NIAID
BIODEFENSE
Preparing Through Research

NIAID Biodefense Research Agenda for Category B and C Priority Pathogens

Progress Report

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health
National Institute of Allergy and Infectious Diseases

Introduction

The nation's ability to detect and counter bioterrorism depends largely on information generated through biomedical research on pathogenic microbes and the immune response to these microbes. A small amount of biodefense research had been conducted for years by civilian agencies such as the National Institutes of Health (NIH). It was the anthrax attacks of 2001, however, that revealed significant gaps in the nation's overall preparedness against potential agents of bioterrorism.

In response to the new sense of urgency to understand anthrax and other potential bioterrorist threats, in early 2002, NIH's National Institute of Allergy and Infectious Diseases (NIAID) developed the *NIAID Strategic Plan for Biodefense Research*. (See NIAID's Web site, biodefense.niaid.nih.gov.) The purpose of the strategic plan is to guide the implementation of basic and translational biodefense research and to engage partners in academia, industry, and other private and public-sector entities to develop biodefense-related diagnostics, therapeutics, and vaccines.

To help implement the recommendations outlined in the strategic plan, NIAID convened several panels of experts to provide objective guidance on the Institute's future biodefense research agenda. The first panel prioritized NIAID research needs for Category A pathogens, those that are most deadly and pose the biggest threat to public health. The second group helped establish research priorities for Category B and C priority pathogens, which, in general, cause less disease and fewer deaths than Category A agents and are more difficult to disseminate or disperse in populations. The third group focused on research related to innate and adaptive immune factors important for host protection against any or all potential bioterror pathogens. The recommendations of all three panels have provided valuable guidance to NIAID on developing new research initiatives and modifying existing grant and contract solicitations, enabling the Institute to implement immediate, intermediate, and long-term priorities for its basic and applied biodefense research portfolio.

In August 2003, the *NIAID Biodefense Research Agenda for CDC Category A Agents Progress Report* was released. It outlined scientific advances made for each Category A agent and described the programs and activities initiated by NIAID to address immediate research needs identified in the Category A research agenda.

Significant progress also has been made since the January 2003 release of the *NIAID Biodefense Research Agenda for Category B and C Priority Pathogens;* these scientific advances and programmatic activities are described in this new progress report. Specific topics include: progress made towards meeting general recommendations in the areas of research, product development, and research resources, which are crucial for building a robust biodefense infrastructure; and scientific advances and research activities related to the immediate goals in the research agenda. In keeping with these goals, this progress report focuses on basic and applied research aimed at developing pathogen-specific diagnostics, therapeutics, and vaccines, as well as progress in understanding the biology

of Category B and C microbes and the host response to them. The report also summarizes scientific progress in the area of immune-based strategies for biodefense.

The panel that developed the *NIAID Biodefense Research Agenda for Category B and C Priority Pathogens* also identified several important areas that needed examination and consideration by various experts to overcome obstacles to product development in critical biodefense areas. This report describes progress in addressing recommendations in these areas, including re-examining the lists of Category A-C agents in light of new scientific information, exploring the role of industry in the biodefense research agenda, and considering ways to diagnose and defend against genetically modified organisms.

Clearly, virtually all the fruits of NIAID biodefense activities—including research results, intellectual capital, laboratory resources, and countermeasures in the form of diagnostics, therapeutics, and vaccines—can be applied to emerging, re-emerging, and deliberately released microbes alike. Experience demonstrates that knowledge developed to understand one pathogen invariably applies to others. Research on microbial biology and on the pathogenesis of organisms with potential for bioterrorism will undoubtedly lead to enhanced understanding of other more common and naturally occurring infections such as HIV/AIDS, West Nile virus, malaria, tuberculosis, and those caused by multi-drug resistant microbes. Finally, new insights into mechanisms that regulate the human immune system will have positive spin-offs for preventing, diagnosing, and treating illnesses such as cancer, immune-mediated neurological diseases, and allergic and hypersensitivity diseases, and for preventing the rejection of transplanted organs.

Progress on General Recommendations

An expert panel convened in October 2002 to prioritize the research agenda for Category B and C priority pathogens made several recommendations that apply to all areas of emerging infectious diseases and biodefense research. These general recommendations were grouped into 1) areas of research, 2) product development, and 3) research resources.

Areas of Research

Recommendation: Apply structure-based design, comparative genomics, and structural biology information to the development of new diagnostics and broadly based, cross-reactive therapeutics.

- The National Institute of Allergy and Infectious Diseases (NIAID) continues support of the Pathogen Functional Genomics Resource Center (PFGRC*).* The PFGRC is undertaking comparative genomic analyses of human pathogens to identify genetic variations within and between species and strains to define characteristic biosignatures that would be useful for rapid and accurate identification. (The Institute for Genomic Research (TIGR), Rockville, MD)

- In fiscal year 2004, NIAID will establish a biodefense proteomics research program, Identifying Targets for Therapeutic Interventions Using Proteomic Technology, to develop and enhance innovative proteomic technologies and methodologies. By applying this knowledge to understanding human pathogens and host cell proteomes, scientists may discover and identify novel targets for drugs, vaccines, diagnostics, and immunotherapeutics against microbes considered potential agents of bioterrorism.

- With the National Institute of General Medical Sciences, NIAID is co-funding the Protein Structure Initiative, a federal, university, and industry effort to make the three-dimensional, atomic-level structures of most proteins easily obtainable from corresponding DNA sequences. The ultimate goal is to enhance identification of promising new structure-based medicines and develop better therapeutics for treating both genetic and infectious diseases.

Recommendation: Evaluate inducers of innate immunity for use as first-line therapies for biodefense.

- In fiscal year 2004, NIAID will support development of new therapies for use soon after infection and for use as adjuvants with new vaccines under the new Innate Immune Receptors and Adjuvant Discovery initiative. Natural and synthetic ligands will be screened for their ability to stimulate innate immune receptors.

- NIAID continues to encourage and support research on immune protective mechanisms against infection with Category A-C pathogens under the Biodefense

and Emerging Infectious Diseases Research Opportunities initiative. For example, research focusing on the development of technology for probing innate immunity is currently under way. (Scripps Research Institute, La Jolla, CA)

Recommendation: Develop approaches to enhance the effectiveness of vaccines in immunologically compromised populations, including the elderly.

- In fiscal year 2004, NIAID will establish several Population Genetics Analysis programs to expand current knowledge of the immunological mechanisms that defend against infectious diseases caused by Category A-C priority pathogens. The objective of these programs is to identify associations between specific immune response gene polymorphisms and either susceptibility to infection or quality of response to vaccination.

- The NIAID Hyperaccelerated Award/Mechanisms in Immunomodulation Trials program has been expanded to include immunological studies in the context of vaccine trials for biodefense agents. These studies will provide information important for improvements in vaccine design and safety.

- Investigators at the NIAID Cooperative Centers for Translational Research on Human Immunology and Biodefense are developing new ways to obtain information from single immune cells. The goal is to be able to test very small tissue and blood samples representing diverse populations. Several projects under these centers focus on immunologically compromised/vulnerable individuals such as young children, the elderly, cancer patients after severe bone marrow ablation, and atopic dermatitis patients at risk for complications from the smallpox vaccine. Improved techniques could help researchers determine the immune mechanisms responsible for strong versus weak immune responses to vaccines.

Recommendation: Examine the pathogenesis of microorganisms transmitted through aerosolization in immunized and immunologically naïve animal models.

- In fiscal year 2003, NIAID provided support for the construction of two National Biocontainment Laboratories (NBLs) and nine Regional Biocontainment Laboratories (RBLs). These laboratories will provide increased capacity for conducting aerosol challenge experiments in animal models.

- Under the *In Vitro* and Animal Models for Emerging Infectious Diseases and Biodefense initiative, NIAID has access to various resources for preclinical testing of new therapies and vaccines, including non-human primate models, for Category A-C priority pathogens. Among the activities available are safety, toxicology, and pharmaceutical testing in small and large animals, including the capability for conducting aerosol challenge studies.

- In December 2003, NIAID hosted a workshop entitled Aerosol Challenge Technology and Applications in Biodefense. Participants included experts in the field of aerosol challenge biology as well as NIAID contractors and grantees in an effort to expand the field and harmonize technologies.

Recommendation: Develop integrated approaches to understand the factors that lead to the natural emergence of infectious diseases to distinguish them from diseases that emerge through an intentional release of an infectious agent.

- The NIAID PFGRC is undertaking comparative genomic analyses between species and strains of several NIAID Category A-C priority pathogens to define genetic variations for discovery of important molecular differences.

- In fiscal year 2004, NIAID will establish eight to ten Bioinformatics Resource Centers to develop and maintain comprehensive relational databases. Researchers will be able to query these databases and collect, store, display, annotate, and analyze data. Genomic, functional genomic, structural, and related data will be available about microorganisms responsible for emerging and re-emerging infectious diseases, including the NIAID Category A-C priority pathogens.

- NIAID staff members participate in a number of federal agency working groups and committees to develop a coordinated effort to address serious gaps in the comprehensive genomic analysis, functional genomics, and bioinformatics of microorganisms considered agents of bioterrorism. This coordinated approach can lead to new forensic methodologies that will enable scientists to distinguish between naturally emerging infectious diseases and those caused by agents of bioterrorism.

Recommendation: Develop methods for rapid detection of antimicrobial susceptibility/resistance.

- In fiscal year 2003, NIAID awarded 18 grants under the Innovative Approaches for Combating Antimicrobial Resistance initiative. Among the goals of this initiative is enhanced understanding of mechanisms of resistance and the spread of resistance genes. Examples of research being conducted under this initiative include:

    - Using genomics to identify antibiotic sensitivity genes (University of Colorado at Boulder)
    - Validating mathematical models for predicting resistance (University of California, San Francisco)
    - Rapidly evaluating drug resistance in pathogenic fungi (Public Health Research Institute, Newark, NJ)
    - Studying resistance in isolates of *Mycobacterium tuberculosis* (Stellenbosch University, Tygerberg, South Africa)
    - Identifying mechanisms of Ciprofloxacin resistance (University of Michigan)

- NIAID-supported researchers have developed a rapid, reliable polymerase chain reaction assay for testing antibiotic susceptibility in *Coxiella burnetii*-infected macrophages. The results represent a promising alternative to traditional susceptibility testing methodologies for intracellular bacteria such as *C. burnetii*.

- NIAID continues to support the development of diagnostics and novel therapeutic and preventive measures to minimize infection with resistant pathogens; prevent the acquisition of resistance traits; and control the spread of resistance factors and resistant pathogens in hospital settings under a number of initiatives, including the Biodefense and Emerging Infectious Diseases Research Opportunities initiative.

Recommendation: Expand research on polymicrobial interactions and the consequence of co-infections.

- In fiscal year 2003, NIAID awarded 23 grants under the Impact of Microbial Interactions on Infectious Diseases initiative. Among the goals of this initiative is the development of novel, exploratory approaches for examining polymicrobial interactions. Examples include:

    - Identify immune determinants to bacterial and viral co-infection (The Wistar Institute, Philadelphia, PA)
    - Study quorum sensing, bacterial interaction, and disease (Tufts University)
    - Evaluate *M. tuberculosis* interactions with environmental mycobacteria and the effects on vaccine efficacy (Ohio State University)
    - Study susceptibility to secondary bacterial infection as a result of influenza infection (Wayne State University)
    - Investigate enteropathogenic and toxigenic *Escherichia coli* interactions (The Research Foundation of The State University of New York, Albany)
    - Study secondary infections in relationship to *M. tuberculosis* infection (University of Wisconsin-Madison)

- In October 2003, NIAID staff co-organized an American Society of Microbiology Conference on Polymicrobial Diseases to provide an update on this emerging topic and to foster interdisciplinary collaborations.

- A session entitled Immunity in Polymicrobial Infections was held at the 2004 meeting of the American Society for Microbiology. It focused on the complex interaction between multiple infectious agents and their collective influence on host immunity. NIAID staff were among those leading the session.

Recommendation: Identify host-response profiles for early detection of presymptomatic infections.

- In fiscal year 2003, NIAID awarded five Cooperative Centers for Translational Research on Human Immunology and Biodefense to support basic, clinical, and applied research on human immune responses to Category A-C priority pathogens or their products. Each center includes a large component that focuses on developing and applying new assays to facilitate the study of human immune responses. Additional center awards are planned for fiscal year 2004.

- NIAID staff participated in a trans-National Institutes of Health (NIH) workshop, Imaging Technology and the Study of Immune Function, in April 2003. Participants presented recent progress in imaging techniques as applied to immune responses and identified opportunities for application to human immunity research.

Recommendation: Investigate mechanisms by which organisms evade host immune responses.

- NIAID intramural researchers discovered a genome-wide protective response used by certain bacteria to prevent destruction by the human immune system. The studies are among the first to identify complex genetic programs in bacteria that promote disease by circumventing human innate immunity.

- NIAID continues to encourage and support research on microbial immune evasion and enhancement under the Biodefense and Emerging Infectious Diseases Research Opportunities initiative.

- In June 2003, NIAID convened an expert panel on Antiviral Innate Immunity: Recognition, Defenses, Evasion, and Biodefense Strategies, with the goal of identifying research needs and establishing the basis for future research programs in the areas of innate immune activation by viruses, viral evasion mechanisms, and antiviral therapies.

Product Development

Recommendation: Involve the Food and Drug Administration (FDA) and industry in the early planning of development of vaccines, diagnostics, and therapeutics for biodefense.

- NIAID has established working groups, consisting of representatives from FDA, the Centers for Disease Control and Prevention (CDC), and pharmaceutical manufacturers, at an early stage of a product's development. One goal of each working group is to identify and address the development of animal models for testing candidate biodefense products.

- NIAID is implementing a process to obtain FDA review and comment early during product development, so that the necessary follow-on studies can be more quickly identified and initiated.

- NIAID is actively seeking input from both FDA and industry regarding the types of products needed for biodefense. This information is being used to plan and execute product development.

- In fiscal year 2004, NIAID will make several awards under the initiative Assessing Safety of Cell Substrates and Vaccine Components to advance the development of assays to better characterize vaccine cell substrates. Data generated from these assays may allow vaccine manufacturers and FDA to make more informed risk-benefit analyses regarding use of new cell substrates in vaccine manufacturing.

Recommendation: Develop new models that facilitate industry participation in the development of products for biodefense.

- In May 2004, NIAID held a workshop entitled Biological Assays – Development, Validation and Long Term Maintenance, which was taught by experts in assay validation. This workshop taught companies working on biodefense products how to develop and validate assays.

- NIAID regularly conducts current Good Manufacturing Practices (cGMP) and GLP[1] audits of manufacturing and testing facilities that are producing and testing biodefense products being used in clinical trials. These audits provide the companies with valuable guidance on steps needed to validate assays, and to manufacture and test products in a manner compliant with FDA guidelines.

- NIAID has created several novel mechanisms to encourage biodefense research and product development activities by the private sector. Ongoing initiatives used to specifically engage industry in this effort include Challenge Grants: Biodefense Partnerships; and Cooperative Research for the Development of Vaccines, Adjuvants, Therapeutics, Immunotherapeutics, and Diagnostics for Biodefense.

- In fiscal year 2003, NIAID established the Biodefense and Emerging Infections Research Resources Repository to acquire, authenticate, and store Category A-C priority pathogens and related reagents, and to make them available to scientists for research and product development. The repository also provides a means to receive input and advice from scientists regarding reagent needs, characterization requirements and quality control,

[1] cGMPs are regulations issued by the FDA that specify methods, equipment, facilities, and controls required for producing, packaging, handling, and holding drugs and other products for clinical use. GLPs are FDA regulations that establish standards for conducting and reporting non-clinical laboratory studies that are used to support or are intended to support applications for research or marketing permits for regulated products.

proprietary and ethical issues, training and technology transfer needs, and access to information. (American Type Culture Collection)

Recommendation: Develop vaccines and immune-based therapies for emerging pathogens, including those that are broadly protective.

