NIAID BIODEFENSE
Preparing Through Research

NIAID Biodefense Research Agenda for CDC Category A Agents

2006 Progress Report

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

National Institute of Allergy and Infectious Diseases Biodefense Research Agenda for CDC Category A Agents

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Introduction

In 2002, the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH), convened a Blue Ribbon Panel on Bioterrorism and Its Implications for Biomedical Research. This panel of experts came together to provide guidance on the Institute's biodefense research agenda, which was published soon afterward. The panel included researchers from academia, industry, government, civilian agencies, and the military.

In 2003, NIAID released its first progress report on accomplishments toward the goals outlined in the Research Agenda. Since that time, extraordinary progress has been made to advance scientific knowledge of these potentially deadly pathogens. To demonstrate the enormity of the research efforts conducted over the last several years, the 2006 progress report details many examples of scientific accomplishments organized according to the areas of emphasis specified in the Research Agenda: biology of the microbe, host response, vaccines, diagnostics, and therapeutics. The achievements made meet all of the immediate goals outlined in the Research Agenda.

The areas of research described above are components of the Biodefense Research Pathway (Figure 1), a process through which basic and applied research and advanced product development generate products: vaccines, diagnostics, and therapeutics. Academia, industry, and government are partners along the pathway. NIAID has expanded support for all stages of the biodefense research pathway, as demonstrated by the advances described in the attached report.

While this report focuses on specific pathogens, NIAID's biodefense research is generating scientific discoveries, new technologies, and expanded resources with broader applications for improving global public health. Studies of microbial biology and the pathogenesis of organisms with bioterror potential will lead to a better understanding of other more common and naturally occurring infectious diseases. For instance, advances in biodefense research are likely to have an enormous positive impact on our ability to diagnose, treat, and prevent major infectious killers such as malaria, tuberculosis, and HIV/AIDS. Furthermore, NIAID biodefense research promises to enhance the understanding of molecular and cellular mechanisms of the immune system, which may help in the search for new ways to treat and prevent immune-mediated diseases such as diabetes and rheumatoid arthritis. New insights into the mechanisms of regulation of the human immune system will advance research on cancer, neurologic diseases, and allergic and hypersensitivity diseases.

While it is impossible to capture the true breadth of the NIAID biodefense research portfolio and accomplishments therein, the activities cited in this report most clearly demonstrate the determination and steadfastness of the Institute toward achieving the goal of developing new therapies, diagnostic tests, and vaccines.

BIODEFENSE RESEARCH PATHWAY

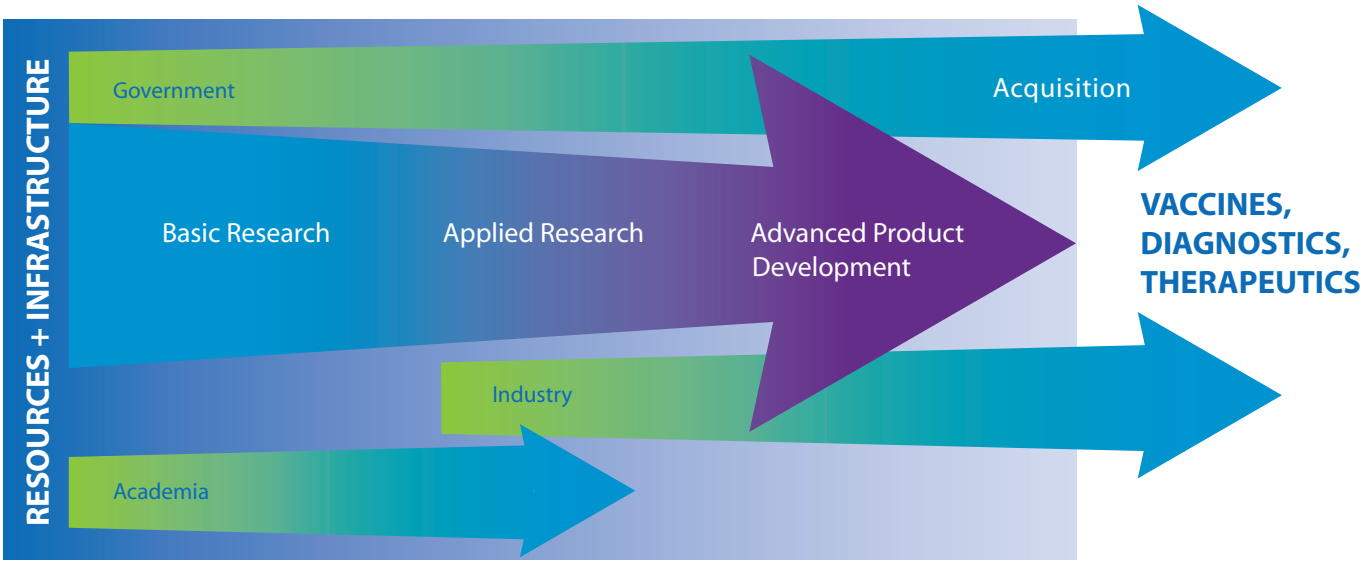

Figure 1. In the **Biodefense Research Pathway**, concepts discovered through basic research are tested in practical settings and may generate candidate products: vaccines, diagnostics, and therapeutics. Academia participates in basic and applied research; industry in applied research and advanced product development. Government supports the entire process, including acquiring completed products. Resources and infrastructure provide a foundation. Knowledge gained along the way leads to new ideas to be explored further.

For a list of, and links to, NIAID strategic plans, research agendas, and progress reports, visit http://biodefense.niaid.nih.gov

Progress on General Recommendations of the Blue Ribbon Panel

Recommendation: Develop Regional Centers of Excellence for Bioterrorism and Emerging Infectious Diseases Research.

- The National Institute of Allergy and Infectious Diseases (NIAID) completed a national network of 10 Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases (RCEs) in 2005. Each Center comprises a consortium of universities and complementary research institutions serving a specific geographical region. The Centers, located throughout the United States, will build and maintain a strong scientific infrastructure supporting multifaceted research and development activities that promote scientific discovery and translational research capacity required to create the next generation of therapeutics, vaccines, and diagnostics for biodefense and emerging infectious diseases. In the event of a national biodefense emergency, the RCEs will provide facilities and support to first-line responders. For more information about the RCEs, see Appendix A.

Recommendation: Expand the capacity to conduct Phase I, II, and III evaluations of candidate vaccines and treatments for agents of bioterrorism.

- The NIAID Vaccine Research Center (VRC) has completed the first human clinical trial of a DNA vaccine designed to prevent Ebola infection. The vaccine, composed of three DNA plasmids, was well tolerated and elicited both humoral and cellular immune responses at all doses. In parallel, nonhuman primate challenge studies have refined the design of the final Ebola vaccine products. The DNA plasmid and recombinant adenovirus (rAd) products are currently being manufactured for clinical testing.

- The VRC has evaluated modified vaccinia Ankara (MVA) as an alternative smallpox vaccine. Two Phase I clinical trials directly testing the capacity of MVA to protect against a vaccinia (Dryvax®*) challenge were performed in both vaccinia-naïve and vaccinia-immune subjects. Injection of two or more doses of MVA was shown to attenuate the clinical reaction to Dryvax and improve the vaccinia-specific immune responses. (*Dryvax® is a registered trademark of Wyeth.)

- NIAID intramural scientists have continued to develop and test vaccine formulations for each dengue subtype, and to improve formulations based on clinical data. The goal is to combine the best formulation for each subtype into a tetravalent dengue vaccine. Through a contract with the Johns Hopkins Bloomberg School of Public Health Center for Immunization Research, these dengue vaccine formulations are being evaluated in clinical trials. Vaccine candidates against West Nile virus and tick-borne viral encephalitis are also being tested.

- Between 2003 and 2006, NIAID Vaccine and Treatment Evaluation Units (VTEUs) conducted 14 Phase I and II clinical trials of vaccines and therapeutics for several Category A agents, including anthrax, smallpox, and tularemia.

- NIAID physician researchers initiated a clinical protocol in 2002 to study the natural history of anthrax. The goal is to look at the infectious disease process over time, from initial infection through the clinical course and beyond recovery. A small number of anthrax survivors from the 2001 attacks have enrolled. Because the medical literature on anthrax does not include any findings regarding long-term complications in survivors, information gained in this study will be valuable to patients and doctors.

- NIAID has established the Food and Waterborne Diseases Integrated Research Network (FWDIRN) to support multidisciplinary research to facilitate development and preclinical evaluation of products to rapidly identify, prevent, and treat food and waterborne diseases that threaten public health. For a list of network sites, see Appendix A.

- NIAID has established the Respiratory Pathogens Research Network to develop a focused and coordinated basic and clinical human respiratory pathogens research program under which preclinical research activities and Phase I clinical trials can be conducted. The network consists of a Viral Respiratory Pathogens Research Unit, a Bacterial Respiratory Pathogens Research Unit, and a Bacterial Respiratory Pathogens Reference Laboratory. Research themes include understanding microbial pathogenesis and host/pathogen interactions and identifying correlates of protection and

genetic factors that may influence susceptibility to infection. For a list of network sites, see Appendix A.

- NIAID has expanded the Collaborative Antiviral Study Group (CASG) by approximately 20 percent. A clinical protocol has been developed for the treatment of smallpox with cidofovir in the event of an outbreak or release. A Phase I clinical trial to assess initial safety, tolerability, and pharmacokinetics of a new oral derivative of cidofovir in people infected with cytomegalovirus or adenovirus is planned to begin in 2006-2007.
- In order to support increased clinical research activities, NIAID has expanded contracts for regulatory support, assay development, immunology quality assurance and quality control, and clinical trial management.

Recommendation: Expand nonhuman primate capability to evaluate new therapeutic and vaccine products.

- NIAID has expanded its capacity to support development of reagents for analyzing the immune response, technologies for immune monitoring, and high-throughput techniques for major histocompatibility complex (MHC) typing in nonhuman primate disease models through the Reagent Development for Monitoring Immunity in Non-Human Primates program. Participating sites include:
  - Nonhuman Primate Reagent Research, Beth Israel Deaconess Medical Center, Boston, MA
  - Nonhuman Primate MHC Typing Development, University of New Mexico, Albuquerque, NM
  - Nonhuman Primate MHC Typing Development, University of Wisconsin, Madison, WI
- The VRC has completed the first preclinical studies of a Marburg Angola strain vaccine in non-human primates. These studies demonstrated that a glycoprotein (GP) vaccine delivered by DNA prime/rAd boost, or by DNA alone, provides uniform protection against lethal challenge with the highly pathogenic Angola strain of Marburg virus. This strain was responsible for 90 percent mortality in infected individuals during a recent outbreak in Africa.
- The VRC is collaborating with scientists in the New England RCE to conduct preclinical studies in nonhuman primates testing the antiviral activity of cathepsin inhibitors. Cathepsins play an essential role in the ability of the Ebola virus to enter and infect cells. Inhibitors of cathepsins effectively block viral entry.
- The *In Vitro* and Animal Models for Emerging Infectious Diseases and Biodefense program, established in 2003 and 2004, supports the development, validation, and use of various small animal and nonhuman primate models to evaluate therapeutic, diagnostic, and preventive candidate compounds for Category A-C bacteria and viruses, and to test their efficacy. Awards were made in 2003; for a list of awardees see Appendix B or visit the NIAID Web site at www.niaid.nih.gov/biodefense/research/biodawards.htm.
- With funding from the RCE program, other NIAID grants and contracts, and the Department of Homeland Security (DHS), a nonhuman primate aerosol biology core has been established. The core is developing aerosol models for plague, tularemia, poxviruses, hantaviruses, and anthrax. (Lovelace Respiratory Research Institute, Albuquerque, NM)
- To complement existing models of anthrax post-exposure prophylaxis, NIAID is collaborating with the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) to develop an African green monkey symptomatic model of inhalational anthrax for evaluating potential toxin therapeutics in a treatment setting. Researchers will use rapid diagnostic assays in conjunction with telemetry to monitor the animals for signs of disease, determining the most informative parameters and likely time frame for intervention. Once developed, the African green monkey will be compared in a head-to-head study with rhesus macaques and cynomolgus macaques to determine if all three species are useful therapeutic models. This information could be useful in considering potential therapeutics for licensure using the Food and Drug Administration's (FDA's) Animal Efficacy Rule.
- NIAID, in collaboration with USAMRIID, is developing and testing vaccines for pneumonic plague, the form of plague that poses the highest concern for biodefense. A non-human primate parenteral challenge model of bubonic plague has been developed that is available for future use through this collaboration. Once a vaccine is developed that is effective against pneumonic plague, the nonhuman primate model may be used to test the vaccine for efficacy against bubonic plague. Bubonic is the form of plague that is of highest concern in the developing world.
- The intramural program continues a research support contract that provides nonhuman primates for biodefense and emerging infectious diseases research. In addition, NIAID expanded its nonhuman primate capability with the completion of the C.W. Bill Young Center for Biodefense and

Emerging Infectious Diseases on the National Institutes of Health (NIH) campus. The Center has a large vivarium, with Biosafety Laboratory (BSL)-3 housing for nonhuman primates.

Recommendation: Attract new scientific disciplines to counter-bioterrorism research, and expand the research training of a new cohort of investigators.

- NIAID has been identifying gaps in biodefense research and expanding programs in targeted efforts to fill these gaps. This includes attracting new investigators with expertise in a variety of different research areas related to microbial pathogenesis and infectious diseases. NIAID continues to offer training in biodefense research through institutional training grants, fellowships, mentored career awards, and special training programs at the RCEs. These programs train postdoctoral fellows, physician scientists, and veterinarians in several areas critical to the biodefense research effort, including pathogenesis, host response, translational research, product development, and clinical research. From 2003 through 2006, NIAID awarded 49 Training Grant Awards, 37 Career Development Awards, and 21 Fellowship Awards specifically focused on biodefense.
- The National Biosafety and Biocontainment Training Program (NBBTP) Fellowship was established through a partnership between the NIH Division of Occupational Health and Safety and NIAID. The NBBTP is a fellowship that prepares individuals for professional careers in biosafety and biocontainment. Additional information can be found at www.nbbtp.org/nbbtp_program.htm.
- NIAID has created a new Biodefense Research Section (BRS) laboratory at the VRC, prompted by the expansion of VRC/NIAID biodefense research efforts in the area of Ebola and other viral hemorrhagic fevers (VHFs). The BRS conducts research on VHFs and other biodefense agents, including research on the immune response to such agents, disease pathogenesis, and the utility of various animal models for human vaccine product development. This BRS laboratory promotes interdisciplinary efforts in biodefense research.
- NIAID has expanded intramural biodefense investigations, which has provided new opportunities in NIAID laboratories for postdoctoral research in select agent biology and pathogenesis, and in development of vaccines, diagnostics, and therapeutics for biodefense. Since 2002, new intramural research projects focused on anthrax, poxviruses, tularemia, dengue-associated antibody-dependent disease enhancement, and vectors for vaccines against highly pathogenic viruses have been initiated. These new or expanded research areas involving Category A pathogens and diseases are providing dozens of opportunities for postdoctoral training and for new investigators to work in NIAID labs.
- 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 has continued to sponsor and participate in a number of scientific meetings and training workshops that bring together top scientists and decision makers to assess the current state of research, identify potential roles for NIAID and other participants, and shape future research agendas. Activities have included:
  - Workshop on Development of Broad Spectrum Therapeutics, 2006
  - Monoclonal Antibody Therapeutics for Biodefense and Emerging Infectious Diseases Workshop, 2006
  - Advanced Product Development for Multiplex Infectious Disease Diagnostics, 2005 (www.niaid.nih.gov/research/topics/diagnostics/PDF/adv_prod.pdf)
  - Annual ASM Biodefense Research Meetings, 2004-2006 (www.asmbiodefense.org)
  - NIAID 2004 Summit on the State of Anti-Infective Development (www.niaid.nih.gov/research/topics/antimicrobial/PDF/anti_infective_mtg_2004.pdf)
  - NIAID Tularemia Workshop, 2004

  In addition, NIAID has established a section of the Biodefense Web site devoted to meetings and symposia related to biodefense, and a section that provides information about funding opportunities (http://biodefense.niaid.nih.gov).

Recommendation: Expand extramural and intramural research and clinical infrastructure, including construction and renovation of BSL-3/4 laboratories.

- NIAID has established a network of 2 National Biocontainment Laboratories (NBLs) and 13 Regional Biocontainment Laboratories (RBLs) . When construction of these facilities has been completed, the NBLs and RBLs will complement and support the research activities of NIAID's RCEs. The NBLs will serve as national and regional resources for research on biodefense and emerging infectious disease agents that require BSL-2/3/4 biocontainment. The RBLs will serve as regional resources for research

requiring BSL-2/3 biocontainment. These laboratory facilities are being designed and built using the strictest federal standards and incorporating multiple layers of safety and security to protect laboratory workers and the surrounding environment.

- Under the Extramural Research Facilities Improvement Program co-sponsored by NIAID and the National Center for Research Resources, seven awards were made to upgrade existing BSL-2 and BSL-3 facilities. For more information, see Appendix A or visit www.niaid.nih.gov/biodefense/research/2005awards.
- Through the RCE program, money has been given to a number of universities to add or upgrade BSL-2 and BSL-3 facilities.
- In May, 2006, NIAID dedicated its new BSL-2/3 facility on the NIH campus, the C.W. Bill Young Center for Biodefense and Emerging Infectious Diseases (www.niaid.nih.gov/about/organization/dir/building33). The Center will house several NIAID programs, including research on respiratory viruses such as influenza and avian influenza; respiratory bacteria such as multi-drug-resistant tuberculosis and anthrax; insect-borne viruses such as West Nile and dengue; immunology of infectious diseases; and the development of vaccines for infectious diseases. Construction continues at the NIAID integrated research facilities (IRFs) in Montana and Frederick, Maryland. The Montana IRF is scheduled to open in 2007.

Recommendation: Expand the availability of animal models for preclinical research.

- In the NIAID intramural program, several animal models have been developed for preclinical investigations of Category A pathogens. These include a flea-to-mouse model of plague transmission for vaccine studies; mouse models for investigations of anthrax toxin pathology and treatment; and immunodeficient mice for MVA safety studies. These studies involve extensive collaborations with scientists from other U.S. and international institutions, expanding the availability of these resources far beyond NIAID.
- The ***In Vitro* and Animal Models for Emerging Infectious Diseases and Biodefense** program, awarded in FY2003 and FY2004, provides a range of resources for preclinical testing of new therapies and vaccines. Under this program, small animal and nonhuman primate models will be developed and validated. Specific ongoing projects include:
  - **Anthrax:** developing recombinant Protective Antigen (rPA) animal models for pre- and post-exposure prophylaxis; refining spore process development; refining rabbit and nonhuman primate models; testing toxin therapeutics; evaluating efficacy and pharmacokinetics/safety for antibiotics; developing therapeutic models; developing alternative nonhuman primate models.
  - **Plague:** evaluating antibiotic efficacy; developing small animal models; developing alternative nonhuman primate models.
  - **Tularemia:** developing alternative nonhuman primate models and small animal models.
  - **Smallpox:** developing MVA animal models, MVA assays, and immunocompromised mouse models; testing drug/vaccine efficacy against monkeypox.
  - **Botulism:** developing animal models and assays for botulinum neurotoxin therapeutics.

  For additional information, including site locations, see Appendix A or www.niaid.nih.gov/biodefense/research/biodawards.htm.
- Under a coordinated network of contracts, NIAID supports the development of animal models and screening of compounds for activity against orthopoxviruses (murine models of vaccinia, cowpox, and ectromelia) and respiratory viruses (murine models of influenza A and B).
- Existing resources that support animal model development for emerging viral infections were expanded to include several VHFs and encephalitides. The new models are:
  - Bunyavirus: Punta Toro virus in hamsters (as a model for Rift Valley fever (RVF) virus)
  - Bunyavirus: Punta Toro virus in mice
  - Arenavirus: Pichinde virus in hamsters
  - Flavivirus: Banzi virus in mice
  - Togavirus: Semliki Forest virus in mice

Recommendation: Develop rapid, inexpensive, and broad-based clinical diagnostics approaches using genomics and proteomics.

- Through a variety of initiatives, including a comprehensive genomics program, NIAID has established a solid foundation for diagnostic research that would have been inconceivable even three years ago. NIAID-supported investigators are

now developing improved molecular assays and methods for detecting Category A agents, including sensitive and specific multiplex polymerase chain reaction (PCR) methods that can simultaneously detect multiple pathogens in a single assay. Continuing efforts include clinical validation of these methods as well as studies to improve sample processing and preparation, decrease time to diagnosis, and develop instrumentation and platforms for point-of-care assays. See Appendix A for a list of NIAID genomic and proteomic resources, including site locations.

- NIAID-supported scientists have developed and demonstrated the ability of a real-time PCR assay to simultaneously detect the four bacterial agents that cause anthrax, plague, tularemia, and melioidosis. This assay is specifically designed to test sterile body fluids, and can be easily adapted to the wide range of PCR machines that are commonly used in hospital and biomedical research settings. The assay uses molecular beacons that fluoresce in different colors in the presence of complementary DNA from each of the tested target pathogens. Development of this technology raises the possibility that highly multiplexed PCR assays can be designed to serve as 'molecular blood cultures,' replacing current culture-based techniques for detecting pathogens in blood samples.
- NIAID/RCE-supported scientists have developed a multiplexed diagnostic system using 64 distinct Masscode tags and reverse transcription polymerase chain reaction (RT-PCR). This system has been shown to be able to identify up to 22 viral respiratory pathogens in a single Mass Tag PCR from clinical specimens.
- In response to the need to develop improved diagnostics for biodefense, NIAID-supported small business investigators have greatly expanded research efforts through the Small Business Biodefense Program. Using technologies such as fluorescence correlation, immunoassays, biochips, and Micro-Electro-Mechanical Systems (MEMS) sensors, scientists have developed improved methods for detecting multiple biodefense pathogens and toxins.
- NIAID established a network of Proteomics Research Centers (PRCs) in FY2004 to use proteomic technologies to discover targets for potential candidates for the next generation of vaccines, therapeutics, and diagnostics. For more information on the PRCs, see description on page 10.
- See information below for details on NIAID's genomic resources applicable to diagnostics.

Recommendation: Encourage structural genomics and proteomics for the targeted development of drugs, vaccines, and diagnostics.

- NIAID continues to make a significant investment in the genomic sequencing of microorganisms considered potential agents of bioterrorism, including Category A organisms. As of May 2006, NIAID completed sequences for 109 genomes including 89 bacteria, 8 fungi, 10 protozoan parasites, and 2 invertebrate vectors of infectious diseases. For example, with Institute support, the complete genome of *Bacillus anthracis* (Ames strain) has been sequenced, as have six additional strains of *B. anthracis*, two strains of *B. cereus*, and a strain of *Yersinia pestis*. NIAID is supporting a variety of genome sequencing projects including several for *Francisella tularensis* and *Y. pestis*.
- To develop the next generation of medical diagnostics and to discover potential targets for vaccines and therapeutics, NIAID has established comprehensive state-of-the art resources for genomics research. A key component of this program is making data, reagents, and resources rapidly available to the scientific community for use in basic and applied research to discover new targets. NIAID's genomics program includes:
  - **Pathogen Functional Genomics Resource Center (PFGRC).** NIAID has expanded the PFGRC to provide additional resources and reagents for basic and applied research on microorganisms responsible for emerging and re-emerging infectious diseases, including those considered agents of bioterrorism. The PFGRC continues to offer an extensive portfolio of DNA microarrays to scientists worldwide through an updated online request process. The PFGRC has produced 33 different organism-specific DNA microarrays and has distributed more than 30,000 DNA microarrays. In addition, the Center has produced full protein expression clone sets for seven microorganisms, has custom clone sets available for 20 microorganisms, and has distributed more than 30,000 clones to 60 investigators.

    The PFGRC has developed comparative genomic platforms useful both for novel gene discovery and for identifying sequence variation between different organisms and strains. NIAID is collaborating with DHS on developing resequencing chip technology to identify genetic polymorphisms between strains of *F. tularensis* and gene discovery chip-based technology for identifying novel genes among different organisms. PFGRC has also developed technologies for protein arrays and comparative

microbial protein profiling. A project comparing proteomes of different strains of *Y. pestis* is under way.

For more information and a list of available microarrays, visit www.niaid.nih.gov/dmid/genomes/pfgrc.

– **Microbial Sequencing Centers (MSCs).** NIAID's MSCs, awarded in 2003, offer a rapid and cost-efficient way to produce high-quality genome sequences of pathogens and invertebrate vectors of infectious diseases, including NIAID Category A agents. NIAID is now supporting more than 36 sequencing projects, including projects for these Category A agents: *B. anthracis*, *Y. pestis, F. tularensis,* and dengue. For a list of center locations, see Appendix A or visit www.niaid.nih.gov/dmid/genomes/mscs.

– **Proteomics Research Centers (PRCs).** In 2004, NIAID established seven Biodefense PRCs to develop and enhance innovative proteomic technologies and apply them to understanding pathogen and host cell proteomes. The ultimate goal is to identify novel targets for the next generation of drugs, vaccines, diagnostics, and immunotherapeutics against microorganisms considered agents of bioterrorism. In FY2005 and FY2006, 1947 potential protein targets for vaccines, therapeutics, and diagnostics were identified and 4077 protein expression clone reagents were developed that can be used as screening tools for potential targets. For a list of center locations and information about accessing reagents, see Appendix A or visit www.niaid.nih.gov/dmid/genomes/prc or www.proteomicsresource.org.

– **Bioinformatics Resource Centers (BRCs).** In 2004, NIAID established eight BRCs that help scientists easily access genomic data for a variety of pathogens, including NIAID Category A agents. Each BRC is composed of multidisciplinary teams of scientists working to develop new computational tools and interfaces to analyze and interpret genomic data. BRC staff also maintain genome sequence data released by the MSCs and other national and international sequencing efforts. The BRC Central Web site is a repository that links to each of the eight BRCs (www.brc-central.org). For a list of center locations, see Appendix A or visit www.niaid.nih.gov/dmid/genomes/brc.

– **NIAID's Viral Bioinformatics Resource Center** (formerly the Poxvirus Bioinformatics Resource Center) provides sequencing and functional comparisons of orthopox genes, as well as genes from other Category A-C virus families.The Center designs and maintains a relational database to store, display, annotate, and query genome sequences, structural information, phenotypic data, and bibliographic information; and serves as a repository of well-documented viral strains. This information is important for the development of drugs and vaccines. For example, sequence analyses of the variola DNA polymerase showed that this enzyme is highly conserved among all poxviruses. Thus, an antiviral drug that targets this enzyme and is effective in animal models infected with various poxviruses may also work against variola.

- In FY2004, NIAID awarded six contracts to support the Population Genetics Analysis Program: Immunity to Vaccines/Infections. Focusing on the NIAID Category A-C pathogens, the centers conduct case-control studies to identify associations between specific immune response gene polymorphisms/genetic variations and susceptibility to infection or response to vaccination. Studies include examining host response to immunization against smallpox and anthrax as well as susceptibility to infection by influenza, tuberculosis, encapsulated bacteria, and West Nile virus. Once genetic polymorphisms are identified, structure-function studies of the relevant proteins will be conducted. For more information, see Appendix A.

- In FY2003, NIAID initiated support for large, multidisciplinary efforts focused on the proteomics of more than one microorganism. Since then, the Biodefense Proteomics Collaboration has developed high-throughput proteomic technologies to identify changes in cellular levels of immunomodulatory proteins after viral infection. Recent progress includes creating a novel proteomics system to measure changes in NF-kB levels, developing a new method to study RNA binding of the Venezuelan equine encephalitis virus, and determining the physical structure of domain III of West Nile virus. (University of Texas Medical Branch-Galveston)

Recommendation: Encourage industry participation to ensure the availability of rapid, sensitive, and licensed diagnostics to hospital clinical laboratories.

- NIAID has created several novel mechanisms to encourage research and development of diagnostics by the private sector. These initiatives include Biodefense Partnerships; the Cooperative Research for the Development of Vaccines, Adjuvants, Therapeutics, Immunotherapeutics, and Diagnostics for Biodefense Program; and the Small Business Biodefense Program. For a complete list of awards, see Appendix B.

Recommendation: Expand partnership opportunities with other agencies and governments.

- On July 21, 2004, President Bush signed into law **Project BioShield**, which provides new tools to improve medical countermeasures protecting Americans against a chemical, biological, radiological, or nuclear (CBRN) attack. Project BioShield is a comprehensive effort overseen jointly by the Department of Health and Human Services (DHHS) and DHS, and involving other Federal agencies, including NIH, as appropriate. Project BioShield will:
  - ensure that resources are available to pay for "next-generation" medical countermeasures;
  - expedite the conduct of NIH research and development on medical countermeasures; and
  - give FDA the ability to make promising treatments quickly available in emergency situations.
- NIAID is a major participant in the National Inter-Agency Genomics Sciences Coordinating Committee (NIGSCC). This committee, composed of representatives from many Federal agencies, coordinates Federal efforts to address the most serious gaps in the comprehensive genomic analysis of microorganisms considered potential agents of bioterrorism. The NIGSCC has focused on Category A agents and provided the Centers for Disease Control and Prevention (CDC) with new technologies, including Affymetrix-based microarrays, for sequencing additional smallpox viral strains. The committee also provided bioinformatics expertise for analysis of genomic sequencing data.
- NIAID has established collaborations with several Department of Defense (DoD) entities focused on biodefense applications.
  - With the Joint Vaccine Acquisition Program, NIAID is coordinating development of botulinum neurotoxin and plague vaccines and exploring future joint acquisition projects.
  - NIAID is meeting regularly with the Defense Threat Reduction Agency (DTRA) and Defense Advanced Research Projects Agency (DARPA) to ensure program synergy and reduce scientific overlap in research and development programs.
  - NIAID has established a cooperative program with USAMRIID to conduct mutually agreed upon research projects related to biodefense. For details concerning specific research projects, please refer to individual chapters in this report.
- NIAID and CDC, along with other DHHS agencies, participate regularly in several interagency working groups of technical experts to discuss and develop countermeasures for Category A agents.
- NIAID is participating in the Federal Bureau of Investigation (FBI)-sponsored Scientific Working Group on Microbial Genetics and Forensics. Other participants include Federal agency officials and scientists with expertise in genomics, bioinformatics, microbiology, and infectious diseases. The working group's mission is to define criteria and coordinate the development and validation of microbial forensic methods that will support criminal investigations. The group has developed and implemented guidelines that provide laboratories engaged in microbial forensics with a framework for developing and executing quality assurance programs.
- NIAID and DoD are collaborating in development of overseas field sites for the testing of new therapeutics, vaccines, and diagnostics against Category A-C priority pathogens in endemic areas.
- NIAID's RCEs have established partnerships to collaborate with state and local agencies on a variety of activities including:
  - Conducting training courses for laboratory personnel from academia and industry.
  - Establishing protocols for emergency response and communications in the case of a bioterrorism event or disease outbreak.
  - Conducting emergency response drills and other training exercises.
  - Informing the public health and medical communities of the RCEs' role as an expert resource on scientific issues pertaining to biodefense.

