIAID Resources for Biodefense Research program is forging new collaborations between immunologists and infectious disease experts and is reinvigorating research in the general area of immune protection against pathogenic infection.

The terrorist attacks on the United States in the fall of 2001 helped mobilize and expand federal support for research on a broad range of infectious agents that might be used as terrorist weapons. Many of these pathogens were not well studied in the past, in part because of limited research facilities and appropriate model systems with which to safely conduct rigorous studies. In February 2002, the National Institute of Allergy and Infectious Diseases (NIAID) of the US National Institutes of Health (NIH) published a comprehensive Strategic Plan for Biodefense Research (http://www2.niaid.nih.gov/biodefense/research/strat_plan.htm) for conducting basic and 'translational' work for the development of new vaccines, drugs, immunotherapies and diagnostic assays focused on 'biothreat' agents classified as category A, B or C (http://www2.niaid.nih.gov/biodefense/bandc_priority.htm). These categories define pathogens such as *Bacillus anthracis*, *Variola major* and *Francisella tularensis* that can be readily transmitted person to person with high mortality rates (category A); those associated with food, water and other safety threats, such as salmonella, *Vibrio cholerae* and *Rickettsia prowazekii*, that are relatively easy to disseminate and result in moderate morbidity rates (category B); and emerging pathogens such as Nipah and West Nile viruses, as well as pathogens such as the influenza virus, that might be readily engineered for increased morbidity and mortality (category C). As the NIH institute with a primary focus on immunology and infectious disease, the NIAID received input from a multitude of expert sources for the creation of the Strategic Plan for Biodefense Research. Expert panel recommendations for implementation of the biodefense research program, and summaries of progress so far, are available at the following website: http://www2.niaid.nih.gov/biodefense/research/strat_plan.htm. One common recommendation was that research resources in a variety of areas be made available to the research community (**Fig. 1**).

Many new research and product development grants and contracts were awarded by the NIAID to both academic and commercial laboratories in 2003. Funded projects range from basic studies in areas such as innate immunity and microbial pathogenesis to clinical trials of new vaccine candidates. Training programs and small business grants targeting biodefense research were also expanded. Given the comprehensive nature of this research effort, it is clear that its benefits will extend beyond the prevention of bioterrorism to affect public health in geographical areas where category A, B or C pathogens are naturally endemic, are recently emergent or are re-emerging in the human population. Generalizable advances such as new vaccine platforms, drug targets, antigen-nonspecific immunotherapies and diagnostic tools will also lead to improvements in the treatment or prevention of immune-mediated and inflammatory diseases. These efforts, then, should support a broad spectrum of biomedical applications.

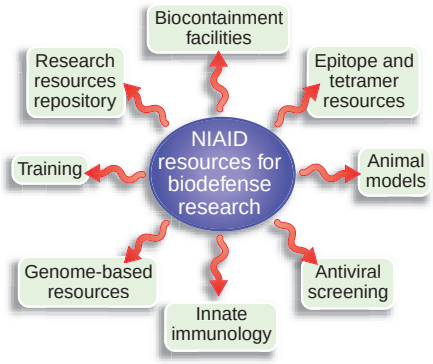

Figure 1 Resources for biodefense research. These research areas are supported by a variety of resource programs designed to facilitate research on dangerous pathogens for the research community.

How to work with dangerous bugs

For a variety of reasons, many of the pathogens of interest for biodefense have not been studied extensively using modern molecular methods of biomedical research. Thus, large gaps exist in our understanding of pathogenesis, host response and immune evasion strategies. Research facilities designed to safely contain highly pathogenic organisms such as Ebola virus and *F. tularemia* have never been available to most investigators. Therefore, when the NIAID began the large expansion of its biodefense research program in 2003, an early priority was the construction of biosafety level (BSL)-3 and BSL-4 laboratories to safely handle highly infectious and deadly pathogens. Sites for nine BSL-3 and two BSL-4 facilities were funded across the US (**Table 1**). These laboratories will serve the general scientific community on a 'user fee'

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basis and will also serve the newly established Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases Research (RCEs). The RCE program itself funds basic, translational and product development research in eight different consortia and offers opportunities for collaborations with non-RCE scientists in a variety of areas (**Table 1**). Additional BSL-3 and RCE sites will be funded in fiscal year 2005. This integrated BSL-RCE endeavor will expand considerably the professional expertise and specialized infrastructure needed for cutting-edge research in the area of biodefense.