The development of vaccines and immune-based therapies for biodefense is supported under many of NIAID's new biodefense initiatives including Challenge Grants: Biodefense Partnerships; Cooperative Research for the Development of Vaccines, Adjuvants, Therapeutics, Immunotherapeutics, and Diagnostics for Biodefense; Regional Centers of Excellence (RCEs) for Biodefense and Emerging Infectious Diseases; and the Small Business Biodefense Program. These goals are also supported under several of NIAID's research resources, including the Vaccine and Treatment Evaluation Units, *In Vitro* and Animal Models for Emerging Infectious Diseases and Biodefense, and the Biodefense and Emerging Infections Research Resources Repository**.** Examples of recently funded research in these areas include:

- Development of improved *Brucella* vaccines
- Development of vaccines for *Burkholderia* species
- Development of outer membrane protein-based subunit vaccines for Q fever
- Development of attenuated strains of the endemic typhus bacterium for potential use in vaccines
- Discovery of T- and B-cell antigens for a model bacterium similar to the causative agent of Rocky Mountain spotted fever
- Development of replicon vaccines for eastern, western, and Venezuelan equine encephalitis viruses
- Development of a non-replicating subunit vaccine for West Nile virus
- Development of neutralizing human monoclonal antibodies for eventual immunization and/or treatment of Crimean-Congo hemorrhagic fever and West Nile virus infection
- Development of a multivalent vaccine for *Shigella flexneri*
- Development of new vaccines that generate a broad immunity against divergent influenza strains

- NIAID's Vaccine Research Center (VRC), in collaboration with Vical Inc., is developing a DNA vaccine candidate for West Nile virus. Immunogenicity studies have shown elicitation of significant antibody titers, and challenge studies have demonstrated substantial protection in vaccinated mice. A Phase I clinical trial is planned for late 2004 to assess safety and immunogenicity of the vaccine in healthy volunteers.

- NIAID intramural scientists are developing a number of vaccines and immunotherapies against Category B and C agents, as well as some Category A agents. For example:

  - Live attenuated pandemic influenza A virus vaccines

- Vaccines against West Nile virus, multiple toxins of *Clostridium botulinum*, and other Category B and C priority pathogens
- A live, attenuated tetravalent dengue virus vaccine
- Humanized monoclonal antibodies against dengue virus

Recommendation: Develop new, broadly applicable therapeutic agents.

- The development of therapeutics for biodefense is supported under many of NIAID's new biodefense initiatives including Challenge Grants: Biodefense Partnerships; Cooperative Research for the Development of Vaccines, Adjuvants, Therapeutics, Immunotherapeutics, and Diagnostics for Biodefense; and the Small Business Biodefense Program. Examples of recently funded research in this area include:

  - A novel platform to discover biodefense therapeutics
  - Broad-spectrum therapeutics for influenza
  - Pathogen-specific drug targets for weaponized bacteria
  - Gram-negative sepsis and pharmacophore-based therapies
  - New therapies for pathogenic *E. coli* diseases
  - RNA interference as a potential therapeutic for RNA virus infections
  - Immunotherapies for biodefense
  - Novel synthetic Toll-like receptor ligands for biodefense
  - Drug development for multi-drug resistant tuberculosis
  - Novel antiviral agents for Lassa fever, Nipah virus, Hendra virus, Sin Nombre virus, and West Nile virus
  - DerG (immunomodulator) treatment of viral encephalitis

- By expanding existing resources and developing new ones, NIAID has enhanced its capabilities to evaluate potential therapeutics. Resources include:

  - The *In Vitro* and Animal Models for Emerging Diseases and Biodefense program, which provides preclinical testing for new as well as licensed therapeutics. This network includes capabilities for *in vitro* screening of antimicrobial activity; clinical isolate panels for selected bacterial pathogens; small animal models; non-human primate models; and safety/toxicology and pharmacology testing for therapeutics.

  - A coordinated network of contracts under which potential therapeutics can be evaluated for activity against Category A-C viruses in both *in vitro* and animal model screens.

Research Resources

Recommendation: Establish cGMP and GLP facilities capable of producing monoclonal antibodies, vaccines, and other drugs and immunotherapeutics for preclinical development and clinical trials.

- NIAID's VRC is currently developing a Vaccine Pilot Plant facility, leased and operated by a contractor, that can produce vaccines for clinical trials. The facility will be operated according to cGMP, and its flexible design will allow vaccines to be manufactured using a wide variety of technology platforms.

- NIAID has expanded its ability to access facilities using cGMPs and GLPs to develop, manufacture and characterize pilot lots of vaccines, antibodies and therapeutics for evaluation in Phase I clinical studies. Recently, NIAID awarded contracts for production of cGMP pilot lots of an inactivated influenza vaccine, severe acute respiratory syndrome (SARS) coronavirus vaccines, and a human monoclonal antibody against coronavirus S protein.

- Several of the NIAID NBLs and RBLs awarded in fiscal year 2003 are planning to have the flexibility to accommodate GLP space within their facilities. In addition, one of the RBLs is planning to have a cGMP suite.

Recommendation: Develop and standardize functional assays for measurement of human immunity.

- NIAID has established five Cooperative Centers for Translational Research on Human Immunology and Biodefense. Each center includes a large component focused on developing and applying new assays to facilitate the study of human immune responses.

- Through NIAID repositories, the Institute is making characterized reagents available to scientists involved in biodefense research activities for use in standardized assays of immune function.

Recommendation: Establish genomics and proteomics resources for identification and comparison of new or emerging pathogens, including those that are genetically engineered.

- NIAID has made a significant investment in establishing a comprehensive genomics program. Among the funded and planned resources are:

    - The **Pathogen Functional Genomics Resource Center** (PFGRC), which provides and distributes to researchers a wide range of genomic and related resources and technologies for functional analyses of microbial pathogens, including potential agents of bioterrorism, and invertebrate vectors of

infectious diseases. In fiscal year 2004, additional genomic resources, including protein expression clone sets and DNA microarrays, will be available (see www.niaid.nih.gov/dmid). In addition, the PFGRC is undertaking comparative genomic analyses to identify genetic variations and relatedness within and between species that define characteristic pathogen biosignatures. These biosignatures can be used for rapid and accurate pathogen identification in forensics and other applications. (TIGR, Rockville, MD)

- **Microbial Sequencing Centers** that allow for rapid and cost-efficient production of high-quality microbial genome sequences. Genomes to be sequenced include microorganisms considered agents of bioterrorism, related organisms, clinical isolates, closely related species, and invertebrate vectors of infectious diseases. Comparative genomic analysis will provide critical data enabling identification of genetic polymorphisms that correlate with phenotypic characteristics such as drug resistance, morbidity, and infectivity. (TIGR, Rockville, MD and the Massachusetts Institute of Technology)

- **Bioinformatics Resource Centers** that will develop, populate, display, store, and continuously update comprehensive, relational, multi-organism databases. The databases will focus on microorganisms responsible for emerging and re-emerging infectious diseases, including those considered potential agents of bioterrorism. They will provide scientists with easy access to a large amount of genomic, functional genomic, structural, and related data. Access to these data will facilitate comprehensive analyses of genomes to help identify critical pathogen-specific molecular markers for forensic strain identification. Approximately eight Bioinformatics Resource Centers are expected to be awarded in fiscal year 2004, complementing the research efforts under way at the Microbial Sequencing Centers.

- **Proteomics Research Programs: Identifying Targets for Therapeutic Interventions Using Proteomic Technology,** under which innovative proteomic technologies and methodologies will be developed and enhanced. By using this knowledge to understand pathogens and/or host cell proteomes, scientists may discover and identify novel targets for the next generation of drugs, vaccines, diagnostics, and immunotherapeutics against microbes considered potential agents of bioterrorism.

- The **NIH Influenza Genomics Project**, which NIAID recently initiated in collaboration with the NIH National Library of Medicine, TIGR, and academic partners. This project will build capacity for rapid and complete genomic sequencing of both human and avian influenza viruses and for developing bioinformatics tools. The availability of complete genomic sequences of several thousand known human and animal influenza viruses, and the capacity to rapidly sequence those that emerge in the future, is critical for understanding the overall molecular evolution of influenza viruses and

genetic correlates of virulence and severe disease. This information will be a critical resource for basic and applied research on influenza, including vaccine development. All sequence information will be immediately released to the scientific community through Genbank.

- The NIAID intramural research program has established a proteomics facility and enhanced its genomics capabilities to support biodefense and emerging infectious diseases initiatives. These resources are being used to analyze multiple strains of a number of bacterial and viral pathogens.

Recommendation: Ensure adequate numbers of BSL-3 facilities with aerosol challenge capacity.

- In fiscal year 2003, NIAID provided support for the construction of two NBLs and nine RBLs. The NBL construction program provides funding to design, construct, and commission comprehensive, state-of-the-art biosafety laboratory (BSL)-4, BSL-3, and BSL-2 facilities, as well as associated research and administrative support space. The RBL construction program provides funding for similar facilities containing BSL-3 and BSL-2 labs. These laboratories will provide increased capacity for aerosol challenge experiments in animal models.

- Recent facility renovations have expanded the capacity of NIAID intramural scientists to conduct aerosol challenge studies in BSL-3 laboratories. Preconstruction activities and/or construction of three new integrated research facilities that will provide additional clinical, laboratory, and animal biocontainment capacity are under way.

- NIAID has established a cooperative program with the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) to conduct research projects related to biodefense, including pathogenesis and immune response studies. Under the agreement, NIAID is supporting efforts to increase USAMRIID's aerosol challenge capability by increasing containment facilities and animal holding space.

Recommendation: Establish small animal and non-human primate models for emerging infectious diseases.

- The *In Vitro* and Animal Models for Emerging Diseases and Biodefense program supports development, validation, and use of various small animal and non-human primate models to screen new therapeutic, diagnostic, and preventive compounds for Category A-C bacteria and viruses, and to test their efficacy.

- NIAID supports a coordinated network of contracts under which potential therapeutics can be evaluated for activity against Category A-C viruses in both *in vitro* and animal model screens. These resources are being expanded to incorporate additional animal models.

- In fiscal year 2004, NIAID will initiate the Development of Immune Monitoring Reagents and MHC (Major Histocompatibility Complex) Typing Technologies for Non-Human Primates program to develop, evaluate, produce, and distribute new or improved non-human primate immune monitoring and immune modulating reagents needed to advance preclinical studies of vaccine and immunotherapeutic candidates into non-human primates.

- NIAID intramural scientists are developing animal models of emerging infectious diseases such as SARS.

Recommendation: Develop a network of centralized repositories for reagents and clinical specimens for emerging and biothreat infections and encourage new strategies to facilitate the shipment of infectious biological samples.

- The Biodefense and Emerging Infections Research Resources Repository, initiated in fiscal year 2003, is acquiring, authenticating, and storing Category A-C priority pathogens and related reagents, and making them available to the scientific community for research and product development. It will also produce and dispense reagents, such as DNA clones, body fluids and cells, synthetic peptides, and monoclonal and polyclonal antibodies. In addition, the repository staff have been developing procedures to ensure compliance with safety and security regulations for transport of select agents. (American Type Culture Collection)

- The NIH/NIAID Tetramer Facility provides custom synthesis and distribution to researchers from around the world of soluble MHC-peptide tetramer reagents that can be used to stain antigen-specific T cells. This facility was recently expanded to produce MHC-peptide tetramer reagents specific for T cells recognizing the Category A-C priority pathogen antigens. (Emory University)

Recommendation: Identify and develop potential field sites in appropriate endemic areas to study natural history, develop diagnostics, evaluate interventions, and acquire clinical materials.

- In fiscal year 2003, NIAID expanded its International Collaboration in Infectious Disease Research network of overseas endemic field sites to include natural history, diagnostic, and pathogenesis studies for a number of Category A-C pathogens.

- NIAID is working with Department of Defense laboratories to identify potential overseas field sites for testing new therapeutics, vaccines, and diagnostics for Category A-C priority pathogens in endemic areas.

- NIAID is investigating options for developing foreign research sites in locations where select biodefense pathogens are endemic.

Recommendation: Attract new scientific disciplines, such as computational biology and bioinformatics, to biodefense research and expand the research training of a new cohort of investigators.

- NIAID recently made available new and expanded programs to support training and career development of young scientists in biodefense research. Approximately 30 percent of training grant applications received in fiscal year 2003 were specifically focused on training in biodefense.

- The expansion of NIAID intramural biodefense research has created new opportunities for postdoctoral research in select agent biology and pathogenesis, as well as in development of vaccines, diagnostics, and therapeutics for biodefense.

- An integral component of the Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases program includes training of a new generation of science professionals to perform biodefense research activities, including computational biology and bioinformatics.

- NIAID staff regularly present the Institute's biodefense research agenda at scientific meetings and workshops to raise awareness of and interest in available funding opportunities. In addition, NIAID co-sponsored meetings with the American Society for Microbiology in 2003 and 2004 entitled Future Directions for Biodefense Research: Development of Countermeasures, at which new biodefense research and training programs were discussed. Additional information regarding the Institute's research activities, funding opportunities, scientific resources, and training opportunities can be found on the NIAID Web site (biodefense.niaid.nih.gov).

- In June 2003, NIAID, in cooperation with the National Academy of Sciences, held a workshop to bring together scientists from diverse research areas such as virology, chemistry, cell biology, structural biology, and drug development to share ideas and identify unique approaches for the discovery and development of new therapies of importance for biodefense.

Inhalational Bacteria

The Category B and C bacteria with the potential to infect via the aerosol route include *Brucella* species, *Burkholderia mallei* and *pseudomallei, Coxiella burnetii,* and select *Rickettsia* species. Most of these organisms cause zoonotic diseases or infections, which are diseases or infections that may be transmitted from vertebrate animals (e.g., rodents, birds, and livestock) to humans. Different bacteria infect humans through different routes, including ingestion, inhalation, or arthropod-mediated transmission. All of these agents, however, are believed to be capable of causing infections following inhalation of small numbers of organisms. Consequently, these agents are of special concern for biodefense because they may be weaponized for more effective dispersion as aerosols.

Scientific Progress

Since the publication of the *NIAID Biodefense Research Agenda for Category B and C Priority Pathogens* in January 2003, important progress has been made in understanding the basic biology of these inhalational bacteria as well as the development of strategies for prevention, treatment, and diagnosis of associated infections.

Discovery of antigens and cross-protective immunity may lead to development of vaccines against multiple species of *Rickettsia*. National Institute of Allergy and Infectious Diseases (NIAID)-supported investigators have discovered T-cell antigens for *Rickettsia conorii*, a bacterium similar to the causative agent of Rocky Mountain spotted fever, *Rickettsia rickettsii*. This group has also shown that immunization with some rickettsial species confers broad protection against other rickettsial species. Both of these discoveries could provide important information for future vaccine development for *R. rickettsii* and related infections.
(Feng HM and Walker DH, Cross-protection between distantly related spotted fever group rickettsiae, *Vaccine* 2003;21:3901-3905; Li Z et al., Identification of CD8 T-lymphocyte epitopes in OmpB of *Rickettsia conorii*, *Infect Immun* 2003;71:3920-3926)

Quick and reliable assay determines whether certain inhalational bacteria succumb to antibiotics. NIAID-funded researchers recently developed a rapid, reliable polymerase chain reaction (PCR) assay for testing antibiotic susceptibility in *C. burnetii*-infected macrophages. This assay represents a promising alternative to traditional antibiotic testing methodologies for intracellular bacteria such as *C. burnetii.*
(Brennan RE and Samuel JE, Evaluation of *Coxiella burnetii* antibiotic susceptibilities by real-time PCR assay, *J Clin Microbiol* 2003;41:1869-1874)

Sequencing and cloning of Q fever pathogen pave the way for improved diagnostics and vaccines. A multi-institutional team of investigators, funded in part by NIAID, completed the genome sequence of *C. burnetii*, the causative agent of Q fever. The *C. burnetii* genome sequence will allow investigators to more easily study genes that may be involved in causing disease, and should also help identify targets for improved diagnostics and potential vaccine candidates.
(Seshadri R et al., Complete genome sequence of the Q-fever pathogen *Coxiella burnetii*, *Proc Natl Acad Sci U S A* 2003;100:5455-5460)

Progress made toward Q fever vaccines. NIAID-funded researchers have identified and cloned multiple immunogenic proteins from *C. burnetii* that react with infection-derived antibodies. These proteins are logical candidates for future vaccines against Q fever. (Zhang G et al., Identification and cloning of immunodominant antigens of *Coxiella burnetii*, *Infect Immun* 2004;72:844-852)

Programmatic Progress in Addressing Immediate Goals

Goal: Investigate the mechanisms by which the intracellular inhalational bacteria survive.

- NIAID supports sequencing of virulent and avirulent strains of *B. mallei*. This research will allow sequences to be compared in order to identify bacterial genes conferring virulence. (The Institute for Genomic Research (TIGR), Rockville, MD)

- NIAID intramural scientists found subtle differences in the modifications of a surface lipopolysaccharide expressed by *B. mallei*, which causes glanders, and *B. pseudomallei*, which causes melioidosis. Investigators are conducting additional research to better understand how these differences might influence the bacteria's ability to evade the host immune system.