  For updated information about the RCEs, see page 5.
- NIAID staff participate in an Interagency Working Group that coordinates science and technology efforts by Federal agencies on biodefense diagnostics. The working group is a subcommittee of an Office of Science and Technology Policy/Homeland Security Council (HSC)-led interagency working group. In 2004, NIAID and other Federal agencies

in this group generated a gap analysis of biodefense medical diagnostics that identified critical needs in medical diagnostics.

- NIAID coordinates genomic and post-genomic initiatives, including those related to biodefense, with other Federal agencies through participation in the Microbe Project Interagency Working Group (IAWG), which has developed the following:
  - A coordinated, interagency five-year action plan on microbial genomics, including functional genomics and bioinformatics (www.ostp.gov/html/microbial/start.htm);
  - guiding principles for sharing pre-publication microbial DNA sequence data to help agencies implement specific data release plans; and
  - a comprehensive list of microorganisms considered potential agents of bioterrorism that identifies species, strains, and clinical and environmental isolates that have been sequenced, are currently being sequenced, or should be sequenced.
- In FY2004, the IAWG supported a workshop, An Experimental Approach to Genome Annotation, that was coordinated by the American Society of Microbiology. The colloquium brought together bioinformaticians and experimental biologists. Participants discussed issues involved in annotating microbial genome sequences that have been completed or will be completed in the next few years. (Report available at www.asm.org/Academy/index.asp?bid=32664.)

Recommendation: Develop a centralized repository for reagents and clinical specimens for agents of bioterrorism.

- NIAID established the Biodefense and Emerging Infections Research Resources Repository (BEI Resources, www.beiresources.org) in FY2003 to provide reagents and information to scientists studying NIAID Category A, B, and C priority pathogens and emerging infectious disease agents. Available reagents include reference pathogen isolates, inactivated antigens and genomic DNA or RNA extraction, positive serum references and standards of human and animals, cell lines, expression clones, recombinant proteins, peptides, toxins, monoclonal and polyclonal antibodies, and other diagnostic tools. The BEI Resources program reflects a coordinated effort between NIAID, CDC, the U.S. Department of Agriculture (USDA), USAMRIID, and American Type Culture Collection (ATCC). These materials are widely used to support basic biodefense research projects as well as preclinical and nonclinical development of vaccines and therapeutics candidates against Category A-C agents. (ATCC, Manassas, VA)

Recommendation: Develop procedures and cGMP facilities capable of producing monoclonal antibodies, vaccines, and other immunotherapies for Phase I and II clinical studies.

- The VRC established a cGMP Vaccine Production Plant. Vaccines produced at this facility will be evaluated in Phase I and II clinical trials. In addition, the facility incorporates design features that will allow conversion to larger scale operations capable of providing sufficient quantities of material for Phase III trials, if necessary. The first product, currently being manufactured at this cGMP facility, is an Ebola DNA vaccine.
- The VRC established the National Vaccine, Immune, T-Cell, and Antibody Laboratory (NVITAL) in collaboration with NIAID's Division of Acquired Immune Deficiency Syndrome (DAIDS) and the Henry M. Jackson Foundation to create added immune assay capacity and to accelerate the immunologic testing of candidate vaccines, including those for Category A agents.
- NIAID scientists are working with MacroGenics, Inc. to isolate and characterize human-like neutralizing monoclonal antibodies to anthrax and vaccinia virus.
- NIAID has developed the capability to produce sufficient quantities of monoclonal antibodies against diseases caused by agents of bioterrorism through several different mechanisms, as described below:
  - NIAID used expedited authorities granted under Project BioShield to support development of a monoclonal antibody-based therapy for botulinum neurotoxin serotype A. (Xoma, Berkeley, CA)
  - Through the FWDIRN, NIAID supports botulinum toxin research and the development of small molecule toxin inhibitors and monoclonal antibody-based therapies. (Tufts University)
  - The NIAID VRC developed overlapping peptides to the envelope protein and nucleoprotein of Ebola virus. These peptides will be produced and validated, and will be used in ELISA assays by the centralized immunology laboratory.

– VRC scientists have characterized the neutralization mechanism of a human monoclonal antibody that protects guinea pigs from lethal Ebola Zaire virus challenge. This accomplishment suggests that such antibodies, generated in cell culture, might be promising candidates for immunoprophylaxis of Ebola virus infection.

- NIAID awarded two contracts to develop, manufacture, and characterize a cGMP pilot lot of MVA vaccine for evaluation in a Phase I clinical study. cGMP-compliant pilot lots of MVA have been successfully manufactured and this material has been tested in Phase I clinical trials in healthy individuals as well as individuals with human immunodeficiency virus (HIV) and atopic dermatitis. (Bavarian Nordic, Denmark; Acambis, Inc., Cambridge, MA)
- NIAID continued to support advanced development of MVA by awarding two contracts in 2004 to manufacture cGMP material for use in Phase II clinical trials. Contracts cover development, process validation, and cGMP manufacturing of MVA, including formulation, vialing, and release and stability testing. Both manufacturers successfully completed this process including cGMP manufacturing of 500,000 doses. Phase II clinical studies in healthy individuals and individuals with HIV and atopic dermatitis have begun. Additional Phase II studies in healthy individuals and individuals with HIV and atopic dermatitis are expected to begin in 2007. (Bavarian Nordic, Denmark; Acambis, Inc., Cambridge, MA)
- NIAID continues support of advanced development and production of an rPA vaccine for anthrax. Contracts were awarded to support production, testing, and evaluation of rPA vaccine including Phase II clinical trials. Proof-of-concept aerosol challenge efficacy studies in rabbits and nonhuman primates have been successfully completed for both rPA vaccine candidates. New facilities and processes for large-scale cGMP manufacturing are being validated. (VaxGen Inc., Brisbane, CA; Avecia, U.K.)
- Phase II clinical trials of rPA are nearing completion. (Avecia, U.K.)
- A NIAID-supported reference reagent repository has been expanded to include reagents needed to develop and test improved vaccines, diagnostics, and therapeutics, including those for Category A agents. Reagents are available to scientists through BEI Resources (www.beiresources.org).

Recommendation: Enhance adjuvant discovery and rational design of Toll system mediators.

- The NIAID intramural program is conducting several studies of Toll-like receptors on mammalian cells and the pathogen ligands with which they interact. These basic studies provide the fundamental knowledge base that informs the rational design of Toll system mediators.
- NIAID has established a research development program on Innate Immune Receptors and Adjuvant Discovery. Scientists at five participating sites are conducting research to discover and characterize new adjuvant candidates based upon innate immunological principles. The ultimate goal is to enhance the potency, longevity, and safety of specific vaccines for biodefense, and to enhance nonspecific immunity for immediate protection against acute infectious threats. (See Appendix A for a list of sites.)

Recommendation: Identify and characterize innate and adaptive immune responses that occur after exposure to agents of bioterrorism and enhance basic research on mucosal immunology.

- In FY2005, NIAID established a research program, Immune Function and Biodefense in Children, Elderly, and Immunocompromised Populations, to develop novel methods to protect or treat these special populations from bioterror threats. Research includes identifying biological mechanisms responsible for increased susceptibility to infection or decreased effectiveness of vaccines in these populations, as well as testing treatments designed to increase safety or efficacy. (See Appendix B for a list of awardees.)
- To address the specific problems encountered by atopic dermatitis patients when innoculated with the smallpox vaccine, NIAID established an Atopic Dermatitis and Vaccinia Immunization Network in FY2004. The sites involved are:
  – Clinical Studies Consortium, National Jewish Medical Center, Denver, CO
  – Animal Studies Consortium, Children's Hospital Boston, MA
  – Statistical and Data Coordinating Center, Rho Federal Systems Division, Inc., Chapel Hill, NC
- In FY2005, NIAID awarded four contracts to establish Immune Modeling Centers to develop mathematical modeling packages, validated in experimental systems, for simulating host immune responses to infection and vaccines. Two of the

centers focus on modeling innate and adaptive pulmonary immunity to infection. (University of Pittsburgh; University of Rochester)

- In 2005, NIAID's intramural program began a multidisciplinary research initiative, Program in Systems Immunology and Infectious Disease Modeling (PSIIM), to further understand how the immune system interacts with pathogens. PSIIM emphasizes quantitative, computer-based, microscopic and macroscopic modeling of immune functions, integration of these modeling efforts with data sets derived from global analyses of cell components, and development and application of advanced imaging methods to analyze immune responses *in vivo*. A major focus will include devising predictive models of immune behavior after exposure to Category A-C pathogens and following vaccine administration. For more information, see the Immunity and Biodefense section.
- NIAID established a network of eight Cooperative Centers for Translational Research on Human Immunology and Biodefense. The network, established in FY2003, supports an interactive group of investigators studying various aspects of immunity to biodefense pathogens and developing new technologies to facilitate clinical immunology research. (See Appendix A for a list of participating centers.)
- Under the Biodefense and Emerging Infectious Diseases Research Opportunities initiative, NIAID is encouraging research on protective mechanisms against infection with the CDC Category A-C priority pathogens. One grant is focused on the development of technology for probing innate immunology. It is hoped that a knowledge base of innate immune system activity will be developed to aid in identifying genetic changes and proteins that are triggered by encounters between innate immune cells and infectious pathogens. (Scripps Research Institute, La Jolla, CA)

Recommendation: Establish MHC-peptide and B-cell epitope databases that may be used to further define immune responses, including the identification of relevant immune polymorphisms, and maximize such responses.

- NIAID has established a centralized Immune Epitope Database and Analysis Program. This program offers a public, online, searchable database of antibody binding sites (B-cell epitopes) and antigenic MHC-binding peptides (T-cell epitopes). Information and data analysis tools are also provided to help scientists identify novel vaccine candidates and immunotherapeutic strategies for a variety of infectious agents, including Category A priority pathogens. The Web site for the freely available database and analysis tools is www.immuneepitope.org.
- In 2004, NIAID established the Large Scale Antibody and T-Cell Epitope Discovery Program. This network of 13 sites conducts research to discover novel antibody and T-cell epitopes for a variety of infectious agents, including Category A pathogens. By operating on a large scale, the network takes advantage of new technologies such as computer-based algorithms that predict epitope sites, genome-wide scanning, structural genomics, MHC-peptide tetramer technology, and synthetic peptide laboratory screens. The program also supports development of new or improved high-throughput screening methods for epitope discovery. (See Appendix A for a list of sites.)

Anthrax

Bacillus anthracis, the agent that causes anthrax, has several characteristics that make it a formidable bioterrorist threat. These characteristics include its stability in spore form, its ease of culture and production, its ability to be aerosolized, the seriousness of the disease it causes, and the lack of sufficient vaccine for widespread use.

Human anthrax has three major clinical forms: cutaneous, inhalational, and gastrointestinal. If left untreated, all three forms can result in septicemia and death. Early antibiotic treatment of cutaneous and gastrointestinal anthrax is usually curative; however, even with antibiotic therapy, inhalational anthrax is a potentially fatal disease. Although case-fatality estimates for inhalational anthrax are based on incomplete information, the historical rate is considered to be high (about 75 percent) for naturally occurring or accidental infections, even with appropriate antibiotics and all other available supportive care. However, the survival rate after the recent intentional exposure to anthrax in the United States was 60 percent for the first 10 cases.

SCIENTIFIC PROGRESS

Biology of the Microbe

Three-dimensional structure of anthrax toxin complex solved. Using an intensive X-ray beam to determine the position of atoms in a crystal form of the protein complex, National Institute of Allergy and Infectious Diseases (NIAID)-supported scientists have mapped the three-dimensional structure of one of anthrax toxin's protective antigen (PA) proteins "docked" to one of two human anthrax cell receptors. The resulting image revealed how anthrax toxin enters human cells. This finding provides new approaches for the discovery of anthrax antitoxins, and should aid in designing cancer therapeutics.

(Santelli E et al., Crystal structure of a complex between anthrax toxin and its host cell receptor, *Nature* 2004;430(7002):905-908)

New mouse model for pulmonary anthrax. NIAID-supported investigators have developed and standardized a new, relatively inexpensive mouse model for studying inhalational anthrax. It permits investigators to examine the kinetics for dissemination of infection after exposure to spores as well as pertinent issues and variables related to pathogenesis. This is a major advance since until now the ability to conduct such studies was greatly limited by the short supply of nonhuman primates.

(Lyons CR et al., Murine model of pulmonary anthrax: kinetics of dissemination, histopathology, and mouse strain susceptibility, *Infect Immun* 2004;72:4801-4809)

Scientists discover new combinations of anthrax toxin components. It is known that *B. anthracis* exerts harmful and often deadly effects on its hosts through the action of two proteins—edema factor (EF) and lethal factor (LF)—that combine with the protective antigen protein to form anthrax toxin. Although a considerable amount is known about these three proteins, much remains to be learned about their basic biology so that therapies can be developed. Recently, NIAID-supported scientists at the New England Regional Center of Excellence for Biodefense and Emerging Infectious Diseases (RCE) have shown that protective antigen can bind edema factor and lethal factor at the same time, forming a greater variety of toxin complexes than were previously known. This finding could have implications in developing antitoxin therapies.

(Pimental RA et al., Anthrax toxin complexes: heptameric protective antigen can bind lethal factor and edema factor simultaneously, *Biochem Biophys Res Commun* 2004;322:258-262)

Sortase enzymes may serve as a new target for anthrax therapies. The Gram-positive bacteria *B. anthracis* and *Staphylococcus aureus* have enzymes called sortases that anchor bacterial surface proteins to the cell walls. These enzymes may be essential to bacterial survival in their hosts. The fact that they are located on the surface of the bacteria makes these enzymes an attractive potential target for therapies. As a first step in developing those therapies, NIAID-supported scientists from the Great Lakes RCE and Argonne National Laboratory solved the structure of one of those sortases. Knowing the high-resolution crystal structure of Sortase B will facilitate further exploration of this protein and potential drug target.

(Zhang R et al., Structures of Sortase B from *Staphylococcus aureus* and *Bacillus anthracis* reveal catalytic amino acid triad in the active site, *Structure* 2004;12:1147-1156)

The Cre/loxP system proves useful for manipulating the *B. anthracis* genome. NIAID scientists demonstrated the usefulness of a gene engineering method, called Cre/loxP site-specific recombination, to disrupt *B. anthracis* genes. Their experiments showed that the method can yield genetic modifications without the permanent establishment of antibiotic resistance markers, which are typically inserted into genes targeted for disruption. Removal of the antibiotic resistance markers assures that cell physiology will not be altered by known or unrecognized activities of the resistance genes or the antibiotic drugs themselves. This gene-disruption method may make *B. anthracis* more acceptable for biotechnology applications such as the production of recombinant protective antigen and LF. Using the system, the scientists showed that 34 *B. anthracis* genes were not essential for growth of *B. anthracis.*

(Pomerantsev AP et al., Genome engineering in *Bacillus anthracis* using Cre recombinase, *Infect Immun* 2006;74(1):682-693)

Structure-based drug design targets the anthrax PA pore. Scientists from NIAID, National Institute of Child Health and Human Development, and Innovative Biologics, Inc. (Manassas, VA) demonstrated a structure-based approach to disabling the anthrax toxin. The method focuses on the PA, which has a seven-sided pore to facilitate LF and EF transport into the cytosol. Guided by the shape and chemistry of the PA pore, the investigators synthesized small cyclic molecules to find a compound that might block it. One such compound had a high-affinity interaction with the PA pore and blocked LF and EF entry into cells at subnanomolar concentrations. The compound completely protected the highly susceptible Fischer F344 rats from lethal toxin. This approach may serve as the basis for a structure-directed drug discovery program to find new and effective treatments for anthrax.

(Karginov VA et al., Blocking anthrax lethal toxin at the protective antigen channel by using structure-inspired drug design, *Proc Natl Acad Sci USA* 2005;102(42):15075-15080)

Researchers unravel anthrax genomes. *B. anthracis* is the microorganism that causes anthrax and is responsible for the illness and deaths associated with the deliberate exposure of civilian populations in the United States to this agent. Comparing the genome sequences of two strains of *B. anthracis* (the Ames strain and a clinical isolate from a patient in Florida exposed to anthrax), investigators identified four novel DNA sequence differences at the single nucleotide level not seen before using other molecular genotyping methods. These differences in sequence were used as genetic markers to screen a set of *B. anthracis* isolates. Analysis of the data revealed that the Florida isolate was likely to have been derived from the Ames strain. This study provides strong evidence to support the use of genome-based analysis in finding genetic variation at the single nucleotide polymorphism (SNP) level for forensic strain identification.

(Read TD et al., Comparative genome sequencing for discovery of novel polymorphisms in *Bacillus anthracis, Science* 2002;296:2028-2033)

Complete genome of anthrax bacteria is sequenced. Most recently, the complete genetic blueprint of *B. anthracis* has been solved. Investigators found a number of genes encoding proteins that *B. anthracis* may use to enter host cells and therefore may be important targets for vaccines and drugs. Using comparative genomics, investigators compared an Ames isolate with two closely related *Bacillus* bacteria and found remarkably little difference, highlighting the similarity of *B. anthracis* to related pathogens not associated with anthrax. Additional comparisons revealed similarities between genes of *B. anthracis* and pathogens that infect insects, suggesting that a recent ancestor of *B. anthracis* also may have infected insects. Information obtained from these efforts will help researchers to better understand the disease-causing capabilities of *B. anthracis* and to design new vaccines and treatments.

(Read TD et al., The genome sequence of *Bacillus anthracis* Ames and comparison to closely related bacteria, *Nature* 2003;423:82-86)

Host Response

Steps toward a third-generation anthrax vaccine. There is considerable evidence that live bacterial vectors that present foreign proteins to human immune systems are effective in generating immunity. NIAID-supported scientists from the Mid-Atlantic RCE developed a *Salmonella* serovar Typhi-based vaccine that expresses the PA of *B. anthracis* as part of a fusion protein that is exported from the vector. This initial study shows promise in generating an immune response to protective antigen.

(Galen JE et al., Adaptation of the endogenous *Salmonella enterica* serovar Typhi *clyA*-encoded hemolysin for antigen export enhances the immunogenicity of anthrax protective antigen domain 4 expressed by the attenuated live-vector vaccine Strain CVD 908-htrA, *Infect Immun* 2004;2:7096-7106)

Discovery of the role of LRP6 in anthrax toxicity. A collaborative study by scientists from Stanford University and the NIAID intramural program resulted in the discovery of a

potential new target for prophylaxis and treatment of anthrax toxicity. The researchers inactivated genes randomly in human cells and then identified those genes whose inactivation caused toxin resistance. This approach uncovered LRP6, a member of a gene family encoding receptor-related proteins (LRPs) on the cell surface that aid in the delivery of a variety of ligands into the cytoplasm. The LRP6 protein, previously known as a mediator in embryonic development and cell proliferation, was shown to have a role in the delivery of anthrax toxins into cells. Antibodies directed against LRP6 protected cell cultures from killing by anthrax lethal toxin, suggesting that targeting of LRP6 may prove useful in protecting against the effects of accumulated toxin.

(Wei W et al., The LDL receptor-related protein LRP6 mediates internalization and lethality of anthrax toxin, *Cell* 2006;124:1141-1154)

Pathology associated with anthrax edema toxin (ET) described. NIAID scientists conducted the first comprehensive study of ET-induced pathology in an animal model. Highly purified ET caused death in BALB/cJ mice at lower doses and more rapidly than previously seen with the other major *B. anthracis* virulence factor, lethal toxin (LT). To understand the pathological processes in this model, the investigators conducted gross and microscopic analyses of timed tissue harvests with concomitant blood chemistry analyses and measured various cytokines and physiological parameters. These studies detail the extensive pathological lesions caused by ET and suggest that it causes death due to multiple organ failure.

(Firoved AM et al., *Bacillus anthracis* edema toxin causes extensive tissue lesions and rapid lethality in mice, *Am J Pathol* 2005;167(5):1309-1320)

Anthrax toxin lethality in mice is cytokine-independent. *B. anthracis* LT is the major virulence factor of anthrax and reproduces most of the laboratory manifestations of the disease in animals. NIAID scientists studied LT toxicity in two mouse strains. Timed histopathological analysis identified bone marrow, spleen, and liver as major affected organs in both mouse strains. LT induced hepatic dysfunction, hypoalbuminemia, and vascular/oxygenation insufficiency. There was no evidence of disseminated intravascular coagulation or renal dysfunction. Fifty cytokines were analyzed. Results show that LT kills mice by hypoxia-induced liver failure and death is not mediated by cytokine release, as was previously thought.

(Moayeri M et al., *Bacillus anthracis* lethal toxin induces TNF-alpha-independent hypoxia-mediated toxicity in mice, *J Clin Invest* 2003 Sep;112(5):670-682)

***B. anthracis* physiology and toxin activity further elucidated.** Recent findings by NIAID-supported investigators have revealed new details about anthrax pathogenesis and host response that may suggest potential targets for therapeutic intervention.

- *B. anthracis* requires siderophore biosynthesis for growth in macrophages and for virulence.

  (Cendrowski S et al., *Bacillus anthracis* requires siderophore biosynthesis for growth in macrophages and mouse virulence, *Mol Microbiol* 2004;51:407-417)

- Anthrax LT inhibits the transcriptional induction of inflammatory cytokines.

  (Dang O et al., Cutting edge: anthrax lethal toxin inhibits activation of IFN-regulatory factor 3 by lipopolysaccharide, *J Immunol* 2004;172:747-751)

- The membrane insertion of anthrax PA and cytoplasmic delivery of LF occur at different stages of the endocytic pathway.

  (Abrami L et al., Membrane insertion of anthrax protective antigen and cytoplasmic delivery of lethal factor occur at different stages of the endocytic pathway, *J Cell Biol* 2004;166:645-651)

Key features in the pathogenesis of anthrax identified; research may yield an antitoxin. The anthrax bacterium causes illness and death by releasing toxins that kill cells and damage organs. Few people survive when the microbe spreads throughout the body, as is the case in the severe, inhalational form of the disease. This is because the toxin remains active in the bloodstream for several days, even if antibiotics kill the bacteria that are producing it. In one study, researchers elucidated how the toxin binds to and enters healthy cells, and how it disrupts a cell's internal communications network. They identified key structures on the toxin molecule that could lead to the development of nontoxic analogs (decoys) to block the lethal effects of toxins, thereby resulting in a new approach for treating the infection.

(Pannifer AD et al., Crystal structure of the anthrax lethal factor, *Nature* 2001;414:229-233)

Molecular mechanisms by which anthrax evades immune systems uncovered. NIAID-supported scientists and those from NIAID's Division of Intramural Research have discovered one strategy that anthrax uses to avoid the host's immune reaction. In this research, the investigators deter-

mined that the critical virulence factor, anthrax LT, targets the mitogen-activated protein (MAP) kinase intracellular signaling pathway in dendritic cells, an important class of antigen presenting cell in lymph tissues. This blocks the stimulation of antigen-specific T cells and both T- and B-cell immunity are reduced. These data suggest a role for LT in suppressing host immunity during *B. anthracis* infections.

(Agrawal A et.al., Impairment of dendritic cells and adaptive immunity by anthrax lethal toxin, *Nature* 2003;424:329-334)

Vaccines

Multiple doses of new rPA anthrax vaccine increase antibody response. NIAID-supported scientists conducted a randomized, double-blind, Phase I clinical trial of a new recombinant protective antigen (rPA102) anthrax vaccine. Immunogenicity and tolerance of ascending doses of rPA102 were compared with that of Anthrax Vaccine Adsorbed (AVA). Responses after two injections of rPA102 were similar to those measured after two injections of AVA. The third rPA102 vaccination substantially increased the antibody response. No clinically serious or dose-related toxicity or reactogenicity was observed. This study provides important initial human safety and immunogenicity data on the rPA vaccine and is the first peer-reviewed publication of clinical data on this vaccine.

(Gorse G et al., Immunogenicity and tolerance of ascending doses of a recombinant protective antigen (rPA102) anthrax vaccine: a randomized, double-blinded, controlled, multicenter trial, *Vaccine* 2006; 24(33-34):5950-5959)

New patch delivery system for anthrax vaccine. NIAID-supported scientists have designed a novel vaccine delivery system using a dry adhesive patch that simplifies administration and improves tolerability of a subunit anthrax vaccine. The technology, called transcutaneous immunization (TCI), is a needle-free technique that delivers antigens and adjuvants to potent epidermal immune cells. Preclinical studies of anthrax TCI vaccine patches showed that the patches stimulated robust and functional immune responses that protected against lethal aerosol challenge in a mouse model. Demonstration of immune responses in mouse lung tissue following patch vaccine administration suggests that the patch may also be protective against challenge with aerosolized anthrax spores. A formulated, pressure-sensitive, dry adhesive patch, which is stable and can be manufactured on a large scale, elicited comparable immunoglobulin G and TNA responses, suggesting that an anthrax vaccine patch is feasible and should advance into clinical evaluation.

(Kenney RT et al., Induction of protective immunity against lethal anthrax challenge with a patch, *J Infect Dis* 2004;190(4):774-782)

Anthrax monoclonal antibody may have potential as a vaccine. NIAID-supported investigators have demonstrated that monoclonal antibodies specific for the surface poly-D-glutamic acid capsule of *B. anthracis* are able to confer protective immunity, thereby indicating their potential for use in a vaccine.

(Kozel TR et al., mAbs to *Bacillus anthracis* capsular antigen for immunoprotection in anthrax and detection of antigenemia, *Proc Natl Acad Sci USA* 2004;101(14):5042-5047;

Wang TT et al., Induction of opsonic antibodies to the gamma-D-glutamic acid capsule of *Bacillus anthracis* by immunization with a synthetic peptide-carrier protein conjugate, *FEMS Immunol Med Microbiol* 2004;40(3):231-237)

Dominant-negative inhibitor may serve as both an anthrax vaccine and therapy. The currently available anthrax vaccine is based on PA, a central component of anthrax toxin. Vaccination with PA raises no direct immune response against anthrax bacteria and, being a natural toxin component, PA may carry some risks when used immediately following exposure to *B. anthracis*. NIAID-supported scientists at the New England RCE have developed a new, dually active vaccine candidate that may be used both pre- and post-anthrax exposure, and that targets both the anthrax toxin and bacteria. This vaccine, made by adding an additional molecule called PGA to a dominant negative inhibitory (DNI) mutant of anthrax PA, elicits the production of antibodies specific for both bacilli and toxins. The DNI portion of the vaccine also has been shown to directly inhibit anthrax toxin, making it potentially useful as a therapeutic.

(Aulinger BA et al., Combining anthrax vaccine and therapy: a dominant-negative inhibitor of anthrax toxin is also a potent and safe immunogen for vaccines, *Infect Immun* 2005;73(6):3408-3414)

Anthrax vaccine made in a tobacco plant. NIAID scientists collaborated with University of Central Florida (UCF) investigators to demonstrate the efficacy of a plant-based anthrax vaccine in mice. The UCF scientists developed the vaccine by expressing anthrax PA in transgenic tobacco chloroplasts. Macrophage lysis assays showed that plant-derived PA was equal in potency to PA produced in *B. anthracis*. Mice immunized with partially purified, chloroplast-derived- or *B. anthracis*-derived PA with adjuvant survived (100 percent) challenge with lethal doses of toxin. This work demonstrates the

immunogenic and protective properties of plant-derived anthrax vaccine antigen.

(Koya V et al., Plant-based vaccine: mice immunized with chloroplast-derived anthrax protective antigen survive anthrax lethal toxin challenge, *Infect Immun* 2005; 73(12):8266-8274)

Diagnostics

Rapid polymerase chain reaction (PCR)-based diagnostic for anthrax, plague, tularemia, and melioidosis. Rapid detection of pathogens in patient blood samples is a critical diagnostic need for biodefense. NIAID-supported scientists have developed and demonstrated the ability of a real-time PCR assay to simultaneously detect the four bacterial agents that cause anthrax, plague, tularemia, and melioidosis. This PCR assay is specifically designed to test sterile body fluids, and can be easily adapted to the wide range of PCR machines that are commonly used in hospital and biomedical research settings. The assays use 'molecular beacons' that fluoresce in different colors in the presence of complementary DNA from each of the tested target pathogens. The fluorescence can then be detected by specialized instruments. The development of this technology raises the possibility that highly multiplexed PCR assays can be designed to serve as 'molecular blood cultures,' replacing current culture-based techniques for detecting pathogens in blood samples.

(Varma-Basil M et al., Molecular beacons for multiplex detection of four bacterial bioterrorism agents, *Clin Chem* 2004;50(6):1060-1062)

Therapeutics

Scientists design potent anthrax toxin inhibitor. In a novel approach to anthrax therapeutics, scientists have designed an inhibitor that blocks anthrax toxin from attaching to receptors on the surface of host cells. The inhibitor, known as a "functionalized liposome," is a fatty bubble studded with small proteins that binds to multiple sites on the two host receptors for anthrax toxin. Binding of the liposome to the receptors blocks internalization of the toxin into the host cell, which is necessary for the toxin to produce its toxic effects. Without the ability to enter host cells, the toxin is rendered effectively neutralized. In animal studies, the new inhibitor was shown to be many times more potent than current therapies and is especially promising as a countermeasure to antibiotic-resistant strains or as a potential adjunct to antibiotic therapy. The general concept could also apply to designing inhibitors for other pathogens.

(Rai Pet al., Statistical pattern matching facilitates the design of polyvalent inhibitors of anthrax and cholera toxins, *Nat Biotechnol* 2006;24(5):582-586;

Basha S et al., Polyvalent inhibitors of anthrax toxin that target host receptors, *Proc Natl Acad Sci USA* 2006; 103(36):13509-13513)

Human monoclonal antibody protects against inhalation anthrax in three animal models. NIAID-supported scientists demonstrated that a human monoclonal antibody (AVP-21D9) to anthrax PA protects mice, guinea pigs, and rabbits against anthrax. AVP-21D9 combined with ciprofloxacin protected mice and guinea pigs against challenge with anthrax spores, while AVP-21D9 given alone soon after challenge protected rabbits against challenge and rechallenge. Analysis of sera from various surviving animals showed development of species-specific PA antibodies that correlated with protection against reinfection. The effectiveness of human anti-PA antibody alone or in combination with low ciprofloxacin levels may lead to improved strategies for prophylaxis or treatment following inhalation anthrax infection.

(Peterson JW et al., Human monoclonal anti-protective antigen antibody completely protects rabbits and is synergistic with ciprofloxacin in protecting mice and guinea pigs against inhalation anthrax, *Infect Immun* 2006;74(2): 1016-1024)

Antibody fragment protects guinea pigs against inhalational anthrax. NIAID-supported scientists engineered antibody fragments that recognize the PA component of the *B. anthracis* exotoxin. When conjugated to polyethylene glycol, these antibody fragments confer significant protection against inhalation anthrax. The speed and lower manufacturing cost of these antibody fragments may represent a unique approach for mounting a rapid therapeutic response to emerging pathogen infections.

(Mabry R et al., Passive protection against anthrax by using a high-affinity antitoxin antibody fragment lacking an Fc region, *Infect Immun* 2005; 73(12):8362-8368)

Development of anthrax toxin-neutralizing antibodies. NIAID-supported scientists have generated potent anthrax toxin neutralizing human monoclonal antibodies from the peripheral blood of persons vaccinated with the AVA vaccine. The anti-anthrax toxin human monoclonal antibodies isolated were evaluated for neutralization of anthrax LT *in vivo* in a rat bolus toxin challenge model. Three antibodies were shown to protect rats from anthrax LT at low dose. These potent anti-

bodies may be attractive candidates for prophylaxis and/or treatment of humans against anthrax bioterrorism toxins.