Research resource repository

Of course, not all biodefense research requires confrontation with dangerous bugs. Much work can be done with attenuated strains or recombinant proteins. To provide a comprehensive source of reagents and information for investigators new to these pathogens, the NIAID established a repository in 2003 to collect, authenticate, store and distribute relatively unique and 'quality-assured' reagents to the research community. Included are DNA clones, purified proteins, synthetic peptides, cells and antibodies. This repository resides at the American Type Culture Collection under contract to the NIAID, and it will offer additional services in the future, such as the storage of select agents for research institutions unwilling or unable to provide their own protected facility. The repository will also collect, develop and distribute information about the reagents and pathogens, will help standardize their use and will develop and disseminate methods to facilitate technology transfer and commercialization. Interested parties should check the Biodefense and Emerging Infections Research Resources Repository website at http://www.beiresources.org for current and future resources.

Genes, proteomics and epitopes

The NIAID made a substantial investment in the sequencing of microbial genomes over the last 5 years and recently funded two Microbial Genome Sequencing Centers at The Institute for Genomic Research and the Massachusetts Institute of Technology. The Microbial Genome Sequencing Centers will support rapid and cost-efficient sequencing of additional microorganisms, including potential agents of bioterrorism, as well as invertebrate vectors of infectious disease. DNA sequencing services can be requested at http://www.niaid.nih.gov/dmid/genomes/mscs.

In September 2004, several contracts will be awarded under the Population Genetics Analysis Program: Immunity to Infection or Vaccination. This program will describe single-nucleotide polymorphisms of human immune response genes and will associate them with adverse reactions or resistance to infection by or vaccination against category A, B and C pathogens and their toxins. Verified results will be published for the research community and should prove generally useful for application to immune-mediated diseases as well as infectious diseases.

Table 1 NIAID Regional Centers for Biodefense Research

Region	RCE	BSL-3 facility	BSL-4 facility	Institution	Location
New England	X			Harvard Medical School	Boston, Massachusetts
			X	Boston University	Boston, Massachusetts
Northeast	X			New York State Department of Health	New York, New York
		X		University of Medicine and Dentistry of New Jersey	Newark, New Jersey
Mid-Atlantic	X			University of Maryland	Baltimore, Maryland
		X		University of Pittsburgh	Pittsburgh, Pennsylvania
Southeast	X	X		Duke University	Durham, North Carolina
		X		University of Alabama	Birmingham, Alabama
		X		University of Tennessee	Memphis, Tennesses
Great Lakes	X	X		University of Chicago	Chicago, Illinois
Western	X		X	University of Texas Medical Branch	Galveston, Texas
		X		Tulane University	New Orleans, Louisiana
Midwest	X			Washington University	St. Louis, Missouri
		X		University of Missouri	Columbia, Missouri
Northwest	X			University of Washington	Seattle, Washington
		X		Colorado State University	Fort Collins, Colorado

X indicates that a facility has this qualification. More information is available at http://www2.niaid.nih.gov/biodefense/research/resources.htm.

The NIAID Pathogen Functional Genomics Resource Center at The Institute for Genomic Research provides resources and reagents such as pathogen-related microarrays, protein expression clones, genotyping resources and bioinformatics services free of charge to the investigator (http://pfgrc.tigr.org). This center also provides training in emerging technologies related to functional genomics. In addition, several Proteomics Research Centers were funded in June 2004, each with a focus on characterizing pathogen or relevant host cell proteomes to facilitate the discovery of targets for the next generation of vaccines, therapeutics and diagnostics for biodefense. A network of Bioinformatics Resource Centers was also funded recently to facilitate data integration from genomic, proteomic, microarray and other studies involving category A, B and C pathogens and host immune responses. Access to these data will be facilitated by a user-friendly online interface and state-of-the-art analysis tools for the research community. These centers were not limited to specific geographical regions.

The NIH Tetramer Facility, which began operations 5 years ago, was recently expanded to provide peptide–major histocompatibility complex (MHC) tetramer reagents that identify T cells that specifically recognize antigens from category A, B and C agents. This facility, located at Emory University, provides both standard and customized tetramers to investigators in many areas of research. Tetramers are provided free of charge (although the investigator must provide the peptide and pay shipping charges). Human, mouse and monkey MHC tetramers are available. Most are MHC class I, but several MHC class II and mouse monomeric CD1d proteins are now available. Additional CD1d-based reagents are in development (http://www.emory.edu/WHSC/TETRAMER/).