- NIAID intramural scientists have initiated studies to identify those genes that are up- or down-regulated in an intracellular environment and allow *B. pseudomallei* to resist bactericidal immune activity. *B. pseudomallei* is known to resist the bactericidal activity of both reactive oxygen and nitrogen intermediates, as well as to survive and multiply within several mouse and human macrophage cell lines.

- NIAID intramural scientists have developed methods to isolate different forms of the Q fever pathogen, *C. burnetii*, that display environmental stability. Isolation of these variants will allow researchers to define the kinetics of *C. burnetii* development and the biological properties of the variant forms in terms of infectivity, antibiotic sensitivity, and resistance to environmental insult.

- NIAID intramural scientists have isolated several novel *C. burnetii*-secreted proteins that are likely critical for survival and replication of the organism. Further identification of these proteins may yield new targets for vaccines or improved therapies.

Goal: Further characterize the mechanisms by which the inhalational bacteria are taken up into cells and cause infection.

- Several NIAID-supported researchers are conducting studies to better understand the basic biology of *Brucella* species. Studies include:

- Gene expression in *Brucella*-infected macrophages (Virginia Bioinformatics Institute at Virginia Tech)
- The relationship between gene expression in stationary-phase bacteria and virulence (East Carolina University)
- Research on a *Brucella* gene required for interactions with host macrophages (Texas A&M University)

- NIAID supports basic research on glanders, including regulation of *B. mallei* virulence genes (TIGR, Rockville, MD) and examination of the role of quorum sensing in *B. pseudomallei* virulence. (University of Cincinnati)

- NIAID supports multiple studies that seek to understand how the epidemic typhus bacterium, *Rickettsia prowazekii*, survives within host cells. Researchers are investigating transport mechanisms for moving essential metabolites from the host cell cytoplasm into the bacterium's intracellular compartment; conducting genetic analysis of *R. prowazekii* pathogenesis; and comprehensively analyzing the bacterium's genomes and proteomes. This research has led to recent publications on how *R. prowazekii* utilizes cellular transport mechanisms for survival. (University of South Alabama)

- NIAID-supported researchers are studying the pathogenic mechanisms of the Q fever bacterium. (Texas A&M University)

Goal: Develop appropriate animal models for all the inhalational bacterial diseases, including models that incorporate aerosol challenge.

- NIAID-supported researchers are developing a guinea pig model of aerosolized *C. burnetii* (Q fever) infection. (Texas A&M University)

- The new *In Vitro* and Animal Models for Emerging Infectious Diseases and Biodefense program supports development, validation, and use of various relevant, small animal and non-human primate models to screen for and test efficacy of new therapeutic, diagnostic, and preventive compounds for Category A-C bacteria and viruses.

Goal: Identify promising drug and vaccine candidates for preclinical development.

- NIAID-supported investigators are evaluating several vaccine candidates for Q fever and Rocky Mountain spotted fever. (Texas A&M University and The University of Texas Medical Branch-Galveston (UTMB))

- NIAID intramural researchers are initiating studies to identify and characterize outer-membrane proteins from *B. pseudomallei* and *B. mallei* strains grown under a variety of environmental conditions for use as potential vaccine candidates.

Goal: Identify and develop potential field sites in appropriate endemic areas to study natural history, acquire clinical materials, develop diagnostics, and evaluate interventions for inhalational bacteria.

- NIAID is investigating options for developing foreign research sites in locations where select biodefense pathogens are endemic.

- In fiscal year 2003, NIAID expanded its International Collaboration in Infectious Disease Research network of overseas endemic field sites to include natural history, diagnostic, and pathogenesis studies for a number of Category A-C priority pathogens.

- NIAID is working with Department of Defense laboratories to identify potential overseas field sites for testing new therapeutics, vaccines, and diagnostics for Category A-C priority pathogens in endemic areas.

- NIAID is investigating options for developing foreign research sites in locations where select biodefense pathogens are endemic.

Goal: Evaluate efficacy of antimicrobials in animal models of the inhalational bacterial diseases.

- NIAID-funded researchers have developed a rapid, reliable PCR assay for testing antibiotic susceptibility in *C. burnetii*-infected macrophages. This assay represents a promising alternative to traditional antibiotic testing methodologies for intracellular bacteria such as *C. burnetii*. (Texas A&M University) (Also included under Scientific Progress section.)

- The *In Vitro* and Animal Models for Emerging Infectious Diseases and Biodefense program supports development, validation, and use of various relevant, small animal and non-human primate models to screen for and test efficacy of new therapeutic, diagnostic, and preventive compounds for Category A-C bacteria and viruses.

Goal: Initiate and/or develop rapid diagnostic tests for these pathogens including point-of-care diagnostics.

- NIAID-supported researchers have identified several immunogenic antigens from *C. burnetii*. Based on these proteins, the investigators are currently developing recombinant antigens for use in creating rapid diagnostic tools for Q fever. (Texas A&M University)

- NIAID-supported researchers are developing new diagnostic methods for acute rickettsial infections focusing on *Rickettsia typhi* and *R. prowazekii*. The approach

is to detect antigens using highly sensitive techniques such as electrochemi-luminescence and tyramide signal amplification coupled with enzyme-labeled fluorescence amplification technology. These researchers are also exploring the use of host protein signatures as specific markers of the early host response to rickettsial disease. (UTMB)

Goal: Develop microarrays for functional genomics studies of inhalational bacteria.

- NIAID intramural scientists have developed a custom microarray representing 21 genomes from seven different pathogens, including 2,104 open reading frames from *C. burnetii*.

- NIAID supports microarray analysis of gene expression in *B. mallei*. (TIGR, Rockville, MD)

Goal: Initiate and/or complete the genomic sequencing of representative members and strains of the inhalational bacteria and compare them to detect differences that correlate with pathogenesis and virulence.

- NIAID supports research to complete and annotate the genome sequence of virulent and avirulent strains of *B. mallei*. This research will allow sequences to be compared in order to identify bacterial genes conferring virulence. (TIGR, Rockville, MD)

- NIAID-supported researchers recently completed the genome sequence of *Brucella suis*. The *B. suis* genome reveals fundamental similarities between animal and plant pathogens and symbionts. (TIGR, Rockville, MD)

- A multi-institutional team of investigators, supported in part by NIAID, completed the genome sequence of *C. burnetii* (also included under Scientific Progress section).

Arthropod-Borne Viruses

Category B and C arthropod-borne viruses (arboviruses), which are important agents of viral encephalitides and hemorrhagic fevers, include the following:

- **Alphaviruses**: Venezuelan equine encephalitis (VEE) virus, eastern equine encephalitis (EEE) virus, and western equine encephalitis (WEE) virus

- **Flaviviruses**: West Nile virus (WNV), Japanese encephalitis (JE) virus, Kyasanur forest disease virus, tick-borne encephalitis (TBE) virus complex, and yellow fever (YF) virus

- **Bunyaviruses**: California encephalitis virus, La Crosse (LAC) virus, and Crimean-Congo hemorrhagic fever (CCHF) virus

Arthropod vectors such as mosquitoes, ticks, and sandflies are responsible for the natural transmission of most viral encephalitides and hemorrhagic fever viruses to humans and animals in all areas of the world, including the United States. Importantly, many of these viruses also pose a serious health risk from intentional exposure as bioterrorist weapons due to their extreme infectivity following aerosolized exposure. Vaccines or effective specific therapeutics are available for only very few of these viruses.

Scientific Progress

Since publication of the *NIAID Biodefense Research Agenda for Category B and C Priority Pathogens*, significant progress has been made in research on arbovirus vaccines, and on vector biology and control strategies.

Vaccines against West Nile virus perform well in animal models.

- Over the past three years, the National Institute of Allergy and Infectious Diseases (NIAID) has supported the preclinical development of a live, attenuated recombinant vaccine for WNV. This vaccine was created by replacing several genes of the well-established YF 17D vaccine virus with those of WNV. Preclinical testing of this WNV vaccine candidate (ChimeriVax-West Nile) has demonstrated safety, efficacy, and protection against disease in animal models (mice and non-human primates). Currently further development of this vaccine candidate, including Phase I clinical trials in healthy adults, is under way. (Acambis, Cambridge, U.K.)

- NIAID intramural scientists have developed two chimeric WNV vaccine candidates that have shown promising results in animal models. Despite their high level of attenuation, the vaccines induced moderate to high titers of neutralizing antibodies in monkeys and horses and prevented viremia in monkeys challenged with WNV. Additional equine studies are nearing completion. Phase I clinical

trials of one of these chimeric vaccines are planned for 2004.
(Pletnev AG et al., Molecularly engineered live-attenuated chimeric West Nile/dengue virus vaccines protect rhesus monkeys from West Nile virus, *Virology* 2003;314:190-195)

Recombinant Japanese encephalitis virus vaccine developed; clinical trials ongoing. NIAID provided support for initial research and preclinical development of a new JE candidate vaccine. The live-attenuated, recombinant, chimeric vaccine was created by replacing several of the envelope genes of the YF 17D vaccine virus with those of JE virus. Clinical development of this vaccine candidate (ChimeriVax-JE) is ongoing with industrial and World Health Organization support. Two Phase II clinical trials have been completed, and a third is under way to expand safety and efficacy data and investigate the duration of immunity.
(Monath, TP et al., Chimeric live, attenuated vaccine against Japanese encephalitis (ChimeriVax-JE): phase 2 clinical trials for safety and immunogenicity, effect of vaccine dose and schedule, and memory response to challenge with inactivated Japanese encephalitis antigen, *J Infect Dis*. 2003 Oct 15;188(8):1213-1230)

Studies of arthropod biology and ecology identify key elements to controlling mosquito-borne diseases. An important goal in studying arthropod-borne viral infections is to better understand the role of mosquitoes and other viral vectors in the transmission of the viruses. These advances in the basic understanding of the mosquito vector may lead to the development of new strategies for controlling the spread of disease.

- NIAID-supported researchers recently identified the role of a gene that regulates egg production of *Aedes aegypti* (the mosquito carrier of dengue and YF) in response to blood meals. This discovery may reveal new strategies for reducing the ability of this mosquito species to reproduce.
  (Attardo GM et al., RNA interference-mediated knockdown of a GATA-factor reveals a link to anautogeny in the mosquito, *Aedes aegypti*, *PNAS* 2003;100:13374-13379)

- NIAID-supported researchers used microarrays to identify genes in the mosquito midgut that are induced or repressed in response to blood meals. These genes may be important targets in the development of new insecticide strategies.
  (Sanders HR et al., Blood meal induces global changes in midgut gene expression in the disease vector, *Aedes aegypti*, *Insect Biochem Mol Biol* 2003;33:1105-1122)

- NIAID-supported researchers have developed a mosquito cell line that transcribes double-stranded RNA from the dengue virus. This type of RNA is thought to induce mosquito cells to resist infection by dengue virus. This cell line will serve as a model for studying the evolutionary balance between the mosquito and dengue virus infection and could lead to new strategies for preventing transmission of disease.
  (Adelman ZN et al., RNA silencing of dengue virus type 2 replication in transformed C6/36 mosquito cells transcribing an inverted-repeat RNA derived from the virus genome, *J Virol* 2002;76:12925-12933)

Programmatic Progress in Addressing Immediate Goals

Goal: Expand research on the pathogenesis and biology of arthropod-borne viral infections in animal models.

- NIAID established two Emerging Viral Diseases Research Centers under which multidisciplinary research teams are focused on zoonotic, arthropod-borne, and other emerging viral pathogens.

    - Researchers are developing and characterizing hamster models of mosquito-borne Punta Toro and Rift Valley fever (bunyaviruses), as well as WNV and YF (flaviviruses) for eventual use in preclinical evaluation of newly developed vaccines or drug therapies. They are also developing animal models to characterize the pathogenesis of newly recognized animal or human viral pathogens. (The University of Texas Medical Branch-Galveston (UTMB))

    - Scientists are studying the biology, epidemiology, diagnosis, treatment, and prevention of WNV. This includes developing and characterizing mouse and avian models for WNV, and establishing a mouse model for VEE (alphavirus). (Health Research Inc./New York State Department of Health)

Goal: Expand research on immune responses to these viral infections, their correlation with protection from disease, and potential for immune enhancement of disease severity.

- In fiscal year 2003, under the Collaborative Antiviral Study Group (CASG), NIAID initiated a Phase I/II clinical trial to characterize the natural history of severe WNV infection. (University of Alabama, Birmingham)

- NIAID-supported researchers are examining human immune responses to YF, JE, and WNV, and mouse immune responses to chimeric YF/JE and YF/WNV vaccines in order to assess the role of T cells in pathogenesis and protection. (University of Massachusetts Amherst)

- NIAID-supported researchers are assessing host control of arboviral (WNV) infections via toll-like-receptor-mediated responses and/or other potential host determinants of disease. (Yale University)

Goal: Initiate and/or advance the development of vaccines.

- With NIAID support, researchers are developing EEE, WEE, and VEE replicon vaccines (single-cycle virus-like particle replicating vaccines) that express viral

genes on an attenuated VEE vector platform. (AlphaVax Human Vaccines, Inc., Research Triangle Park, NC)

- NIAID is supporting the development of several non-replicating subunit WNV vaccines including:

  - A vaccine for use in humans, targeted toward populations that are at greater risk of infection, such as the elderly (Hawaii Biotech Inc., Aiea, HI)

  - A vaccine for use in horses, with possible further development for use in humans (L2 Diagnostics LLC, New Haven, CT)

- Collaboration between NIAID Vaccine Research Center intramural researchers and Vical Inc. has led to the rapid development of a promising DNA vaccine candidate for WNV. Immunogenicity studies have shown that the vaccine elicits significant antibody titers and challenge studies have demonstrated that it provides substantial protection in vaccinated mice. Following preclinical toxicity testing, NIAID plans a Phase I clinical trial for late 2004 to assess the safety and immunogenicity of the vaccine candidate in healthy volunteers.

- The NIAID Hyperaccelerated Award/Mechanisms in Immunomodulation Trials program was recently expanded to include immunological studies in the context of vaccine trials for biodefense (including WNV and other arbovirus vaccines), which should generate important information for vaccine design and safety.

- NIAID intramural scientists developed a Langat/dengue chimeric vaccine using sequences from Langat virus and dengue type 4 virus. Langat virus is a naturally avirulent member of the tick-borne flavivirus group that is closely related to the virulent TBE virus. This chimeric vaccine was found to be attenuated and efficacious in monkeys against Langat virus. Further, immune serum from immunized monkeys passively protects mice against TBE. Clinical evaluation is planned for 2004.

- NIAID intramural scientists have constructed a candidate TBE vaccine virus using several genes from TBE inserted into a dengue backbone with a genetic deletion that confers additional attenuation. This vaccine will be compared to a Langat/dengue chimeric vaccine and an inactivated TBE vaccine in non-human primate studies in 2004.

Goal: Determine correlates of immunity and evaluate potential for vaccine-induced cross-reactive immunity and immune enhancement of disease severity.

- In June 2003, NIAID convened an expert panel on Antiviral Innate Immunity: Recognition, Defenses, Evasion, and Biodefense Strategies. The goal was to identify research needs in the areas of innate immune activation by viruses, viral

evasion mechanisms, and antiviral therapies, in order to establish the basis for future research programs in these areas.

Goal: Expand the *in vitro* and *in vivo* screening capability for effective antiviral drugs.

- In fiscal year 2003, NIAID expanded its *in vitro* and *in vivo* antiviral screening programs. The capacity of the *in vitro* screening assays has increased more than five-fold so that in fiscal year 2003 approximately 1,500 compounds were evaluated against one or more arboviruses, including WNV, YF, VEE, Punta Toro, dengue, and Pichinde. The capacities for *in vivo* animal model screening were expanded to include Punta Toro, Pichinde, Banzi, Semliki Forest, and WNV. Compounds that show initial *in vitro* activity (evidenced by reduced viral cytopathogenic effect or growth in cell culture) are then tested in animal models of disease. (Utah State University)

- NIAID is supporting research to identify new targets for antiviral therapeutics that will facilitate screening of combinatorial chemistry libraries and testing of potential antibody therapies to identify activity against alphaviruses, flaviviruses, and bunyaviruses. Examples include:

  - Evaluation of drug targets for WNV and YF (University of Houston)

  - Identification of inhibitors of WNV and dengue virus (Health Research Inc./New York State Department of Health)

Goal: Initiate and/or develop rapid diagnostic tests for these pathogens including point-of-care diagnostics.