(Sawada-Hirai R et al., Human anti-anthrax protective antigen neutralizing monoclonal antibodies derived from donors vaccinated with anthrax vaccine adsorbed, *J Immune Based Ther Vaccine* 2004;2(1):5)

Potent anthrax-neutralizing monoclonal antibodies developed. A team of NIAID scientists and colleagues from the Protein Biophysics Resource of the National Institutes of Health (NIH) developed and characterized monoclonal antibodies (mAbs) that can neutralize the toxin of *B. anthracis*. The antibodies bind to the portion of a PA molecule that binds to the cellular receptor with an affinity that is 20- to 100-fold higher than the affinity of PA for its receptor, and higher than the affinity of any other similar antibody reported to date. The mAbs, derived from the bone marrow of an experimentally immunized chimpanzee, protected against anthrax toxin in cell culture and also *in vivo* in a rat model. Chimpanzee antibodies are virtually identical to human antibodies and this study suggests that they may replace human antibodies for clinical applications.

(Chen Z et al., Efficient neutralization of anthrax toxin by chimpanzee monoclonal antibodies against protective antigen, *J Inf Dis* 2006;193:625-633)

Treatment with anthrax PA monoclonal antibody may be useful even after onset of septic shock. NIH scientists and colleagues from Human Genome Sciences (Rockville, MD) investigated the clinical effect in a rat model of administering a monoclonal antibody to anthrax LT (PA-MAb) after the onset of shock. Previous animal studies found that the antibody improved survival when administered close to the time of *B. anthracis* LT bolus or live bacterial challenge. The scientists studied the effects of PA-MAb versus placebo administered at specified times after the initiation of a 24-hour LT infusion. At each treatment time, survival rates were greater for PA-MAb than for placebo, although improvement was decreased at later treatment times. PA-MAb also significantly increased mean blood pressure and heart rate. These findings suggest that PA-MAb may reduce morbidity and mortality due to LT even if administered after the onset of septic shock resulting from infection with *B. anthracis*.

(Cui X et al., Late treatment with a protective antigen-directed monoclonal antibody improves hemodynamic function and survival in a lethal toxin-infused rat model of anthrax sepsis, *J Infect Dis* 2005;191(3):422-434)

Furin-inhibiting molecule protects against anthrax toxemia. The anthrax toxin protective antigen precursor is activated by cleavage by a protease called furin. NIAID-supported scientists developed the compound hexa-D-arginine (D6R) as a furin inhibitor and showed its ability to block the effects of bacterial toxin using *Pseudomonas aeruginosa* exotoxin A, which also requires furin processing. (Louisiana State University (LSU), Baton Rouge). Following this work, NIAID scientists collaborated with LSU investigators to study D6R as a potential therapeutic for anthrax toxemia. Their findings in cells and in two animal models demonstrated the efficacy of this furin inhibitor against anthrax toxemia and suggest that D6R represents a reasonable lead compound for further development.

(Sarac MS et al., Protection against anthrax toxemia by hexa-D-arginine *in vitro* and *in vivo*, *Infect Immun* 2004;72(1):602-605)

Study reveals clues to development of improved antibody-based anthrax toxin therapies. NIAID scientists created a panel of anthrax toxin PA variants and determined which individual amino acid residues were critical for interactions with the cellular receptor and with a neutralizing MAb. This MAb, known as 14B7, is one of a group of MAbs that neutralize anthrax toxin by inhibiting the binding of PA to cells. The amino acid residues identified in this work may provide targets for directed antitoxins. Additionally, amino acid changes resulting from the neutralizing action of 14B7 imply that MAb therapies should include a mixture of antibodies to different PA epitopes. The PA variant panel used in this study could also be useful in evaluating potential therapeutics directed against the binding domain of PA.

(Rosovitz MJ et al., Alanine-scanning mutations in domain 4 of anthrax toxin protective antigen reveal residues important for binding to the cellular receptor and to a neutralizing monoclonal antibody, *J Biol Chem* 2003;278(33): 30936-30944)

Scientists discover an anthrax toxin receptor. Through genetic analysis, scientists identified a protein on the surface of mammalian cells, the anthrax toxin receptor (ATR), and identified the specific region on ATR to which the toxin attaches. With this information they were able to produce a soluble version of this as well as another receptor with the toxin-binding domain. When these soluble receptors are mixed with mammalian cells in the presence of anthrax toxin, they act as decoys to absorb anthrax toxin before it can attach to target cells to produce its lethal effects.

(Bradley KA et al., Identification of the cellular receptor for anthrax toxin, *Nature* 2001;414:225-229)

PROGRAMMATIC PROGRESS IN ADDRESSING IMMEDIATE GOALS

GOAL: Establish capacity for the development and production of pilot lots of candidate anthrax vaccines.

- In September 2002, NIAID awarded two contracts for the development and testing of next-generation anthrax vaccines based on rPA, including production of pilot lots. These contracts have led to successful manufacture of clinical grade pilot lots of rPA vaccine, preclinical testing in rabbits, successful rabbit and nonhuman primate (NHP) spore inhalation proof-of-concept studies, and Phase I clinical trials. (Avecia, U.K.; VaxGen Inc., Brisbane, CA)

GOAL: Conduct Phase I and II trials with rPA anthrax vaccine candidates and alternative adjuvant formulations.

- NIAID is now supporting intermediate-scale manufacture of rPA vaccine, as well as development of multiple rabbit models and NHP models. In addition, several Phase I human clinical studies have been completed. (Avecia, U.K.; VaxGen, Inc., Brisbane, CA)
- Phase II trials of rPA are nearing completion. (Avecia, U.K.)
- NIAID is also soliciting for a third-generation anthrax vaccine to enhance the efficacy of the rPA-based second-generation vaccine. Enhancements may include the use of antigens other than PA, novel adjuvants, and novel inoculation strategies. This will support pilot-scale cGMP manufacturing, preclinical and nonclinical studies, and options for Phase I and II clinical trials.

GOAL: Expand clinical capability to accelerate Phase I and II testing of candidate vaccines.

- NIAID is supporting advanced development of rPA vaccine candidates including assay and facility validation; production and release of cGMP consistency lots; fill/finish of vaccine; and clinical evaluation in multiple Phase II trials. (Avecia, U.K.; VaxGen, Inc., Brisbane, CA)
- NIAID established an animal studies group that includes representatives from NIAID, the Food and Drug Administration (FDA), and other government agencies. The group designs study protocols for both rabbit and NHP models to define correlates of protection for post-exposure prophylaxis and general-use prophylaxis indications. Studies are being performed for the rPA vaccine manufacturers under a separate NIAID contract. (Battelle, Columbus, OH)
- NIAID has expanded its Vaccine and Treatment Evaluation Unit (VTEU) network and used these resources to conduct clinical trials on anthrax vaccines.
  - A Phase I clinical trial for a DNA-based vaccine against anthrax was conducted at the Baylor College of Medicine and University of Rochester VTEUs. (Vical Inc., San Diego, CA)
  - A Phase I clinical trial of an rPA protein-only vaccine candidate was conducted at the University of Maryland VTEU. This trial provided proof-of-concept for the protein-only vaccine approach.
- In order to support increased clinical research activities, NIAID has expanded contracts for regulatory support, assay development, immunology quality assurance and quality control, and clinical trial management.

GOAL: Screen existing FDA-approved antimicrobials and immunomodulators for efficacy against anthrax.

- Under the *In Vitro* and Animal Models for Emerging Infectious Diseases and Biodefense contracts, awarded in FY2003, approved antibiotics are being evaluated for post-exposure efficacy against inhalational anthrax in animal models (small animals as well as NHPs). Currently, animal model development is ongoing, along with pharmacokinetic analysis, in order to bridge animal dosing to human pharmacokinetic parameters. (Health Protection Agency, U.K.; Southern Research Institute, Birmingham AL; SRI International, Menlo Park CA; and University of Texas Medical Branch-Galveston)
- Under the *In Vitro* and Animal Models for Emerging Infectious Diseases and Biodefense contracts, and a cooperative agreement with the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), NIAID is conducting studies to determine whether the course of antibiotic therapy can be decreased by vaccinating subjects with the rPA vaccine candidates currently under development. Two NIAID-sponsored projects demonstrate that the combination of antibiotics plus anthrax vaccines provide complete protection against death in anthrax-infected animals.
  - In NHPs infected by aerosolized anthrax exposure, antibiotic therapy in combination with immediate post-infection BioThrax® vaccination provided protection

against death in 100 percent of animals tested. (USAMRIID, Fort Detrick, MD)

– In rabbits infected with aerosolized *B. anthracis*, the combination of short duration antibiotic with two vaccinations, started a half day after exposure, resulted in statistically significant increases in survival over antibiotic treatment alone. This animal model was effective in demonstrating efficacy of three vaccines: BioThrax which is currently licensed for pre-exposure use, as well as two rPA vaccines in development. Development of this model overcomes a significant hurdle in the licensure process for a post-exposure indication, and is applicable to vaccines as well as other therapeutics that may be used in combination with antibiotics.

GOAL: Conduct comparative genomic sequencing of selected *Bacillus* strains to detect subtle differences in the pathogenesis and virulence associated with antigens or other factors.

- The Pathogen Functional Genomics Resource Center (PFGRC) completed the genome sequencing of a number of different species, strains, clinical isolates, and nearest neighbors of *B. anthracis*. In addition, comparative genomic analysis has been conducted and genetic variations and relatedness within and between species identified. Sequencing projects have been completed for the *B. anthracis* strains, Kruger B and Western North America strain, and a related species, *B. cereus*. In addition, new genomic software tools have been developed to enhance comparative genomic analyses. (See also Scientific Progress section, Complete genome of anthrax bacteria is sequenced.) (The Institute for Genomic Research [TIGR], Rockville, MD)

- NIAID, in collaboration with the Department of Energy and the Office of Naval Research, recently completed sequencing of the Ames strain of *B. anthracis*. In addition, four more strains of *B. anthracis* (France CNEVA 9066, A01055, Vollum A4088, and Australian 94 A0039) and two *B. cereus* strains (ATCC 10987 and G9241) have been completed. (TIGR, Rockville, MD)

  The PFGRC has developed tools, technologies, and software for detecting genetic variation among different strains and clinical isolates, providing potential genetic signatures for diagnosis. In addition, PFGRC is using DNA microarray technology to identify polymorphisms for a number of *Bacillus* strains. (TIGR, Rockville, MD)

GOAL: Establish a centralized immunology laboratory to develop and validate tests required for the licensure of anthrax vaccines.

- With assistance from NIAID staff and consultants, NIAID-supported researchers have developed and are validating serological assays required for the licensure of anthrax vaccines. (Battelle, Columbus, OH)

- NIAID is collaborating with the Centers for Disease Control and Prevention (CDC) for the development of immunogenicity assays to be used for evaluation of a second-generation anthrax vaccine based on rPA. CDC is establishing standard reagents and validating a number of critical serologic assays that will be used to evaluate samples from small animals, NHPs, and humans.

- In April 2002, NIAID co-sponsored a workshop entitled Anthrax Vaccines that focused on anthrax vaccine history, histology of infection, animal models, human immune responses, assay validation, and approaches to establishing surrogate markers of immunity for licensing an anthrax vaccine. Co-sponsors included FDA, the Joint Vaccine Acquisition Program (JVAP), and the Department of Defense (DoD).

GOAL: Develop and evaluate *in vivo* transmission and spore germination models.

- Under the *In Vitro* and Animal Models for Emerging Infectious Diseases and Biodefense contracts, NIAID-supported investigators have developed and standardized a new mouse model for studying inhalational anthrax that enables examination of the kinetics of dissemination of infection after exposure to spores as well as specifics of pathogenesis. See also Scientific Progress section, New mouse model for pulmonary anthrax.

GOAL: Identify and characterize new virulence and pathogenicity factors.

- NIAID has funded more than 50 research projects on the identification of virulence factors to better understand the pathogenesis of *B. anthracis*. This includes research on the interactions between *B. anthracis* and macrophages; spore surface antigens and how spores survive and germinate within host cells; the characterization of anthrax toxins and how they produce their lethal effects; plasmids and their association with virulence and the production of toxins; as well as the genetic basis for all of these processes. Among the most significant findings are the following, each of which suggest potential targets for therapeutic intervention:

– *B. anthracis* requires siderophore biosynthesis for growth in macrophages and for virulence.

– Anthrax LT inhibits the transcriptional induction of inflammatory cytokines.

– The membrane insertion of anthrax PA and cytoplasmic delivery of LF occur at different stages of the endocytic pathway.

See also Scientific Progress section, *B. anthracis* physiology and toxin activity further elucidated.

- NIAID-supported scientists are using proteomics techniques to identify suitable vaccine candidates and virulence factors that can be exploited for use in rapid and sensitive diagnostic procedures.

GOAL: Identify targets within innate and adaptive pathways that can be used to modulate infection.

- NIAID established a network of seven Biodefense Proteomics Research Centers (PRCs) in FY2004 (see Appendix A). These Centers support development and application of innovative proteomic technologies to help scientists better understand proteomes of pathogens and host cells, with the goal of developing targets for therapeutic development, including immunotherapies. Anthrax-specific projects being conducted through the PRCs include:

  – The use of proteomics and genomics to document the protein and gene expression patterns during all phases of the *B. anthracis* life cycle, with a particular focus on its germination and growth inside host macrophages. Scientists determined that approximately 490 host genes were expressed differently when the host was infected with *B. anthracis*. These genes/proteins represent host targets that may potentially be exploited for vaccines and therapeutics. (University of Michigan)

  Conducting screening for interacting host/pathogen proteins using industrial-scale yeast two-hybrid analysis. This project covers five Category A pathogens, including *B. anthracis*. Scientists identified 15 specific interacting host proteins for this pathogen, 7 for the protective antigen, and 8 for lethal factor. Work is ongoing to look for further interactions and to test the 15 identified interacting proteins as potential targets for therapeutics and/or diagnostics. (Myriad Genetics, Inc., Salt Lake City, UT)

GOAL: Identify and characterize innate and adaptive immune responses that occur after initial exposure to anthrax, including responses associated with spore germination.

- NIAID physician-researchers initiated a clinical protocol in 2002 to study the natural history of anthrax. The goal is to look at the infectious disease process over time, from initial infection through the clinical course and beyond recovery. A small number of anthrax survivors from the 2001 attacks have enrolled. Because the medical literature on anthrax does not include any findings regarding long-term complications in survivors, information gained in this study will be valuable to patients and doctors.

- NIAID-supported investigators have demonstrated that monoclonal antibodies specific for the capsular material of *B. anthracis* can confer protective immunity, thereby indicating potential for use as a vaccine. See also Scientific Progress section, Anthrax monoclonal antibody may have potential as a vaccine.

- NIAID awarded grants to develop a knowledge base of innate immune system activity. As one example, researchers at Scripps Research Institute in La Jolla, CA, will identify genetic changes and proteins that are triggered by encounters between innate immune cells and infectious pathogens and will study anthrax toxin interactions with innate immune receptors.

Smallpox

Smallpox, which is caused by the virus variola major, is considered one of the most dangerous potential biological weapons because it is easily transmitted from person to person, no effective therapy exists, and few people carry full immunity to the virus. Although a worldwide immunization program eradicated smallpox disease in 1977, small quantities of smallpox virus still exist in two secure facilities in the United States and Russia. However, it is likely that unrecognized stores of smallpox virus exist elsewhere in the world.

The symptoms of smallpox infection appear approximately 12 days (the range is from 7 to 17 days) after exposure. Initial symptoms include high fever, fatigue, headache, and backache. A characteristic rash, which is most prominent on the face, arms, and legs, follows in two to three days. The rash starts with flat red lesions (a maculopapular rash) that evolve into vesicles. Unlike chickenpox, the lesions associated with smallpox evolve at the same rate. Smallpox lesions become filled with pus and begin to crust early in the second week after exposure. Scabs develop, separate, and fall off after approximately three weeks. Individuals are generally infectious to others from the time immediately before the eruption of the maculopapular rash until the time scabs are shed. Smallpox spreads directly from person to person, primarily by aerosolized saliva droplets expelled from an infected person. Contaminated clothing or bed linens also can spread the virus. The mortality of smallpox infection is approximately 30 percent, and patients who recover frequently have disfiguring scars.

On December 13, 2002, President George W. Bush announced a plan to protect Americans against the threat of a smallpox bioterrorist attack. Under the plan, the Department of Health and Human Services (DHHS) is working with state and local governments to form volunteer "Smallpox Response Teams" that can provide critical services in the event of attack. To ensure that these teams can mobilize immediately in an emergency, health care workers and other critical personnel are being asked to volunteer to receive the smallpox vaccine. In addition, the Department of Defense (DoD) has vaccinated certain military and civilian personnel who are or may be deployed in high-threat areas.

SCIENTIFIC PROGRESS

In the years since publication of the *NIAID Biodefense Research Agenda for CDC Category A Agents* in February 2002, significant progress has been made in understanding the variola virus and how it causes disease, and in developing countermeasures against its intentional release. Key advances are included below.

Biology of the Microbe

Understanding of poxvirus pathogenesis has improved. Vaccinia virus, the immunizing agent used to help eradicate smallpox, encodes over 20 genes that regulate the immune response to the virus in the infected host. There has been substantial progress in understanding these genes' pathogenic effects and mechanisms.

- **Vaccinia E3L gene may play a role in helping the virus overcome host defenses.** During poxvirus infection, the virus takes over protein production machinery of the host cell to make the proteins it needs to survive. One way that host cells defend themselves against infection is by turning off their own protein production so that the virus no longer has a source of needed proteins. A National Institute of Allergy and Infectious Diseases (NIAID)-supported study has revealed that the vaccinia E3L gene may allow the virus to overcome this host defense mechanism. The E3L gene is used by the virus to prevent the host cell from shutting down its protein production, thereby ensuring that the host will continue making viral proteins needed for viral survival.

  (Langland JO et al., Inhibition of PKR by vaccinia virus: role of the N- and C-terminal domains of E3L, *Virology* 2004;324:419-429)

- **Vaccinia gene K1L may assist virus in overcoming host immune response.** NIAID-supported scientists have discovered that a vaccinia gene known to be involved in pathogenesis plays a role in the host's immune response. The K1L gene has been shown to inhibit expression of a cellular gene involved in the host's inflammatory response to infection. This suggests that by reducing host immune responses, the K1L gene may enable the vaccinia virus to survive in the host for longer periods of time. Since the K1L gene has been known to be deleted in the attenuated vac-

cine strain modified vaccinia Ankara (MVA), this mechanism may partially explain the reduced pathogenicity of this vaccine strain.

(Oie KL et al., Cowpox virus and other members of the orthopoxvirus genus interfere with the regulation of NF-kappa B activation, *Virology* 2001;288:175-187;

Shisler JL et al., The vaccinia virus K1L gene product inhibits host NF-kappaB activation by preventing I kappaB alpha degradation, *J Virol* 2004;78:3553-3560)

- **Biological function of poxvirus enzyme revealed.** All poxviruses have a highly conserved DNA topoisomerase. Although the structure and catalytic activity of this enzyme were well studied, little was known about its biological function. NIAID scientists found that the primary, perhaps only, role of the poxvirus topoisomerase is to increase early transcription, which takes place within virus cores in the cytoplasm of infected cells. Because the topoisomerase functions early in infection, drugs capable of penetrating the virus core and irreversibly damaging DNA by trapping nicked DNA-topoisomerase intermediates could make potent antiviral agents.

  (Da Fonseca F and Moss B, Poxvirus DNA topoisomerase knockout mutant exhibits decreased infectivity associated with reduced early transcription, *Proc Natl Acad Sci USA* 2003;100(20):11291-11296)

- **Role of E3L gene in poxvirus attenuation uncovered.** NIAID-supported researchers determined that deletion of the vaccinia gene E3L, which is part of the interferon gamma evasion system in poxviruses, attenuates the virus to such an extent that it appears to be nonpathogenic in severe combined immunodeficiency (SCID) mice. This suggests that E3L-deleted viruses may be the basis for safer vaccines if MVA proves not to be as efficacious as hoped.

  (Xiang Y et al., Deletion of vaccinia gene E3L makes virus apparently nonpathogenic in SCID mice, *J Virol* 2002;76:5251-5259)

- **New understanding of E3L protein structure offers promise for anti-smallpox drugs.** In another study, NIAID-supported researchers found that the domain of the E3L protein that binds to Z-DNA is important for pathogenesis; that is, Z-DNA binding per se is important for pathogenesis. This result has two important implications. Because variola virus contains a protein with a similar Z-DNA binding motif, Z-DNA binding could be a useful target for new anti-smallpox drugs. These results also may provide an opportunity to gain some insight into the role of Z-DNA in normal cells, which is currently not well understood .

  (Kim Y-G et al., A role for Z-DNA binding in vaccinia virus pathogenesis, *Proc Natl Acad Sci USA*, 2003;100:6974-6979)

- **Role of interleukin-4 (IL-4) gene in mousepox pathogenesis revealed.** Researchers have also made strides in understanding how these viruses' pathogenic potential can be altered either to decrease or increase virulence. Australian scientists showed that introducing the murine IL-4 gene into ectromelia (mousepox) virus increased the virus's pathogenicity to the extent that standard vaccines were no longer protective. (This work was supported by the Australian government.) Although publication of these results generated concern within the scientific community, they provide both an impetus and an opportunity to develop countermeasures against such engineered viruses. Recent experiments by NIAID-supported scientists have shown that a combination therapy is capable of protecting mice against these engineered viruses: the combination of two doses of the standard smallpox vaccine, which provides a greater immune response than the normal single dose, and the antiviral drug cidofovir, provided protection for both susceptible and genetically resistant mice.

  (Jackson RJ et al., Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox, *J Virol* 2001;75:1205-1210)

  As of 2006, NIAID-supported scientists have expanded on previous research to identify mechanisms by which IL-4 increases pathogenicity of ectromelia virus. Research is ongoing to explore other potential methods of therapy that could be used generally as countermeasures for infection by poxviruses that have been engineered for increased virulence. Recent experiments have shown that a combination of two antiviral drugs (cidofovir and ST-246), but neither drug alone, protects mice against the highly pathogenic IL-4 ectromelia. (Saint Louis University)

Researchers gain better understanding of poxvirus entry into cells and virion formation and release. A productive infection by vaccinia virus involves entry into the cell; expression and replication of the genome; defense against specific and nonspecific host immune mechanisms; and assembly and release of infectious, enveloped, virus particles. Researchers have identified poxvirus proteins needed for these processes and have better defined the mechanisms by which poxvirus

virions are formed in and released from infected cells. Greater understanding of these processes should lead to the identification of new targets for antiviral therapy and to a clearer picture of how the virus spreads from cell to cell in the infected host. Advances made by NIAID intramural scientists include:

- Identification of a novel redox system encoded by virus that is responsible for forming disulfide bonds in virion proteins.

  (Senkevich TG et al., Complete pathway for protein disulfide bond formation encoded by poxviruses, *Proc Natl Acad Sci USA* 2002;99:6667-6672)

- Identification of virion-associated proteases.

  (Byrd CM et al., The vaccinia virus I7L gene product is the core protein proteinase, *J Virol* 2002;76:8973-8976)

- Additional studies conducted by NIAID scientists have further characterized the role of virion-associated proteases in vaccinia infection.

  (Ansarah-Sobrinho C and Moss B, Vaccinia virus G1 protein, a predicted metalloprotease, is essential for morphogenesis of infectious virions but not for cleavage of major core proteins, *J Virol* 2004;78:6855-6856;

  Ansarah-Sobrinho C and Moss B, Role of the I7 protein in proteolytic processing of vaccinia virus membrane and core components, *J Virol* 2004;78:6335-6343)

- Determination of the pathway by which virion membranes are formed and infectious virions exit from the cell.

  (Szajner P et al., Vaccinia virus G7L protein interacts with the A30L protein and is required for association of viral membranes with dense viroplasm to form immature virions, *J Virol* 2003;77:3418-3429)

- Additional studies conducted by NIAID scientists have advanced knowledge regarding viral assembly pathways and proteins involved in virion formation.

  (da Fonseca FG et al., Vaccinia virus mutants with alanine substitutions in the conserved G5R gene fail to initiate morphogenesis at the nonpermissive temperature, *J Virol* 2004;78:10238-10248;

  Resch W, Weisberg AS, Moss B, Vaccinia virus nonstructural protein encoded by the A11R gene is required for formation of the virion membrane, *J Virol* 2005;79(11):6598-6609;

  Szajner P et al., A complex of seven vaccinia virus proteins conserved in all chordopoxviruses is required for the association of membranes and viroplasm to form immature virions, *Virology* 2004;330:447-459;

  Szajner P et al., External scaffold of spherical immature poxvirus particles is made of protein trimers, forming a honeycomb lattice, *J Cell Biol* 2005 Sep 12;170(6):971-981;

  Ward BM and Moss B, Vaccinia virus A36R membrane protein provides a direct link between intracellular enveloped virions and the microtubule motor kinesin, *J Virol* 2004;78:2486-2493)

- NIAID scientists have identified poxvirus proteins necessary for viral entry into cells. These proteins are conserved in all poxviruses analyzed to date, indicating that the mechanism of poxvirus entry into cells is also conserved.

  (Senkevich TG, Moss B, Vaccinia virus H2 protein is an essential component of a complex involved in virus entry and cell-cell fusion, *J Virol* 2005;79(8):4744-4754;

  Townsley AC, Senkevich TG, Moss B, The product of the vaccinia virus L5R gene is a fourth membrane protein encoded by all poxviruses that is required for cell entry and cell-cell fusion, *J Virol* 2005;79(17):10988-10998;

  Townsley AC, Senkevich TG, Moss B, Vaccinia virus A21 virion membrane protein is required for cell entry and fusion, *J Virol* 2005;79(15):9458-9469;

  Senkevich TG et al., Poxvirus multiprotein entry-fusion complex, *Proc Natl Acad Sci USA* 2005;102(51):18572-18577;

  Ojeda S, Senkevich TG, Moss B, Entry of vaccinia virus and cell-cell fusion require a highly conserved cysteine-rich membrane protein encoded by the A16L gene, *J Virol* 2006;80(1):51-61)

Host Response

NIAID scientists develop a high-throughput test to measure immune response induced by smallpox vaccines. Although development of a lesion at the site of scarification with vaccinia virus is sufficient to confirm the potency of the present smallpox vaccine, a battery of immunological assays is necessary to evaluate new ones. The plaque reduction assay, the classical method of determining neutralizing antibody titers, is cumbersome, labor-intensive, and impractical for large numbers of samples. NIAID scientists developed a 96-well plate, semi-automated, flow cytometric assay that uses a recombinant vaccinia virus expressing enhanced green fluorescent protein. The new assay is 5- to 10-fold more sensitive than the standard plaque reduction assay and is faster and less expensive to perform.

(Earl PL, Americo JL, Moss B, Development and use of a vaccinia virus neutralization assay based on flow cytometric detection of green fluorescent protein, *J Virol* 2003;77(19):10684-10688)

Immune response to the vaccinia virus has been further characterized. Although the previously licensed smallpox vaccine was given to hundreds of millions of people in the past, routine vaccination was halted before the research tools needed to characterize the immune response to vaccinia became available. This lack of understanding of the details of the immune response to vaccination has hampered the development of new vaccines. Progress in this area is now beginning to emerge.

- **Recombinant vaccinia vaccine induces protective immunity.** A key element in the design of new, safer smallpox vaccines is identifying vaccinia-encoded proteins that induce protective immunity. NIAID intramural investigators have recently shown that optimal protective immunity requires immunization with outer membrane proteins of both major forms of infectious virions, intracellular mature virus (IMV) and extracellular enveloped virus (EEV). In their experiments, these scientists demonstrated that while immunization with recombinant vaccines containing either IMV or EEV proteins provided partial immunity, recombinant vaccines containing combinations of both IMV and EEV proteins provided complete protection to mice immunized and subsequently challenged with virus. These studies suggest the feasibility of a multiprotein vaccine for smallpox.

  (Fogg C et al., Protective immunity to vaccinia virus induced by vaccination with multiple recombinant outer membrane proteins of intracellular and extracellular virions, *J Virol* 2004;78:10230-10237)

- **MVA protects mice from lethal vaccinia challenge with molecularly modified virus expressing IL-4.** IL-4 is known to enhance virulence of poxviruses. Thus, scientists at NIAID's Vaccine Research Center (VRC) conducted preclinical studies of MVA vaccination in mice, followed by challenge with a vaccinia expressing murine IL-4. In unimmunized mice, nasal challenge with naturally occurring vaccinia caused significant, but self-limited illness, while recombinant vaccinia expressing murine IL-4 resulted in a lethal infection. Immunization with MVA prevented illness and reduced virus titer in mice challenged with either type of vaccinia. MVA induced a dose-related neutralizing antibody and vaccinia-specific T-cell response. These data support the continued development of MVA as an alternative candidate vaccine for smallpox.

  (McCurdy LH et al., Modified vaccinia Ankara immunization protects against lethal challenge with recombinant vaccinia expressing murine IL-4, *J Virol* 2004;78:12471-12479)

- **A critical step forward in refining the mouse model for studies of basic immunology of poxvirus protection.** NIAID intramural scientists described poxvirus determinants recognized by mouse CD8+ T cells and their biological relevance in protection against lethal poxvirus infections. They identified five peptide determinants that account for approximately half of the vaccinia-specific CD8+ T-cell response in mice and showed that the primary immunodominance hierarchy is greatly affected by the route of vaccinia infection and the poxvirus strain used. MVA failed to induce responses to two of the defined determinants. These findings have important implications for understanding poxvirus immunity in animal models and for evaluating immune responses to poxvirus vaccines in humans. Understanding route-dependent effects on immunogenicity is critical to interpreting vaccine trials, such as comparing intramuscular immunization with MVA to standard scarification with Dryvax.

  (Tscharke DC et al., Identification of poxvirus CD8+ T cell determinants to enable rational design and characterization of smallpox vaccines, *J Exp Med* 2005;201(1):95-104)

- **Three-dimensional structure of a potent target for poxvirus neutralizing antibodies determined.** NIAID intramural scientists determined the three-dimensional structure and shape of the poxvirus L1 protein, a molecule that is conserved throughout the poxvirus family and is nearly identical in vaccinia virus and variola virus. L1 is a myristoylated envelope protein that is a potent target for neutralizing antibodies and an important component of current experimental vaccines. The L1 structure reveals a novel protein fold with a hydrophobic cavity located adjacent to its amino terminus. The cavity would be capable of shielding the myristate moiety, which is essential for virion assembly. The structure of L1 is a step in elucidating molecular mechanisms common to all poxviruses that may stimulate the design of safer vaccines and new anti-poxvirus drugs.