For the creation of tetramers or similar reagents, the antigenic epitopes presented by MHC-restricting elements must be identified. To aid in this effort, the NIAID supports the HLA Ligand/Motif Database developed at the University of Oklahoma Health Sciences Center (http://hlaligand.ouhsc.edu/LigandDB/servlet/GenerateFormServlet?form_type=index). To continue and expand this effort, the NIAID recently established a more comprehensive Immune Epitope Database and Analysis Program at the La Jolla Institute for Allergy and Immunology. This database will be accessible in prototype form in mid-2005 and should be fully operational in 2006. It will centralize known epitope information for both T cells and antibodies (excluding human immunodeficiency virus data that are

available through a separate database), will incorporate new epitopes as they are verified, will provide state-of-the-art tools to help investigators locate and analyze information and will develop new models to facilitate epitope prediction.

Translating research into products

In vitro screening assays and preclinical animal models are highly valuable tools for the identification and characterization of lead candidates for product development and for the exploration of mechanisms of pathogenesis, immune response and preventive or therapeutic activities of candidate products. For many biodefense applications, challenge studies cannot be done in humans and investigators must complete efficacy, toxicity and pharmacokinetic studies in animals to obtain data needed for US Food and Drug Administration approval under the Animal Efficacy Rule (http://www.fda.gov/OHRMS/DOCKETS/98fr/053102a.pdf).

A centralized service for *in vitro* antiviral drug screening is now available for evaluating the toxicity and potential efficacy of therapeutic candidates to treat human viral infections. At present, screening assays are replication based and are low throughput. With no cost to the drug sponsor, screens are done at one of two sites: Utah State University or the University of Alabama, Birmingham. The viruses that can be screened can be found at http://www2.niaid.nih.gov/biodefense/research/resources.htm.

Many of the category A, B and C pathogens have not been studied in sufficient depth to know which specific animal models are most likely to faithfully mimic the human situation. The NIAID *In Vitro* and Animal Models for Emerging Infectious Diseases and Biodefense Program was established in late 2003 to address some of these needs through multiple contract awards. This program will provide targeted screening of potential vaccines or therapeutics using *in vitro* assays, small animals and nonhuman primates to test safety and efficacy. Candidate vaccines, therapeutics and diagnostics will be drawn from basic research as well as more advanced product development programs. At present, studies directed toward the urgent need for licensure of next-generation vaccines have tapped the mature capacity of the program, and efforts are underway to expand capacity. Early candidate screening should be available to qualified investigators by early 2005. Eligibility and procedures for access will be announced on the NIAID website at http://www2.niaid.nih.gov/biodefense/research/resources.htm. The program will include services such as Good Laboratory Practice studies in both small animals and nonhuman primates, clinical isolate panels for certain bacteria, and safety and pharmacology testing for therapeutics.

Opportunities

The opportunities created by this large investment in biodefense research and product development extend well beyond the immediate goals, and the specialized resources created to facilitate targeted research will undoubtedly enrich many areas of investigation. The research community is encouraged to follow the NIAID Biodefense website at http://www2.niaid.nih.gov/biodefense to be informed of developing resources and new opportunities.

In addition to the NIAID research resource repository, BSL-3 and BSL-4 facilities, *in vitro* and animal model screening programs, gene sequencing support, functional proteomics reagents, MHC tetramers and bioinformatics support now available, centralized public information sources will become increasingly comprehensive to provide the research community with validated data in areas such as immune response gene associations with outcomes of infection and/or vaccination, protein expression data from a variety of human cell types, and small molecules or biologics that modify innate and adaptive immune responses *in vivo*.

Training opportunities are available at the pre- and postdoctoral level (http://grants1.nih.gov/grants/guide/notice-files/NOT-AI-03-046.html), as are opportunities for specific product development through the NIAID Biodefense Small Business Innovation Research program, which allows a considerably higher budget and longer time frame than conventional NIH Small Business Innovation Research awards (http://grants.nih.gov/grants/guide/pa-files/PAS-02-149.html). Investigator-initiated basic research grants are also funded under the biodefense program, and awards may include pilot project R21 grants, single-project R01 grants and multiproject P01 program project grants (http://grants1.nih.gov/grants/guide/pa-files/PA-04-119.html). Although subject to change, several new biodefense initiatives are planned for fiscal year 2005. These new programs would focus on mechanisms of immune evasion by human pathogens, mathematical modeling of immune response and regulation, and immunity in special populations such as infants, the elderly and immunocompromised people (http://www.niaid.nih.gov/ncn/budget/concepts/c-ait0903.htm).