- In fiscal year 2003, NIAID established the World Reference Center for Emerging Viruses and Arboviruses, under which viral infection in clinical and veterinary samples is identified using serology, molecular (nucleic acid or protein) assays, and electron microscopy techniques. (UTMB)

- NIAID is supporting research to enable and expand capabilities for developing rapid diagnostics of WNV and other arboviruses. Examples include:

  - Use of biophotonics to detect WNV and WNV antibodies (Platypus Technologies, Madison, WI)

  - Development of an immunoassay for WNV (Health Research Inc./New York State Department of Health)

- Development of a microchip to diagnose WNV infections (Northwestern University)

Goal: Initiate and/or complete the genomic sequencing of representative members and strains of the arthropod-borne viruses and compare them to detect differences that correlate with pathogenesis and virulence.

- NIAID is supporting comparative genomic analyses of several arthropod-borne viruses (WNV, YF, EEE, WEE, VEE) for identifying genetic differences and variations that may correlate with pathogenicity, virulence, vector competence (in various mosquitoes/species, ticks, etc.), and transmission. (The Institute for Genomic Research (TIGR), Rockville, MD)

Goal: Exploit genomic information to design new vaccines and diagnostics.

- Genomic and proteomic information for several arboviruses, including EEE, WEE, VEE, and WNV, has facilitated development of several new candidate vaccines. (AlphaVax Human Vaccines, Inc., Research Triangle Park, NC; Acambis, Cambridge, U.K.; University of Kansas)

- NIAID-supported researchers are developing rapid diagnostic assays (diagnostic microarrays) for WNV utilizing genomic and proteomic information. (Northwestern University)

Goal: Develop human or humanized antibody preparations for passive immunization against arboviruses.

- In fiscal year 2003, under the CASG, NIAID initiated Phase I and II randomized, placebo-controlled clinical trials to assess safety, tolerability, and potential efficacy of intravenous immunoglobulin G (Omr-IgG-am). This product, which contains high anti-WNV antibody titers, is administered to patients with, or at risk for, progression to WNV encephalitis and/or myelitis. (University of Alabama, Birmingham)

- NIAID-supported researchers are developing neutralizing human monoclonal antibodies for eventual immunization against and/or treatment of CCHF, WNV, and other infections. (Regional Center of Excellence, University of Maryland)

- NIAID intramural scientists are developing humanized monoclonal antibodies that neutralize WNV, JE, and TBE.

Goal: Expand research on vector biology, ecology, and vector control methods.

- Under NIAID's Emerging Viral Diseases Research Centers, scientists are investigating factors that influence disease emergence, geographic spread, and control mechanisms for WNV, JE, LAC, and other arboviruses. (UTMB and Health Research Inc./New York State Department of Health)

- NIAID has expanded the *Aedes aegypti* sequencing project to include generation of a draft whole-genome sequence. Understanding the mosquito genome will facilitate efforts to develop novel control strategies. (TIGR, Rockville, MD)

- NIAID is supporting several ecology and epidemiology studies of WNV, EEE, WEE, and VEE. Examples include:

    - Characterization of the roles of several vectors of WNV responsible for trafficking between various animal reservoirs (University of Alabama, Birmingham)

    - Characterization of the role of various mosquito species as vectors of viral encephalitis agents (University of Illinois)

    - The relationship among dengue and WNV virus strains and arthropod vectors in the Yucatan Peninsula with the goal of identifying subsets of the population responsible for most of the human disease in that area (University of Colorado)

    - Identification of patterns of movement of several arboviruses (Health Research Inc./New York State Department of Health; UTMB; Harvard University)

- Research to better understand the role of vectors in disease transmission as well as enhanced control strategies is supported under many of NIAID's new biodefense initiatives.

    - Development of a lethal trap for *Aedes* species mosquitoes (Tulane University)

    - Development of a novel method of controlling culex vectors of WNV (University of California, Davis)

    - Development of a new repellant for mosquitoes (Uniformed Services University of the Health Sciences)

Goal: Assess the availability of licensed vaccines and vaccine candidates, including production capacity and regulatory status.

- NIAID staff participate in the Biodefense Vaccines and Immunologics Interagency Working Group of the Office of Science and Technology Policy, Executive Office of the President. The group is assessing the availability of unlicensed vaccines that were developed by the Department of Defense.
- The development of new vaccines for arboviruses that may have improved safety or efficacy over vaccines currently licensed for these viral diseases is encouraged under several of NIAID's new biodefense initiatives including the Partnerships for Biodefense and Small Business Biodefense programs.

Goal: Initiate development of standardized reagents for use with non-murine animal models of disease.

- NIAID is supporting the development of hamster models and reagents for research on flaviviruses and hantaviruses. The hamster-specific anti-cytokine and anti-cell surface marker antibodies will enable and/or enhance the study of viral pathogenesis in the hamster model of infection. The generated antibodies will be made available to researchers upon request. (UTMB)

- Cytotoxic T-cell responses and T-helper responses appear to be critical components of immune responses to members of the flavivirus family. The Biodefense and Emerging Infections Research Resources program has initiated the synthesis of overlapping peptide sets of five virion proteins of the West Nile virus. The arrayed sets of 227 unique peptides will be made available to scientists conducting research on viral pathogenesis, immunological responses and vaccine evaluation in a variety of animal models and humans.

Goal: Attract and train new investigators in laboratory and field-based investigation specific to arboviruses.

- The NIAID Emerging Viral Diseases Research Centers include support to train pre- and post-doctoral investigators in laboratory and field-based arbovirology research. (UTMB and Health Research Inc./New York State Department of Health)

- The NIAID World Reference Center for Emerging Viruses and Arboviruses includes support to train professional and technical personnel in emerging arbovirus identification and characterization, and to investigate and diagnose disease outbreaks. (UTMB)

Goal: Identify and develop potential field sites in appropriate endemic areas to study natural history, acquire clinical materials, develop diagnostics, and evaluate interventions for arboviruses.

- Under the Emerging Viral Diseases Research Centers, research is conducted on the emergence, maintenance, and spread of disease among natural hosts and vectors for WNV, EEE, VEE, LAC, and other arboviruses in endemic areas. (UTMB and Health Research Inc./New York State Department of Health)

- NIAID is supporting the collection of clinical materials from arbovirus-infected individuals, and is developing and distributing reagents and diagnostics to researchers under the World Reference Center for Emerging Viruses and Arboviruses. (UTMB)

- NIAID is conducting Phase I and II clinical trials at 35 U.S. sites to characterize the natural history of severe WNV infection and assess safety, tolerability, and potential efficacy of intravenous immunoglobulin G (Omr-IgG-am). (University of Alabama, Birmingham)

Toxins

The Category B toxins include ricin toxin from the plant *Ricinus communis*, epsilon toxin of *Clostridium perfringens*, and Staphylococcal enterotoxin B (SEB). These protein toxins are among the most toxic biologic agents known, second only to the botulinum neurotoxins, which are Category A agents. Delivery of these toxins may occur by a variety of modalities including contamination of food and water, and inhalational exposure to aerosols — all methods pose a potential major public health threat from the perspective of bioterrorism. For example, as recently as February 2004, a small amount of ricin powder found in the U.S. Senate mailroom forced closure of buildings and disrupted Senate operations.

No licensed vaccines or specific therapies against ricin toxin, *C. perfringens* epsilon toxin, or SEB are currently available for use in humans.

Scientific Progress

Since publication of the *NIAID Biodefense Research Agenda for Category B and C Priority Pathogens* in January 2003, progress has been made in understanding these dangerous toxins and how they cause disease, and in developing medical countermeasures against them. Although not directly supported by the National Institute of Allergy and Infectious Diseases (NIAID), the following scientific advances are recent key developments in the search for countermeasures for these toxins.

Studies of gene expression may lead to new targets for clinical intervention. Investigators at U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) recently completed studies that profile gene expression in the lungs of mice exposed to ricin through the inhalational route. Results of these and other related studies may lead to identification of new targets for clinical interventions.
(DaSilva L et al., Pulmonary gene expression profiling of inhaled ricin, *Toxicon* 2003;41:813-822)

New recombinant Staphylococcal enterotoxin vaccine candidate is found protective in mice. Department of Defense (DoD) researchers have purified a recombinant SEB vaccine candidate and found that it elicits an immune response in mice, and protects mice against a lethal challenge with the native toxin. This is an important milestone in the clinical development pathway of this candidate vaccine.
(Coffman JD et al., Production and purification of a recombinant Staphylococcal enterotoxin B vaccine candidate expressed in *Escherichia coli*, *Protein Expression and Purification* 2002;24:302-312)

Programmatic Progress in Addressing Immediate Goals

Goal: Collaborate with other agencies to determine research gaps related to the Category B toxins.

- In April 2004, NIAID convened experts from academia, government, and industry to discuss developing countermeasures for ricin. The meeting focused on determining ideal candidates for new or next generation countermeasures and identifying technical opportunities and obstacles to developing such products.

Goal: Evaluate potential countermeasures for the Category B toxins.

- In fiscal year 2003, seven new awards were made under NIAID's biodefense initiatives for research that is key to developing vaccines, diagnostics, and therapeutics for ricin toxin, epsilon toxin of *C. perfringens*, and SEB. Recently funded research includes:

    - Development of a recombinant ricin vaccine based on the A chain of the toxin (University of Texas Southwestern Medical Center)

    - Optimization of antibodies specific for ricin for both therapeutic and diagnostic purposes (Diversa Corporation, San Diego, CA)

    - Development of anti-ricin monoclonal antibodies and characterization in *in vitro* and *in vivo* models (Louisiana State University Health Sciences Center)

    - Development of a microencapsulated ricin toxin vaccine candidate (Dor Biopharma, Lake Forest, IL)

    - Development of *C. perfringens* Type B-D virulence plasmid (University of Pittsburgh)

    - Comparative genomic analysis of *C. perfringens* (The Institute for Genomic Research (TIGR), Rockville, MD)

    - Development of gastrointestinal immunity to ricin. (Children's Hospital Boston)

    - Development of transgenic cattle for production of human polyclonal neutralizing antibodies against SEB (University of Massachusetts)

    - Control of enterotoxin gene expression in *Staphylococcus aureus* (Kansas State University)

- The DoD is supporting further development and current manufacture according to current Good Manufacturing Practices of a recombinant SEB vaccine candidate, including development of animal models. This should allow for clinical evaluation of this vaccine candidate.

Goal: Attract and train new investigators.

- NIAID recently made available new and expanded programs for supporting training and career development for young scientists in biodefense research. Approximately 30 percent of the training grant applications received in fiscal year 2003 were specifically focused on training in biodefense.

- An integral component of the Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases program includes training a new generation of science professionals to perform biodefense research activities, including computational biology and bioinformatics.

- Through the Biodefense and Emerging Infectious Diseases Research Opportunities initiative, NIAID encourages new investigators to become involved in exploratory, innovative biodefense research that may contribute to the development of diagnostics, therapies, or prevention strategies for Category A-C priority pathogens.

Food- and Waterborne Pathogens

In the United States, public health surveillance activities, along with sewage and water treatment infrastructure and food safety regulations, are the first defense against deliberate contamination of food or water. The centralized production and wide, rapid distribution of food products has increased the risk for outbreaks of disease that can affect large geographic regions of the country. Globalization of the food supply increases the potential for exposure to a greater variety of foodborne pathogens. Clearly, food and water are potentially important routes for the dissemination of infectious agents by bioterrorists. A troubling outbreak of salmonellosis in Oregon in 1984 illustrates this point: investigations revealed that members of a religious cult had deliberately contaminated salad bars in area restaurants, resulting in 751 reported cases of illness. In 2003, there were large and small outbreaks of food- and waterborne diseases all over the world. In the United States, green onions contaminated with hepatitis A virus caused over 550 cases in Pennsylvania, believed to be linked to similar outbreaks in Georgia and Tennessee. Contamination (confirmed or suspected) of beef with Shiga toxin-producing *Escherichia coli*, usually *E. coli* O157:H7, resulted in several multi-state meat recalls. There were also major epidemics of cholera in Liberia leading to an estimated 35,000 cases.

Enteric infections can result from bacterial, viral, or protozoal contamination of food and water. In this chapter, the unique goals and recommendations of each class of organism are addressed in separate sections. The approach used to prioritize the research activities for this large category of pathogens is based on several criteria including availability (e.g., ease of propagation), inoculum size needed, stability in the environment, lethality, degree of incapacitation caused by the disease, possibility of secondary transmission, and availability of countermeasures (e.g., vaccines and therapeutics).

Scientific Progress

Since publication of the *NIAID Biodefense Research Agenda for Category B and C Priority Pathogens*, significant progress has been made in understanding the basic biology of and host response to food- and waterborne pathogens, which has important implications for development of countermeasures.

On/off switches in bacterial genes help elucidate mechanisms of human *E. coli* infection. National Institute of Allergy and Infectious Diseases (NIAID)-supported researchers are studying a system called quorum sensing that allows pathogenic enteric *E. coli* (as well as other bacteria) to sense their environment and communicate with one another. This group recently found that the hormone epinephrine (Epi), normally used by the mammalian host for cell-to-cell communication, can turn on bacterial genes. Further, they identified a signalling compound that can induce the same bacterial gene expression as Epi. This finding may provide important insight into the development of new therapies for treating enteric *E. coli* infections.

(Sperandio V et al., Bacteria-host communication: the language of hormones, *Proc Natl Acad Sci U S A* 2003;100:8951-8956)

New strain of transgenic mouse is resistant to intestinal infections. Defensins, small proteins produced naturally by intestinal cells of humans, are capable of killing bacteria. NIAID-supported scientists have now developed a strain of transgenic mouse that produces human defensin 5 (HD5) in their intestines. When these mice were fed a virulent pathogen, *Salmonella typhimurium*, at a level many times over that which would be lethal to non-transgenic mice, all survived the challenge. When infection was introduced by a route that bypasses the intestine, and the bacteria were not exposed to HD5, the transgenic mice were just as susceptible to lethal infection as their non-transgenic counterparts. The ability of HD5 to kill *Salmonella* may lead to the development of new therapies against infection with this pathogen in humans. In addition, this transgenic mouse model can be used to study the antimicrobial properties of other naturally produced peptides.
(Salzman NH et al., Protection against enteric salmonellosis in transgenic mice expressing a human intestinal defensin, *Nature* 2003;422:522-526)

Unique structure in cholera bacteria responsible for attachment to cells identified. The cholera bacterium *Vibrio cholerae* has a system for attaching to human epithelia that is closely related to the systems used by *Pseudomonas aeruginosa* (pneumonia), *Neisseria gonorrhea, N. meningitidis* (meningitis), and several pathogenic *E. coli* species, along with numerous plant pathogens. This key system is called the type IV pilus, and understanding its structure is critical to developing small molecules that can prevent it from binding to epithelial cells, as well as developing vaccines and therapeutics for several diseases. With NIAID support, researchers have accomplished the difficult, longstanding goal of purifying the type IV pilus of *V. cholerae,* crystallizing it, and uncovering its fine structure at the atomic level. The crystal structure of the cholera type IV pilus reveals the secrets of its strength, flexibility, and multi-functionality. These results provide information critical to the development of new drugs and diagnostics for this pathogen.
(Craig L et al., Type IV pilin structure and assembly: X-ray and EM analyses of *Vibrio cholerae* toxin-coregulated pilus and *Pseudomonas aeruginosa* PAK pilin, *Mol Cell* 2003;11:1139-1150)

Immunogenic cholera proteins identified. Patients recovering from cholera are immune to subsequent infection from the same strain of cholera. The precise Vibrio protein immunogen (or antigen) responsible for conferring lasting immunity is not known. NIAID-supported researchers in Bangladesh and the United States used a genetic system to force *V. cholerae* to express proteins, including some that are only expressed in humans with the disease. This panel of proteins was probed using antibodies obtained from the blood of patients recovering from cholera to identify those proteins that induced an antibody response. The investigators found that several proteins involved in attaching cholera to the intestine surface were immunogenic, providing important information to inform the development of improved cholera vaccines and therapeutics.
(Hang L et al., Use of *in vivo*-induced antigen technology (IVIAT) to identify genes uniquely expressed during human infection with *Vibrio cholerae*, *Proc Natl Acad Sci U S A* 2003;100:8508-8513)

Genome sequence of the apicomplexan, *Cryptosporidium parvum*, completed. The complete genome sequences of two strains of *C. parvum* provide insights into the biology of this species and its ability to cause disease. Currently there are no effective drugs for this infection; the genome information should accelerate drug discovery. The completion of genome sequences for the *C. parvum* type 1 isolate, which has primarily a human tropism, and the type 2 isolate, which is transmitted between numerous mammals, including humans, will facilitate research to better understand the differences in transmission and host specificity.
(Abrahamsen MS et al., Complete genome sequence of the apicomplexan, *Cryptosporidium parvum, Science* 2004;304:441-510)

Reliance on oral transmission helps to explain distribution and virulence of *Toxoplasma gondii.* A recent analysis supported by NIAID shows that the three major types of *T. gondii* infecting people today are the evolutionary result of a single genetic cross between two ancestral parental strains of the parasite within the last 10,000 years. The subsequent expansion of these types to account for more than 95 percent of human *T. gondii* infections may be from successive oral transmission, bypassing the sexual phase of the life cycle in a carnivorous mammal. The three types also show enhanced infectivity in mice compared to other strains of parasite that occur much less frequently. Therefore, although the parasite has a complex life cycle, oral transmission seems to be a major factor determining distribution and virulence.
(Su C et al., Recent expansion of Toxoplasma through enhanced oral transmission, *Science* 2003;299:414-416)

A vaccine based on an *Entamoeba histolytica* virulence factor provides protection against intestinal amoebiasis in a rodent model. Studies in mice indicate that immunization with the amebic Gal/GalNAc lectin, an *E. histolytica* factor that mediates parasite adherence to the surface of the colon, can prevent the development of intestinal lesions. The vaccines used in the studies were either the native lectin purified from the parasite or a recombinant protein expressed from the gene encoding the lectin. These studies also demonstrated an association between the degree of protection with the level of anti-lectin IgA in the mice. This association correlated with previous results in children that indicate that fecal anti-lectin IgA levels are associated with protection from intestinal reinfection. The further development of this candidate vaccine antigen is being pursued in an effort to block both invasive amoebiasis disease and parasite transmission.
(Houpt E et al., Prevention of intestinal amoebiasis by vaccination with the *Entamoeba histolytica* Gal/GalNAc lectin, *Vaccine* 2004; 22:611-617)

Programmatic Progress in Addressing Immediate Goals

In fiscal year 2003, NIAID established the Food- and Waterborne Diseases Integrated Research Network (FWDIRN). The network is focused on multidisciplinary research on all food- and waterborne pathogens, and toxins, to facilitate the development and evaluation of products to rapidly identify, prevent, and treat food- and waterborne diseases that threaten public health. The network includes five separate units:

- Immunology Research Unit (IRU), University of Maryland
- Clinical Research Unit (CRU), University of Maryland
- Microbiology Research Units (MRU), Tufts University and Michigan State University
- Zoonoses Research Units, Cornell University and Washington State University
- Coordinating and Biostatistics Center, The Emmes Corporation, Rockville, MD

Food- and Waterborne Bacteria

Goal: Accelerate clinical development of existing *Shigella* vaccine candidates.