  (Su HP et al., The 1.51-Angstrom structure of the poxvirus L1 protein, a target of potent neutralizing antibodies, *Proc Natl Acad Sci USA* 2005;102:4240-4245)

- **Rational vaccine design for smallpox through characterization of poxvirus peptides.** Dryvax, the only licensed vaccine against smallpox, uses live vaccinia virus and has associated complications that make it inappropriate for use in some populations. One possible approach to making a safer smallpox vaccine would be to use synthesized protein

peptides from the virus, rather than the whole live virus. These protein peptides, or epitopes, are recognized by the immune system and targeted by antibodies, killer T cells, or both. However, the specific epitopes from vaccinia and variola viruses that activate killer T cells to establish protective immunity remain largely unknown. To identify the epitopes recognized by human killer T cells, NIAID-funded researchers employed a multistep process involving computational tools to predict the epitopes, and laboratory tests to measure the killer T-cell responses against these epitopes in individuals vaccinated with the Dryvax vaccine. Multiple epitopes were discovered from viral proteins involved in virulence and viral gene regulation. Some of the novel epitopes were recognized by T cells from multiple vaccinated individuals, indicating that these epitopes may be promising as vaccine candidates or as diagnostic tools.

(Oseroff C et al., HLA class I restricted responses to vaccinia recognize a broad array of proteins mainly involved in virulence and viral gene regulation, *Proc Nat Acad Sci USA* 2005;102:13980-13985)

- **Newly identified vaccinia epitopes recognized by human killer T cells.** Protein antigens from pathogens are processed into small fragments called epitopes by specialized cells called antigen presenting cells (APC). Immune responses in T cells are initiated when T cells recognize foreign/pathogen epitopes that are presented on the surface of the APC. NIAID-supported researchers identified 16 new epitopes from vaccinia proteins that are recognized by killer T cells. The epitopes were verified and shown to be vaccinia-specific by functional assays using killer T cells from recently vaccinated individuals. The novel epitopes were from proteins spanning all stages of the viral cycle: early, intermediate, and late. In addition, the viral proteins represented a wide range of functions such as enzymes, transcription factors, structural proteins, and proteins involved in immune evasion. Many of the epitopes were conserved among a number of vaccinia strains. This work may aid the development of new vaccines and protective therapies against vaccinia and related poxviruses.

  (Jing L. et al., Diversity in the acute CD8 T cell response to vaccinia virus in humans, *J Immunol* 2005;175:7550-7559)

- **Requirement for both antibodies and killer T cells to combat poxviruses.** This study focuses on the importance of using a host-specific pathogen when studying host immune response. The orthopoxvirus ectromelia, which is in the same group as the smallpox variola virus, is a highly pathogenic virus that naturally infects mice, causing mousepox. NIAID-supported researchers demonstrated that both killer T cells and antibodies produced by B cells were critical to control viral infection in the natural host. This work showed that even though killer T cells were necessary for viral control, they alone were not sufficient to eliminate this highly pathogenic virus. The killer T-cell responses were effective during the early stages of infection, but were not able to clear the virus without the aid of subsequent antibody production. These findings are important for the development of vaccines and therapeutics for human poxviruses and many other cytopathic viruses.

  (Fang M et al., Antibodies and CD8+ T cells are complementary and essential for natural resistance to a highly lethal cytopathic virus. *J Immunol* 2005; 175:6829-6836)

- **Humanized monoclonal antibody to vaccinia B5 protein neutralizes vaccinia and smallpox viruses and protects mice against vaccinia.** Chimpanzee antibody fragments or Fabs, that recognize the B5 envelope glycoprotein of vaccinia virus were isolated and converted into complete humanized monoclonal Abs (mAbs). One mAb inhibited the spread of both vaccinia and variola *in vitro*, protected mice from subsequent intranasal challenge with virulent vaccinia, protected mice when administered two days after challenge, and provided significantly greater protection than that afforded by vaccinia immune globulin. These mAbs may be useful in prevention and treatment of vaccinia-induced complications from vaccination against smallpox and may also be effective in immunoprophylaxis and immunotherapy of smallpox.

  (Chen Z et al., Chimpanzee/human mAbs to vaccinia virus B5 protein neutralize vaccinia and smallpox viruses and protect mice against vaccinia virus, *Proc Natl Acad Sci USA* 2006;103(6):1882-1887)

- **Defective Immune System Response to Smallpox Vaccine Linked with Overproduction of IL-4 and IL-3.** NIAID-supported scientists have identified a defect in the immune response of people with atopic dermatitis that puts them at risk of developing serious complications following smallpox vaccination. The researchers showed that LL-37, an immune system protein, is critical for controlling replication of vaccinia virus. Overproduction in skin cells of inflammation-promoting molecules IL-4 and IL-3 hampers LL-37 activity in those with atopic dermatitis, limiting their ability to control viral replication. These findings may lead

to new approaches that would allow people with atopic dermatitis to safely be vaccinated against smallpox.

(Howell MD et al., Cytokine milieu of atopic dermatitis skin subverts the innate immune response to vaccinia virus, *Immunity* 2006;24(3):341-348)

- **Study shows previous smallpox vaccination may provide long-lasting protection.** While most U.S. citizens over 30 years of age have been vaccinated against smallpox, the extent of their immunity is unclear. A small but significant study of such people showed that the duration of cell-mediated immunity in previously vaccinated individuals might be greater than previously thought. Vaccinia-specific CD8+ lymphocyte response in people vaccinated 6 to 35 years earlier averaged greater than one-half that of recently vaccinated individuals (4 percent of CD8+ lymphocytes responded to vaccinia vs. 6.5 percent in the recently vaccinated). This NIAID-supported study suggests that previous smallpox vaccination, even if it occurred many years ago, may provide at least some protection.

  (Frelinger JA et al., Responses to smallpox vaccine, *N Engl J Med* 2002; 347:689-690)

- **Scientists identify a poxvirus epitope that induces T-cell immune response.** Researchers in Germany have identified a vaccinia T-cell epitope that is conserved in variola. Vaccinated mice and humans both had specific T-cell responses to this epitope, and in vaccinated mice, CD8+ responses specific to this epitope correlate with protection against lethal intranasal challenge with vaccinia. MVA-vaccinated mice have the same level of CD8+ responses to this epitope as Dryvax-vaccinated mice. (Work was supported by German government.)

  (Drexler I et al., Identification of vaccinia virus epitope-specific HLA-A*0201-restricted T cells and comparative analysis of smallpox vaccines. *Proc Natl Acad Sci USA* 2003;100:217-222)

- **Scientists identify additional poxvirus peptides that induce T-cell immune response.** NIAID-funded investigators identified two additional CD8+ T cell epitopes that are both highly conserved in vaccinia and variola. T cells recognizing these epitopes represented a relatively large percent of all vaccinia-reactive cells, and were still detected as long as three years after immunization.

  (Terajima M et al., Quantitation of CD8+ T cell responses to newly identified HLA-A*0201-restricted T cell epitopes conserved among vaccinia and variola (smallpox) viruses, *J Exp Med* 2003;197:927-932)

Vaccines

Potential correlates of reactogenicity to the smallpox vaccine. Vaccinia virus, the component of the smallpox vaccine that elicits a protective immune response, also can cause adverse reactions in some vaccinees. Researchers at one of NIAID's Vaccine and Treatment Evaluation Units (VTEUs) conducted a study to determine which components of the immune response may be involved in protective immunity or adverse vaccine responses. Researchers examined cytokines present in the serum of volunteers receiving the Aventis Pasteur smallpox vaccine (APSV) and demonstrated that all vaccine recipients showed a burst of interferon-gamma in the serum at one week post-vaccination which then declined with time. However, individuals who reported one or more adverse reactions to vaccination also had increased serum levels of the cytokines TNF-alpha, interleukin (IL)-2, IL-4, and IL-10 at one week post-vaccination, and these cytokines remained elevated after several weeks. The results of this study demonstrate an association between certain adverse events resulting from APSV smallpox vaccination and increased cytokine levels, suggesting the possibility that more serious adverse events resulting from smallpox vaccination may also be associated with cytokine level alterations.

(Rock MT et al., Adverse events after smallpox immunizations are associated with alterations in systemic cytokine levels, *J Infect Dis* 2004;189:1401-1410)

Longevity of immunity against smallpox after vaccination. NIAID-supported researchers assessed antibody- and antigen-specific B cells present in human serum in persons who had received Dryvax smallpox vaccination 1 to 50 years prior to the study. The researchers found that virus-specific antibody was present in a fraction of the circulating IgG and that persisting memory B cells sampled were still able to respond to revaccination even 50 years after the original immunization. These studies demonstrated stable and equivalent B-cell memory and serum antibody levels present at 10-year increments over 50 years, following an initial decline that occurred immediately after the acute phase vaccination response had ended.

(Crotty S et al., Cutting edge: long-term B cell memory in humans after smallpox vaccination, *J Immunol* 2003;171:4969-4973)

Modified vaccinia ankara (MVA) may be a safer, effective alternative for persons who cannot receive the current U.S.-licensed smallpox vaccine. Approximately 25 percent of the population cannot receive Dryvax because they are at increased risk for post-vaccine complications. NIAID scientists and their collaborators compared immunization with

MVA and Dryvax in a monkey model. After two doses of MVA or one MVA dose followed by Dryvax, the immune response was equivalent or higher than that induced by Dryvax alone. After challenge with monkeypox virus, unimmunized animals developed greater than 500 pustular skin lesions and became gravely ill or died, whereas vaccinated animals were healthy and asymptomatic, except for a small number of transient skin lesions in animals immunized only with MVA. These findings are important steps in the evaluation of MVA as a replacement vaccine or pre-vaccine for those with increased risk of severe side effects from Dryvax.

(Earl PL et al., Immunogenicity of a highly attenuated MVA smallpox vaccine and protection against monkeypox, *Nature* 2004;428:182-185)

Existing supply of smallpox vaccine can be expanded to protect more Americans. Although smallpox was eradicated worldwide through a successful immunization program, and authorized samples of the virus are contained in only two laboratories in Russia and the United States, unauthorized sources of the virus are believed to exist, increasing the likelihood that smallpox could be intentionally released. The supply of smallpox vaccine available may be insufficient to adequately vaccinate all U.S. residents with the recommended dose. Thus, it is important to determine whether the current supply of the vaccine could be diluted to quickly increase the available number of doses and still provide effective protection.

An NIH-supported clinical study indicates that the existing U.S. smallpox vaccine supply—15.4 million doses—could successfully be diluted at least five times and retain its potency, effectively expanding the number of individuals it could protect. The study compared the effectiveness of full-strength smallpox vaccine to that of 5-fold- and 10-fold-diluted vaccine in 680 adults age 18 to 32 with no history of smallpox vaccination. More than 97 percent of all participants in the study responded with a vaccine "take," a blister-like sore at the injection site that serves as an indirect measure of the vaccine's conferred immunity. Most importantly, the investigators found no significant difference in the take rate of the three doses.

(Frey SE et al., Clinical responses to undiluted and diluted smallpox vaccine, *N Engl J Med* 2002;346:1265-1274)

Diagnostics

Evaluation of methods to detect the smallpox vaccine virus in specimens from patients who were vaccinated. NIH scientists analyzed three different methods to detect the smallpox vaccine virus in specimens from patients who were vaccinated: a shell vial cell culture assay that detects infectious virus, a polymerase chain reaction (PCR) test that detects virus DNA, and a direct fluorescent antibody test (DFA) that detects virus proteins. The PCR test was the most sensitive (100 percent), the cell culture test was next (89 percent), and the DFA was the least sensitive (40 percent). The researchers concluded that the shell vial culture may be useful to determine whether a lesion or dressings are infectious or to obtain an isolate for antiviral susceptibility testing. DFA can provide a result in a few hours when the specimen is properly collected and is more likely to be positive in primary vaccinees. Indirect immunofluorescence with vaccinia monoclonal antibodies (which do not cross-react with HSV-1 or other poxviruses) could be used in the shell vial assay or directly on smears from lesions to increase specificity. Real-time PCR is rapid, specific, and more sensitive than shell vial culture and DFA.

(Fedorko DP et al., Comparison of methods for detection of vaccinia virus in patient specimens, *J Clin Microbiol* 2005;43(9):4602-4606)

Therapeutics

Anticancer drugs offer promising therapy for smallpox. Over the past several decades, thousands of promising anticancer drugs have been developed by pharmaceutical companies to interfere with signal transduction cascades. Two different studies conducted by NIAID-supported researchers have shown that the anticancer drugs CI-1033 and Gleevec each inhibit a late step in the orthopoxvirus life cycle by interfering with biochemical signaling pathways within the host cells. Since viruses are simple in their makeup, having only a small number of genes, they rely on being able to co-opt host cell machinery and cellular processes to replicate and spread. The anticancer drugs CI-1033 and Gleevec interfere with host cell processes limiting spread of the virus and preventing poxvirus-induced disease. This therapeutic approach may prove generally efficacious in treating other infectious diseases that rely on similar host-cell enzymes. Because the drug targets host, not viral, targets, this strategy is much less likely to cause drug-resistance compared to conventional antimicrobial therapies. Based on these findings, anticancer drugs may have promise as antipoxvirus therapeutics. In addition, these new studies suggest that anticancer drugs may more broadly represent a largely untapped source of potential antiviral drugs that could be used against a variety of viruses, and therefore merit further exploration.

(Reeves PM et al., Disabling poxvirus pathogenesis by inhibition of Abl-family tyrosine kinases, *Nature Medicine* 2005; 11:731-739;

Yang H et al., Antiviral chemotherapy facilitates control of poxvirus infections through inhibition of cellular signal transduction, *J Clin Invest* 2005;115:379-387)

Antipoxvirus compound, ST-246, shown to be promising therapy for smallpox. Currently, there is no Food and Drug Administration (FDA)-approved drug for the prevention or treatment of smallpox infection and the use of available vaccines for mass immunizations is not recommended due to the risk of complications in certain individuals. Concerns over the use of variola as a biological weapon have prompted renewed interest in development of small molecule therapeutics that target the virus.

A compound known as ST-246 is active against multiple orthopoxviruses both *in vitro* and in small animal models of disease. ST-246 acts upon a vaccinia gene that encodes a protein required to produce extracellular virus. In animal experiments, oral administration of ST-246 protected mice from lethal vaccinia virus and ectromelia virus infections, completely protected golden ground squirrels from a lethal dose of monkeypox, and inhibited vaccinia virus-induced tail lesions in mice. These results demonstrate that an inhibitor of extracellular virus formation can protect against orthopoxvirus-induced disease in animal models. NIAID scientists are beginning expedited Phase I trials of ST-246.

(Yang G et al., An orally bioavailable antipoxvirus compound (ST-246) inhibits extracellular virus formation and protects mice from lethal orthopoxvirus challenge, *J Virol* 2005;79:13139-13149)

Pill form of cidofovir developed for treatment of smallpox. The public faces a potential health threat from bioterrorist use of dangerous pathogens. The most feared agent in a biological terrorist attack is smallpox virus. Cidofovir has been identified as a potential treatment for smallpox and vaccine complications. This drug is of particular interest as a potential therapy for smallpox because it has already been approved for treatment of cytomegalovirus infection. In its current form cidofovir must be given intravenously, a complicated approach to treating a large civilian population in an emergency situation. Development of a cidofovir pill would provide a more practical solution. Hostetler and colleagues developed a chemical process by which some lipids, or fats, are attached to the drug so it can be more easily taken up into cells following oral administration. Once in the cells, the lipid can be removed so that pure cidofovir is available. The researchers demonstrated that the modified drugs can significantly reduce the growth of virus in tissue culture. These drugs have been tested in animals.

(Kern ER, Enhanced inhibition of orthopox virus replication *in vitro* by alkoxylalkyl esters of cidofovir and cyclic cidofovir, *Antimicrob Agents Chemother* 2002;46:991-995)

PROGRAMMATIC PROGRESS IN ADDRESSING IMMEDIATE GOALS

GOAL: Expand the existing supply of live and attenuated vaccines, with particular emphasis on vaccines with reduced reactogenicity.

- A safer alternative to the current FDA-licensed vaccine, Dryvax, is needed for inducing orthopoxvirus immunity in the general population. Therefore NIAID scientists at the VRC evaluated MVA in two randomized, placebo-controlled, double-blind clinical trials. Healthy vaccinia-naïve or vaccinia-immune adult volunteers received MVA or placebo and were later vaccinated with Dryvax. Vaccination with two or more doses of MVA prior to Dryvax resulted in attenuated clinical signs of lesion formation in more than 96 percent of vaccinia-naïve volunteers, decreased magnitude and duration of viral shedding, and improved immune response. These findings support the potential use of MVA as a vaccine to safely improve immunity to orthopoxviruses in the general population, and for use prior to inoculation in first responders and laboratory workers.
- NIAID has awarded two contracts for advanced development of a next-generation live attenuated smallpox vaccine that can be safely administered to individuals contraindicated for Dryvax. (Bavarian Nordic A/S, Denmark; Acambis, Inc., Cambridge, MA)
- NIAID supports efforts to develop and validate animal models to evaluate safety and efficacy of MVA. These animal models will be important for consideration for licensure of MVA under FDA's animal rule. (Acambis, Inc., Cambridge, MA; Battelle, Columbus, OH; Bavarian Nordic A/S, Denmark; Southern Research Institute, Birmingham, AL; Health Protection Agency, U.K.; IIT Research Institute, Chicago, IL; Lovelace Respiratory Research Institute, Albuquerque, NM; University of Illinois at Chicago).
- NIAID supports the development of several assays to enable characterization of MVA itself as well as to assess immunogenicity in animals and humans following vaccination with MVA. (Acambis, Inc., Cambridge, MA, Bavarian Nordic A/S, Denmark; Cellular Technology Ltd., Cleveland, OH; Focus Diagnostics, Cypress, CA; University of Texas Medical Branch-Galveston (UTMB))

- NIAID established an Atopic Dermatitis and Vaccinia Immunization Network in 2004 to aid in understanding the specific problems encountered by atopic dermatitis patients when innoculated with the smallpox vaccine. Network participants include:
  - Clinical Studies Consortium, National Jewish Medical and Research Center, Denver, CO
  - Animal Studies Consortium, Children's Hospital Boston
  - Statistical and Data Coordinating Center, Rho Federal Systems Division Inc., Chapel Hill, NC

GOAL: Conduct Phase I and II trials with new candidate smallpox vaccines, with particular emphasis on the cell culture vaccines currently under development.

- NIAID awarded contracts in 2004 to support the intermediate-scale advanced development of MVA vaccine as well as clinical evaluation in Phase II trials including trials in special populations (e.g., human immunodeficiency virus (HIV) and atopic dermatitis). (Bavarian Nordic A/S, Denmark; Acambis, Inc., Cambridge, MA)
- NIAID has supported multiple Phase I clinical trials that demonstrated initial safety and immunogenicity of MVA vaccines. So far, MVA has been administered to more than 1,000 healthy individuals under NIAID-funded clinical trials. NIAID is also supporting ongoing Phase I trials in special populations that are contraindicated for Dryvax (e.g., those with HIV or atopic dermatitis). Phase II trials to further evaluate safety and immunogenicity of MVA vaccine in healthy individuals and individuals with HIV and atopic dermatitis have also been initiated. (Bavarian Nordic A/S, Denmark; Acambis, Inc., Cambridge, MA)
- NIAID has funded additional Phase I clinical studies in healthy adults to further study the safety and immunogenicity of MVA. (St. Louis University VTEU, Harvard RCE)
- NIAID awarded contracts to two companies to develop, manufacture, and conduct safety trials of MVA vaccine candidates. (Bavarian Nordic A/S, Denmark; Acambis, Inc., Cambridge, MA).

GOAL: Initiate and expand clinical trials of existing smallpox vaccines.

- Since 2002, NIAID has conducted nine clinical trials with both Dryvax and APSV smallpox vaccines to evaluate the safety and determine the optimal dose for potential use of these vaccines to protect the public in case of a public health emergency. Initial trials determined that limited supplies of Dryvax smallpox vaccine could successfully be diluted at least five-fold and retain its potency. The following sites were involved in these clinical trials: Baylor College of Medicine; Duke University; Kaiser Permanente of Northern California; University of California, Los Angeles; Saint Louis University; Stanford University; University of Cincinnati; University of Iowa; University of Maryland, Baltimore; University of Rochester; and Vanderbilt University.

GOAL: Determine the correlates of immunity for smallpox vaccines through the detailed evaluation of immune responses to Dryvax.

- NIAID intramural scientists developed a high-throughput test to measure immune response induced by smallpox vaccines. This semi-automated assay is 5- to 10-fold more sensitive than the standard plaque reduction assay and is faster and less expensive to perform. See also Scientific Progress section, NIAID scientists develop a high-throughput test to measure immune response induced by smallpox vaccines.
- NIAID is conducting and supporting studies to determine the correlates of immunity in samples collected from volunteers who have been administered Dryvax and other vaccine candidates. This work has included the development of high-throughput virus neutralization assays, which have been optimized and are presently being validated. ELISA assays have also been developed and validated to determine general immune responses to vaccinia virus. Lastly, ELISPOT and internal cytokine staining assays to measure cellular responses to vaccines are under development.
- The cytotoxic T-lymphocyte response of human Dryvax vaccine recipients was measured by ELISPOT as part of one of the NIAID-supported Epitope Discovery contracts. Forty-eight immune epitopes, derived from 35 different vaccinia proteins, were identified and shown to be recognized by T cells from multiple individuals. Viral gene regulation and virulence factors were the most immunogenic viral proteins, whereas viral structural proteins were the least immunogenic. Most of the T-cell epitopes were conserved among two other vaccinia strains, as well as variola major, suggesting a role for them in diagnostic applications and cross-protection.

GOAL: Develop a centralized immunology laboratory to validate assays required for the licensure of smallpox vaccines.

- Through a contract expansion, NIAID is assessing the quality and validity of assays being done on clinical specimens collected through human clinical trials. Included as part of the laboratory support are the following activities: assessing a clinical site's ability to reliably perform immunological assays (proficiency testing); supporting comparative evaluations of cytometric instruments, methods, and reagents; facilitating the development, standardization, and assay characterization of immunological assays for implementation in multi-center investigations; acquiring, characterizing, storing, documenting, and disbursing quality-control materials and reagents; disseminating quality-assessment technical and scientific data; and maintaining a computerized system that supports the Quality Assessment Program. Once validated, immunology assays will be used to generate data to support licensure of smallpox vaccines. (UTMB and Focus Diagnostics, Cypress, CA)

GOAL: Fully characterize activity of cidofovir against poxviruses and ensure that an adequate supply of the drug is available to treat complications from vaccination.

- A clinical protocol to assess activity of cidofovir as a treatment for complications related to smallpox vaccine has received institutional review board (IRB) approval in the VTEUs. This protocol is active should it be needed for response to a public health emergency.

GOAL: Develop animal models for studying smallpox pathogenesis.

- NIAID has expanded existing resources that support animal-model development for emerging viral infections including smallpox. For example:
  - NIAID is supporting development of a nonhuman primate model for testing smallpox vaccines. The primary focus is to determine monkeypox optimal doses and to fully characterize monkeypox pathogenesis using four challenge routes: intravenous, intratracheal, intranasal, and aerosol.
  - NIAID is further evaluating safety of the smallpox vaccines in various immunocompromised/deficient mouse models and time to protection in nonhuman primates. These studies are assessing the efficacy of two different antiviral products: cidofivir and ST-246, a potent, antipoxvirus compound that is administered orally. (Battelle, Columbus, OH; Health Protection Agency, U.K.; IIT Research Institute, Chicago, IL; Lovelace Respiratory Research Institute, Albuquerque, NM; Southern Research Institute, Birmingham, AL; University of Illinois at Chicago)
  - U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) and Centers for Disease Control and Prevention (CDC) researchers have developed two models for studying smallpox in cynomolgus monkeys. After infection with monkeypox virus, these animals die of a disease that is very similar to human smallpox, but which progresses over a shorter period of time. Both models will likely be used in evaluation of new vaccines and therapies.

GOAL: Expand *in vitro* and *in vivo* screening capability for oral antivirals, immunotherapies, and replacements for VIG.

- NIAID-supported researchers have shown that at least two, and possibly many more, anticancer drugs that target cellular signaling pathways inhibit the release of the EEV form of infectious virions from cells and are effective as poxvirus antivirals in animal studies. (Emory University School of Medicine; Pennsylvania State University; Dana Farber Cancer Institute, Boston, MA)
- NIAID-supported scientists are completing the preclinical development of a candidate oral derivative of cidofovir necessary in order to conduct anticipated clinical trials. (Chimerix, Inc., Durham, NC)
- NIAID has expanded its *in vitro* and *in vivo* antiviral screening contracts. To date, nearly 2,000 compounds, including most of the licensed antivirals, have been evaluated for antipoxvirus activities in cell culture. So far cidofovir and its orally active derivatives appear to be the best candidates for treatment of both smallpox disease and vaccine complications. Seven additional compounds displayed antiviral activity in cell culture and are being further evaluated in animal models.
- NIAID has fostered development of compounds with a mechanism of action different from cidofovir's via rational drug design and high-throughput screens. New targets for screens include viral DNA polymerase and processivity factor; topoisomerase; viral core protein protease; S-adenosylhomocysteine hydrogenase; inosine monophosphate dehydrogenase; E3L Z-DNA binding protein; mRNA capping enzyme; H1 phosphatase; and F10 kinase.
- NIAID intramural scientists developed humanized monoclonal antibodies specific to vaccinia virus B5 protein. These mAbs neutralize vaccinia and smallpox viruses and

protect mice against vaccinia virus. They may be useful in preventing and treating complications of smallpox vaccination and in immunoprophylaxis and immunotherapy of the disease. See also Scientific Progress section, Humanized monoclonal antibody to vaccinia B5 protein neutralizes vaccinia and smallpox viruses and protects mice against vaccinia.

- NIAID-supported researchers have developed a new approach to antibody therapy for smallpox. This approach involves production of monoclonal antibodies directed against virus-encoded pathogenesis factors rather than against of one of the viral gene products that is required for replication. The specific targets of this new therapeutic approach are variola virus-encoded growth factor, which is released from infected cells and stimulates cells that express the Epidermal Growth Factor Receptor, and a variola-encoded protein that inhibits complement. Studies in mice have shown that both antibodies greatly increase the protective effect of a conventional neutralizing monoclonal antibody when the two types of antibodies are combined.

GOAL: Validate current diagnostics for orthopoxvirus infections and other vesicular rashes (e.g., chickenpox).

- NIH scientists analyzed three different methods to detect the smallpox vaccine virus in specimens from patients who were vaccinated: a shell vial cell culture assay that detects infectious virus, a PCR test that detects virus DNA, and a direct fluorescent antibody test (DFA) that detects virus proteins. Based on the speed and sensitivity of each method, the researchers identified the usefulness of each of these methods for different clinical and laboratory applications. See also Scientific Progress section, Evaluation of methods to detect the smallpox vaccine virus in specimens from patients who were vaccinated.

- Bioinformatics tools are being used to develop forensic assays that may be capable of determining whether variola virus has been genetically modified. Multiple probes that align with different portions of the viral genome are being developed. Comparative sequencing has also paved the way for the development of rapid DNA-based diagnostic tests to differentiate between smallpox and other diseases with which it might initially be confused. Further, in collaboration with Affymetrix, microarray/chip approaches to sequencing viral genomes are being set up to rapidly examine viral genomes.

- NIAID has worked with USAMRIID and CDC to develop real-time PCR assays to detect and distinguish between smallpox and other orthopox viruses. These assays were critical for the diagnosis of monkeypox during the outbreak that occurred in the United States during 2003.

GOAL: Conduct comparative genomic sequencing of additional poxvirus strains to detect potential differences in pathogenesis and virulence.

- The Viral Bioinformatics Resource Center (formerly the Poxvirus Bioinformatics Resource Center) provides sequencing and functional comparisons of orthopox genes; designs and maintains a relational database to store, display, annotate and query genome sequences, structural information, phenotypic data, and bibliographic information; and serves as a repository of well-documented viral strains. The center began as a collaborative effort led by two academic centers (St. Louis University and the University of Alabama at Birmingham) supported by two federal agencies (Defense Advanced Research Projects Agency and the National Institutes of Health (NIH)). Collaborators include the CDC, American Type Culture Collection, USAMRIID, the Medical College of Wisconsin, and the University of Victoria. The center provides functional annotation and comparisons of orthopox viral genes, as well as genes from other Category A-C virus families.

- To date more than 30 distinct isolates of variola virus and 5 strains of vaccinia virus have been sequenced by CDC, NIAID, and other groups. In addition, NIAID has supported the sequencing of other important poxviruses. These include ectromelia (mousepox), rabbitpox, camelpox, and monkeypox viruses. The monkeypox virus sequence is of particular significance because monkeypox is known to infect humans and cause a disease that appears to be a mild form of smallpox. The availability of these sequences should provide a better understanding of poxvirus pathogenesis and differences between different isolates and strains.

- By comparing the genomes of variola virus and vaccinia virus, scientists have identified more than 20 potential targets for neutralizing antibodies that could stop smallpox infection.

- NIAID intramural scientists cloned the entire vaccinia virus genome in a bacterial artificial chromosome, enabling the modification or deletion of vaccinia genes or the addition of foreign DNA via methods developed for bacterial systems. This advance will promote the development of

genetically engineered and recombinant vaccinia viruses for use as vaccines and vectors.

GOAL: Identify and characterize host factors and viral proteins that are involved in the production and maintenance of the two forms of infectious orthopoxviruses: intracellular mature virus (IMV) and extracellular enveloped virus (EEV).

- NIAID scientists, along with scientists from Spain and England, have begun to tease apart the mechanism by which intracellular orthopoxvirus virions are transported to the cell surface and are released into the extracellular environment. These investigators have shown that two viral proteins, A33R and A36R, first interact with each other and then with the cellular microtubule system. The interaction with the microtubule system results in the movement of virions to the cell surface. Since release of poxvirus virions from infected cells is an important step in the spread of virus throughout the body of an infected animal or human, these new data will inform the design of new vaccines and therapies. Additional studies have shown that viral proteins interact with host actin filaments to facilitate the release of virions from the cell surface, a process that appears to be regulated by host cellular signaling kinases.
- NIAID-supported scientists have shown that one of the first steps in the formation of new orthopox virions in infected cells is insertion of the viral protein A14 into cellular membranes. This membrane-bound protein then helps to recruit additional components into the developing virion structure. The function of A14 was shown to be regulated by certain modifications, including the addition of a phosphate group to the protein by another viral protein, F10 kinase. The kinase, as well as another viral protein identified by these researchers that removes phosphate additions from proteins, represent important new targets for development of anti-poxvirus drugs.
- NIAID scientists have discovered and characterized a novel poxvirus-encoded enzyme system that harnesses cellular energy carrier molecules to alter the structure of virion proteins while they are assembled into new virions. This newly discovered enzyme system also represents a potentially very important new target for antiviral drug development.

Plague

Plague is caused by the bacterium *Yersinia pestis*. Its potential for use as a biological weapon is based on methods that were developed to produce and aerosolize large amounts of bacteria and on its transmissibility from person to person in certain of its forms. An additional factor is the wide distribution of samples of the bacteria to research laboratories throughout the world. Infection by inhalation of even small numbers of virulent aerosolized *Y. pestis* bacilli can lead to pneumonic plague, a highly lethal form of plague that can be spread from person to person. Natural epidemics of plague have been primarily bubonic plague, which is transmitted by fleas from infected rodents.

Symptoms of pneumonic plague, including fever and cough, resemble those of other respiratory illnesses such as pneumonia. Symptoms appear within one to six days after exposure and lead rapidly to death. If untreated, pneumonic plague has a mortality rate that approaches 100 percent. Early, aggressive antibiotic treatment can be effective against plague, but there is no plague vaccine, licensed or investigational, available in the United States.