- A protocol is under development for a Phase I study of a *Shigella flexneri* vaccine candidate. This candidate is a promising attenuated strain with well-defined sites of genetic alteration to optimize immunogenicity and minimize reactogenicity. This candidate is a serotype 2a strain of *S. flexneri* and is considered to be a critical component of a multivalent vaccine to provide cross-protection to all the known serotypes. (Vaccine and Treatment Evaluation Unit (VTEU), University of Maryland)

- NIAID has increased its capacity to conduct clinical trials of promising vaccine candidates, including those for diseases caused by food- and waterborne pathogens.

    - The CRU of the FWDIRN is available to conduct clinical evaluation of vaccine candidates. (University of Maryland)

    - NIAID has expanded its VTEU network by approximately 60 percent to provide additional capacity for conducting clinical evaluation of vaccine candidates.

Goal: Evaluate licensed antimicrobials for treatment of *Shigella* and Shiga toxin-producing *E. coli* (STEC) infections.

- Under the FWDIRN, NIAID is supporting projects designed to evaluate licensed antimicrobials for treating *Shigella* and STEC infections. Projects to develop improved animal models are also planned.

- The *In Vitro* and Animal Models for Emerging Infectious Diseases and Biodefense program supports development, validation, and use of various relevant, small animal and non-human primate models. The models can be used to screen new therapeutic, diagnostic, and preventive compounds for Category A-C bacteria and viruses, and to test their efficacy.

Goal: Expand research on pathogenesis of understudied food- and waterborne bacteria including *Campylobacter, Listeria,* and non-typhoidal *Salmonella* species.

- In fiscal year 2003, under NIAID's biodefense initiatives, 34 grants were awarded focused on host defense, pathogenesis, vaccine development, and diagnostics for *Campylobacter*, *Listeria,* and non-typhoidal *Salmonella*. Examples include:
    - Development and use of functional genomic tools to study the interaction of *Campylobacter jejuni* with the host intestinal tract (Oklahoma State University)
    - Development and use of proteomic and microarray tools to characterize the function and regulation of *C. jejuni* proteins that are induced at 37^{o}C (Medical College of Georgia)
    - Elucidation of the molecular mechanisms used by *Listeria monocytogenes* to escape destruction by the phagocytic vesicle and cause disease in the host (University of California at Berkeley)
    - Examination of the unique features employed by *L. monocytogenes* to survive in the hostile conditions encountered in the environment, transmission vehicles, and the host (Cornell University)
    - Development of *Salmonella* infection in calves as a model of non-typhoidal salmonellosis in humans (Texas A&M University)
    - Study of effects of host factors on the ability of *Salmonella* to maintain replication and DNA repair functions *in vivo* (University of Washington)
- Under the FWDIRN, several pathogenesis studies have been initiated, including:
    - Development of a small animal model for the study of intestinal colonization and enteritis caused by *Campylobacter*
    - Development of multilocus sequence typing of *Campylobacter* strains
    - Profiles of the emergence of *Salmonella enteritidis*
    - Studies of the virulence of antibiotic-resistant strains of *S. enteritidis*
- NIAID's Pathogen Functional Genomics Resource Center (PFGRC) will be making slide microarrays for *L. monocytogenes*, *V. cholerae*, and *S. typhimurium* available to researchers in fiscal year 2004, facilitating the identification and expression patterns of virulence genes. (The Institute for Genomic Research (TIGR), Rockville, MD)

- Through the FWDIRN, NIAID is supporting the STEC Reference Center that provides investigators with STEC strain sets and genetic information pertaining to the strains. This resource will assist in expanding research on these under-studied organisms. (MRU, Michigan State University)

Goal: Study innate immune responses and their role in combating infection with food- and waterborne bacteria.

- A project to study the innate immune response to *Shigella* lipopolysaccharide (LPS) has been initiated under the FWDIRN. Human blood samples collected from subjects previously infected with *Shigella* or with previous exposure to *Shigella* vaccines, will be used to provide information on genetic determinants of immune responses to LPS, considered a major protective antigen for shigellosis.

- In fiscal year 2003, under NIAID's biodefense initiatives, grants were awarded that focused on innate immune responses to food- and waterborne bacteria. Examples include:

    - Development of the fruit fly as a model to study innate immune responses to *Salmonella* and *Listeria* (Stanford University)

    - Study of the mechanism by which *Salmonella* evades phagocytosis by host immune cells (University of Colorado Health Science Center)

    - Examination of the possible induction of chemokines by *C. jejuni* during intestinal infection (Medical College of Wisconsin)

Goal: Develop improved diagnostic assays for enteric Category B agents that focus on detection of virulence factors, such as direct detection of toxins.

- In fiscal year 2004, the NIAID-supported PFGRC will provide slide microarrays for *L. monocytogenes*, *V. cholerae*, and *S. typhimurium* to research scientists. These microarrays will facilitate the identification of new targets to expedite the development of diagnostics and appropriate therapies.

- The Bioinformatics Resource Centers for Biodefense and Emerging/Re-emerging Infectious Diseases, planned for award in fiscal year 2004, will provide the research community with access to databases of genomic information that can be queried for a number of Category B agents. This information will be important for identifying putative targets for new diagnostics.

Goal: Identify potential field sites, including overseas sites where food- and waterborne diseases are endemic, to test new vaccines, diagnostics, and therapeutics.

- NIAID has identified several field sites with potential usefulness for clinical evaluation of countermeasures for food- and waterborne diseases.

    - Beira, Mozambique, is a potential site for future field trials of vaccines, diagnostics, and therapeutics for enteric diseases. The World Health Organization, International Vaccine Institute, Medecins Sans Frontieres/Doctors Without Borders, and the Mozambique Ministry of Health recently conducted a cholera vaccine demonstration project at this site.

    - The Armed Forces Research Institute of Medical Sciences (AFRIMS) in Thailand continues to develop and test vaccines against enteric pathogens such as *Shigella* species, enterotoxigenic *E. coli*, and *Campylobacter* species. AFRIMS resources include a clinical trial unit, an immunology laboratory, and a non-human primate colony that are suitable for modeling *Shigella* infection and for evaluating *Shigella* vaccine efficacy.

Goal: Develop syndrome-based diagnostic tests that can identify pathogens (bacteria, viruses, protozoa) in patients presenting with diarrhea or fever.

- NIAID is supporting the development of emerging genomic and non-genomic technologies, suitable for creating the next generation of medical diagnostics for enteric pathogens. Recent awards under NIAID's Biodefense initiatives include:

    - Development of multiplexed polymerase chain reaction (PCR)-based technology for detecting multiple pathogens in one assay (Medical College of Wisconsin)

    - Development of an optical detector system using nanotechnology for rapid detection of multiple pathogens in a miniature, portable PCR device (Lynntech, Inc., College Station, TX)

    - Development of a novel diagnostic assay that integrates amplification and real-time detection of nucleic acids from multiple pathogens on a microarray chip, eliminating multiple steps resulting in faster detection (Nanogen, Inc., San Diego, CA)

    - Development of an immunogenetic laboratory-on-a-chip apparatus for highly specific and quantitative detection of pathogens using microfluidic technologies (University of California, Berkeley)

- Identification of pathogen-specific diagnostic signatures using high density DNA microarrays for the development of chip-based diagnostic assays for multiple pathogens (Perlegen Sciences, Inc., Mountain View, CA)

Food- and Waterborne Viruses

Goal: Pursue Phase I testing of candidate calicivirus vaccines.

- Researchers have developed a transgenic tomato that expresses the Norwalk capsid protein and Phase I studies are planned for fiscal year 2004. Oral plant-based vaccines offer the potential for new safe and inexpensive vaccines against diseases for which a protective antigen has been identified and can be cloned in the plant of interest. (Cornell University/Boyce Thompson Institute for Plant Research)

Goal: Characterize the available challenge pool for Norwalk virus that can be used in future vaccine efficacy studies.

- The evaluation of a new challenge pool of Norwalk virus inocula has been initiated under the VTEU network. This study will provide a characterized pool of Norwalk virus, which is necessary for the clinical evaluation of Norwalk vaccine candidates. (Baylor College of Medicine)

- NIAID-funded researchers are continuing to select volunteers as subjects for evaluating the norovirus challenge pool. Identifying persons at risk for infection may be important in determining the target population. (VTEU, Cincinnati Children's Hospital Medical Center)

Goal: Develop and evaluate rapid, broadly reactive diagnostics for identifying caliciviruses, including those capable of distinguishing animal and human caliciviruses.

- Under the FWDIRN, a new project has been initiated to develop a comprehensive diagnostic panel for detection of enteric viruses in clinical samples. The diagnostic panel will include the category B enteric viruses, human caliciviruses (Norwalk virus), and hepatitis A, as well as astroviruses, rotavirus, and enteric adenoviruses. The project will provide the necessary reagents for detecting and distinguishing these viruses at the antigen and nucleic acid level. (MRU, Tufts University)

Goal: Investigate immune responses to Norwalk virus and other caliciviruses.

- Based on a recent discovery of a strain of calicivirus that is infectious in mice, NIAID is supporting research to study calicivirus immunity and pathogenesis in a mouse model. (Washington University School of Medicine)

- NIAID intramural scientists are working to develop an infectivity assay for noroviruses that will allow monitoring for the presence of infectious virus in the environment and establish the parameters of adaptive and innate immunity.

- NIAID intramural scientists continue their efforts to develop prevention and treatment strategies to combat Norwalk virus and other caliciviruses. Recently, they isolated and characterized an active recombinant norovirus enzyme with both proteinase and polymerase activities. This enzyme offers a unique target for development of anti-calicivirus drugs**.**

Food- and Waterborne Protozoa

Goal: Expand the understanding of the relationship of parasitic genotypes to virulence and disease severity.

- NIAID is supporting the construction and distribution to researchers of genomic resources for *T. gondii* and *E. histolytica.* This should enable analysis of genes involved in virulence and disease outcome for these two pathogens.

    - NIAID-funded investigators are constructing microarrays for *T. gondii* and *E. histolytica*. (Stanford University)

    - Under the PFGRC, distribution of microarrays for gene expression studies and Gateway™ clones for functional analysis of genes and proteins are planned. (TIGR, Rockville, MD)

Goal: Evaluate currently available therapies for use against a broad number of enteric protozoa.

- Together with the National Institute of Child Health and Development, NIAID is supporting a Phase I/II study of nitazoxanide for treatment of chronic diarrhea caused by *C. parvum* in HIV-infected infants, children, and adolescents in South Africa and Thailand.

Goal: Evaluate validated candidate vaccine antigens (e.g., *T. gondii* p30, *E. histolytica* Gal/GalNAc lectin, and serine-rich *E. histolytica* protein (SREHP)) in clinical studies.

- NIAID-funded researchers are assessing the protective efficacy of the Gal/GalNAC lectin. A recent scientific advance (see Scientific Progress) supports its further development as a leading vaccine candidate for *E. histolytica.* (University of Virginia)

Goal: Complete sequencing of protozoa currently under way.

- NIAID-sponsored genome sequencing projects are nearing completion for the following food- and waterborne protozoa.

    - *Giardia lamblia* (Marine Biological Laboratory, Woods Hole, MA)
    - *T. gondii* (TIGR, Rockville, MD)
    - *E. histolytica* (TIGR, Rockville, MD)

Emerging Infectious Diseases

The National Institute of Allergy and Infectious Diseases (NIAID) is the primary institute at the National Institutes of Health (NIH) that conducts and supports biomedical research on emerging and/or re-emerging infectious human pathogens, including the agents of bioterrorism. It was once believed that infectious diseases could be conquered. However, new infectious diseases continue to emerge and this goal remains elusive.

In addition to the continual discovery of new human pathogens, old infectious disease enemies are re-emerging. Natural genetic variations, recombinations, and adaptations allow new strains of pathogens to appear to which the immune system has not been previously exposed and is therefore not primed to recognize (e.g., influenza). Furthermore, human intervention plays a big role in re-emergence. Increased and sometimes imprudent use of antimicrobial drugs and pesticides has led to the development of resistance, allowing many diseases to make a comeback (e.g., tuberculosis (TB) and food- and waterborne infections). Moreover, many important diseases have never been adequately controlled on either the national or international levels. Infectious diseases that have posed ongoing health problems in developing countries are re-emerging in the United States (e.g., food- and waterborne infections, dengue hemorrhagic fever, and West Nile virus (WNV)).

New human diseases can also emerge from animal pathogens (e.g., hantavirus). Organisms that are highly infective, transmissible, and virulent and that can circumvent our current armamentarium of antimicrobial drugs and/or vaccines represent potential biothreats. Multi-drug resistant tuberculosis (MDR-TB) and influenza offer two examples of such organisms on the current NIAID list of Category C priority pathogens (see Appendix 1).

Influenza

Influenza A is a major pathogen of both humans and animals, and recent advances in genetic engineering have raised concerns about the use of influenza as a biological threat agent. While epidemics of influenza result in approximately 30,000 deaths each year in the United States, the sudden emergence of a novel influenza virus could result in global outbreaks of disease (pandemics) in which morbidity and mortality rates would significantly increase. The devastating impact of the 1918 influenza A pandemic, which killed an estimated 21 million people worldwide and more than 500,000 in the United States, provides a stark illustration of potential consequences of the emergence of new strains of influenza viruses in humans, or the theoretical case of deliberate manipulation and release of a highly pathogenic influenza virus. Between January and mid-March 2004, H5N1 influenza viruses were identified in an unprecedented spread in poultry in a number of Asian countries. During this same time frame, 34 human cases of H5N1 influenza were identified in Vietnam and Thailand, resulting in 23 reported deaths.

Scientific Progress

Since the January 2003 publication of the *NIAID Biodefense Research Agenda for Category B and C Priority Pathogens*, advances have been made in several areas including elucidating the basic biology of influenza, understanding how new influenza viruses emerge, and developing new vaccines.