SCIENTIFIC PROGRESS

In the years since publication of the *NIAID Biodefense Research Agenda for CDC Category A Agents* in February 2002, significant progress has been made in understanding *Y. pestis* and how it causes disease, and in developing countermeasures. Key advances made in basic research on this pathogen are outlined below.

Biology of the Microbe

Hypervirulence of *Y. pestis* likely resulted from poor transmissibility from fleas to mammals. National Institute of Allergy and Infectious Diseases (NIAID) intramural scientists determined that the competence of the rat flea *Xenopsylla cheopis* as a vector for *Y. pestis* is poor compared to vectors of other arthropod-borne agents. The number of *Y. pestis* organisms required in a blood meal to successfully infect fleas was high, and the transmission efficiency of *Y. pestis* from *X. cheopis* was low and irregularly distributed. The results suggest that the evolutionary change from oral to flea-borne transmission led to the emergence and continued maintenance of a hypervirulent *Y. pestis* clone. This hypervirulent clone represents an adaptation to the high infectious threshold for fleas and the low transmission efficiency from fleas to mammals. This discovery may also have implications for disease-control strategies.

(Lorange EA et al., Poor vector competence of fleas and the evolution of hypervirulence in *Yersinia pestis*, *J Inf Dis* 2005;191:1907-1912)

Genome sequence for the organism that causes bubonic and pneumonic plague has been completed. NIAID-supported investigators have completed the genome sequence of the KIM strain of *Y. pestis*, which was associated with the second pandemic of plague, including the Black Death. The publicly accessible genome sequence will provide a valuable research resource to the scientific community for identifying new targets for vaccines, drugs, and diagnostics for this deadly pathogen, considered an agent of bioterrorism.

(Deng W et al., Genome sequence of *Yersinia pestis* KIM, *J Bacteriology* 2002;184:4601-4611)

Single gene change led to deadly plague organism. The plague organism, *Y. pestis*, is highly infectious for humans and considered to be a bioterrorism threat. A relatively benign ancestor of *Y. pestis* picked up a gene for phospholipase D (PLD) from an unrelated organism only about 1,500-20,000 years ago. NIAID scientists discovered that this gene is required for the plague bacillus to survive in the midguts of fleas. Acquisition of the PLD gene thus explains how *Y. pestis* evolved from a form that caused only a mild intestinal illness, acquired through ingestion, to become a much more deadly pathogen transmitted by fleas. This work illustrates how microbes continually re-invent themselves to emerge as novel or more virulent agents of human disease.

(Hinnebusch BJ et al., Role of Yersinia murine toxin in survival of *Yersinia pestis* in the midgut of the flea vector, *Science* 2002;296:733-735)

Genes in the yersiniabactin iron transport system have been identified. Nine genes and their respective products have been identified in the yersiniabactin (Ybt) iron transport system, a virulence determinant for *Y. pestis*. A more complete analysis of these genes and their products or interactions may advance

the development of novel therapeutic approaches for the treatment of plague.

(Perry RD et al., Iron and heme acquisition storage systems in *Yersinia pestis, Recent Res Dev Microbiol* 2001;5:13-27)

Host Response

Plague bacteria shown to target immune cells during infection. *Y. pestis* injects Yersinia effector proteins (Yop) into host cells using the type III secretion pathway during infection. NIAID-supported scientists have conducted studies using plague-infected mice to identify specific cell types most often targeted by the bacteria. They demonstrated that the Yop proteins are most frequently injected into immune cells, specifically dendritic cells, macrophages, and neutrophils. *Y. pestis* may make use of this mechanism to target and disable host immune responses during infection.

(Marketon MM et al., Plague bacteria target immune cells during infection, *Science* 2005;309:1739-1741)

New rat model of bubonic plague offers new tool for development of interventions. Bubonic plague in humans follows transmission by infected fleas and is characterized by swollen and very tender lymph glands, accompanied by pain, fever, chills, headache, extreme exhaustion, and sometimes life-threatening blood infection and organ damage. NIAID intramural researchers developed a model of bubonic plague using the inbred Brown Norway strain of *Rattus norvegicus* to characterize the progression of infection and the host immune response after *Y. pestis* is administered through the skin. The clinical signs and pathology in the rat closely resembled bubonic plague in humans. Understanding disease progression in this rat infection model should facilitate further investigations into the molecular pathogenesis of bubonic plague and the immune response to *Y. pestis* at different stages of the disease.

(Sebbane F et al., Kinetics of disease progression and host response in a rat model of bubonic plague, *Am J Pathol* 2005;166:1427-1439)

Vaccines

Mouse model mimics real-world bubonic plague infection. An experimental plague vaccine proved 100 percent effective when tested in a new mouse model for plague infection developed by NIAID intramural scientists at Rocky Mountain Laboratories (RML). The scientists developed their model to mimic the natural transmission route of bubonic plague through the bites of infected fleas. The flea-to-mouse model provides a more realistic test setting than previously used models, enabling a better assessment of a vaccine's ability to protect against a real-world challenge.

(Jarrett C et al., Flea-borne transmission model to evaluate vaccine efficacy against naturally acquired bubonic plague, *Infect Immun* 2004;72:2052-2056)

New subunit plague vaccine gives increased protection in mice and reduces immune suppression. While immunization of animals with LcrV protein has been shown to elicit protective immunity, it can also suppress host defense mechanisms. NIAID-supported scientists have developed a new subunit vaccine for plague using an altered LcrV protein. This vaccine has been shown to increase protection in mice while reducing the level of immune suppression.

(Overheim KA et al., LcrV plague vaccine with altered immunomodulatory properties, *Infect Immun* 2005;73:5152-5159)

Diagnostics

New rapid diagnostic test for pneumonic plague can be used in most hospitals. NIAID-funded scientists developed a six-hour test to assist in the diagnosis of plague in patients presenting with pneumonia symptoms at a hospital. This will allow health care providers to identify and isolate the pneumonic plague patient away from other patients, as well as enable the health care providers to use appropriate precautions to protect themselves. The diagnostic test can be used for both blood and sputum samples, which raises the possibility that the test may not require a blood sample from a patient.

(Gomes-Solecki MJC et al., LerV capture enzyme-linked immunosorbent assay for detection of *Yersinia pestis* from human samples, *Clinical and Diagnostic Laboratory Immunology* 2005;12:339-346)

PROGRAMMATIC PROGRESS IN ADDRESSING IMMEDIATE GOALS

GOAL: Accelerate the search for candidate *Y. pestis* vaccines.

- Investigators at the NIAID-supported Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases (RCEs) are exploring a variety of options to develop new plague vaccines. These include:
  - Targeting dendritic cells for effective T-cell based protection and enhanced antibody responses to a particular *Y. pestis* protein. Scientists have constructed a fusion protein that gives partial protection in mice. (Northeast

Biodefense Center, New York State Department of Health)

- Testing the ability of novel adjuvants to stimulate the immune system to protect against aerosolized *Y. pestis*, and to enhance subunit and live attenuated *Y. pestis* vaccines. (WWAMI (WA, AK, OR, ID) RCE, University of Washington)
- Studying the humoral and T-cell responses to killed or live *Y. pestis* vaccine in mice, as well as to live, attenuated vaccine in humans to identify major antigens of the organism. (Western RCE, University of Texas Medical Branch-Galveston)
- Using a novel antigen, the YcsF protein, to create a vaccine against *Y. pestis*. (Rocky Mountain RCE, Colorado State University)
- Producing recombinant proteins and cDNA vaccines from large outer membrane proteins to evaluate them for protection against unencapsulated *Y. pestis*. (Southeast RCE, Duke University)
- Using Psn as a vaccine candidate with various adjuvants. (Southeast RCE, Duke University)
- Evaluating the potential for a variety of vaccine vectors to be used to deliver a recombinant LcrV (low-calcium-response V or V antigen) subunit vaccine. The systems under investigation include adenovirus vectors, Newcastle disease virus vectors, vesicular stomatitis virus, a Salmonella type III secretion system, and live attenuated mycobacterial vectors. (Northeast Biodefense Center, New York State Department of Health)

- NIAID-supported researchers are using a genome-wide screen to identify candidate antigens for inclusion in a *Y. pestis* vaccine. Potential candidates are being cloned and expressed for further evaluation. The most promising antigens will be assessed for their ability to confer protection to mice against aerosol challenge. (Chiron Corporation, Emeryville, CA)
- Through the Challenge Grants program, NIAID-supported researchers are working on developing an intranasal plague vaccine that uses a novel adjuvant called Protollin. (ID Biomedical Corporation of Washington, Seattle)
- NIAID intramural scientists completed a study of the efficacy of a recombinant F1-V fusion protein plague vaccine to protect mice from bubonic plague, which is transmitted to mice via flea bites in the laboratory. The results showed that the F1-V vaccine was protective against bubonic plague infection in mice, and that the similarity of the flea-to-mouse transmission model used in the study to natural plague infection makes it a desirable model for use in future plague vaccine studies. See also Scientific Progress section, Mouse model mimics real-world bubonic plague infection. This bubonic plague mouse model is being used to evaluate the efficacy of a new live attenuated plague vaccine developed at Montana State University.
- NIAID programs that encourage applications for the development of plague vaccines include: Biodefense Partnerships; the Cooperative Research for the Development of Vaccines, Adjuvants, Therapeutics, Immunotherapeutics, and Diagnostics for Biodefense Program; and the Small Business Biodefense Program.
- NIAID has established a cooperative program with U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) to conduct mutually agreed upon research projects related to biodefense. This includes developing and testing vaccines for pneumonic plague, the form of plague that poses the highest concern for biodefense. A nonhuman primate parenteral challenge model of bubonic plague has been developed that is available for future use through this collaboration. Once a vaccine is developed that is effective against pneumonic plague, the nonhuman primate model may be used to test the vaccine for efficacy against bubonic plague. Bubonic is the form of plague that is of highest concern in the developing world.
- NIAID awarded a contract in 2004 to support the advanced development stages for a candidate plague vaccine that is based on a mixture of two independently expressed and purified recombinant antigens (F1 and V) of *Y. pestis* that are key stimulators of the human immune response. The contract provides for Good Manufacturing Practice (GMP) pilot lot manufacture and Phase I human clinical testing of the vaccine in healthy adults. (Avecia, U.K.)
- NIAID intramural scientists are collaborating with intramural scientists at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) to identify candidate antigens from *Y. pestis* for development of third-generation plague vaccines.

GOAL: Establish capacity for the development, refinement, production, and testing of pilot lots of candidate *Y. pestis* vaccines.

- In 2004, the U.S. Food and Drug Administration (FDA) and NIAID co-hosted a workshop on animal models and correlates of immunity for plague vaccines that helped define the direction of product development for plague vaccine candidates.
- A new mouse model and a new rat model of bubonic plague, both developed by NIAID intramural scientists, will facilitate testing of candidate plague vaccines. See also Scientific Progress section, New rat model of bubonic plague offers new tool for development of interventions, and mouse model mimics real-world bubonic plague infection.
- During the past few years, USAMRIID investigators have conducted preclinical safety and efficacy studies on an F1-V fusion protein vaccine, while scientists in the United Kingdom have been conducting similar studies on an F1+V combined subunit vaccine. Plans for development of these products include: comparative studies to assess immunogenicity in various nonhuman primate animal models, as well as in Phase I human trials; development and evaluation of surrogate markers of protective immunity generated after immunization; optimization of the immunization regimen to enhance the degree and duration of protective immunity for these and other promising vaccine candidates; validation of assays used for the assessment of protective immunity; and optimization and scale-up production of current GMP product.

GOAL: Encourage exploration of new targets leading to the development of *Y. pestis*-specific chemotherapeutics and/or entities with novel modes of action (e.g., Ybt, TTSS, LcrV).

- NIAID-supported scientists are researching the rational design of inhibitors of *Y. pestis* protein chain elongation factor, EF-Tu. (Molsoft, LLC, La Jolla, CA)
- Several new NIAID grant programs encourage the identification of new targets for the development of therapeutics for Category A agents including *Y. pestis*. These include the Cooperative Research for the Development of Vaccines, Adjuvants, Therapeutics, Immunotherapeutics, and Diagnostics for Biodefense Program; the Small Business Biodefense Program; and Biodefense and Emerging Infectious Diseases Research Opportunities.
- NIAID scientists completed pilot testing of the susceptibility of *Y. pestis* to a novel class of antimicrobial compounds being developed by researchers at Duke University. These compounds target bacterial enzymes required for the biosynthesis of lipopolysaccharide, the major component of the outer membrane of Gram-negative bacteria. Results of the tests showed that 6 of the 11 compounds tested had some antibacterial activity against *Y. pestis*. This information may be useful for future research toward plague therapeutics.

GOAL: Screen existing FDA-approved antimicrobials and immunomodulators for efficacy against *Y. pestis*.

- In collaboration with USAMRIID and FDA, NIAID supported the testing of licensed antibiotics for efficacy against pneumonic plague. Studies are currently under way to determine the pharmacokinetics and toxicity of five licensed drugs—gentamycin, doxycycline, ciprofloxacin, levofloxacin, and ceftriaxone—in African green monkeys exposed to aerosolized *Y. pestis*. Testing of gentamycin and ciprofloxacin has been completed. The data are being prepared for FDA evaluation. Testing of the other three antibiotics is ongoing. (USAMRIID; SRI International, Menlo Park, CA; Lovelace Respiratory Research Institute, Albuquerque, NM; Battelle Memorial Institute, Columbus OH)

GOAL: Develop rapid, inexpensive, and broad-based clinical diagnostic approaches for plague.

- NIAID is supporting research to distinguish *Y. pestis* strains based on single nucleotide polymorphisms, differences in one nucleotide in the genome sequence. (Perlegen Sciences, Mountain View, CA)
- NIAID is supporting development of a rapid, sensitive and fully automated *Y. pestis* diagnostic (Cellex Biosciences Inc., Minneapolis, MN) and polymer-based point-of-care diagnostics for *Y. pestis*. (Nomadics, Stillwater, OK)
- Several new NIAID grant programs encourage the development of diagnostics for the Category A agents including plague. These include: Biodefense Partnerships; the Small Business Biodefense Program; and the Cooperative Research for the Development of Vaccines, Adjuvants, Therapeutics, Immunotherapeutics, and Diagnostics for Biodefense Program.
- Under the NIAID Bioinformatics Resource Centers program, an Enteropathogen Resource Center was established in 2004. This center provides genomic sequences, annotations, and related biological data for biodefense-related organisms, including *Y. pestis*. (University of Wisconsin)

GOAL: Develop standards for validation and comparison of potential plague diagnostics.

- In collaboration with USAMRIID, NIAID is supporting the development and standardization of pneumonic- and bubonic-plague animal models, and the development, standardization, and transfer of these research resources to a central repository. For example, the African green monkey model of pneumonic plague developed in collaboration with USAMRIID may be applicable to validate and compare plague diagnostics.
- Since establishing the Biodefense and Emerging Infections Research Resources Repository in 2003, NIAID has supported the acquisition, authentication, storage, and distribution of 15 reference strains of *Y. pestis* through the Biodefense and Emerging Infections Research Resources Repository Program. Five isolates are available, with the remainder in progress. In addition, work is ongoing to make monoclonal antibodies for plague research available to the community.

Botulism

Botulinum toxin, which is produced by the spore-forming anaerobic bacterium *Clostridium botulinum*, is a highly toxic substance that presents a major threat from intentional exposure. The toxin is highly lethal, and easy to produce and release into the environment. Botulinum toxin is absorbed across mucosal surfaces and irreversibly binds to peripheral cholinergic nerve synapses. Seven antigenic types (A-G) of the toxin exist. All seven toxins cause similar clinical presentation and disease; botulinum toxins A, B, and E are responsible for the vast majority of foodborne botulism cases in the United States.

Exposure to the toxin induces symptoms that include muscle paralysis; difficulty in speaking, swallowing, or seeing; and, in severe cases, the need for mechanical respiration. People exposed to the toxin require immediate and intensive supportive care and treatment. The onset and severity of symptoms depend on the rate and amount of toxin absorbed into circulation. With foodborne exposure, incubation varies from 2 hours to 8 days but is generally limited to 72 hours. Symptoms subside when new motor axon twigs reinnervate paralyzed muscles, a process that can take weeks or months in adults.

SCIENTIFIC PROGRESS

Since publication of the *NIAID Biodefense Research Agenda for CDC Category A Agents* in February 2002, significant progress has been made in understanding *C. botulinum* neurotoxins (BoNT) and how they cause disease, and in developing medical countermeasures to protect exposed individuals. Key advances made in basic research on the organism and the neurotoxin are outlined below.

Biology of the Microbe

New information about the botulinum neurotoxin light chain opens door for accelerated antitoxin research. The development of high-throughput screening assays for neurotoxin inhibitors has previously been limited by the inability to produce an active, recombinant form of the botulinum neurotoxin light chain molecule. National Institute of Allergy and Infectious Diseases (NIAID)-funded researchers have now identified portions of the light chain that contribute to solubility, stability, and catalysis of the toxin. This information has allowed them to successfully express and purify active recombinant type A neurotoxin, which may be used in the development of high-throughput assays for potential inhibitors of the toxin.

(Baldwin MR et al., The C-terminus of botulinum neurotoxin type A light chain contributes to solubility, catalysis, and stability, *Protein Expr Purif 2004;37:187-195)*

A new serotype A subtype is characterized. Two subtypes of botulinum neurotoxin serotype A, designated A1 and A2, were identified in the mid-1990s. NIAID-supported researchers have, for the first time, described the distinct gene cluster of the BoNT/A2 neurotoxin. This analysis has allowed identification of conserved BoNT cluster genes and may provide insight into the formation or structure of the botulinum neurotoxin complexes, as well as the evolutionary history of the BoNT/A clusters.

(Dineen SS et al., Nucleotide sequence and transcriptional analysis of the type A2 neurotoxin gene cluster in *Clostridium botulinum*, *FEMS Microbiol Lett* 2004;235:9-16)

Sequencing of the *C. botulinum* Hall strain A bacterium genome has been completed. The genome was determined to be approximately 3.9 megabase pairs in size, with a G+C content of approximately 28.2 percent. There is also a plasmid of 16,344 base pairs. Genomic information is critical to the development of effective countermeasures against *C. botulinum* neurotoxins.

(The Wellcome Trust Sanger Institute, available at www.sanger.ac.uk/Projects/C_botulinum)

Research provides a better understanding of botulinum toxin entry into cells. Researchers have determined the role of gangliosides as receptors for neurotoxin entry. In addition, the mechanism used by the botulinum neurotoxin heavy chain to chaperone or carry the light chain across the endosomal membrane into the cytosol has also been further delineated. With this information researchers can develop drugs to block or disrupt toxin entry.

(Koriazova LK and Montal M, Translocation of botulinum neurotoxin light chain protease through the heavy chain channel, *Nature Structural Biology* 2002;10:13-18)

Vaccines

Animals are protected after immunization with A and F toxins. Animals immunized intranasally were completely protected from intraperitoneal challenge with active *C. botulinum* neurotoxin A.

(Park JB and Simpson LL, Inhalational poisoning by botulinum toxin and inhalational vaccination with its heavy-chain component, *Infect Immun* 2003;71:1147-1154)

In other work, animals immunized with the heavy chain of botulinum neurotoxin expressed in *Salmonella enterica* serovar Typhimurium were partially protected from intraperitoneal challenge of active *C. botulinum* type F toxin.

(Foynes S et al., Vaccination against type F botulinum toxin using attenuated *Salmonella enterica* var Typhimurium strains expressing the BoNT/F HC fragment, *Vaccine* 2003;21:1052-1059)

Researchers have developed new methods for the expression and purification of recombinant catalytically active, nontoxic endopeptidase derivatives of *C. botulinum* neurotoxin type A. The recombinant protein was immunogenic in animals and induced neurotoxin neutralizing antibodies and thus could be used as an alternative to toxoid for producing antitoxin in animals.

(Chaddock JA et al., Expression and purification of catalytically active, non-toxic endopeptidase derivatives of *Clostridium botulinum* toxin type A, *Protein Expr Purif* 2002;25:219-228)

These results provide important information regarding host response following immunization with vaccines against botulinum toxins.

PROGRAMMATIC PROGRESS IN ADDRESSING IMMEDIATE GOALS

NIAID established the Food and Waterborne Diseases Integrated Research Network (FWDIRN) in 2003. The network consists of six sites that conduct multidisciplinary research on all food and waterborne pathogens (bacteria, viruses, and protozoa) and toxins. The goal is to facilitate development and evaluation of products to rapidly identify, prevent, and treat food and waterborne diseases that threaten public health. The Microbiology and Botulism Research Unit is the FWDIRN site that focuses specifically on botulism. (Tufts University School of Veterinary Medicine)

GOAL: Process, produce, and conduct Phase I and II trials with the heptavalent equine antitoxin.

- In 2005, the Centers for Disease Control and Prevention (CDC) completed the processing of existing lots of bulk equine plasma to produce additional doses of heptavalent and monovalent antitoxin. NIAID is testing the pharmacokinetics of the toxin in rodent models through the FWDIRN. (Immunology Research Unit, University of Maryland, Baltimore)
- CDC initiated a new program for producing equine antitoxin in 2004. Horses have been immunized against all seven serotypes of neurotoxin. Plasma will be collected and processed to produce additional doses of heptavalent antitoxin. NIAID staff participate in the interagency working group guiding this process.

GOAL: Scale up production and Phase I testing of three human monoclonal antibodies to toxin A.

- NIAID is supporting fast-track development of a monoclonal antibody-based therapy for botulinum neurotoxin serotype A using the authorities granted under Project BioShield. The monoclonal antibodies have been engineered onto human compatible frameworks and will be manufactured as clinical grade material. (Xoma, Berkeley, CA)
- NIAID has developed the capability to produce sufficient quantities of monoclonal antibodies against diseases by agents of bioterrorism through several other mechanisms, including NIAID grants and the FWDIRN.
- A combination of three monoclonal antibodies, which neutralize botulinum neurotoxin serotype A with a potency 90 times greater than human hyperimmune globulin, has been identified.

  (Nowakowski A et al., Potent neutralization of botulinum neurotoxin by recombinant oligoclonal antibody, *Proc Nat Acad Sci USA* 2002;99:11346-11350)

GOAL: Develop and test human monoclonal antibodies to toxins B, C, D, E, F, and G.

- NIAID is supporting research on human compatible botulinum neurotoxin polyclonal antibodies specific to serotypes A, B, and E produced in transgenic rabbits. (SRI International, Menlo Park, CA)
- NIAID is developing panels of human monoclonal antibodies to botulinum toxin serotypes B and E. (University of California, San Francisco)

- NIAID supports several grants to identify and validate therapeutic monoclonal antibodies that neutralize neurotoxin serotypes B-G.

GOAL: Produce and conduct Phase I and II trials of recombinant vaccine for serotypes A and B.

- A recombinant vaccine to protect against serotypes A and B of botulinum neurotoxin has been developed by the Department of Defense (DoD). DoD began Phase I clinical evaluation of this vaccine in 2005.

GOAL: Develop recombinant vaccines against neurotoxins A, B, C, D, E, F, and G.

- Using authorities granted under Project BioShield, NIAID is supporting advanced process development of a recombinant botulinum neurotoxin serotype E vaccine including manufacture of a clinical grade lot for evaluation in preclinical studies. (DynPort Vaccine Company LLC, Frederick, MD)
- NIAID is supporting research on vaccines for botulinum neurotoxin through a wide variety of biodefense-specific grant programs. Projects include:
  - Development of a vaccine derived from recombinant protein fragments of the neurotoxin (DynPort Vaccine Company LLC, Frederick, MD)
  - Novel vaccine development approaches such as constructing alphavirus replicon particles that express protective, nontoxic fragments of the neurotoxin or immune enhancing entities *in vivo* (AlphaVax Inc., Research Triangle Park, NC)
  - Co-delivery of inactive botulinum neurotoxin genes with immune-enhancing genes in DNA vaccine constructs (Thomas Jefferson University)
  - Novel formulation strategies for multivalent protein vaccines to improve vaccine stability and shelf life. For example, water soluble sugar glasses created by a vitrification process offer a solid, crystalline vaccine product that allows for transport and storage without refrigeration. (DynPort Vaccine Company LLC, Frederick, MD)
  - Development of a noncatalytic BoNT light chain vaccine (Medical College of Wisconsin)
- Several NIAID initiatives encourage the development of vaccines for Category A agents including botulinum neurotoxin. These include: Biodefense Partnerships; the Small Business Biodefense Program; and the Cooperative Research for the Development of Vaccines, Adjuvants, Therapeutics, Immunotherapeutics, and Diagnostics for Biodefense Program.
- In November 2002, NIAID convened a Botulinum Toxin Expert Panel to formulate specific goals regarding the development of second-generation therapies, vaccines, and diagnostics for biodefense. Discussions included the challenges posed by the rapid development of countermeasures, including vaccines, for *C. botulinum* toxins. (For a meeting summary, visit www.niaid.nih.gov/biodefense/pdf/bot_toxins.pdf)

GOAL: Develop rapid and inexpensive diagnostics for botulism toxins and their genes for use in multiple settings.

- Research that exploits the enzymatic activity of the BoNT has led to the development of an *in vitro* assay with sensitivity for serotypes A and E that exceeds the present mouse bioassay by 10-fold, and for serotypes B and F that matches the standard mouse bioassay. Initial results suggest that this new enzyme-fluorescence test is compatible with human specimens. Future research plans include a cooperative effort to develop a robust, chip-based automated assay that will replace the mouse bioassay. (Health Protection Agency, U.K.)

  Through a variety of initiatives, NIAID is supporting projects to discover new targets and develop diagnostics that can be used to screen clinical samples for botulinum neurotoxin and other Category A pathogens. Projects include:
  - Pathogen and Genetic Element Detection Using Microarrays (Affymetrix, Inc., Santa Clara, CA)
  - Acoustic Detection of Viruses, Bacteria, and Toxins (Akubio, Ltd., U.K.)
  - Rapid Immunoassays for Detecting Pathogenic Bacteria (EIC Laboratories, Inc., Norwood, MA)
  - Rapid Turn-around Multiplex Testing: Bioweapon Agents (EraGen Biosciences, Madison, WI)
  - Advanced Detection Technologies for Biochips (InDevR, LLC, Boulder, CO)
  - Multivariate Pathogen Diagnostic Products (Isis Pharmaceuticals, Inc., Carlsbad, CA)
  - Multiplex PCR Detection of CDC 'A' Bioterrorism Agents (Medical College of Wisconsin)
  - Multiplexed POC Diagnostic System for Bio-Threat Agents (Meso Scale Diagnostics, Gaithersburg, MD)

- Recombinant Antigen Multiagent Diagnostic Assays (Tulane University)
- Multiplex Detection of Microbial Pathogens (Sandra W, LLC, Manassas, VA)
- A Multiplexed Diagnostic Platform for Bioagent Detection (University of California Lawrence Livermore National Laboratory)
- Multiplexed Detection of Bioterror Agents (Weill Medical College of Cornell University)

- In May 2003, NIAID convened an expert panel to focus on the development of next-generation diagnostics for botulinum neurotoxins. The panel discussed the most promising technical opportunities for the development of new diagnostics in the short, medium, and long term. Workshop participants identified botulinum toxins as exceptionally potent potential bioweapons on the basis of their extreme toxicity at small doses. Participants highlighted the need for new, faster, cost-effective diagnostics suitable for use in the field with sensitivity comparable to the present mouse bioassay. One promising project has met the needed performance requirements, and collaboration with an industrial partner is underway to develop a commercially available test. A summary of the expert panel can be found at www.niaid.nih.gov/Biodefense/PDF/bot+toxins+mtg.pdf.

GOAL: Develop effective cell culture systems to study toxin binding, internalization, and protease activity.

The NIAID grant program, Biodefense and Emerging Infectious Diseases Research Opportunities, encourages research on basic biology of *C. botulinum* neurotoxin. Projects specific to botulinum that have been supported under this grant program include:

- Botulinum neurotoxin substrate specificity (Vanderbilt University)
- High-throughput drug screen against botulinum neurotoxin (Vanderbilt University)
- Intracellular inhibitors of botulinum neurotoxins (Vanderbilt University)
- Identification of botulinum toxin membrane targets (University of Georgia)
- Development of genetic tools for *C. botulinum* (University of Wisconsin)

ADDITIONAL PROGRESS

- Several approaches are being taken to identify inhibitors that block the activity of the neurotoxin after it has entered the neuronal cell, with the goal of developing BoNT therapeutics. Projects include:
  - A computational approach called dynamic pharmacophore modeling to identify computational leads (Microbiology and Botulism Research Unit, FWDIRN, Tufts University)
  - Developing novel drug carrier systems to deliver inhibitors to the interior of peripheral cholinergic nerve cells (Microbiology and Botulism Research Unit, FWDIRN, Tufts University)
  - Target-guided botulinum neurotoxin inhibitor discovery (Microbiology and Botulism Research Unit, FWDIRN, Tufts University)
  - Monoclonal antibody-based therapies for botulinum toxin serotype A and B (Microbiology and Botulism Research Unit, FWDIRN, Tufts University)
  - Identification of a compound that accelerates neurotoxin degradation within the neuronal cell (Tufts University, Boston, MA)
  - Identification of peptides that inhibit the protease activity of the neurotoxin (Georgetown University, Washington, DC)
  - A broad, long-acting inhibitor of botulinum neurotoxin (Biorexis Pharmaceutical Corporation, King of Prussia, PA)
  - High-throughput screens for botulinum toxin therapeutics (Veritas, Inc., Rockville, MD)
  - High-throughput screening combined with mechanism- and structure-based design (The Scripps Research Institute, La Jolla, CA)
- NIAID convened an Expert Panel on Botulinum Therapeutics in 2004 to identify technical opportunities for development of novel post-exposure therapeutics for botulinum neurotoxins. The panel noted the need for therapeutics against botulism and recommended studying the structure of the toxin in combination with the target substrate. The panel also recommended performing genetic microarray studies of cultured neuronal cells intoxicated with botulinum toxin. (Report posted at www.niaid.nih.gov/Biodefense/PDF/ Report+BoNT.pdf)

– Scientists at NIAID's FWDIRN are using X-ray crystallography to reveal the physical interaction between botulinum neurotoxin serotype A and its substrate or inhibitor. (Microbiology and Botulism Research Unit, FWDIRN, Tufts University)

– FWDIRN scientists are carrying out a proteomic analysis of cultured cells intoxicated with BoNT/A. This will help to determine which genes are turned on by intoxication by revealing the protein profile of cells responding to toxin exposure. (Microbiology and Botulism Research Unit, FWDIRN, Tufts University)

Tularemia

Tularemia is a potential bioterrorist agent because of its high level of infectivity (as few as 10 organisms may cause disease) and its ability to be aerosolized. *Francisella tularensis*, which causes tularemia, is a nonspore-forming, facultative intracellular bacterium that can survive at low temperatures for weeks. Two strains of the organism have been characterized—type A, which is found in North America, is more virulent than type B, which is found in Europe and Asia. The disease is not transmitted from person to person; it spreads naturally from small mammals, arthropod vectors, or contaminated food, soil, or water to humans. Natural infection can also occur after inhalation of airborne particles. It is not known how this pathogen survives in nature to produce sporadic outbreaks in endemic areas.