Characterization of 1918 flu virus provides basis for battling emerging pandemic flu strains. NIAID-funded scientists have generated and characterized recombinant influenza viruses containing up to five genes of the 1918 virus strain. The recombinant viruses containing the two major surface viral glycoproteins, HA and NA, of the 1918 viral strain were highly lethal in mice, suggesting that these genes might have a critical role in the virulence of this virus. Mice that were immunized with an inactivated influenza vaccine were completely protected from lethal challenge. This study provides important information on the pathogenicity of a pandemic virus and on the identification of vaccine strategies against emerging pandemic influenza strains.
(Tumpey T et al., Pathogenicity and immunogenicity of influenza viruses with genes from the 1918 pandemic virus, *Proc Natl Acad Sci U S A* 2004;101:3166-3171)

Identification of H9N2 virus from ducks in southern China; virus may play a role in the emergence of a pandemic. NIAID-funded investigators have discovered that H9N2 viruses that circulate in chickens in southern China are transmitted back to domestic ducks where the virus undergoes a high degree of gene reassortment. Some of these new viruses generated in the duck have been found to have an amino acid sequence in the HA gene that makes them potentially compatible with the receptor in human cells. This discovery raises the possibility that a new pandemic virus might emerge from aquatic birds in southern China.
(Li KS et al., Characterization of H9 subtype influenza viruses from the ducks of southern China: a candidate for the next influenza pandemic in humans? *J.Virol.* 2003;77:6988-6994)

Generation of influenza vaccine master strain that grows efficiently in tissue cultured cells. Using reverse genetics, NIAID-supported scientists have generated an influenza vaccine reference strain that has improved viral rescue and growth properties in African green monkey kidney (Vero) cells, a suitable cell-based system for vaccine production. This improvement in generating vaccine reference strains is important for the reproducible generation of high yielding vaccine candidates by reverse genetics and might eventually result in more rapid manufacturing of influenza vaccines.
(Ozaki H et al., Generation of high yielding influenza A viruses in African green monkey kidney (Vero) cells by reverse genetics. *J Virol* 2004;78:1851-1857)

Novel potential target for new anti-influenza drugs identified. The mechanism by which the eight RNA genome segments of the influenza virus selectively assemble into new viral particles in an infected cell is not known. Recently, NIAID-supported researchers identified specific genetic targeting signals (one on each of the eight genome segments) that selectively interact, leading to the incorporation of the segments into newly forming virus particles. This is a significant step forward in elucidating the biology

of the virus and might provide an important target for developing new anti-influenza drugs.
(Fujii Y et al., Selective incorporation of influenza virus RNA segments into virions, *Proc Natl Acad Sci U S A* 2003;100:2002-2007)

Characterization of flu virus protein provides potential target for antiviral drugs. NIAID-supported investigators recently demonstrated that the previously uncharacterized BM2 protein of influenza B viruses is a membrane protein that possesses ion channel activity. The ion channel activity of the M2 protein of influenza A virus is required for viral replication and is the target of the licensed antiviral drug amantidine. Because amantidine is only effective against influenza A, the identification and characterization of the influenza B ion channel is an important step in developing antiviral drugs against this virus.
(Mould J et al., Influenza B virus BM2 protein has ion channel activity that conducts protons across membranes, *Developmental Cell* 2003;5:175-184)

Novel genomic-based antiviral approach against influenza virus developed. In 2003, NIAID-supported researchers demonstrated that short interfering RNAs specific for conserved regions of influenza virus genes potently inhibit influenza replication in tissue culture and virus production in the lungs of infected mice. These results serve as the foundation for further development of this novel antiviral approach to treating influenza infection in humans.
(Ge Q et al., RNA interference of influenza virus production by directly targeting mRNA for degradation and indirectly inhibiting all viral RNA transcription, *Proc Natl Acad Sci U S A* 2003;100:2718-2723)

Progress made in identifying influenza T-cell epitopes. NIAID-supported researchers have developed a new method to identify CD8 T-cell epitopes for candidate vaccines against influenza. A novel computer algorithm was used to determine the sequence of potential epitopes. The algorithm, which was successful in predicting influenza epitopes in an animal model, has the potential to improve vaccine design for human immunization.
(Zhong W et al., Genome-wide characterization of a viral cytotoxic T lymphocyte epitope repertoire, *J Biol Chem* 2003;278:45135-45144)

U.S. Food and Drug Adminstration (FDA) approves FluMist, a new influenza vaccine with the potential to induce broadly protective immune responses. For more than 30 years, NIAID has supported the development of an intranasal, cold-adapted, live-attenuated influenza vaccine, including clinical trials to evaluate its effectiveness in children. In June 2003, FDA approved FluMist for healthy people five to 49 years of age. An advantage of FluMist is its ease of administration. FluMist may also be able to induce a broader cross-protective immune response against divergent influenza strains.
(MedImmune, Inc., Gaithersburg, MD)

Programmatic Progress in Addressing Immediate Goals

Goal: Expand animal influenza surveillance activities, including natural history studies, on emergence of pandemic strains.

- In 1999, NIAID initiated a program to conduct influenza surveillance in wild birds, live bird markets, and pigs in Hong Kong; examine the molecular basis of transmission of influenza viruses between animal species and humans; and identify avian influenza viruses suitable for use in vaccine development. (St. Jude Children's Research Hospital)

  In fiscal year 2003, the program was expanded to:

  - Increase animal influenza surveillance sites in Asia. NIAID-supported investigators detected the re-emergence of highly pathogenic avian H5N1 influenza viruses in Hong Kong in 2003 and are now assisting agricultural and public health authorities to control the current widespread outbreak of highly pathogenic 2004 H5N1 influenza virus in Asia. (Hong Kong University)

  - Support production of reference reagents that can be used to detect the 2004 H5N1 influenza virus in Asia. These reagents include purified recombinant HA protein and monoclonal and polyclonal antibodies against the 2004 H5N1 virus. (Collaborative effort with FDA and Centers for Disease Control and Prevention)

  - Support a World Health Organization training course in March 2004 to strengthen diagnosis and surveillance of animal influenza in the Asia-Pacific region.

Goal: Develop high-growth vaccine viruses for selected avian influenza subtypes.

- NIAID-supported investigators generated high-growth reference strains suitable for vaccine production against the H5N1 and H7N7 influenza viruses that caused human outbreaks in 2003. A reference strain suitable for vaccine production against a 2004 human clinical isolate from Vietnam has recently been developed and is being characterized. (St. Jude Children's Research Hospital)

Goal: Produce and evaluate pilot lots of vaccine against avian influenza viruses with pandemic potential.

- NIAID-supported investigators are conducting Phase I and Phase II clinical trials to evaluate increasing doses of an inactivated vaccine made using one of the H9N2 influenza viruses that infected two children in Hong Kong in 1999. The trial, which started in October 2003, is evaluating the safety and

immunogenicity of two doses of the H9N2 vaccine. Results from this study will provide important information about the dosage level of a vaccine with the novel hemagglutinin H9 that may need to be administered in the event of a pandemic. (Vaccine and Treatment Evaluation Unit (VTEU), Baylor College of Medicine)

- NIAID intramural scientists and their collaborators are using an attenuated live virus strategy to develop pandemic influenza vaccines. The investigators generated a reassortant virus that contains the hemagglutinin and neuraminidase genes from a chicken influenza virus isolated in Hong Kong and six internal gene segments from a live-attenuated influenza virus. A pilot lot of this H9N2 influenza vaccine has been manufactured, and a Phase I clinical trial is planned for 2004.

- NIAID is currently supporting the production of small, pilot lots of investigational inactivated vaccine against the 2004 H5N1 virus. Evaluation of the vaccine in Phase I and Phase II clinical trials is planned for 2004. (Aventis, Strasbourg, France; Chiron Corporation, Emeryville, CA)

- NIAID intramural scientists are working with MedImmune, Inc. to develop candidate live attenuated pandemic influenza vaccines. They have obtained H5N1 influenza viruses from the current avian outbreak in Asia and will use reverse genetics to engineer an H5N1 influenza vaccine strain.

Goal: Expand support for the preclinical development of influenza vaccine candidates including strategies to enhance the immune response.

- In 2003, NIAID expanded its support for preclinical research activities on influenza and other acute respiratory pathogens. These activities include studying viral pathogenesis, developing strategies to optimize the immune response, identifying and validating correlates of protection, and identifying host factors that influence susceptibility to infection. This program also supports Phase I and Phase II clinical trials of candidate vaccines and therapeutics (Viral Respiratory Pathogens Research Unit (VRPRU), Baylor College of Medicine).

- NIAID recently awarded several grants for developing vaccines that induce broadly protective immunity against divergent influenza strains. These projects focus on developing novel influenza vaccines that target conserved viral proteins, like M2 and NP, rather than the highly variable HA protein that is the main target of the currently licensed influenza vaccines. These vaccine candidates have demonstrated efficacy by protecting mice from lethal challenges with influenza virus. The advantage of these types of vaccines is that they would not need yearly updating.

    - Universal Influenza Matrix 2 Subunit vaccine (Molecular Express, Inc., Los Angeles, CA)

- Universal Influenza A vaccine (Apovia, Inc., San Diego, CA)

- M2-based Influenza Type A Virus Vaccine (Wistar Institute, Philadelphia, PA)

- ISS-linked NP vaccine to control pandemic flu outbreak (Dynavax Technologies Corporation, Berkeley, CA)

- In 2003, NIAID awarded a grant for comparison of immune responses to FluMist (live attenuated influenza vaccine), inactivated influenza vaccine, and natural influenza infection in adults and children five to nine years of age. Investigators will study the role of humoral immunity (antibodies), cell-mediated immunity, and innate immunity in the response to influenza infection or vaccination. This study will provide important information about immunological mechanisms of protection, and might lead to the design of better vaccines. (Stanford University)

Goal: Continue to support the development of alternatives to egg-based vaccines, including cell culture-based platforms.

- In 2003, NIAID supported the production of the first trivalent baculovirus-expressed influenza vaccine and its evaluation in a Phase II clinical study. The vaccine was tested for safety and its ability to increase antibody levels in healthy subjects 65 years of age and older. This vaccine is produced in a large-scale tissue-culture system, which is an attractive alternative to the current egg-based manufacturing platform. (Protein Sciences Corporation, Meriden, CT; VTEUs, Baylor College of Medicine and University of Rochester)

- In 2003, NIAID awarded a grant for developing a novel microcarrier-based tissue culture system to grow influenza viruses for vaccine manufacturing. (SoloHill Engineering, Inc., Ann Arbor, MI)

- NIAID is supporting studies for the development and clinical evaluation of DNA-based influenza vaccines as well as a novel delivery method (gene gun). (PowderJect Vaccines, Inc., Madison, WI)

Goal: Expand research to identify host genetic factors that influence susceptibility to influenza disease.

- In 2003, NIAID supported a small clinical study to assess the role of genetic polymorphism in the interleukin-6 promoter in the human response to experimental infection with influenza virus. These studies might help to explain the variety of influenza disease outcomes observed in different individuals. (VTEU, University of Rochester)

- In 2003, NIAID-funded investigators initiated a project to develop an *in vitro* assay to identify genes that regulate immune responses to acute respiratory viruses, including influenza. Validation of the assay technology via testing in influenza vaccine studies is planned. (VRPRU, Baylor College of Medicine)

Multi-Drug Resistant Tuberculosis

Multi-drug resistant tuberculosis (MDR-TB) is an emerging public health threat. *Mycobacterium tuberculosis* (Mtb), the causative agent of TB, is spread from person to person by airborne droplets expelled from the lungs when a person with TB coughs, sneezes, or speaks. Outbreaks may therefore occur in closed settings and under crowded living conditions such as homeless shelters and prisons.

It is estimated that one-third of the world's population (1.86 billion people) is infected with Mtb, and 16.2 million people have TB disease. In 1995, the year with the highest TB casualty rate to date, nearly 3 million people worldwide died from the disease. While MDR-TB currently represents a small percentage of all U.S. TB cases, large regional clusters of MDR-TB cases exist globally, with the potential to spread widely.

Identification of both drug-sensitive and drug-resistant Mtb is time-consuming and not easily implemented in resource-poor settings. Since treatment of MDR-TB requires more expensive and less well-tolerated second-line antibiotics for up to two years, an outbreak of MDR-TB would place immense strain on the public health infrastructure of even medically advanced countries. Estimates of the average cost of medical care for a single patient with MDR-TB in the United States can be as high as $180,000. Epidemics of MDR-TB would likely result in casualty rates similar to those seen when TB is not treated.

Scientific Progress

Since publication of the *NIAID Biodefense Research Agenda for Category B and C Priority Pathogens* in January 2003, important progress has been made in basic microbe biology, understanding the immune response to TB infection, and evaluating vaccines and therapeutics.

Understanding the immune response to *M. tuberculosis* helps scientists design vaccine candidates. A detailed understanding of the immune response against TB is crucial for designing optimized vaccine candidates as well as appreciating all aspects of pathogenesis in the various forms of TB disease. Research in this area has been hampered by a lack of suitable animal models that faithfully replicate disease analogous to humans. A number of recent scientific advances by NIAID-supported investigators may have a positive impact on vaccine development for TB.

- A cynomolgus macaque model has recently been developed that appears to faithfully replicate most aspects of human TB disease. This model has proven valuable for dissecting the molecular details of granuloma biology during Mtb infection, and it is being used to evaluate advanced vaccine candidates. It also has the potential to allow real-time, non-invasive monitoring of development and resolution of TB disease.
  (Capuano SV 3rd et al., Experimental *Mycobacterium tuberculosis* infection of cynomolgus macaques closely resembles the various manifestations of human *M. tuberculosis* infection, *Infect Immun* 2003;71:5831-5844; Fuller CL et al., In situ study of abundant expression of proinflammatory chemokines and cytokines in pulmonary granulomas that develop in cynomolgus macaques experimentally infected with *Mycobacterium tuberculosis*, *Infect Immun* 2003;71:7023-7034)

- Remarkable progress has been made in the study of CD1 antigen presentation in TB. CD1 molecules present glycolipids, which represent unique antigenic determinants on mycobacteria, to the host immune system. CD1 molecules straddle the divide between the innate immune response and the more classic Major Histocompatibility Complex (MHC) class I and II molecules. In addition, differences in the distribution of CD1 molecules among host species may explain differences in immune responses among various animal models of human disease.
  (Roura-Mir C and Moody DB, Sorting out self and microbial lipid antigens for CD1, *Microbes Infect* 2003;12:1137-1148)

- CD1 responses comprise a significant component of the immunological memory to TB. In addition, individuals with active disease have reduced levels of TB-specific CD1 T cells. Levels increase, however, following initiation of TB therapy, suggesting that maintaining an active CD1 response could be a critical component in the development of a vaccine for TB.
  (Ulrichs T et al., T cell responses to CD1-presented lipid antigens in humans with *Mycobacterium tuberculosis* infection, *Infect Immun* 2003;71:3076-3087)

- In an animal model of TB, vaccination with mycobacterial glycolipid resulted in protection against experimental TB disease that is qualitatively similar to that seen with Bacille Calmette-Guerin (BCG) vaccination. This suggests that vaccines containing glycolipids have the potential to elicit CD1 specific protection and long lasting immunity.
  (Dascher CC et al., Immunization with a mycobacterial lipid vaccine improves pulmonary pathology in the guinea pig model of tuberculosis, *Int Immunol* 2003;15:915-925)

- Currently, there is limited understanding of the human response to vaccination with BCG, the most widely distributed vaccine in the world. Investigators have now initiated more detailed studies of the human immune response to BCG vaccination to further guide development and clinical evaluation of new vaccines.
  (VTEU, St. Louis, MO)

Three new TB vaccines hold promise for more effective alternatives to the traditional BCG vaccine. The major goal of TB research is an improved vaccine that protects against adult pulmonary disease. The current BCG vaccine does not offer this

protection although it does reduce some complications of pediatric TB. Encouragingly, the past year has seen the introduction of three different candidate vaccines for evaluation in humans.

- Over the past five years, NIAID has supported the advanced development of adjuvanted Mtb72f (Corixa Corporation, Seattle, WA/GlaxoSmithKline Biologics (GSK), Research Triangle Park, NC) and rBGC30 (University of California, Los Angeles). Both of these candidates recently received FDA approval for Phase I human trials to be initiated in 2004. The preclinical development of Mtb72f was supported by a NIAID challenge grant highlighting the value of government/private partnerships.
  (Horwitz MA and Harth G, A new vaccine against tuberculosis affords greater survival after challenge than the current vaccine in the guinea pig model of pulmonary tuberculosis, *Infect Immun* 2003;71:1672-1679)

- Although not supported by NIAID, a third TB vaccine candidate, MVA85A, is undergoing initial Phase I testing in Europe.
  (Goonetilleke NP et al., Enhanced immunogenicity and protective efficacy against *Mycobacterium tuberculosis* of Bacille Calmette-Guerin vaccine using mucosal administration and boosting with a recombinant modified vaccinia virus Ankara, *J Immunol* 2003;171:1602-1609)

Comparing sequences of mycobacterial genomes reveals unique drug targets and antigens for candidate vaccines. To date, five mycobacterial genomes have been fully sequenced and annotated. With six additional mycobacterial species undergoing sequencing, the potential to identify and reveal unique drug targets as well as candidate antigens for vaccine design will be realized.

- NIAID is supporting genotyping of Mtb strains in endemic countries and border areas of the United States. To refine models of disease transmission, this has now been expanded to include MDR-TB strains. Investigators have recently completed the genotypic analysis of MDR-TB isolates from Mexico. This study indicated that drug resistance may not be predictable by strain identification alone but may require more detailed genetic analysis.
  (Ramaswamy SV et al., Genotypic analysis of multidrug-resistant *Mycobacterium tuberculosis* isolates from Monterrey, Mexico, *J Med Micro* 2004;53:107-113)

- The sequencing of M. *bovis*, a close relative of Mtb and the progenitor of the BCG vaccine, was completed in 2003 with support from European funding agencies. This has provided insight into the genetic basis for attenuation of a potential human pathogen, *M. bovis,* resulting in a non-virulent, but immunogenic vaccine strain. Genomic analysis will permit identification of relevant features of a vaccine candidate that will have to be retained to elicit human immune responses, as well as features that need to be eliminated to render a vaccine candidate safe.
  (Garnier T et al., The complete genome sequence of *Mycobacterium bovis*, *Proc Natl Acad Sci U S A* 2003;100:7877-7882)

Gene identified that may contribute to drug resistance in tuberculosis.
Understanding the mechanisms by which Mtb develops drug resistance may help in the identification of new targets for therapeutic intervention.

- NIAID intramural scientists have identified an Mtb gene crucial for both the survival of Mtb in the laboratory as well as virulence of the organism in mice. This gene (dnaE2) can induce genetic mutations that allow the organism to adapt to the host environment and may contribute directly to the emergence of drug resistance *in vivo*.
  (Boshoff HI et al., DnaE2 polymerase contributes to *in vivo* survival and the emergence of drug resistance in *Mycobacterium tuberculosis*, *Cell* 2003;113:183-193)

Advances in TB drug development may lead to improved treatment regimens.

- The *Global Alliance for TB Drug Development* has licensed PA-824 from Chiron Corp. (Emeryville, CA) and is preparing to seek regulatory approval for Phase I clinical testing later this year. The preclinical development of this candidate is being supported in part by NIAID.

- NIAID has supported the development of a gamma interferon-knockout mouse aerosol infection model for rapid screening of drug candidates against MDR-TB and drug-sensitive Mtb. This model integrates evaluation of microbiological activity and drug levels, and allows for rapid activity assessment of lead molecules against Mtb strains grown in the host.
  (Lenaerts AJM et al., Rapid *in vivo* screening of experimental drugs for tuberculosis using gamma interferon gene-disrupted mice. *Antimicrobial Agents and Chemotherapy 2003*;47:783-785)

Clinical trials likely to determine potential role for fluoroquinolones in TB treatment. Fluoroquinolones have been advocated for treatment of MDR-TB where first-line therapy has failed. However, there have been little or no clinical data to guide their rational introduction into treatment regimens. In addition, limited concordance between *in vitro* and *in vivo* efficacy, as well as limitations of existing animal models, have hampered selection of the optimal fluoroquinolone candidate and optimal dosing regimen. A recent clinical trial comparing two fluoroquinolones showed that levofloxacin was superior to ofloxacin for treatment of MDR-TB. Additional trials are now under way at NIAID's Tuberculosis Research Unit (TBRU). (Case Western Reserve University)

Programmatic Progress in Addressing Immediate Goals

Goal: Exploit genomic and proteomic information to identify new targets for vaccine, drug, and diagnostics development.

- NIAID is supporting the sequencing of two additional mycobacterial genomes, *Mycobacterium avium* and *Mycobacterium smegmatis*. By the end of 2004, at least eight full genomic sequences will be available in the public domain for mycobacterial species. Availability of sequences from multiple mycobacterial species will allow refinement of model systems and serve as the prerequisite for

using comparative genomics and comparative biology to identify intervention targets unique to Mtb. (The Institute for Genomic Research (TIGR), Rockville, MD)

- Mtb microarrays, used to understand the genetic basis of microbial physiology, are being made available to the worldwide research community through NIAID's Pathogen Functional Genomics Resource Center and the Tuberculosis Research Materials and Vaccine Testing contract. (TIGR, Rockville, MD; Colorado State University)

- NIAID-supported researchers in the TB Structural Genomics Consortium are determining three-dimensional structures of more than 40 Mtb proteins. These structures are especially useful in the computational modeling process used in drug design. The TB Structural Genomics Consortium is co-funded with the National Institute of General Medical Sciences and, at the beginning of fiscal year 2004, consisted of scientists from 65 institutions in 14 countries. (Los Alamos National Laboratories)

- NIAID has expanded the capacity to screen potential drug candidates for Mtb in both *in vitro* assays and in animal models. In addition, these screening tools have been enhanced by state-of-the-art robotics and automated facilities for high-throughput screening for promising drug targets. (Colorado State University; Southern Research Institute, Birmingham, AL)

- NIAID is supporting a public/private partnership to study a new class of drugs (beta-sulfonylcarboxamides) with activity against MDR-TB. Investigators are exploring the mechanism of action of this class of chemicals and its potential to be integrated into drug regimens currently used to treat MDR-TB. (Johns Hopkins University; FASgen, Inc., Baltimore, MD)

Goal: Develop and standardize animal models that better predict vaccine and drug efficacy in humans.

- NIAID is supporting studies to determine the biological function of important Mtb gene products in various animal hosts. These results will yield potential targets for future drug and vaccine development efforts. In addition, as part of these studies, scientific tools will be developed to expedite and refine translational research activities that will be made available to the broader TB research community. (Johns Hopkins University)

- NIAID is planning a new resource to determine pharmacological correlates for anti-TB drug efficacy. This resource will impact future drug development efforts in terms of streamlining human clinical drug trials.

Goal: Develop faster, more robust microbiological and serological diagnostics for drug-sensitive Mtb and MDR-TB.

- NIAID recently awarded several grants for the development of novel, rapid diagnostics for MDR-TB as well as drug-sensitive Mtb. Some of the technologies may also have the potential to help define and track human responses to therapy and vaccination. Examples include:

    - A novel breath test to detect volatile organic compounds providing a signature of active TB and possibly Mtb infection. (Menssana Research, Inc., Fort Lee, NJ)

    - A novel cartridge design to facilitate concentration and preparation of clinical specimen samples from smear-negative TB patients for detection and resistance determination by polymerase chain reaction. (University of Medicine and Dentistry of New Jersey; Cepheid, Sunnyvale, CA)

    - Miniarray technology for rapid detection of mycobacteria and discrimination of mycobacterial species. (Weilin Biotechnology, Inc., Norcross, GA)

    - Detection of viable, drug-resistant Mtb via bioluminescent phage. (Sequella, Incorporated, Rockville, MD)

Goal: Increase capacity for testing vaccine candidates in standardized animal models.

- In fiscal year 2003, NIAID expanded its Tuberculosis Research Materials and Vaccine Testing program to provide increased capacity for testing additional promising vaccine candidates and vaccine/adjuvant combinations. This resource played a significant role in preclinical development of the Corixa Corporation/GSK vaccine candidate, which is scheduled to enter Phase I clinical trials in 2004. (Colorado State University) (See listing under Scientific Progress section.)

Goal: Expand the infrastructure for conducting clinical trials for therapeutics and vaccines, including education and training of personnel in high-burden countries.

- NIAID has expanded the TBRU via the addition of sites in Manila, Philippines, and Cape Town, South Africa. Expansion to Manila is expected to expedite an ongoing clinical study to evaluate the use of surrogate markers in patients who respond well to therapy. At the Cape Town site, the response of very young children to BCG vaccination will be examined in the context of HIV infection. (Case Western Reserve University)

- NIAID is supporting a clinical research study in Uganda to address the question of whether immediate treatment of HIV infection with a punctuated course of antiretroviral drugs will improve the outcomes for patients with active tuberculosis. Tuberculosis relapses will be evaluated to detect treatment failures and associated strains will be genotyped for patterns of MDR-TB. (Case Western Reserve University; University of California, San Francisco)

- NIAID intramural scientists established a collaboration with colleagues from Yonsei University and Masan National Tuberculosis Hospital in Busan, South Korea, for the study of MDR-TB in South Korea. The collaborators will study the basic biology underlying the development of drug resistance and evaluate novel anti-TB agents. Clinical studies will be conducted at Masan Hospital, the referral center for TB treatment failures in South Korea, with the largest population of inpatient MDR-TB victims anywhere in the world.

Immunity and Biodefense

Both the innate and adaptive immune systems play critical roles in protecting against pathogens, and direct manipulation of the immune system can help protect individuals against infection by bioterrorist threat agents. Early innate responses normally serve as a first line of defense to limit infection; these responses have the potential to be used therapeutically to provide temporary protection early after pathogen exposure. Longer lasting protection can be provided by vaccines that target the adaptive immune system to generate pathogen-specific neutralizing antibodies or T cells that can eliminate or contain infection. The potency of specific vaccines can be increased by developing new adjuvants targeted to innate immune system components that work in conjunction with T cells and B cells to induce stronger or more appropriate responses, and generate more robust memory T and B cells. Therefore, defense against bioterrorism includes developing generalizable immune-based strategies to combat Category B and C priority pathogens.

Scientific Progress

Variant toll-like receptors (TLRs) associated with defective immunity to bacterial infection. Recent results identified specific TLRs as novel targets for bacterial vaccine development. Researchers supported by National Institute of Allergy and Infectious Diseases (NIAID) have linked polymorphisms in a specific TLR gene to defective responses to *Mycobacterium leprae* and *Mycobacterium tuberculosis*, and also found that defects in another TLR gene abolish signaling from flagellin and increase human susceptibility to *Legionella pneumoniae.*

In a related finding, only pyogenic and not other types of bacteria were found to infect children with deficiencies in expression of a protein that transmits signals from some TLRs, demonstrating that certain classes of bacteria are controlled by definable subsets of TLRs.

(Bochud P et al., A Toll-like receptor 2 polymorphism that is associated with lepromatous leprosy is unable to mediate mycobacterial signaling, *J Immunol* 2003;170:3451-3454; Hawn TR et al., A common dominant TLR5 stop codon polymorphism abolishes flagellin signaling and is associated with susceptibility to Legionnaires' disease, *J Exp Med* 2003;198:1563-1572; Picard C et al., Pyogenic bacterial infections in humans with IRAK-4 deficiency, *Science* 2003;299:2076-2079)

New TLR signaling pathway identified

Many TLRs are known to transmit signals into cells by means of a protein called MyD88, in order to activate the cells for specific responses. In addition, MyD88-independent signaling pathways were recently discovered, and NIAID-funded researchers identified a specific gene, called LPS2. LPS2 is important for MyD88-independent signaling, and therefore for activation of cells that bind bacteria and viruses via TLRs on their cell surfaces. These findings provide novel targets for drug development to treat bacterial or viral infection.

(Hoebe K et al., Identification of LPS2 as a key transducer of MyD88-independent TIR signaling, *Nature* 2003;424:743-748)

Different outcomes yielded by different types of DNA vaccination. Findings by NIAID-supported researchers indicate that the magnitude and type of immune response to vaccination can be manipulated by altering the cellular location of the antigen and the route of DNA immunization. Gene gun immunization of DNA vectors that produce cytoplasmic- or transmembrane-expressed antigens induce strong cytotoxic T-cell responses as well as antibody production. In contrast, vectors that produce secreted proteins do not induce cytotoxic T cells, although the antibody response is seen. Intramuscular injection of the vectors produces a somewhat different profile of responses. Because some pathogens are best controlled by antibodies, and some by T-cell mediated responses, these discoveries will aid in designing more effective vaccines for specific pathogens.
(Morel PA et al., DNA immunisation: altering the cellular localisation of expressed protein and the immunization route allows manipulation of the immune response, *Vaccine* 2004;22:447-456)

Memory cytotoxic T cells as key mediators of immune protection against viral infections. The induction of cytotoxic T cells by virus infection results in the generation of both effector and memory cytotoxic T cells. The memory cells induced by vaccination provide protection against subsequent exposure to that virus. NIAID-supported investigators recently discovered that production of memory cytotoxic T-cells is regulated by the IL-15 cytokine. These results suggest that manipulation of IL-15 levels by new vaccine formulations may facilitate the generation of more robust immunization strategies to protect against Category B and C priority pathogens.
(Schluns KS et al., Transregulation of memory CD8 T-cell proliferation by IL15Ralpha^{+} bone marrow-derived cells, *Blood* 2004;103:988-994)

Peptide-Major Histocompatibility Complex (MHC) microarray for detection of antigen-specific T cells advanced. Recent results from NIAID-funded investigators provide a major advance in technology to detect, track, and perform functional analyses of antigen-specific T cells generated after infection, or persisting as memory cells. This has the potential to greatly advance the development of vaccines and immunotherapeutics to combat infectious disease.

Microarrays of MHC-peptide pairs were found to capture specific T cells that recognize only the relevant MHC-peptide configurations. Rare T cells, generated only after immunization, were successfully detected in an *in vivo* test of the technique. This sensitive and specific approach will be useful for epitope discovery as well as to follow T-cell responses during and after infection or vaccination.
(Soen Y et al., Detection and characterization of cellular immune responses using peptide-MHC microarrays, *PLoS Biology* 2003;1:429-438)

Programmatic Accomplishments

- In fiscal year 2003, NIAID awarded five Cooperative Centers for Translational Research on Human Immunology and Biodefense to support basic, clinical, and applied research on human immune responses to Category A-C priority pathogens or their products. (Emory University, Stanford University, Baylor Research Institute, University of

Massachusetts, Dana-Farber Cancer Institute). Each center includes a large component that focuses on developing and applying new assays to facilitate the study of human immune responses. Additional centers are planned for fiscal year 2004.

- NIAID is supporting the development of new adjuvants with three contracts awarded under the program entitled Innate Immune Receptors and Adjuvant Discovery (Montana State University; NovaScreen, Inc., Hanover, MD; Corixa Corporation, Seattle, WA). It is expected that promising candidates for new vaccine adjuvants and immunotherapies will be developed through these programs. NIAID will award additional contracts under this program in fiscal year 2004.

- Within the Large Scale Discovery of Antibody and T-Cell Epitopes program, NIAID supports the comprehensive identification of epitopes for Category A-C priority pathogens, and development of new methods to predict epitopes, as the basis for new vaccine development. (La Jolla Institute for Allergy and Immunology, San Diego, CA; University of Copenhagen; The Scripps Research Institute, La Jolla, CA; The University of Oklahoma Health Sciences Center; Benaroya Research Institute at Virginia Mason, Seattle, WA)

- NIAID has awarded an Immune Epitope Database and Analysis Program contract to design, develop, populate, and maintain a publicly accessible and comprehensive database containing antibody and T-cell epitopes for Category A-C priority pathogens and their products. (La Jolla Institute for Allergy and Immunology, San Diego, CA)

- The National Institutes of Health (NIH) Tetramer Facility was expanded to produce MHC-peptide tetramer reagents specific for T cells recognizing Category A-C priority pathogen antigens. (Emory University)

- In June 2003, NIAID convened an expert panel on Antiviral Innate Immunity: Recognition, Defenses, Evasion, and Biodefense Strategies. The goal was to identify research needs in the areas of innate immune activation by viruses, viral evasion mechanisms, and antiviral therapies, in order to establish the basis for future research programs in these areas.

- In June 2003, NIAID convened a workshop on Mathematical Models of Immunity: Extrapolation to Human Responses to Emerging Infectious Diseases. Experts in both modeling and immunology explored mechanisms by which interdisciplinary research in this area could advance the development of new vaccines and immunotherapeutic approaches to biodefense.

- NIAID staff participated in a trans-NIH workshop, Imaging Technology and the Study of Immune Function, in April 2003. Participants presented recent progress in imaging techniques as applied to immune responses, and identified opportunities for application to human immunity research.

Additional Biodefense Considerations

At the meeting of the expert panel, it was recognized that a number of issues warranted additional discussion with other agencies and/or the scientific community. The issues discussed by the panel included recommendations for additions, deletions, and changes to the National Institute of Allergy and Infectious Diseases (NIAID) lists of Category A-C priority pathogens, the role of industry in the biodefense research agenda, and the consequences of genetically modified organisms. Progress in these areas is outlined below.