Tularemia can take one of several forms, depending on the route of exposure. The disease resulting from the inhalation of airborne *F. tularensis* is of major concern with respect to biodefense since it results in an acute, nonspecific illness beginning three to five days after respiratory exposure; in some cases, pleuropneumonia develops after several days or weeks. If untreated, the disease could lead to respiratory failure. Treatment with antibiotics reduces mortality for naturally acquired cases by 2 percent to 60 percent. A live-attenuated tularemia vaccine developed by the Department of Defense (DoD) has been administered under an investigational new drug (IND) application to thousands of volunteers. To date, use of this vaccine has been limited to laboratory and other high-risk personnel.

SCIENTIFIC PROGRESS

Since publication of the *NIAID Biodefense Research Agenda for CDC Category A Agents* in February 2002, progress has been made in understanding *F. tularensis* and how it causes disease, and in developing countermeasures against its intentional release. Key scientific advances are included below.

Biology of the Microbe

Genes discovered that allow survival of *F. tularensis* in cells. National Institute of Allergy and Infectious Diseases (NIAID)-supported researchers have identified several genes that are important for survival of *F. tularensis* in immune system cells called macrophages, and in the free-living amoebas that may serve as their reservoir outside of mammalian hosts. Mutations in the *F. tularensis mglA* and *iglC* genes cause defects for survival and replication within macrophages and amoebas. Moreover, mutants are less virulent in mice than non-mutated strains, suggesting that a common mechanism underlies both survival and virulence of bacteria within these two types of hosts. The researchers showed that the mglA gene is a master regulator of other genes, including *iglC*, that play a role in virulence and intracellular survival. Identifying genes that play key roles in the survival of *F. tularensis* in the environment and in its infection capacity in human hosts may accelerate the effort to develop vaccines and therapies for tularemia.

(Lauriano CM et al., MglA regulates transcription of virulence factors necessary for *Francisella tularensis* intraamoebae and intramacrophage survival, *Proc Natl Acad Sci USA* 2004;101(12):4246-4249)

Researchers develop new tool to aid understanding of tularemia genetics. In the past, studies of the pathogenesis of *F. tularensis* have been hampered by lack of methods to genetically manipulate the organism in order to understand the functions of its genes. Little is currently known about the genes that allow tularemia to cause human disease. In fact, although the live attenuated tularemia vaccine strain has been shown to protect against tularemia infections, the genetic basis for its attenuation remains unknown. NIAID-supported researchers have developed a new method involving the use of transposons or "jumping genes" to genetically manipulate *F. tularensis*. This technique will allow researchers to randomly "knock out" various *F. tularensis* genes to uncover their functions. This is an important step forward for studying all aspects of the organism's biology.

(Kawula TH et al., Use of transposon-transposase complexes to create stable insertion mutant strains of *Francisella tularensis* LVS, *Appl Environ Microbiol* 2004;70(11):6901-6904)

Researchers develop new tool to aid genetic research on *F. tularensis*. Historically, genetic manipulation of *F. tularensis* has been limited by the lack of stable plasmids, or round pieces of DNA, that can be used to shuttle new genes into cells

and can be inherited genetically. NIAID-supported researchers constructed a shuttle plasmid that is stable in both *F. tularensis* and *Escherichia coli*, the species of bacteria most commonly used for laboratory research. This plasmid enters *F. tularensis* and *E. coli* with high efficiency during the process called "transformation," is stably maintained within the cells, and does not alter the ability to grow *F. tularensis* in macrophage host cells in the laboratory. This new tool will enhance genetic characterization of *F. tularensis*, leading to a better understanding of its pathogenesis.

(Maier TM et al., Construction and characterization of a highly efficient Francisella shuttle plasmid, *Appl Environ Microbiol* 2004;70(12):7511-7519)

Host Response

Scientists reveal how *F. tularensis* escapes digestion by immune system cells. Microscopy studies conducted by NIAID-supported scientists revealed that *F. tularensis* bacteria establish infection within cells of the immune system by escaping from the cellular holding compartment, called the phagosome, where they await digestion. During tularemia infection, host cell phagosomes containing *F. tularensis* apparently break down before digestive processes can begin. This allows the pathogen to escape into the cytoplasm of the host cell and establish infection. The capacity of *F. tularensis* to alter the maturation of its phagosome and enter the cytoplasm is likely an important element in its capacity to parasitize immune system cells, and may be a critical factor for consideration in the development of vaccines for tularemia. The immune response to pathogens trapped within phagosomes is based on pathogen engulfment and antibody mechanisms. In contrast, the response to pathogens found in the host cell cytoplasm is mediated by killer T cells. Since *F. tularensis* resides in the host cytoplasm, researchers may be able to develop tularemia vaccines that stimulate the killer T-cell response.

(Clemens DL et al., Virulent and avirulent strains of *Francisella tularensis* prevent acidification and maturation of their phagosomes and escape into the cytoplasm in human macrophages, *Infect Immun* 2004;72(6):3204-3217)

Host defense mechanisms revealed in mouse model. Mice that were infected systemically with the live vaccine strain of *F. tularensis* have been used extensively in recent months to reveal host defense mechanisms against this pathogen. Such studies have demonstrated the critical need for neutrophils and interferon-gamma (INF-ψ) to combat early stages of systemic tularemia. However, these defenses do not appear to combat early pulmonary tularemia. This finding suggests that the effectiveness of particular antibacterial host defenses varies depending on the invasion site.

(Telepnev M et al., *Francisella tularensis* Toll-like receptor-mediated activation of intracellular signalling and secretion of TNF-alpha and Il-1 from murine macrophages, *Cell Microbiol* 2003;5(1):41-52)

Vaccines

Tularemia live vaccine strain (LVS) induces atypical immune response. A study by NIAID-supported investigators showed that tularemia LVS induces an inflammatory immune response in a manner atypical of many other Gram-negative bacteria. Tularemia LVS only activates certain known immune response pathways, leaving other known pathways inactive. The incomplete immune response elicited by live *F. tularensis* bacteria may help establish infection and contribute to the exceptional infectivity and virulence of this pathogen.

(Forestal CA et al., *Francisella tularensis* selectively induces proinflammatory changes in endothelial cells, *J Immunol* 2003;171(5):2563-2570)

Researchers gain new insight into limitations of *F. tularensis* LVS. In order to better understand factors influencing immunity to tularemia, and to establish a baseline for judging efficacy of potential novel vaccines, NIAID-supported researchers studied how mice vaccinated with LVS respond when encountering tularemia-causing bacteria systemically and in aerosol form. They found that immune protection offered by LVS varied depending on the virulence of the bacterial strain used to infect the mice, on the route of administration (systemic or aerosol), and on the genetic backgrounds of the host mice. One of the most important findings was that LVS did not protect one strain of mice against infection via the aerosol route, while it did protect them against systemic infection when tularemia was injected. This suggests that either immune responses elicited by vaccination with LVS are not as well expressed in the lungs, or that the immune responses raised by LVS are less able to combat pulmonary tularemia than systemic tularemia. This new understanding may be crucial for developing effective vaccines for systemic and pulmonary manifestations of the disease.

(Chen W et al., Tularemia in BALB/c and C57BL/6 mice vaccinated with *Francisella tularensis* LVS and challenged intradermally, or by aerosol with virulent isolates of the pathogen: protection varies depending on pathogen virulence, route of exposure, and host genetic background, *Vaccine* 2003;21(25-26):3690-3700)

Conjugating the O-polysaccharide of the lipopolysaccharide (LPS) of *F. tularensis* to bovine serum albumin (BSA) does not change the vaccine's effectiveness. Mice immunized with this conjugate vaccine, but not with BSA alone, were completely protected against intradermal challenge with a highly virulent type B strain of *F. tularensis*; they were only partially protected against aerosol challenge with the same strain. The same vaccination strategy failed to protect against aerosol challenge with a virulent type A strain. This suggests that the O-antigen of *F. tularensis* could be considered as a potential component of a subunit vaccine against type B, but not type A, strains of *F. tularensis*.

(Conlan JW et al., Mice vaccinated with the O-antigen of *Francisella tularensis* LVS lipopolysaccharide conjugated to bovine serum albumin develop varying degrees of protective immunity against systemic or aerosol challenge with virulent type A and type B strains of the pathogens, *Vaccine* 2002;20(29-30): 3465-3471)

Therapeutics

Intranasal combination therapy shows efficacy in treating pulmonary tularemia infections in mice. Inhalational tularemia can lead to development of bronchopneumonia, which is frequently fatal without medical intervention. Thus, development of treatment strategies that directly target the respiratory system may be especially important for medical management of acute aerosol exposure. To this end, NIAID-supported researchers have developed an intranasal strategy for treating pulmonary *F. tularensis* infection in mice that uses the antibiotic gentamicin combined with interleukin-12 (IL-12). The intranasal administration of IL-12 alone promoted bacterial clearance and prolonged survival, but did not prevent death in mice exposed to aerosolized *F. tularensis*. However, intranasal treatment with gentamicin and IL-12 together markedly enhanced the rate of survival (70 to 100 percent) against pulmonary infection compared to the rates of survival for animals treated with antibiotic or IL-12 alone. These findings suggest that IL-12 may be a potent adjunct to antimicrobial drugs in the treatment of pulmonary tularemia.

(Pammit MA et al., Intranasal interleukin-12 treatment promotes antimicrobial clearance and survival in pulmonary *Francisella tularensis* subsp. novicida infection, *Antimicrob Agents Chemother* 2004;48(12):4513-4519)

PROGRAMMATIC PROGRESS IN ADDRESSING IMMEDIATE GOALS

GOAL: Conduct comparative genomic sequencing of selected strains of *F. tularensis*, type A and B, LVS, and *F. novicida*, and develop genetic systems to correlate differences in pathogenesis and virulence.

- Through the Biodefense and Emerging Infectious Diseases Research Opportunities program, NIAID is supporting studies of:
  - Comparative genomics of Francisella (Baylor College of Medicine)
  - Proteomics of *F. tularensis* infection/immunity (National Research Council of Canada, Ottawa, Ontario)
  - Genome biology of *F. tularensis* populations (University of Nebraska, Lincoln)
- As a result of a coordinated Federal effort, genome sequencing projects funded by NIAID, the Centers for Disease Control and Prevention (CDC), the Department of Energy (DoE), and the National Science Foundation (NSF) are ongoing for at least one strain of a bacteria or protozoan considered to be Category A organisms. To date there is a completed or near-completed genome sequence for every bacteria on the Category A list, including *F. tularensis*.
- NIAID's Microbial Sequencing Centers are sequencing additional strains of *F. tularensis*. As soon as sequence data are generated, they will be released to the scientific community via GenBank. (Broad Institute, Cambridge, MA)
- NIAID intramural scientists are conducting microarray experiments using custom Affymetrix GeneChip technology to perform a transcriptome analysis of Francisella genes expressed at various stages of its intracellular cycle. Such an approach will generate a comprehensive picture of the bacterial genes required for Francisella intracellular pathogenesis and lead to the identification of essential virulence determinants.
- NIAID's eight Bioinformatics Resource Centers provide the scientific community with access to genomic and related data for the NIAID Category A-C priority pathogens, pathogens causing emerging and re-emerging infectious diseases, and invertebrate vectors of infectious diseases. One center is focusing on *F. tularensis*. Genomic sequence data will be integrated with information on gene expression, comparative genomics, proteomics, host/pathogen interactions, and pathways data. (Northrop Grumman, Los Angeles, CA)

GOAL: Develop a bacterial repository of Francisella species.

- Since 2003, NIAID has supported the acquisition, authentication, storage, and distribution of approximately 25 species isolates of Francisella through the Biodefense and Emerging Infections Research Resources Repository Program. Some isolates are already available; the remaining ones will be available soon. (American Type Culture Collection, Manassas, VA)

GOAL: Characterize responses of *F. tularensis* to available chemotherapeutics *in vitro* and in animal models of infection and disease.

- Most current research on *F. tularensis* is focused solely on aerosol models of infection and host response. Deliberate contamination of drinking water or the food supply with *F. tularensis* is a viable bioterrorism threat and very little is known about pathogenesis of and immunity to virulent *F. tularensis* infections following ingestion. NIAID-supported researchers are developing a mouse model of oral infection with virulent *F. tularensis* for use in evaluating the effectiveness of vaccines and therapeutics. (National Research Council of Canada, Ottawa)

- NIAID's *In Vitro* and Animal Models for Emerging Infectious Diseases and Biodefense program provides a range of animal models for preclinical testing of new therapies and vaccines against agents of bioterrorism, including *F. tularensis*.

  Projects include:

  – *In vitro* screening for antimicrobial susceptibility of *F. tularensis* (Battelle, Columbus, OH; Health Protection Agency, U.K.; Oklahoma State University, Stillwater; SRI International, Menlo Park, CA)

  – Development of animal models for screening *F. tularensis*:

    - Small animal models (University of Texas Medical Branch-Galveston; Lovelace Respiratory Research Institute, Albuquerque, NM)

    - Nonhuman primate models (Health Protection Agency, U.K.)

GOAL: Develop new techniques to improve conditions for microbe cultures and for rapid determination of drug sensitivity profiles.

- Prior to 2001, tularemia diagnostics were largely based on microbe cultures. Since that time, advances in genomics and polymerase chain reaction (PCR)- and microarray-based technologies have changed the focus of NIAID's tularemia diagnostics portfolio toward these new, more rapid techniques. Currently NIAID is supporting a variety of projects to discover new targets and develop PCR- and microarray-based diagnostic technologies that can be used to screen clinical samples for the presence of *F. tularensis*, as well as several other Category A-C agents. For more information on NIAID-supported diagnostic development, see page 9.

GOAL: Attract scientific researchers with expertise in a diversity of fields (e.g., immunology, microbiology, and lipid biochemistry) to the study of tularemia.

- Through a variety of biodefense initiatives and support of investigator-initiated grants, NIAID continues to attract new researchers with expertise in bacterial pathogenesis and infectious disease research to the tularemia field. A workshop for NIAID-funded tularemia researchers held in 2004 was filled to capacity with senior investigators, junior investigators and trainees. This illustrates the tremendous growth that NIAID has fostered in the tularemia research field in order to accelerate the development of diagnostics, vaccines and therapeutics for this disease.

- Through the expansion of targeted biodefense research programs, NIAID made a total of 24 awards specific to tularemia research in FYs 2003, 2005, and 2006. For more information, see Appendix TK or visit the NIAID Biodefense Research awards pages, www.niaid.nih.gov/biodefense/research.

- NIAID is committed to supporting the Annual Conference on Tularemia from 2005-2010. This conference focuses on mechanisms of *F. tularensis* infection and pathogenesis, as well as vaccine development. It provides an international forum for tularemia researchers to present and discuss their research, exchange ideas, and develop collaborations. All levels of researcher, including new investigators and trainees, are included.

- In 2003, NIAID Division of Intramural Research, in collaboration with the NIH Office of Rare Diseases and Fogarty International Center, sponsored a conference on Epidemiology and Ecology of Q Fever, Tularemia, and Plague. The workshop gathered a worldwide group of experts to present current knowledge and discuss priorities for future research with experts from a variety of scientific disciplines. The aim of the workshop was to learn how ecological and epidemiological studies might serve as

complements to more molecular level and vaccine- and drug-oriented studies.

- The Rapid Response Grant Program on Bioterrorism-Related Research was developed to support innovative research targeted at the design and development of specific diagnostics, therapies, and prevention strategies for Category A biological diseases. This program encouraged investigators from a diversity of disciplines to become involved in biodefense research. Sixty-nine grants were awarded in FY 2002 in response to this initiative; of these, two were for tularemia research.
- NIAID offers new and expanded programs for supporting the training and career development of young scientists in biodefense research through institutional training grants, fellowships, mentored career awards, and special training programs at the Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases (RCEs). For more information, see the General Recommendations section, page 5.
- NIAID continues a concerted effort to encourage investigators to become involved in research on biodefense pathogens through the use of targeted Requests for Applications and Program Announcements such as the Biodefense and Emerging Infectious Diseases Research Opportunities initiative. The response has been encouraging and a number of new grant applications focused on tularemia have been submitted.
- In 2002 NIAID, in collaboration with the Office of Rare Diseases, sponsored an international conference on the current status of vaccines against plague and tularemia. Experts from throughout the world participated in this conference, which also stimulated productive interactions between various groups of investigators.

GOAL: Develop rapid, inexpensive, and broad-based clinical diagnostics approaches for tularemia.

- NIAID is supporting a variety of investigator-initiated grants, cooperative agreements, and RCEs that are working to discover new targets and develop PCR- and microarray-based diagnostic technologies that can be used to screen clinical samples for the presence of *F. tularensis*, as well as several other Category A-C agents. Projects include:
  - Diagnostic Immunomagnetic Bead Concentration Method for Detecting *F. tularensis* within Blood or Tissues (Tufts University)
  - Pathogen and Genetic Element Detection Using Microarrays (Affymetrix, Inc., Santa Clara, CA)
  - Acoustic Detection of Viruses, Bacteria, and Toxins (Akubio, Ltd., U.K.)
  - Rapid Immunoassays for Detecting Pathogenic Bacteria (EIC Laboratories, Inc., Norwood, MA)
  - Rapid Detection of Agents that Cause Respiratory Disease (EraGen Biosciences, Madison, WI)
  - Rapid Turn-Around Multiplex Testing: Bioweapon Agents (EraGen Biosciences, Madison, WI)
  - Advanced Detection Technologies for Biochips (InDevR, LLC, Boulder, CO)
  - Multivariate Pathogen Diagnostic Products (Isis Pharmaceuticals, Inc., Carlsbad, CA)
  - Multiplex PCR Detection of CDC 'A' Bioterrorism Agents (Medical College of Wisconsin)
  - Multiplexed POC Diagnostic System for Bio-Threat Agents (Meso Scale Diagnostics, Gaithersburg, MD)
  - Recombinant Antigen Multiagent Diagnostic Assays (Tulane University)
  - Multiplex Detection of Microbial Pathogens (Sandra W, LLC, Manassas, VA)
  - A Multiplexed Diagnostic Platform for Bioagent Detection (University of California, Lawrence Livermore National Laboratory)
  - Multiplexed Detection of Bioterror Agents (Weill Medical College of Cornell University)
- NIAID's Bioinformatics Resource Centers include one focused on *F. tularensis*. Comparing genomes of different strains of *F. tularensis* may enable scientists to identify specific gene sequences that could be used to test for the presence of one or more *F. tularensis* strains in clinical samples. These types of diagnostics are more rapid and portable than traditional serum agglutination or culture-based diagnostics. (Northrop Grumman, Los Angeles, CA)

ADDITIONAL PROGRESS

- NIAID intramural scientists are using multiple approaches to further characterize Francisella intracellular trafficking, identify genes expressed at various stages of the intracellular cycle, and assess their role in Francisella virulence. Researchers plan to develop human and nonhuman primate macrophage infection models to study the intracellular pathogenesis of human clinical isolates of virulent types A and B *F. tularensis*. An understanding of the molecular mechanisms of intracellular survival and the bacterial determinants involved will help uncover novel targets for the design of vaccine-based and other therapeutics against tularemia.
- NIAID awarded two contracts in FY 2005 to support development of a vaccine against tularemia, including development of assays and animal models necessary to evaluate new tularemia vaccine candidates. (The University of New Mexico, Albuquerque; DynPort Vaccine Company LLC, Frederick, MD)
- NIAID is supporting research to develop tularemia vaccines through a wide variety of biodefense-specific grant programs. Projects include:
  - Scanning the *F. tularensis* proteome for vaccine antigens (University of California, Irvine)
  - Developing a genome-derived, epitope-driven tularemia vaccine (Epivac, Inc., Paris, France)
  - Developing new tularemia vaccine candidates (Umea University, Sweden; University of Rochester)
  - Studying the molecular basis of Francisella virulence and immunity (University of North Carolina, Chapel Hill)
  - Protein expression in strains of *F. tularensis* (University of Tennessee Health Science Center)
  - Effect of aging on immunity to tularemia (University of Texas Health Sciences Center)
  - Characterizing a recently discovered pathogenicity island—a genomic region containing genes required for *F. tularensis* to grow in macrophages and cause disease in mice. The goal of this effort is to determine the role of the encoded proteins in pathogenicity and host responses. (University of Victoria, British Columbia)
- Scientists at NIAID's RCEs are also developing candidate vaccines against tularemia. Projects include:
  - Using a genomics approach, scientists are identifying new protein candidates that can be used with capsular material to construct conjugate vaccines against *F. tularensis*. Resulting vaccine candidates are being tested in animal models. (Harvard University)
  - Researchers are using genetic mutations known to cause attenuation in other pathogens to develop a live, attenuated tularemia vaccine that can be administered orally or nasally. (University of Maryland, Baltimore)
- NIAID began a collaboration with the U.S. Army in 2004 to create a new version of the tularemia vaccine candidate, LVS. NIAID is currently evaluating this vaccine in a Phase I clinical trial. (Baylor College of Medicine Vaccine and Treatment Evaluation Unit, Houston, TX)
- NIAID is supporting immunological analysis of clinical samples collected during the aforementioned LVS Phase I clinical trial. These studies, conducted through NIAID's Food and Waterborne Diseases Integrated Research Network, will help researchers determine optimal dosage for use in further vaccine evaluation. (University of Maryland, Baltimore)
- NIAID has contracted for manufacture of a second lot of the new LVS for use in a possible future clinical trial. (DynPort Vaccine Company LLC, Frederick, MD)
- NIAID is collaborating with U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), to develop methods to detect LVS in blood and skin cultures of people recently vaccinated with the "old" LVS in a clinical trial through USAMRIID's Special Immunizations Program.
- NIAID is supporting development of single-dose disposable inhalers of two antibiotics for immediate, post-exposure protection against tularemia and pneumonic plague. (Nanotherapeutics Inc, Alachua, FL)
- NIAID is supporting studies of:
  - Novel antibacterial agents for treatment of tularemia (Affinium Pharmaceuticals, Toronto)
  - Pathogenesis of and host response to tularemia (University of Texas at San Antonio)
  - Innate immunity to *F. tularensis* (University of Kansas Medical Center)

Viral Hemorrhagic Fevers

Viral hemorrhagic fevers (VHFs) encompass a group of similar diseases caused by four types of viruses:

- **Arenaviruses**, associated with Argentine, Bolivian, and Venezuelan hemorrhagic fevers, Lassa fever, and Sabia-associated hemorrhagic fever;
- **Bunyaviruses**, including those causing Crimean-Congo hemorrhagic fever, Rift Valley fever (RVF), and hantaviral diseases;
- **Filoviruses**, associated with Ebola and Marburg hemorrhagic fevers; and
- **Hemorrhagic flaviviruses**, including those causing yellow fever (YF), dengue hemorrhagic fever, Kyasanur Forest disease, and Omsk hemorrhagic fever.

These viruses pose a risk from intentional exposure because, with very few exceptions, no vaccines or proven treatments exist, and many of the diseases are highly fatal. Natural infections occur when people come in contact with animals or insects that are infected or act as vectors. After human infection occurs, some VHFs can be transmitted from person to person through close contact or contaminated objects, such as syringes and needles.

Initial symptoms of VHFs are nonspecific and include fever, muscle aches, and fatigue. Disease often progresses to bleeding under the skin and from body orifices and internal organs, followed by shock, coma, seizures, and nervous-system malfunction. Symptoms begin between a few days (in Ebola) and several weeks after exposure, depending on the particular virus. Mortality also varies widely among the diseases; often it is quite high. Some of these viruses also cause significant morbidity and mortality in economically important domestic animals.

SCIENTIFIC PROGRESS

Since publication of the *NIAID Biodefense Research Agenda for CDC Category A Agents* in February 2002, significant progress has been made in understanding how the organisms responsible for VHFs cause disease, and in developing countermeasures against their intentional release. Key scientific advances are outlined in this chapter.

Biology of the Microbe

Structural studies of the flavivirus life cycle offer potential tools for drug discovery. The flavivirus family includes several hemorrhagic fever viruses (dengue, yellow fever) and encephalitis viruses (West Nile, Japanese encephalitis, and tick-borne encephalitis). Advances in cryo-electron microscopy have provided important views into the structure of these and other viruses at atomic resolution. The advances even permit examination of viral particles (virions) as they proceed though the life cycle, from initial attachment and entry into the cell, through various stages of replication and assembly, to final exit from the cell. Using this technology, National Institute of Allergy and Infectious Diseases (NIAID)-supported researchers have developed an impressive visual tour of atomic structures and identified conformational changes of virion components during the dengue life cycle. This research has provided insights into viral structure and physiology that may lead to discovery of antiviral or immunotherapeutic drug targets.

(Mukhopadhyay S et al., A structural perspective of the *Flavivirus* life cycle, *Nature Rev Microbiol* 2005;3(1):13-22)

Discovery of Ebola viral entry mechanisms could lead to new antiviral therapies. NIAID-supported scientists, in collaboration with investigators at the NIAID Vaccine Research Center (VRC), discovered that proteins called cathepsins play an essential role in the ability of the Ebola virus to enter and infect cells. They also found that inhibitors of cathepsins effectively block viral entry. This suggests that cathepsins may serve as promising new targets for anti-Ebola virus therapies.

(Chandran K et al., Endosomal proteolysis of the Ebola virus glycoprotein is necessary for infection, *Science* 2005;308(5728):1643-1645)

Development of a clinically relevant small animal model of dengue fever will advance the development of vaccines and therapeutics. The diseases caused by dengue virus (dengue fever, and the more severe presentations of dengue hemorrhagic fever and dengue shock syndrome) make it the most important mosquito-borne viral disease of humans (50 million -100 million infections per year). Research to develop and test dengue vaccines and drug treatments has been ongoing

for many decades, but has been severely hampered by the lack of a clinically relevant animal model of dengue that mimics the disease presentations seen in humans. Scientists have now developed a humanized small animal model of dengue fever that will serve as a clinically relevant model of disease in which to investigate the efficacy of newly developed vaccines and therapeutics to prevent or treat dengue. This model may also prove invaluable for the study of dengue immunopathogenesis.

(Bente DA et al., Dengue fever in humanized NOD/SCID mice, *J. Virol* 2005;79(21):13797-13799)

Alternative animal models enable studies of RVF pathogenesis and therapeutics. RVF is a severe hemorrhagic fever in humans and animals caused by the RVF virus. Maximum level BSL-4 containment facilities are required to conduct research on this disease. In an effort to develop alternative animal models for RVF that can be studied in lower level biocontainment facilities that are more widely available, experimental infections of hamsters were carried out using two less pathogenic bunyaviruses, Punta Toro (PT) and Gabek Forest (GF). Adult golden hamsters inoculated with either PT or GF viruses developed a fatal illness and pathologic changes resembling those of RVF. Therefore, hamster infections with these two viruses provide feasible alternative models for studying the pathogenesis of RVF and testing potential vaccines and therapies.

(Fisher A et al., Induction of severe disease in hamsters by two sandfly fever group viruses, Punta Toro and Gabek Forest [*Phlebovirus, Bunyaviridae*], similar to that caused by Rift Valley Fever virus, *Am J Trop Med Hyg* 2003;69(3):269-276)

Methods developed to study individual proteins from these viruses in regular, low containment laboratories. Investigators have been able to transfer important genes from these viruses to benign vector or carrier viruses where they can be safely expressed in cells to produce proteins. As a result, the function of these proteins can be studied separately and safely, in the absence of the infectious virus particle. These new methods provide a powerful tool for dissecting the virus life cycle, examining virus assembly, and understanding the role of viral proteins in pathogenicity and the interplay of viral proteins with components of the host cell immune response. These new methods will also help open new avenues to develop live attenuated virus vaccines and vaccine vectors.

(Jasenosky LD et al., Ebola virus VP40-induced particle formation and association with the lipid bilayer, *J Virol* 2001;75(11):5205-5214)

Host Response

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

(Sullivan NJ et al., Ebola virus glycoprotein toxicity is mediated by a dynamin-dependent protein-trafficking pathway, *J Virol* 2005;79(1):547-553)

Novel mechanism of antibody-dependent enhancement discovered for Ebola. NIAID-supported researchers determined that infection with the Ebola Zaire virus induces antibodies that enhance viral infectivity. When plasma or serum from convalescing patients was mixed with primate kidney cells, the infection of these cells was enhanced. This enhancement was mediated by antibodies to the viral GP and by complement C1q. This finding raises additional considerations for vaccine development.

(Takada A et al., Antibody-dependent enhancement of Ebola virus infection, *J Virol* 2003;77(13):7539-7544)

Vaccines

Vaccine given post-exposure protects monkeys against deadly Marburg virus. A study by NIAID intramural scientists in collaboration with other researchers has revealed that a vaccine made from an attenuated recombinant vesicular stomatitis virus (rVSV) and administered to five rhesus macaques 20 to 30 minutes after exposure to a high dose of Marburg virus helped all of them survive. Three control monkeys not protected with the vaccine all died within two weeks. This result demonstrates that it may be possible to use rVSV vaccines to treat Marburg and similar viruses, such as Ebola, after infection.

(Daddario-Dicaprio KM et al., Postexposure protection against Marburg haemorrhagic fever with recombinant vesicular stomatitis virus vectors in non-human primates: an efficacy assessment, *Lancet* 2006; 367(9520):1399-1404)

Comparison of live attenuated tick-borne encephalitis virus vaccine candidates. NIAID researchers developed three antigenic, chimeric, live attenuated tick-borne encephalitis virus (TBEV) vaccine candidates and tested them in neuroblastoma cells, mice, and rhesus monkeys. Two vaccine candidate viruses were generated by replacing the membrane precursor and envelope protein genes of dengue type 4 virus (DEN4) with the corresponding genes of the Sofjin TBEV strain. This was done with and without a 30-nucleotide deletion in the 3' noncoding region of the DEN4 part of the chimeric genome. A third vaccine candidate was based on naturally attenuated Langat virus (LGT). The TBEV/DEN4 candidate without the deletion had little attenuating effect in monkeys. However, the TBEV/DEN4 candidate with the 30-nucleotide deletion greatly attenuated the chimeric virus for rhesus monkeys and induced a higher level of antibody against the TBEV than did LGT/DEN4. A single dose of either highly attenuated TBEV/DEN4del30 or LGT/DEN4 vaccine candidate or three doses of an inactivated TBEV vaccine were efficacious in monkeys against wild-type LGT challenge. These results indicate that both TBEV/DEN4del30 and LGT/DEN4 are safe and efficacious in rhesus monkeys and should be further evaluated as vaccine candidates for use in humans.

(Rumyantsev AA et al., Comparison of live and inactivated tick-borne encephalitis virus vaccines for safety, immunogenicity and efficacy in rhesus monkeys, *Vaccine* 2006;24(2):133-143)

Tetravalent dengue vaccine candidates are promising in preclinical testing. NIAID intramural scientists developed multiple attenuated viruses representing all four dengue subtypes for use as components of a tetravalent dengue vaccine. The monovalent vaccine candidates for DEN1 to DEN4 were generated using two distinct recombinant methods and found to be attenuated and immunogenic in mouse and rhesus monkey models. Three tetravalent vaccines based on these four monovalent recombinant vaccines were compared to a tetravalent formulation of wild-type dengue viruses for replication and immunogenicity in animal models. Two of the tetravalent formulations of the recombinant attenuated viruses were found to possess properties of a successful DEN vaccine and can be considered for evaluation in clinical trials.