Recommendations on the NIAID Priority Pathogens List

The list of NIAID Category A, B, and C Priority Pathogens (Appendix 1) closely follows the Centers for Disease Control and Prevention (CDC) Biological Diseases/Agents List (Appendix 2). The NIAID list, however, highlights specific pathogens identified as priorities for additional research efforts as part of the NIAID Biodefense Research Agendas. During the panel's deliberations, a number of specific recommendations related to the NIAID priority pathogens list were made. Since that time, other agents have arisen that should be considered for inclusion, such as the severe acute respiratory syndrome (SARS) coronavirus.

Progress

NIAID and CDC are working together to address recommendations for modifications to the NIAID lists of Category A-C agents/priority pathogens and will use the CDC Critical Agents Evaluation Process ─ the agency's formal process for reviewing priority pathogens ─ to determine appropriate categorization. As part of this process, a meeting of scientific experts is planned for summer 2004 at which recommendations for modifications, additions, or deletions will be evaluated.

Role of Industry in the Biodefense Research Agenda

Over the last 10 years, the number of companies actively involved in developing antimicrobials or vaccines has decreased significantly. The panel expressed concern that while many small companies are conducting important and innovative research, some may have difficulty carrying a candidate through product development to licensure. Thus, the panel recommended the following:

- NIAID should work with industry representatives to develop a new paradigm for collaborations between government and industry, including the need for a clear statement of the highest-priority products and identifying ways to assist industry in developing these products for use.

- Interactions between industry and the U.S. Food and Drug Administration (FDA) should be increased early in the development process.

- Industry should make available existing chemical libraries for screening against biodefense pathogens.

- There should be a coordinated effort to test existing therapies for new indications related to biodefense.

Progress

In September 2000, NIAID convened the Summit on Development of Infectious Disease Therapeutics. The goal of this meeting with industry leaders was to discuss barriers to NIAID-industry collaborations and how to overcome them. Major themes echoed those of similar meetings and included the need for greater flexibility, speed, and certainty in funding, and the need for government to guarantee purchase of products if no market exists. Recommendations from the meeting helped shape a number of NIAID biodefense initiatives under way today, including:

- Biodefense Partnerships: Vaccines, Adjuvants, Therapeutics, Diagnostics, and Resources Program
- Challenge Grants: Biodefense Product Development
- Cooperative Research for the Development of Vaccines, Adjuvants, Therapeutics, Immunotherapeutics, and Diagnostics for Biodefense
- Small Business Biodefense Program

These initiatives have provided mechanisms for funding biodefense product development in areas where there might not otherwise be incentives for industry or academia.

NIAID will sponsor a second summit with representatives from industry in the summer of 2004 to determine how the Institute can most effectively assist pharmaceutical and biotechnology companies in bringing to market efficacious new therapeutics for emerging, re-emerging, and drug-resistant infections, including potential agents of bioterrorism. The summit will focus on the product development pathway, industry business models, relevant NIAID programs, and an examination of the factors affecting product development that are outside NIAID and industry control.

In his 2003 State of the Union Address, President Bush announced Project BioShield ─ a comprehensive effort to develop and make available modern, effective drugs and vaccines to protect against attack by biological and chemical weapons or other dangerous pathogens. Included among the provisions of Project BioShield is the creation of a permanent funding authority to ensure that resources are available to pay for, and thereby to encourage development of, new medical countermeasures. This authority will enable the government to purchase vaccines and other therapies as soon as experts believe that

they can be made safe and effective, thereby providing an incentive for the private sector to develop countermeasures.

Together, United States Army Medical Research Institute of Infectious Diseases and NIAID are coordinating testing in small animal models and non-human primates of a series of licensed antibiotics as therapies for inhalational anthrax, pneumonic plague, and tularemia. NIAID staff members also participate in several working groups of the Weapons of Mass Destruction Medical Countermeasures Policy Group, including the Existing Antimicrobials Working Group, at which this research is discussed and coordinated.

Genetically Modified Organisms

Since the early 1970s, when scientists discovered how to transfer genetic elements from one organism into another, there has been concern that this technology could be used to create new bioweapons. Many virulent factors have their origins in bacteriophages, and they are easily amenable to genetic manipulation. Research, particularly in genomics and immunology, has created a wealth of new knowledge that could be used to produce organisms that have enhanced pathogenicity, infectivity, and transmissibility. The diseases caused by these modified organisms might initially be difficult to diagnose, and they may resist treatment with current antimicrobials. Additionally, currently available vaccines could be rendered ineffective.

Recently, Australian scientists inadvertently created a new virus that had significantly increased virulence for mice by splicing a gene for interleukin-4 into mousepox virus. Addition of the IL-4 gene apparently suppressed the normal immunological response against the mousepox virus infection, and the bio-engineered poxvirus was able to evade vaccine-induced protection.

These findings were recently confirmed and extended at Saint Louis University under NIAID's animal model testing program. Cidofovir, the only available drug thought to be effective against smallpox, did not protect mice against challenge with the IL-4 mousepox virus. In these experiments, neither vaccination nor Cidofovir were effective.

Although these scientists were using a mouse virus, it may be possible to similarly engineer human viruses or other microorganisms, creating new or modified organisms with enhanced pathogenicity, infectivity, and transmissibility. The panel recommended several areas of research that might help counteract these organisms.

- Develop diagnostics for rapid detection of antimicrobial susceptibility/resistance.

- Develop robust genomic tools to detect genetically modified organisms and the presence of virulence factors associated with bacteriophage.

- Initiate and/or complete genomic sequencing of virulent bacteriophages to identify virulence factors and new drug targets.

- Develop multiple and combination approaches to counter effects of genetic modifications that enhance pathogenicity, infectivity, and transmissibility.

Progress

To counter the potential threat posed by genetic modification of microorganisms, rapid diagnostics need to be developed to detect, characterize, and quantify infectious agents. A number of NIAID's biodefense initiatives specifically target the development of emerging genomic and non-genomic technologies that are suitable for developing the next generation of medical diagnostics to detect infections and the presence of infectious agents. NIAID is also supporting development of high-density comparative microarray technology to identify polymorphic loci that may be useful for rapid and specific strain identification and for developing diagnostic signatures. This technology may pave the way for using new platforms for medical diagnostics.

NIAID has made, and continues to make, a significant investment in its genomics program (see Research Resources section under General Recommendations, page 11). This robust and continually evolving program, includes:

- **Microbial Sequencing Centers** at which the genomes of microorganisms considered agents of bioterrorism as well as several relevant invertebrate vectors are being sequenced.

- **Proteomics Research Centers** under which innovative protein technologies and methodologies will be developed and enhanced for application to understanding important proteins of the host and pathogen, including virulence factors.

- The **Pathogen Functional Genomics Resource Center** that is undertaking genomic analysis of selected human pathogens including an examination of polymorphisms for identifying genetic variations and relatedness within and between species.

- The **Influenza Genomics Program** under which the DNA of various influenza virus isolates will be sequenced and the information generated will be released into the public domain for use by researchers.

- **Population Genetics Analysis Programs** that will address genetic polymorphisms in immune responses against infection with selected human pathogens as well as vaccination.

The data produced under these programs will be applied to gaining a more complete understanding of the biology of each pathogen, its ability to cause disease, and new strategies for prevention and treatment. Key to full use of these data are the Bioinformatics Resource Centers for Biodefense and Emerging/Re-emerging Infectious Diseases that will make available to researchers genomic, functional genomic, structural, and related data about emerging and re-emerging pathogens, including those that are genetically modified, through organized, integrated, relational databases that can be queried.

Safe and effective approaches for modulating the innate immune system to induce broad protection against biological pathogens are also needed. NIAID continues to encourage research that takes advantage of the availability of microbial and human genome sequence data and examines functional analyses of gene and protein expression in whole microbial genomes. Microbial genomics will be used together with the human genome sequence to better understand the host immune response and individual genetic susceptibility to pathogens.

NIAID is pursuing the development of novel therapeutic and vaccine strategies that, in concert with new rapid genomic sequencing technologies, could be used to quickly develop drugs and vaccines against new infectious threats.

To develop new therapeutics that would be effective against multiple classes of pathogens, including genetically modified organisms and unknown pathogens, NIAID is supporting efforts by the National Academy of Sciences to bring together experts from industry and academia in an interdisciplinary forum. This will provide an opportunity for scientists to discover new pathways for developing broader spectrum antibiotics, with the ultimate goal of developing a universal antibiotic that could be used to treat a variety of infectious diseases.

NIAID is expanding existing research infrastructure, including the construction of biosafety/biocontainment laboratories throughout the United States as well as development of important research tools, to support a research response that will be quick, safe, and effective.

List of Abbreviations

AFRIMS - Armed Forces Research Institute of Medical Sciences
BCG - Bacille Calmette-Guerin
BSL - Biosafety Laboratory
CASG - Collaborative Antiviral Study Group
CCHF - Crimean-Congo Hemorrhagic Fever
CDC – Centers for Disease Control and Prevention
cGMP – Current Good Manufacturing Practice
CRU – Clinical Research Unit
DoD - Department of Defense
EEE - Eastern Equine Encephalitis
Epi - Epinephrine
FDA – U.S. Food and Drug Administration
FWDIRN – Food- and Waterborne Diseases Integrated Research Network
GLP – Good Laboratory Practice
HD5 – Human Defensin 5
IRU – Immunology Research Unit
JE - Japanese Encephalitis
LAC - La Crosse
LPS - Lipopolysaccharide
MDR-TB - Multi-Drug Resistant Tuberculosis
MHC – Major Histocompatibility Complex
MRU – Microbiology Research Unit
Mtb – *Mycobacterium tuberculosis*
NBL - National Biocontainment Laboratory
NIAID - National Institute of Allergy and Infectious Diseases
NIH – National Institutes of Health
PCR - Polymerase Chain Reaction
PFGRC - Pathogen Functional Genomics Resource Center
RBL - Regional Biocontainment Laboratory
RCE – Regional Center of Excellence for Biodefense and Emerging Infectious Diseases
SARS – Severe Acute Respiratory Syndrome
SEB - Staphylococcal Enterotoxin B
STEC - Shiga Toxin-Producing *Escherichia coli*
TB - Tuberculosis
TBE - Tick-Borne Encephalitis
TBRU - Tuberculosis Research Unit
TIGR - The Institute for Genome Research
TLR - Toll-Like Receptor
VRC – Vaccine Research Center
VEE - Venezuelan Equine Encephalitis
VRPRU - Viral Respiratory Pathogens Research Unit
VTEU - Vaccine Treatment and Evaluation Unit
USAMRIID - United States Army Medical Research Institute of Infectious Diseases
UTMB - University of Texas Medical Branch-Galveston
WEE - Western Equine Encephalitis
WNV - West Nile Virus
YF - Yellow Fever

Related Web Sites

American Society for Microbiology
www.asm.org
Biodefense and Emerging Infections Research Resources Repository
www.beiresources.org

Global Alliance for TB Drug Development
www.tballiance.org

National Biocontainment Laboratories (NBLs) and Regional Biocontainment Laboratories (RBLs)
www.niaid.nih.gov/newsroom/releases/nblscorrect21.htm

NIAID Biodefense Research Agenda for Category B and C Priority Pathogens
www.niaid.nih.gov/biodefense/research/categorybandc.pdf

NIAID Biodefense Research Agenda for CDC Category A Agents
www.niaid.nih.gov/biodefense/research/biotresearchagenda.pdf

NIAID Biodefense Research Agenda for CDC Category A Agents: Progress Report
www.niaid.nih.gov/biodefense/research/category_A_Progress_Report.pdf

NIAID Biodefense Web Site
biodefense.niaid.nih.gov

NIAID Strategic Plan for Biodefense Research
www.niaid.nih.gov/biodefense/research/strategic.pdf

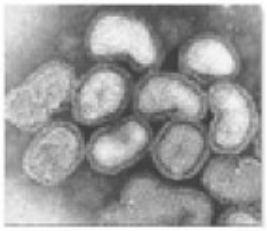
Pathogen Functional Genomics Resource Center (PFGRC)
www.niaid.nih.gov/dmid/genomes/pfgrc/default.htm

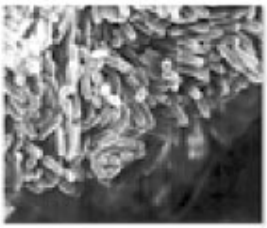
Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases
www.niaid.nih.gov/biodefense/research/rce.htm

Summary of the NIAID Expert Panel on Immunity and Biodefense (June 17, 2002)
www.niaid.nih.gov/publications/pdf/biodimmunpan.pdf

WHO Training Course on Animal Influenza Diagnosis and Surveillance
www.who.int/csr/disease/influenza/chinatraining/en/print.html

A P P E N D I X 1





NIAID CATEGORY A, B, AND C PRIORITY PATHOGENS

NIAID Category A, B, and C Priority Pathogens

Category A

Bacillus anthracis (anthrax)
Clostridium botulinum (botulism)
Yersinia pestis (plague)
Variola major (smallpox) and other pox viruses
Francisella tularensis (tularemia)
Viral hemorrhagic fevers
- Arenaviruses
  - LCM, Junin virus, Machupo virus, Guanarito virus
  - Lassa Fever
- Bunyaviruses
  - Hantaviruses
  - Rift Valley Fever
- Flaviviruses
  - Dengue
- Filoviruses
  - Ebola
  - Marburg

Category B

Burkholderia pseudomallei (melioidosis)
Coxiella burnetii (Q fever)
Brucella species (brucellosis)
Burkholderia mallei (glanders)
Ricin toxin (from *Ricinus communis*)
Epsilon toxin (of *Clostridium perfringens*)
Staphylococcal enterotoxin B
Typhus fever (*Rickettsia prowazekii*)
Food- and Water-borne Pathogens
- Bacteria
  - Diarrheagenic Escherichia coli
  - Pathogenic Vibrios
  - Shigella species
  - Salmonella species
  - Listeria monocytogenes
  - Campylobacter jejuni
  - Yersinia enterocolitica
- Viruses
  - Caliciviruses
  - Hepatitis A

Protozoa

- *Cryptosporidium parvum*
- *Cyclospora cayatenensis*
- *Giardia lamblia*
- *Entamoeba histolytica*
- *Toxoplasma*
- *Microsporidia*

Additional viral encephalitides

- West Nile virus
- LaCrosse
- California encephalitis
- Venezuelan equine encephalitis
- Eastern equine encephalitis
- Western equine encephalitis
- Japanese encephalitis virus
- Kyasanur forest virus

Category C

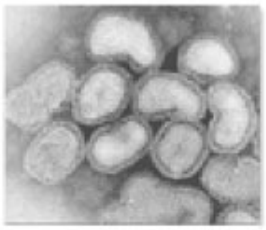
Emerging infectious disease threats such as Nipah virus and additional hantaviruses.

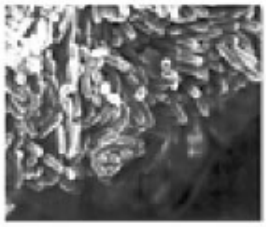
Tickborne hemorrhagic fever viruses

- Crimean Congo Hemorrhagic fever virus

Tickborne encephalitis viruses
Yellow fever
Multi-drug resistant TB
Influenza
Other Rickettsias
Rabies

A P P E N D I X 2





CDC BIOLOGICAL DISEASES/AGENTS LIST

CDC Biological Diseases/Agents List

Category A

Anthrax (*Bacillus anthracis*)
Botulism (*Clostridium botulinum* toxin)
Plague (*Yersinia pestis*)
Smallpox (*Variola major*)
Tularemia (*Francisella tularensis*)
Viral hemorrhagic fevers (filoviruses [e.g., Ebola, Marburg] and arenaviruses [e.g., Lassa, Machupo])

Category B

Brucellosis (*Brucella* species)
Epsilon toxin (of *Clostridium perfringens*)
Food safety threats (e.g., *Salmonella* species, *Escherichia coli* O157:H7, *Shigella*)
Glanders (*Burkholderia mallei*)
Melioidosis (*Burkholderia pseudomallei*)
Psittacosis (*Chlamydia psittaci*)
Q fever (*Coxiella burnetii*)
Ricin toxin from *Ricinus communis* (castor beans)
Staphylococcal enterotoxin B
Typhus fever (*Rickettsia prowazekii*)
Viral encephalitis (alphaviruses [e.g., Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis])
Water safety threats (e.g., *Vibrio cholerae*, *Cryptosporidium parvum*)

Category C

Emerging infectious disease threats such as Nipah virus and hantavirus.

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

National Institute of Allergy and Infectious Diseases

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http://biodefense.niaid.nih.gov