(Blaney JE et al., Recombinant, live-attenuated tetravalent dengue virus vaccine formulations induce a balanced, broad, and protective neutralizing antibody response against each of the four serotypes in rhesus monkeys, *J Virol* 2005;79(9):5516-5528)

Accelerated vaccine for Ebola protects monkeys. Scientists from NIAID's VRC, in collaboration with the U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID), reported that a single shot of a fast-acting, experimental Ebola vaccine successfully protected monkeys after only one month. In this study, the VRC scientists immunized eight monkeys with a single boost injection, consisting of attenuated carrier viruses containing genes for important Ebola antigens. The monkeys were then delivered to USAMRIID where they were injected with an Ebola virus strain obtained from a fatally infected person from the former Zaire in 1995. The single vaccine injection completely protected all eight animals against Ebola infection, even those that received high doses of the virus. This finding suggests that it might be possible to quickly contain Ebola outbreaks with ring vaccination.

(Sullivan NJ et al., Accelerated vaccine for Ebola virus hemorrhagic fever in non-human primates, *Nature* 2003;424(6949):681-684)

Diagnostics

New portable biosensor facilitates rapid detection of dengue virus. NIAID-supported scientists have developed a multi-analyte biosensor for rapid detection of dengue virus. The sensor is based on nucleic acid hybridization and liposome signal amplification. Using a single analysis, this biosensor can rapidly detect any of the four dengue serotypes individually or in mixtures of two. The biosensor demonstrated 92 percent reliability in dengue serotype determination and will be useful for identifying possible dengue infections in clinical samples. The multi-analyte biosensor is portable, inexpensive, and easy to use, and represents an alternative to current more expensive or time-consuming detection methods.

(Zaytseva NV et al., Multi-analyte single-membrane biosensor for the serotype-specific detection of Dengue virus, *Anal Bioanal Chem* 2004; 380(1):46-53)

Development of a novel assay for the detection of human antibodies to Ebola using reverse genetic systems. In this assay, which uses reverse genetics and *de novo* synthesis of negative sense viruses from cloned cDNA, researchers were able to safely determine antibodies that are reactive with all subtypes of Ebola. The assay uses particles prestained with a dye so that detection of binding can be directly determined by visual inspection. The assay is both simple and economical. This accomplishment has particularly helped foster the development of a broad-based, novel assay for the detection of

Ebola. A reverse-genetics system for Crimean-Congo hemorrhagic fever virus is also under development.

(Neumann G et al., Reverse genetics demonstrates that proteolytic processing of the Ebola virus glycoprotein is not essential for replication in cell culture, *J Virol* 2002;76(1):406-410)

Therapeutics

Liposome-DNA complexes induce protective immunity in mice against PT virus, a surrogate for RVF virus. There are currently no Food and Drug Administration-approved antivirals for treatment of hemorrhagic fever viruses such as RVF virus, a member of the Bunyaviridae family. Due to the danger of working with Category A pathogens such as RVF virus, NIAID-supported investigators tested potential antiviral agents using a related virus, PT, which is also a member of the Bunyaviridae family of viruses. In collaboration with a small company, NIAID-supported scientists have recently shown that liposomes complexed to noncoding plasmid DNA protected mice from death when given one day before or one day after PT virus challenge. Remarkably, a single 0.5 microgram dose given to mice 24 hours before challenge protected over 90 percent of the animals from death. A single 1 microgram dose given 24 hours after challenge protected over 80 percent of the mice from death. Although not yet tested against RVF virus, which requires maximum-level containment facilities, the data obtained against PT virus suggest that it might be possible to prevent or treat an RVF infection using this approach.

(Gowen B et al., Protective immunity against acute phleboviral infection elicited through immunostimulatory cationic liposome-DNA complexes, *Antiviral Res* 2006;69(3):165-172)

PROGRAMMATIC PROGRESS IN ADDRESSING IMMEDIATE GOALS

GOAL: Develop animal models that mimic human disease for studying VHF pathogenesis in humans.

- NIAID animal model resources were expanded to include evaluation of several VHFs and encephalitides. The new models are:
  - Hantavirus in hamsters (as a model for Hantavirus-induced cardiogenic shock) (University of Texas Medical Branch-Galveston (UTMB); Lovelace Respiratory Research Institute, Albuquerque, NM)
  - Bunyavirus: PT virus in hamsters (as a model for RVF virus) (Utah State University)
  - Bunyavirus: PT virus in mice (as a model for RVF virus)
  - Arenavirus: Pichinde virus in hamsters (as a model for Lassa and South American hemorrhagic fever viruses)
  - Flavivirus: Banzi Nile virus in mice (as a model for dengue and other flaviviruses)
  - Togavirus: Semliki Forest virus in mice (as a model for encephalitis alphaviruses)

GOAL: Expand the effort to determine the correlates of immunity for VHF vaccines by using appropriate models of natural infection.

- Research to develop and test dengue vaccines and drug treatments has been severely hampered by the lack of an animal model that mimics disease in humans. An important breakthrough has been the recent development of a small animal model of dengue fever in NOD/SCID mice xenografted with human CD34+ cells. Although early in development, this animal model may prove to be invaluable for the study of dengue disease syndromes and potential evaluation of vaccine candidates and therapies. See Scientific Progress section, Development of a clinically relevant small animal model of dengue fever will advance the development of vaccines and therapeutics. (Southwest Foundation for Biomedical Research, San Antonio, TX)
- In FY 2004, a new NIAID intramural project was initiated in which researchers are using the naturally attenuated Langat Tick Borne Encephalitis Virus (TBEV) to study three major areas: 1) interactions between TBEVs and interferon signaling (interferon is used as a therapy for flavivirus infection, however, such treatment often fails); 2) development of improved animal models to study viral mechanisms of pathogenesis and immune responses to infection; and 3) design of DNA vaccines to protect against infection.
- Research with the highly pathogenic RVF virus (bunyavirus) must be conducted in maximum BSL-4 containment, which has hampered progress. Alternative animal models for RVF are thus being pursued to allow study of this disease in more widely available BSL-3 facilities. To this end, alternative animal models of RVF are being developed in hamsters using two less pathogenic bunyaviruses, PT and Gabek Forest viruses. These animal models closely mimic RVF and thus appear to be appropriate for study of RVF pathogenesis and for initial testing of

candidate RVF vaccines and therapies. See Scientific Progress section for details and publication citation. (UTMB)

- Progress is being made on the development and characterization of a YF/hamster model of disease, which appears to correlate with human YF infection. This model is being used to study host responses to the currently licensed live, attenuated YF 17D vaccine as well as effectiveness of potential YF therapeutics. (UTMB)

GOAL: Advance the development of VHF vaccine candidates (e.g., Rift Valley Fever and Junin Fever).

- The VRC has completed the first human clinical trial of a DNA vaccine designed to prevent Ebola infection. The vaccine, composed of three DNA plasmids, was well tolerated and elicited both humoral and cellular immune responses at all doses. In parallel, non-human primate challenge studies have refined the design of the final Ebola vaccine products. The DNA plasmid and recombinant adenovirus (rAd) products are currently being manufactured for clinical testing.
- In FY 2005, a new NIAID intramural project was initiated to study the interactions of flaviviruses with antibodies. An important goal of flavivirus vaccine development is to mimic the antibody response that occurs during natural infection. However, under some circumstances, the presence of antibodies can also increase the efficiency of viral infection. This phenomenon, called antibody-dependent enhancement (ADE) has been linked to a more severe clinical outcome of dengue infection, so a safe dengue vaccine must protect against all four dengue serotypes. NIAID researchers are investigating the mechanisms of antibody-mediated neutralization and ADE to determine the biochemical and cellular factors influencing the outcome of the interaction of flavivirus particles with antibodies.
- NIAID intramural scientists have continued to develop and test vaccine formulations for each dengue subtype, and to improve their formulations based on clinical data. The goal is to combine the best formulation for each dengue subtype into a tetravalent dengue vaccine. Five dengue vaccine formulations are now awaiting investigational new drug (IND) approval or are in Phase I clinical trials. The trials are being conducted in collaboration with The Johns Hopkins Bloomberg School of Public Health Center for Immunization Research. This collaborative effort is also enabling rapid clinical testing of several other vaccine candidates developed by NIAID intramural scientists, including vaccines for several category B and C agents. For example, a Phase I clinical trial of a chimeric, live-attenuated West Nile virus vaccine is completed and results are being analyzed. A Phase I clinical trial of a chimeric vaccine for tick-borne viral encephalitis is under way.
- A Phase I clinical trial of a West Nile virus vaccine developed by NIAID intramural scientists is under way at The Johns Hopkins Bloomberg School of Public Health Center for Immunization Research. The trial is assessing the safety and immunogenicity of a live vaccine composed of an attenuated dengue virus which has had two of its structural genes replaced by the corresponding genes of a West Nile virus.
- A Phase I clinical trial of the safety and immunogenicity of a TBEV (LGT(TP21)/DEN4) developed by NIAID intramural scientists began in 2005. The trial is being conducted by collaborators from The Johns Hopkins Bloomberg School of Public Health and Vanderbilt University.
- In FY 2003, NIAID supported the following areas of VHF vaccine development through various grant programs and other mechanisms:
  - Preclinical development of a non-replicating recombinant dengue subunit vaccine (Hawaii Biotech Inc., Aiea, HI)
  - Design of a virus-like particle vaccine against RVF (Emory University)
  - Evaluation of a dengue-2 DNA vaccine in monkeys (University of Puerto Rico)
  - Development of a chimeric Lassa virus vaccine (University of Maryland Biotechnology Institute)
  - A Kunjin replicon-based vaccine against Ebola (University of Queensland, Brisbane, Australia)
  - A Sindbis-based vaccine against RVF virus (UTMB)
  - A Brucella-based vaccine against Ebola and Lassa viruses (Purdue University)
- In FY 2004, NIAID supported development of the following VHF interventions through various grant programs:
  - A live, attenuated vaccine for RVF virus (UTMB)
  - Further development, including Phase I clinical studies, of a non-replicating recombinant subunit dengue vaccine (Hawaii Biotech Inc., Aiea, HI)

– Development of non-replicating recombinant protein virus-like particle (VLP) vaccines against dengue (Protein Potential LLC, Gaithersburg, MD)

– New adjuvant technologies for a Marburg virus vaccine (Southern Research Institute, Birmingham, AL)

– A polynucleotide vaccine delivery system for a dengue DNA vaccine (Cyto Pulse Sciences, Inc., Columbia, MD)

- In FY 2005, NIAID continued its support for development of vaccines through awards for the following projects:

  – Preclinical development of a recombinant immune complex (RIC) vaccine (prepared in plants) against Ebola (Arizona State University)

  – A genetically engineered, live attenuated vaccine against RVF (UTMB)

  – A Venezuelan Equine Encephalitis-replicon-based vaccine against dengue virus (University of North Carolina, Chapel Hill)

  Note: For further information about the awards described in the previous paragraphs, visit the NIAID Web site at www.niaid.nih.gov/biodefense/research.

GOAL: Establish capacity for the development, refinement, and production of pilot lots of candidate VHF vaccines.

- A Marburg virus (filovirus) vaccine is being developed and a pilot lot is being manufactured using Good Manufacturing Practices (GMP) production. (AlphaVax Human Vaccines, Inc., Research Triangle Park, NC)

GOAL: Develop a centralized immunology laboratory for the validation of tests required for licensure of priority VHF vaccines.

- As candidate vaccines for VHFs are developed, NIAID will expand existing infrastructure in order to meet requirements of licensure.

- The NIAID VRC has developed overlapping peptides to glycoprotein and nucleoprotein for strains of Ebola virus. These peptides will be produced and validated under GMP and will be used in ELISA assays by the centralized immunology laboratory.

GOAL: Screen antibodies to evaluate their possible use as immune therapy for VHFs.

- Monoclonal antibodies are being developed and evaluated for possible prophylaxis and therapy against Ebola virus. (Mapp Biopharmaceutical, Inc., San Diego, CA)

GOAL: Obtain clinical samples from patients with VHFs to help validate potential diagnostics and aid the development of new vaccine therapies.

- NIAID-supported scientists are collecting and using blood and sera from VHF arenavirus patients in Africa as part of an effort to develop and validate multiagent diagnostic immunoassays. Sera and nucleic acids derived from the blood samples will be irradiated to render them non-infectious and then provided to the research community upon request for further immunological and diagnostic studies. (Tulane University)

- A contract with the World Reference Center for Emerging Viruses and Arboviruses includes a provision for collection of reference clinical material from anonymous donors where appropriate. (UTMB)

GOAL: Expand the *in vitro* and *in vivo* screening capability for antivirals against VHFs.

- New antiviral screening tools, including various cell and molecular systems, are being developed to assess potential therapeutic drug activities against Ebola, RVF virus, and hantaviruses. This work is being conducted under Small Business Innovation Research and Small Business Technology Transfer grants as well as other funding mechanisms. For example, NIAID is supporting studies to develop cell-culture based reporter gene expression systems to screen for filoviruses and bunyaviruses. (Apath, LLC, St. Louis, MO)

- Progress on screening compounds for potential antiviral activity has accelerated. The number of compounds evaluated for *in vitro* activity against hemorrhagic fever viruses includes more than 2,100 for Yellow Fever virus, 1,550 for dengue virus, 1,700 for Pichinde virus (model for Lassa and other arenaviruses), and 1,800 for PT virus (model for RVF virus and hantaviruses). A small number of compounds with *in vitro* activity (evidenced by reduced cytopathogenic effect or reduced growth in cell culture) are being evaluated in animal models of disease for Banzi (model for dengue), PT, and Pichinde. (Utah State University)

GOAL: Encourage the exploration of new targets for antiviral therapies against VHFs.

- Using authorities granted by the Project BioShield Act of 2004, NIAID awarded the following two grants for the development of antiviral drugs for Ebola in FY 2005:
  - Development of new antiviral drugs for Ebola infection (Apath, LLC, St. Louis, MO);
  - Development of poly-ICLC for treatment of Ebola (Oncovir, Inc., Washington, DC)
- In FY 2003, NIAID awarded a grant for development of RNA interference (RNAi) as a therapeutic defense against dengue and other agents (Center for Blood Research, Boston, MA)
- NIAID has been supporting large, multidisciplinary efforts focused on the proteomics of more than one microorganism. This includes the Biodefense Proteomics Collaboratory, a multidisciplinary team of scientists from both academia and the biotechnology industry who are using high-throughput proteomic technologies to identify protein targets from arenaviruses and Lassa virus. (UTMB)

GOAL: Complete the genomic sequencing of representative members and strains of the VHFs, and compare them to detect differences in pathogenesis and virulence.

- NIAID is supporting the sequencing of dengue viruses under a project through the Microbial Sequencing Center at the Broad Institute. (Massachusetts Institute of Technology)

ADDITIONAL PROGRESS

- NIAID-funded scientists are developing nucleic acid-based methodologies for rapid, accurate detection of South American arenaviruses. (UTMB)

Immunity and Biodefense

The key strategies of vaccination, immunotherapy, and passive antibody treatment rely upon effective manipulation of the immune system in the face of highly virulent pathogens. New understanding of how the immune system recognizes and takes action against pathogens opens new avenues for devising protective strategies. Most important is the elucidation of the innate immune system's response to microbial invasion and its interaction with the adaptive immune system, leading to the effective development of antibodies and cellular responses that clear the infection and provide long-term protection.

Innate immune defenses are based both upon cells permanently located within tissues and upon the migration of additional cells to the site of infection as needed. The innate immune system network includes Langerhans cells of the skin, tissue dendritic cells and macrophages, and tissue-associated lymphocytes such as natural killer cells and gamma-delta receptor T cells. In addition, many epithelial cells can sense and respond to microbes. Responses include the secretion of antimicrobial peptides that act directly on microbes, as well as the expression of cytokines and chemokines that call additional cells into action, including antigen-specific T and B cells. The key to rapid innate responses is the presence of highly specialized receptors, including the family of receptors known as the Toll-like molecules, which trigger cellular activation in response to various microbial structures. These "pattern-recognition receptors" detect and signal the presence of a broad range of microbes and viruses. For example, ligands of the Toll-like receptors (TLRs) include bacterial lipopolysaccharides, bacterial cell wall teichoic acid, flagellin, cell wall lipoproteins, highly mannosylated polymers, and bacterial nucleic acids that contain unmethylated CpG sequences.

In addition to innate immunity, the host relies on the induction of acquired immune responses to provide long-lived, pathogen-specific defense against infectious diseases, including those caused by agents of bioterrorism. Acquired immunity is distinguished from innate immunity in several ways: specificity, reaction time, and duration of response. Acquired immune responses are directed against specific pathogen components, broadly termed "antigens." Development of a protective response requires exposure to the antigen, either by natural infection or through vaccination strategies, and can take up to two weeks to reach maximum protection. However, once generated, most acquired immune responses persist for the life of the host, a phenomenon known as immune memory. Hallmarks of immune memory include the presence of antibodies against the original antigen and a decrease in the time required for the protective T-cell response after secondary or subsequent exposure to an antigen. Immune memory is the main mechanism by which vaccines provide protection to the host.

SCIENTIFIC PROGRESS

Biology of the Microbe

Immune-evasion strategies determined for anthrax, smallpox, and plague. An understanding of the strategies that pathogens have evolved for evading innate immunity is critical for the rational design of immunotherapeutic approaches. Scientists have now shown that anthrax lethal factor (LF) selectively inhibits a key receptor-signaling pathway needed for macrophage survival, resulting in the death of these key cells of the innate immune system. Variola (smallpox) virus possesses an extraordinarily effective inhibitor of complement, one of the key tools of innate immunity involved in viral clearance. Poxviruses also possess several evasion strategies aimed at defeating interferon, including at least four gene products that counteract interferon. *Yersinia pestis* secretes an outer membrane protein that attaches to the innate immune receptors CD14 and Toll-like receptor 2, triggering an abnormal immune system suppression. The prevalence of these evasion strategies points to the importance of the innate immune system in protection against infection, and underscores the need to search for approaches to bolster innate immunity as a strategy for biodefense.

(Sing A et al., *Yersinia* V-antigen exploits toll-like receptor 2 and CD14 for interleukin 10-mediated immunosuppression, *J Exp Med* 2002;196:1017-1024)

Host Response

Distinguishing bad bugs. The innate immune system provides a first line of defense against infections, mainly by early detection methods that help to coordinate more specific immune responses. Recent studies by National Institute of

Allergy and Infectious Diseases (NIAID)-supported investigators help to unravel one of the pathways that innate immune cells use to discriminate among different types of bacteria. TLRs are one of the major innate immune molecule families involved in early pathogen detection and immune activation. At least 10 TLR molecules have been identified and their fine specificity determined. This team discovered that a cellular protein, CD36, partners with TLR-2 and TLR-6 to distinguish different bacteria based on the types of lipids that the bacteria produce. Understanding this fine specificity may allow investigators to develop novel pathogen detection devices or methods to regulate innate immune responses.

(Hoebe K et al., CD36 is a sensor of diacylglycerides, *Nature* 2005;433:523-527)

Two T-box transcription factors are critical for CD8+ T memory cells. CD8+ T cells are crucial for generating efficient cellular immune responses against viruses. A transcription factor, T-bet, was originally shown to be important for the development of CD8+ T cells. However, studies of T-bet deficient mice showed that CD8+ T-cell cytotoxicity and maintenance are partially independent of T-bet. Recently, NIAID-supported investigators determined that both T-bet and Eomesodermin, a related T-box transcription factor, are essential for CD8+ T-cell cytotoxic function and maintenance of CD8+ T-cell memory against particular virus infections in mice. This work shows that each transcription factor (T-bet and Eomesodermin) has both overlapping and distinct roles in effector functions and memory development of CD8+ T cells. Understanding the development of optimal primary and secondary CD8+ T-cell responses is crucial to developing effective vaccines.

(Intlekofer AM et al., Effector and memory CD8+ T cell fate coupled by T-bet and Eomesodermin, *Nature Immunol* 2005;6:1236-1244)

IFN-gamma production and immunodominant viral epitopes. The production of the cytokine interferon (IFN)-gamma by CD8+ T cells has been shown to be important in effective immune responses in a number of viral infections. Using a mouse model, NIAID-funded researchers demonstrated that the amount of IFN-gamma produced varies among T cells of different antigen specificities. T cells that are specific for immunodominant peptide epitopes produce IFN-gamma more rapidly than T cells that recognize subdominant epitopes. In contrast, once the virus has been cleared, all of the memory T cells respond quickly, whether they recognize dominant or subdominant epitopes. These data demonstrate that IFN-gamma plays a significant role during the primary immune response to virus.

(Liu F et al., The rapidity with which virus-specific CD8+ T cells initiate IFN gamma synthesis increases markedly over the course of infection and correlates with immunodominance, *J Immunol* 2004;173:456-462)

Characterization of memory T cells demonstrates their importance for protective immunity. A subset of memory T cells, called central memory cells (Tcm), expresses lymph node homing receptors but lacks effector T-cell functions until restimulated by antigens and/or cytokines. Further characterization of these cells by NIAID-funded researchers revealed additional subsets, including cell types responsible for countering infection by cytomegalovirus and vaccinia virus. This study supports the growing paradigm that long-term T-cell memory acquisition is a progressive differentiation process that begins in effector T cells after primary immunization and proceeds with time to generate long-lived memory T cells that provide protection upon subsequent encounter with the same pathogen.

(Rivino L et al., Chemokine receptor expression identifies Pre-T helper (Th1), Pre-Th2, and nonpolarized cells among human CD4+ central memory T cells, *J Exp Med* 2004;200:725-735)

Development of new methods for imaging immune cells. NIAID researchers developed new methods for imaging immune cell behavior in various tissues of the body, including the skin, liver, gut, kidney, lymph nodes, and spleen. In addition, they have begun work on imaging in the lungs, a major site of entry of pathogens, including those listed as potential bioterror agents. These new methods are being applied to understand how cells interact during the initiation of normal and pathologic immune responses and how effector cells behave in tissue sites during responses to infections or self-antigens.

(Huang AY et al., Illuminating the landscape of in vivo immunity: insights from dynamic in situ imaging of secondary lymphoid tissues, *Immunity* 2004;21:331-339)

Understanding how memory cells survive may improve vaccines. In studying the mechanisms by which T-cell memory precursors are protected from cell death and induced to differentiate into long-lived memory cells, NIAID-supported investigators identified the serine protease inhibitor 2A (Spi2A) as a protective molecule that increases when certain types of T cells develop into memory cells. Using a mouse

model, these investigators demonstrated that Spi2A inhibits intracellular enzymes, preventing elimination of memory cell precursors. Understanding the mechanisms by which memory cells develop and survive in the body is central to developing new and more effective vaccines.

(Liu N et al., Serine protease inhibitor 2A is a protective factor for memory T cell development, *Nature Immunol* 2003;5:919-926)

Plasmacytoid dendritic cells play a role in antibody production. Dendritic cells (DC) are cells of the immune system that initiate and regulate innate and acquired immune responses to microorganisms. Recent work by NIAID-supported investigators demonstrated that in mice, depletion of a particular type of DCs—plasmacytoid DC subsets—stops production of antigen-specific antibodies. Scientists further demonstrated that plasmacytoid DCs (pDC) are critical in controlling the differentiation of B cells into antibody-secreting plasma cells. This can be at least partially explained because when pDCs encounter viruses and virus-specific T cells, they produce cytokines known to influence B-cell differentiation into plasma cells.

(Jego G et al., Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and IL-6, *Immunity* 2003;19:225-234)

Protein switch for both bacterial and viral infections identified. NIAID-supported researchers have determined that a single protein acts as a key switch point in front-line immune system reactions to both bacterial and viral infections in mice. This finding explains why certain symptoms, such as fever, occur regardless of the cause of infection. The protein, called Trif, is a critical first-line signal for the mouse innate immune system and alerts TLRs to the presence of infectious agents. Once activated by invading pathogens, TLRs relay the alarm to other actors in the immune system. The innate immune system then responds with a surge of chemicals that together causes inflammation, fever, and other responses to infection or injury. This finding provides a new target for the development of broad-spectrum antibiotics.

(Hoebe K et al., Identification of Lps2 as a key transducer of MyD88-independent TIR signaling, *Nature 2003;424(6950):743-748*)

New clues are discovered on how innate immune system is regulated. A full understanding of how innate immune responses are regulated by the body so that they do not result in overwhelming septic shock is a major challenge. The regulatory mechanisms of innate immunity are being defined. For example, the molecule IRAK-M was recently found to negatively regulate TLR signaling.

(Kobayashi K et al., IRAK-M is a negative regulator of Toll-like receptor signaling, *Cell* 2002;110:191-202)

Prophylactic and post-exposure strategies involving innate immune stimulation can prevent or ameliorate bacterial and viral infections in animal models. Researchers recently demonstrated that administration of CpG-containing nucleic acids primed animals to resist lethal infection with intracellular bacteria. Fast-acting, broad-spectrum immunotherapeutics are being developed using knowledge about mechanisms for stimulating Toll-like molecules and other innate immune receptors. The next step will be preclinical development and testing to evaluate whether innate immune stimulation could be safely and effectively manipulated.

(Katze MG et al., Viruses and interferon: a fight for supremacy [Review], *Nat Rev Immunol* 2002;2:675-687)

Stimulation of Toll-like receptors allows immune system to overcome natural suppression. For many years adjuvants have been used to enhance vaccination responses by a process now understood as the innate immune triggering of adaptive immunity. Immature DCs activated via their TLRs undergo a maturation process that includes migration to the local lymph node, expression of co-stimulatory molecules needed for lymphocyte activation, and an enhanced ability to present engulfed protein antigens in the context of major histocompatibility complex (MHC) class I and class II molecules. Recently, investigators have shown that adjuvant activation of innate immune molecules promotes increased survival of activated T cells. Stimulation via TLRs also temporarily relieves natural immune suppressive mechanisms in the body, thus enabling more vigorous responses. This knowledge will help scientists in developing new, more specific adjuvants that are safer and more powerful.

(Sing A et al., Yersinia V-antigen exploits Toll-like receptor 2 and CD14 for interleukin 10-mediated immunosuppression, *J Exp Med* 2002;196:1017-1024)

Cellular protein that plays a critical role in humoral immunity is identified. Humoral immunity is provided by B cells, which differentiate into antibody-producing cells (i.e., plasma cells) upon exposure to antigen. Long-lived plasma cells and memory B cells are vital components of the protection induced by most vaccines. Researchers recently identified a cellular protein, SAP, that is critical for the development of

long-term humoral immunity. Understanding SAP's role in humoral immunity may lead to the manipulation of SAP-mediated signaling pathways for therapeutic benefit.

(Crotty S et al., SAP is required for generating long-term humoral immunity, *Nature* 2003;421:282-287)

A novel protein that plays a critical role in antigen processing identified. Antigen-presenting cells (APCs) rely on internal enzymes to digest, or process, the antigens. Recently, investigators identified a novel protein, gamma interferon-inducible lysosomal thiol reductase (GILT), that plays a critical role in antigen processing. Most proteins are globular in form and are held together by chemical bonds. Normally, GILT breaks down one type of chemical bond that is commonly used by certain viruses, bacteria, and parasites to hold their proteins together. These studies show that immune responses are significantly diminished in GILT-free mice compared to normal mice that have the GILT protein. This work defines a critical antigen-processing component whose function contributes to the development of protective immunity. A clearer understanding of the antigen processing pathway may lead to the design of novel vaccines and therapeutics to combat infectious diseases.

(Maric M et al., Defective antigen processing in GILT-free mice, *Science* 2001;294:1361-1365)

T-cell binding is driven by survival of the fittest. Antigen processing and presentation are critical steps in the development of a protective immune response because T-cell recognition of the peptide-MHC complexes results in T-cell activation and pathogen clearance. Recent studies have shown that T cells that bind very tightly to peptide-MHC prevent weaker-binding T cells from binding and being activated. This competition results in the preferential activation and growth of strong-binding T cells, which may be more efficient at clearing infections from the host compared to weak-binding T cells. These results help explain the observation that responding T-cell populations become less diverse upon second infection with the same microbe, and may be exploited to improve vaccine design and delivery strategies.

(Tangri S et al., Structural features of peptide analogs of human histocompatibility leukocyte antigen class I epitopes that are more potent and immunogenic than wild-type peptide, *J Exp Med* 2001;194:833-846)

Vaccines

Dendritic cell vaccination enhances CD8+ T-cell memory development. To be effective, vaccines must generate a sufficient number of memory T cells that will function efficiently when an individual encounters the pathogen at a later date. DCs are potent activators of immune responses by antigen-specific T cells and there has been a great deal of interest in developing DC-based vaccines for both infectious diseases and tumor therapy. Using a model of *Listeria monocytogenes*, NIAID-supported researchers have shown that priming for CD8+ T cells with a DC vaccine increases the speed at which T-cell memory is developed. This work also demonstrated that priming with DC vaccines allowed boosters to be given sooner and still provide good protective memory T-cell responses. Understanding DC-T-cell interactions, adjuvants, and inflammatory signals required to elicit protective memory responses is essential to design effective vaccines.

(Badovinac VP et al., Accelerated CD8+ T-cell memory and prime-boost response after dendritic-cell vaccination, *Nature Medicine* 2005;11:748-756)

Oligodeoxynucleotides connected with innate immunity against bacteria. The genetic material of bacteria often contains long stretches in which the cytosine-guanine nucleotide pair–CpG–repeats many times. This motif, not found in mammalian DNA, allows the mammalian immune system to recognize and respond to bacterial DNA as foreign material. CpG DNA serves as a potent stimulator of the immune system, which makes it potentially useful as a component for immunotherapeutics. CpG oligodeoxynucleotides (CpG ODN) have been shown to provide broad spectrum defense against pathogens in animal challenge models and are being developed as possible adjuvants for vaccines against pathogens that are potential agents of bioterror. NIAID-funded work recently demonstrated that CpG ODN can also provide protection as a stand-alone immunostimulant when given intra-tracheally prior to challenge with a bacterial pathogen. Study results showed increased numbers of innate immune cells in the lung, enhanced bacterial clearance from the lung, and increased survival in an animal model. This is the first study to demonstrate the effectiveness of CpG ODN against an extracellular pathogen.

(Deng JC et al., CpG oligodeoxynucleotides stimulate protective innate immunity against pulmonary Klebsiella infection, *J Immunol* 2004;173:5148-5155)

Uric acid, a novel signal for innate immune activation, could be a potential new adjuvant. NIAID-supported researchers identified uric acid, which is released from dying cells after

infection, as a powerful signal that activates innate pathways of immunity. Uric acid is normally present in high levels in the body, but remains soluble and invisible to the immune system. When infected cells begin to die, however, their DNA degrades, releasing such high levels of uric acid as to saturate the cellular environment and crystallize. This crystalline form of uric acid has been found to stimulate the immune system, and could lead to a new generation of vaccine adjuvants.

(Shi Y et al., Molecular identification of a danger signal that alerts the immune system to dying cells, *Nature* 2003;425:516-521)

Molecular modifications result in more effective cytotoxic T lymphocyte (CTL) recognition. Another promising approach to enhancing immune responses is to design vaccines that contain specific viral or bacterial peptides that can be recognized by CTLs. Frequently, however, the natural peptides derived from these pathogens do not stimulate T-cell responses efficiently. Investigators have now modified these peptides, resulting in more effective CTL recognition than the natural, unmodified peptide. In addition, in some cases, far less modified peptide was needed to obtain the same immune response as compared with the natural peptide. When tested in mice, the modified peptides efficiently elicited CTLs. These observations suggest that systematic molecular alterations of peptides may prove a viable approach for designing vaccines that elicit potent CTL responses. This finding can also be exploited to design subunit vaccines that will induce long-term protection without the risk of side effects that live or attenuated vaccines can induce.

(Drexler I et al., Identification of vaccinia virus epitope-specific HLA-A*0201-restricted T cells and comparative analysis of smallpox vaccines, *Proc Natl Acad Sci USA* 2003;100:217-222)

Therapeutics

Treating smallpox infection by blocking signaling pathways. After smallpox was eradicated worldwide in the 1970s, immunization programs were largely discontinued. A key challenge now is to develop treatments for smallpox in unvaccinated individuals. Most antiviral agents target specific viral proteins or the process of viral replication. However, NIAID-funded scientists recently took the novel approach of targeting normal cellular signaling pathways that the pox virus usurps for its replication and budding from infected cells. These scientists found that a cancer chemotherapy drug called CI 1033 significantly diminishes the ability of pox viruses to produce infectious viral progeny. The drug inhibits the smallpox growth factor (SPGF), a growth factor produced by poxviruses, from activating a receptor that in turn activates several cell signaling pathways, including signaling cascades that enhance viral replication and release. The research team found that CI 1033 blocks the ability of SPGF to activate the receptor in cultured cells *in vitro*. They also showed that mice treated with CI 1033 before infection and given high levels of a pox virus related to smallpox, survived significantly longer than control mice, and the amount of virus was reduced in infected tissues. This study is the first to demonstrate an effective treatment for acute viral infection by blocking a normal cellular signaling pathway used by a virus to propagate. Such a treatment strategy, which focuses upon the host cell rather than the infecting virus, may make it less likely that the virus would develop drug resistance.

(Yang H et al., Antiviral chemotherapy facilitates control of poxvirus infections through inhibition of cellular signal transduction, *J Clin Invest* 2005; 15:379-387)

Advances in antibody engineering technology provide knowledge to craft more effective and safe passive antibody treatments. Studies of a panel of engineered anthrax toxin-neutralizing antibodies demonstrated that passive transfer of high-affinity antibodies provides post-exposure protection in animal models. These high-affinity antibodies may be of therapeutic value in alleviating symptoms of anthrax toxin in infected individuals, and for prophylaxis to infection. Passive antibody treatment requires the production of large quantities of safe, effective product. In the past, and currently for some applications, antibodies for passive treatment were isolated from immune donors or immune animals, such as horses. Antibodies produced in animals have the potential of inducing an adverse reaction in the recipient. Transgenic animals are being generated that produce "human" polyclonal and monoclonal antibodies in response to immunization to alleviate this problem.

(Maynard J et al., Protection against anthrax toxin by recombinant antibody fragments correlates with antigen affinity, *Nat Biotechnol* 2002;20:597-601)

Passive administration of antibodies can be used to control infections. Recent advances by domestic and international researchers reinforce the idea that passive antibody treatments can be used to control human infections, with both prophylactic and therapeutic applications. For example, administration of a monoclonal antibody that neutralizes vaccinia virus is effective as both a prophylactic and therapeutic treatment of vaccinia-infected mice.

(Ramirez JC et al., Administration to mice of a monoclonal antibody that neutralizes the intracellular mature virus form of vaccinia virus limits virus replication efficiently under prophylactic and therapeutic conditions, *J Gen Virol* 2002;83:1059-1067)

Similarly, monoclonal antibody epitopes have been identified that recognize specific sites on the Ebola virus glycoprotein. Passive administration of a cocktail of these antibodies completely protects mice from lethal Ebola infection.

(Casadevall A, Passive antibody administration [immediate immunity] as a specific defense against biological weapons [Review], *Emerg Infect Dis* 2002;8:833-841)

PROGRAMMATIC ACCOMPLISHMENTS

- NIAID has established eight 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. A large component of each center's work is to develop and apply new assays to facilitate the study of human immune responses. (Emory University; Stanford University; Baylor Research Institute; University of Massachusetts; Dana-Farber Cancer Institute; Mt. Sinai Medical School; Blood Center of Southeastern Wisconsin; Oklahoma Medical Research Foundation)
- In FY 2004, NIAID established a centralized 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. The database and analysis tools are freely available at www.immuneepitope.org. (La Jolla Institute for Allergy and Immunology, San Diego, CA)
- Within the Large Scale Discovery of Antibody and T Cell Epitope Discovery Program, NIAID supports multiple contracts for 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. (For a list of awards, see Appendix B or www.niaid.nih.gov/biodefense/research/2004awards.)
- Recent accomplishments by investigators funded under the Antibody and T Cell Epitope Discovery Program include: detection of cross-reactive anti-botulinum toxin antibodies, epitope mapping in progress (Scripps Research Institute, La Jolla, CA); identification of novel T-cell epitopes for several pathogens including human vaccinia CD4 and CD8 epitopes and Arenavirus CD8 epitopes (La Jolla Institute of Allergy and Immunology); and development of novel/improved algorithms for defining T-cell epitopes that bind to Human Leukocyte Antigens (HLA) class I alleles. (Technical University of Denmark)
- In FY 2005, NIAID awarded five grants to support research on new approaches for vaccine development, focusing on the human innate immune system as a target for immune evasion by Category A-C priority pathogens. (Arizona State University; University of California, Davis; University of Pennsylvania; University of Queensland; University of Texas Medical Branch at Galveston)
- In FY 2005, NIAID created a research program to develop novel methods to protect or treat children, elderly, and immunocompromised populations from bioterror threats. Research includes identifying biological mechanisms responsible for increased susceptibility to infection or decreased effectiveness of vaccines in these populations, as well as testing treatments designed to increase safety or efficacy. (For a list of awards, see Appendix B or www.niaid.nih.gov/biodefense/research/2005awards.)
- In FY 2005, NIAID awarded four contracts to establish Immune Modeling Centers to develop mathematical modeling packages, validated in experimental systems, for simulating host immune responses to infection and vaccines. Each Center also contains strong bioinformatics and training components. Results obtained from these programs will sharpen assessments of vaccine efficacy and responses to other prophylactic and therapeutic treatments, and will advance development of novel or improved vaccines, prophylactics, and immunotherapeutics against emerging and re-emerging infectious diseases. (Duke University Medical Center; Mount Sinai School of Medicine; University of Pittsburgh; University of Rochester)
- NIAID is supporting the development of new vaccine adjuvants and immunotherapies with five contracts awarded under the program Innate Immune Receptors and Adjuvant Discovery. (Coley Pharmaceutical Group, Wellesley, MA; Corixa Corporation, Seattle, WA; Montana State University; NOVASCREEN Biosciences Corporation, Hanover, MD; VaxInnate Corporation, New Haven, CT)
- NIAID established a Population Genetics Analysis Program: Immunity to Vaccines/Infections to correlate immune response gene polymorphisms with outcomes of infection

by Category A-C agents or vaccination against these agents. (For a list of contractors, see Appendix A or www.niaid.nih.gov/biodefense/research/2004awards.)

- The National Institutes of Health (NIH) Tetramer Facility was expanded to produce major histocompatibility complex peptide tetramer reagents specific for T cells recognizing Category A-C priority pathogen antigens. (Emory University)
- For its Non-Human Primate Program, NIAID has developed immune monitoring reagents and MHC typing technologies.
- NIAID investigators, working with scientific collaborators at the U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID), and the Centers for Disease Control and Prevention (CDC), have developed a potentially effective vaccine strategy for Ebola virus infection in non-human primates. In November of 2003, NIAID initiated the first human trial of a DNA vaccine designed to prevent Ebola infection. The study was recently completed. In addition, NIAID is currently testing a fast-acting candidate Ebola vaccine that protects monkeys exposed to the virus one month after immunization. Such a vaccine would be especially useful in an acute outbreak setting. If this vaccine proves similarly effective in humans, it could one day be used to quickly contain Ebola outbreaks with ring vaccination—the same strategy used in the past against smallpox. A second-generation product may also be evaluated that would potentially provide coverage for Marburg and possibly Lassa virus.
- A new NIAID intramural project was initiated to understand and target innate immunity to infectious diseases. Investigators are studying how the structure of each of the human interferon (IFN) alpha family members correlates with its biological functions—including antiviral, antiproliferative, and immunomodulatory activities. Findings will lead to development of novel chimeric IFN-alpha proteins to test as potential antiviral therapeutics for human viral pathogens and select agents.
- To further understand how the immune system interacts with pathogens, NIAID intramural scientists established the Program in Systems Immunology and Infectious Disease Modeling (PSIIM) in 2005. PSIIM teams mathematicians, engineers, computer scientists, biophysicists, biochemists, geneticists, and cell biologists to provide tools and techniques necessary for quantitative, predictive modeling of immune function. These teams of experts integrate micro- and macroscopic modeling with data derived from analyses of cell components. Advanced imaging methods are being developed and applied to analyze immune responses *in vivo*. Accomplishments to date include:
  - Developed first-generation software suite (Simmune) for spatially resolved, multiscale modeling of cell signaling and cell behavior. Software enables complex systems modeling by biologists without needing advanced mathematical skills.
  - Initiated processes to partner with commercial firms to provide Simmune software to biomedical community. Aim to release unsupported free academic version in late 2006.
  - Established relationship with The RNAi Consortium (TRC, Broad Institute, MIT) to help develop NIAID high-throughput RNAi screening capacity for defining molecular networks relevant to immune function and pathogen responses.
- In May 2004, NIAID convened a workshop on Immunization and Vaccination in Special Populations to identify research opportunities and gaps relevant to protection of human subpopulations against bioterrorist threats.
- In June 2004, NIAID convened a workshop on Nanobiology Strategies for Understanding the Immune System to explore opportunities in nanotechnology applicable to development of new vaccines and therapeutics for biodefense.
- In March 2005, NIAID convened a workshop on Innate Immunity to Pathogen-Associated Molecular Patterns of NIAID Category B Protozoa. The goal of the workshop was to identify targets for the development of immune-based countermeasures against these poorly studied organisms.
- In June 2005, NIAID sponsored a workshop on humanized mice to identify opportunities to apply these model systems in vaccine development and define additional models that need to be constructed to aid in testing vaccine or immunotherapeutic candidates.
- NIAID awarded a multicomponent grant to create an "encyclopedia" of innate immunity: a comprehensive and detailed picture of this ancient, essential first line of defense against bacterial and fungal diseases. Under this award, researchers from Scripps Research Institute in La Jolla, California; Rockefeller University in New York; and the Institute for Systems Biology in Seattle are discovering new

ways to study the immune system in living tissue in real time, and to provide materials and information to the scientific community. Knowledge generated could help scientists develop treatments for septic shock, certain autoimmune disorders, and diseases caused by potential agents of bioterrorism.

To date, this program, Systems Approach to Innate Immunity and Inflammation, generated 35 monoclonal antibodies to human and mouse innate immune response genes. At least 10 of these antibodies have been submitted to the NIAID Biodefense Research Resource Repository for public distribution. The research team also produced 56 mutant mouse lines, using random mutagenesis techniques, with defects in immune response genes. Eighteen mutations were mapped to chromosomes and 14 genes were identified, 13 of which are novel genes involved in innate immune responses to viral and bacterial infections. Many of the mutant mice have been deposited in existing mouse repositories (such as Jax, NCRR Mutant Mouse Regional Resource Centers) for public distribution. (Scripps Research Institute, La Jolla, CA; Rockefeller University, NY)

- Administrative supplements were awarded to existing NIAID-supported research programs in FY2002 to support studies on:
  - Genes important for macrophage phagocytosis of anthrax
  - Anti-influenza responses in the elderly
  - Cloning of viral genes that subvert the effects of interferon
  - Protective immune responses in children receiving influenza vaccine
  - Protective effects of a DNA vaccine for anthrax
  - CD8+ T-cell responses to pox and dengue viruses
  - Development of monoclonal antibodies against botulism toxin
  - Characterization of potent anti-influenza T-memory cells in the lung

Appendixes

Category A Progress Report 2006: Appendix A

LIST OF RESOURCES

Atopic Dermatitis and Vaccinia Immunization Network (awarded in 2004):

- Clinical Studies Consortium, National Jewish Medical Center, Denver, CO
- Animal Studies Consortium, Children's Hospital Boston, MA
- Statistical and Data Coordinating Center, Rho Federal Systems Division, Inc., Chapel Hill, NC

Biodefense Proteomics Research Centers (awarded in 2004)

- Albert Einstein College of Medicine
- Caprion Pharmaceuticals, Inc.; University of Montreal
- Harvard Medical School; Massachusetts General Hospital
- Myriad Genetics, Inc; SUNY, Stonybrook; University of California, Los Angeles; Robarts Research Institute
- Pacific Northwest National Laboratory; Oregon Health Sciences University
- University of Michigan; The Scripps Research Institute

For more information, visit www.niaid.nih.gov/dmid/genomes/prc.

Biodefense Proteomics Administrative Center (awarded in 2004)

- Social & Scientific Systems, Inc. (SSS); Virginia Bioinformatics Institute; Georgetown University

For more information, visit www.niaid.nih.gov/dmid/genomes/prc.

Bioinformatics Integration Support Contract (awarded in 2002)

- Northrup Grumman Information Technology

For more information, visit www.niaid.nih.gov/about/organization/dait/bisc.htm.

Bioinformatics Resource Centers (awarded in 2004)

- The Institute for Genomic Research, Rockville, MD
- University of Pennsylvania; University of Georgia
- University of Notre Dame (UND); European Bioinformatics Institute; European Molecular Biology Laboratory; Institute of Molecular Biology and Biotechnology; Harvard University; Purdue University; University of California, Riverside
- University of Chicago (UC); Fellowship for Interpretation of Genomes; University of Illinois, Urbana-Champaign
- University of Alabama Birmingham (UAB); University of Victoria, Canada
- Northrop Grumman; University of Texas Southwestern Medical Center (UTSMC); Vecna Technologies; Amar International
- SRA International; University of Wisconsin, Madison
- Virginia Bioinformatics Institute (VBI); Loyola University Medical Center; Social and Scientific Systems; University of Maryland

For more information, visit www.niaid.nih.gov/dmid/genomes/brc.

Cooperative Centers for Translational Research on Human Immunology and Biodefense (awarded in 2003 and 2004):

- Emory University
- Baylor Research Institute, Dallas, TX
- Oklahoma Medical Research Foundation, Oklahoma City, OK
- University of Massachusetts Medical School
- Blood Center of Southeastern Wisconsin, Milwaukee, WI
- Mount Sinai School of Medicine
- Dana-Farber Cancer Institute, Boston, MA
- Stanford University

For more information, visit www.umassmed.edu/cidvr/CCTRHIB.

Extramural Research Facilities Improvement Program (co-sponsored by NIAID and NCRR, awarded in 2005):

- Colorado State University, Fort Collins
- St. Louis University
- Trudeau Institute
- University of Florida
- University of Massachusetts Medical School
- University of Pennsylvania
- Yeshiva University (Albert Einstein College of Medicine)

For more information, visit www.niaid.nih.gov/biodefense/research/2005awards.

Food and Waterborne Diseases Integrated Research Network (FWDIRN) (awarded in 2003):

- Coordinating and Biostatistical Center, The EMMES Corporation, Rockville, MD
- Immunology Research Unit, University of Maryland, Baltimore
- Microbiology Research Unit, Michigan State University
- Microbiology Research Unit and Botulism Research Center, Tufts University, School of Veterinary Medicine
- Zoonoses Research Unit, Cornell University School of Veterinary Medicine
- Zoonoses Research Unit, Washington State University, College of Veterinary Medicine

For more information, visit http://spitfire.emmes.com/study/fwd.

Immune Epitope Database and Analysis Program (awarded in 2004):

- La Jolla Institute for Allergy and Immunology, La Jolla, CA

For more information, visit www.immuneepitope.org.

Immune Function and Biodefense in Children, Elderly, and Immunocompromised Populations (awarded in 2005):

- Blood Center of Southeastern Wisconsin, Milwaukee, WI
- Children's Hospital of Philadelphia
- Emory University
- Mt. Sinai School of Medicine
- Oklahoma Medical Research Foundation, Oklahoma City, OK
- Oregon Health Sciences University
- University of Rochester
- University of Washington
- Wistar Institute, Philadelphia, PA
- Yale University

For more information, visit www.niaid.nih.gov/biodefense/research/2005awards.

Innate Immune Receptors and Adjuvant Discovery Program (awarded in 2004):

- Coley Pharmaceutical Group, Wellesley, MA
- Corixa Corporation, Seattle, WA
- Montana State University
- NovaScreen Bioscience Corporation, Hanover, MD
- VaxInnate Corporation, New Haven, CT

For more information, visit www.niaid.nih.gov/biodefense/research/2004awards.

***In Vitro* and Animal Models for Emerging Infectious Diseases and Biodefense** (awarded in 2003):

- Battelle Memorial Institute, Columbus, OH
- Cellular Technology, Ltd., Cleveland, OH
- Health Protection Agency (CAMR), Porton Down, UK
- IIT Research Instititute, Chicago, IL
- Lovelace Respiratory Research Institute, Albuquerque, NM
- Oklahoma State University
- Southern Research Institute, Frederick, MD and Birmingham, AL

– SRI International, Menlo Park, CA

– University of Illinois at Chicago

– University of Texas Medical Branch, Galveston

For more information, visit www.niaid.nih.gov/biodefense/research.

Large Scale Antibody and T Cell Epitope Discovery Program (awarded in 2004):

– Benaroya Research Institute at Virginia Mason, Seattle, WA

– Duke University Medical Center

– Imperial College, London, UK

– Johns Hopkins School of Medicine

– La Jolla Institute for Allergy and Immunology, La Jolla, CA

– Oregon Health and Science University

– Scripps Research Institute, La Jolla, CA

– Technical University of Denmark, Lyngby, Denmark

– Torrey Pines Institute for Molecular Studies, San Diego, CA

– University of Copenhagen, Denmark

– University of North Carolina, Chapel Hill

– University of Oklahoma Health Sciences Center

– Vanderbilt University Medical Center

For more information, visit www.niaid.nih.gov/biodefense/research/2004awards.

Microbial Sequencing Centers (awarded in 2004):

– The Institute for Genomic Research, Rockville, MD

– The Broad Institute, Massachusetts Institute of Technology

For more information, visit www.niaid.nih.gov/dmid/genomes/mscs.

Modeling Immunity for Biodefense (awarded in 2005):

– Duke University

– Mount Sinai School of Medicine

– University of Pittsburgh

– University of Rochester

For more information, visit www.niaid.nih.gov/biodefense/research/2005awards.

National Biocontainment Laboratories and Regional Biocontainment Laboratories National Biocontainment (awarded in 2003):

– Boston University

– University of Texas Medical Branch, Galveston

Regional Biocontainment Laboratories
Awarded in 2003:

– Colorado State University, Fort Collins

– Duke University, Durham

– Tulane University, New Orleans

– University of Alabama, Birmingham

– University of Chicago

– University of Medicine and Dentistry of New Jersey

– University of Missouri

– University of Pittsburgh

– The University of Tennessee

Awarded in 2005:

– George Mason University

– Tufts University

– University of Louisville

– University of Hawaii, Manoa

For more information, visit www.niaid.nih.gov/biodefense/research/rbl.htm.

NIH/NIAID Tetramer Core Facility

– Emory University

For more information, visit www.yerkes.emory.edu/TETRAMER.

Pathogen Functional Genomics Resource Center (awarded in 2003):

– The Institute for Genomic Research, Rockville, MD

For more information, visit www.niaid.nih.gov/dmid/genomes/pfgrc.

Population Genetics Analysis Program: Immunity to Vaccines/Infections (awarded in 2004):

- deCode Genetics, Reykjavik, Iceland
- Mayo Clinic, Rochester, MN
- McMaster University
- Research Triangle Institute, Research Triangle Park, NC
- University of Alabama, Birmingham
- University of Washington

For more information, visit www.niaid.nih.gov/biodefense/research/2004awards.

Reagent Development for Monitoring Immunity in Non-Human Primates (awarded in 2004):

- Nonhuman Primate Reagent Research, Beth Israel Deaconess Medical Center, Boston, MA
- Nonhuman Primate MHC Typing Development, University of New Mexico, Albuquerque, NM
- Nonhuman Primate MHC Typing Development, University of Wisconsin, Madison, WI

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

Awarded in 2003:

- **Region I:** New England Regional Center of Excellence for Biodefense and Emerging Infectious Diseases, located at Harvard Medical School
- **Region II:** Northeast Biodefense Center, located at the New York State Department of Health
- **Region III:** Mid-Atlantic Regional Center of Excellence for Biodefense and Emerging Infectious Diseases, located at the University of Maryland, Baltimore
- **Region IV:** Southeast Regional Center of Excellence for Biodefense and Emerging Infectious Diseases, located at Duke University
- **Region V:** Great Lakes Regional Center of Excellence for Biodefense and Emerging Infectious Diseases, located at the University of Chicago
- **Region VI:** Western Regional Center of Excellence for Biodefense and Emerging Infectious Diseases, located at University of Texas Medical Branch
- **Region VII:** Midwest Regional Center of Excellence for Biodefense and Emerging Infectious Diseases, located at Washington University in St. Louis
- **Region X:** WWAMI (WA, AK, OR, ID) Regional Center of Excellence for Biodefense and Emerging Infectious Diseases, located at University of Washington

Awarded in 2005:

- **Region VIII:** Rocky Mountain Regional Center of Excellence, located at Colorado State University (Fort Collins)
- **Region IX:** Pacific-Southwest Regional Center of Excellence, located at University of California, Irvine

For more information, visit www.niaid.nih.gov/biodefense/research/rce.htm or www.rcebiodefense.org.

Respiratory Pathogens Research Network (awarded in 2003):

- Viral Respiratory Pathogens Research Unit, Baylor College of Medicine (Houston)
- Bacterial Respiratory Pathogens Research Unit, University of Iowa (Iowa City)
- Bacterial Respiratory Pathogens Reference Laboratory, University of Alabama (Birmingham)

Vaccine and Treatment Evaluation Units

Established in 1962, the NIAID Vaccine and Treatment Evaluation Units (VTEUs) are a network of university research hospitals across the United States that conduct Phase I and II clinical trials to test and evaluate candidate vaccines for infectious diseases. Through these sites, researchers can quickly carry out safety and efficacy studies of promising vaccines in children, adults, and specific high-risk populations, such as premature infants and the elderly. For more information and a list of sites, visit www.niaid.nih.gov/factsheets/vteu.htm.

Category A Progress Report 2006: Appendix B

NEW NIAID AWARDS (FY03, 04, 05, 06)

ANTHRAX

FY03 Awards:

Advanced Anthrax Vaccine Made with ISS DNA formulations, Dynavax Technologies Corporation

Anthrax Antidote in Animals, University of Texas, Austin

Develop Effective Inhibitors of Anthrax Lethal Factor, Burnham Institute

Development and Testing of Anthrax Toxin Inhibitors, University of Toronto

Development of a Novel Retrogen Vaccine for Anthrax, MithraGen, Inc.

Discovery of New Anti-Bacteremia Vaccines for Anthrax, MacroGenics, Inc.

Hexa-D-Arg: A Furin Inhibitor for Anthrax Biodefense, Molecular Therapeutics, Inc.

Immunotherapy for Pulmonary Anthrax, Planet Biotechnology, Inc.

Molecularly Targeted Vaccines for Anthrax, University of Michigan

A Multicomponent Anthrax Vaccine using Phage T4 Display, Catholic University of America

Non-Invasive Plant Virus Particle-Based Anthrax Vaccines, Dow Chemical Company

A Novel Target for New Anti-Anthrax Drugs, University of Alabama at Birmingham

Novel Therapeutics for *Bacillus anthracis*, University of Illinois at Chicago

Production and Testing of Anthrax Recombinant Protective Antigen (rPA) Vaccine, Avecia, Limited

PTE-Based Drug for Antibiotic-Resistant Anthrax, Influx, Inc.

Therapeutic Antibodies for Lethal Anthrax Infection, Antibody Science, Inc.

Production and Testing of Anthrax Recombinant Protective Antigen (rPA) Vaccine, VaxGen

FY04 Awards:

Anthrax Toxin Entry into Cells, Salk Institute for Biological Studies

Anthrax Vaccination by Targeting Spore Germination, University of Arizona

Anthrax Vaccine Formulations Combining PA/Spore Epitopes, Ligocyte Pharmaceuticals

***B. anthracis*: Passive Immunization with Anticapsular mAB**, University of Nevada, Reno

***B. anthracis* Peptidoglycan Deacatylase as a Drug Target**, University of Alabama at Birmingham

C3d-PA: Efficacy and Cellular Basis as an Anthrax Vaccine, University of California, San Diego

Cell Wall Protein in *B. anthracis* Pathogenesis, Texas A&M University Health Sciences Center

Characterization of Anthrax Toxin Receptor Interactions, University of California, Los Angeles

Conformationally-constrained PA Anthrax Vaccine, University of California, San Diego

Cytokines in Pathogenesis of Anthrax Infection, Public Health Research Institute

Development of Fully Human mAbs as Anthrax Antitoxins, Medarex, Inc.

Dually Active Anthrax Vaccine against Bacilli and Toxins, Brigham and Women's Hospital

The Effects of Interferon on Anthrax Toxicity, Cleveland Clinic Lerner Col/Med-CWRU

Fully Human Anti-Anthrax Toxin MAbs: Product Development, Medarex, Inc.

Function of CMG-2, an Anthrax Toxin Receptor, Texas A&M University Health Sciences Center

Function of the *B. anthracis* Spore Carbohydrate, Kansas State University

Genetics of Susceptibility to Anthrax Toxin *in Vivo*, University of Vermont & St. Agric College

Hexa-D-Arg: A Furin Inhibitor for Anthrax Biodefense, Molecular Therapeutics, Inc.

How Anthrax Lethal Factor Kills Macrophages, University of California, San Diego

Human Antibody Therapeutics against Anthrax, Alexion Antibody Technologies, Inc.

Human Monoclonal IgG for Protection against Anthrax, Scripps Research Institute

Human Antibodies for Exposure/Protection from Anthrax, Avanir Pharmaceuticals

Immunity to *Bacillus anthracis*: spore-host interactions, University of Alabama at Birmingham

Inhibitors of Anthrax Lethal Factor Metalloproteinase, Hawaii Biotech, Inc.

Isolation of New Phage Enzymes to Kill *B. anthracis*, Rockefeller University

Monoclonal Antibody for Treatment of Inhalation Anthrax, Elusys Therapeutics

MHC Tetramers for Epitopes of *B. anthracis* PA, Benaroya Research Institute at Virginia Mason

Plasmid pX02 Replication in *B. anthracis*, University of Pittsburgh at Pittsburgh

Psoralen-Killed, Metabolically-active Anthrax Vaccine, Cerus Corporation

Small Molecule Blockers of *B. anthracis* Toxin, Innovative Biologics, Inc.

Spore Peptidoglycan Degradation in *B. anthracis*, Virginia Polytechnic Institute and State Univ

Structural Determinants of Human Immunity to Anthrax, Children's Hospital and Research Center at Oakland

Virulence Gene Expression by *B. anthracis*, The University of Texas Health Science Center at Houston

FY05 Awards:

Adenovirus-Vectored Nasal Anthrax Vaccine, Vaxin, Inc.

***Bacillus anthracis* Detection with RNA Microchip**, Georgia State University

The Basis of Anthrax-Induced Vascular Damage, Beth Israel Deaconess Medical Center

Click Chemistry-Based Anthrax Lethal Factor Inhibitors, University of California, Los Angeles

Combination Therapies to Counteract Anthrax Toxin, CombinatoRx, Inc.

Delivering Improved Anthrax Vaccine Stability, Avecia, Ltd

Development of Furanones for Treatment of Anthrax, New York University School of Medicine

Development of Screens for *Bacillus anthracis* Targets, MicroBiotiX, Inc.

Early Events During Infection with Anthrax, University of Maryland, Baltimore

Formulation Development for an Intramuscular Administration against Anthrax, Elusys Therapeutics

Human Monoclonal Panel Mimicking Anthrax Immune Globulin, Children's Hospital and Research Center at Oakland

An Improved Targeted Vaccine Strategy against Anthrax, Medarex, Inc.

Intervention against Anthrax Edema Factor (EF), University of Chicago

Killed Rhabdoviruses as Novel Anthrax Vaccines, Thomas Jefferson University

New Generation of Anthrax Prophylaxis and Therapy, AFG Biosolutions, Inc.

PGA Antigenemia for Early Diagnosis of Anthrax, University of Nevada, Reno

Preclinical Development of a Non-invasive Anthrax Vaccine, Ligocyte Pharmaceuticals

A Rapid, Sensitive and Fully Automated Anthrax Test, Cellex, Inc.

Human Monoclonal Panel Mimicking Anthrax Immune Globulin, Children's Research Hospital and Research Center at Oakland

Microsimulation of *Anthracis*-Immune System Interaction, Duke University Medical Center

Primate Model and Pathogenesis of Anthrax Sepsis, Oklahoma Medical Research Foundation

Recombinant Adenovirus Vaccines Against *B. anthracis*, University of Michigan

SecA2-dependent Secretion in *Bacillus anthracis*, University of California, Berkeley

Small Molecule Inhibitors of Anthrax Lethal Factor, MicroBiotiX, Inc.

Small RNA Regulators of the *B. anthracis* Plasmid pXO1, University of South Dakota

Vaccine Hurdle to Anthrax and the Emerging Immune System, University of Medicine and Dentistry of New Jersey

FY06 Awards:

Anthrax Toxins Impair Phagocyte Actin-based Motility, University of Florida

Characterization of Anthrax Lethal Toxin, University of Toronto

Functions of the PcrA Helicase in *Bacillus anthracis*, University of Pittsburgh

Immunological Secretomes of the Early Anthrax Infection, La Jolla Institute for Molecular Medicine

Microfluidic Controlled Gel-Drop Microarrays for Biothreat Diagnostic Devices, Akonni Biosystems, Inc.

New Attenuated Anthrax Vaccine, DMX, Inc

Novel Therapy for Pulmonary Anthrax, Planet Biotechnology, Inc.

Nisin as a Decontaminant for *B. anthracis* Spores on Human Skin, AFG Biosolutions, Inc.

A Novel Rhabdovirus-Based Anthrax Vaccine, Molecular Targeting Technology, Inc.

Potent and Safe Inhibitors of *B. anthracis* Lethal Factor, Burnham Institute

Role of Inter-Alpha Inhibitors in Anthrax Intoxication, ProThera Biologics, LLC

SMALLPOX & OTHER POX VIRUSES

FY03 Awards:

Cell-based Assays Against Vaccinia Virus, Cowpox Virus, University of Alabama at Birmingham

Development and Testing of a Modified Vaccinia Ankara Vaccine, Acambis, Inc.

Development and Testing of a Modified Vaccinia Ankara Vaccine, Bavarian Nordic

Development of an Oral Drug for Smallpox Treatment, Chimerix, Inc.

Development of a Safer Smallpox Vaccine, Arizona State University

Heteropolymer System to Treat Vaccinia Complications, Elusys Therapeutics

High Titer VIG for the Treatment of Smallpox, Omrix Biopharmaceuticals

Inhibiting Poxvirus Phosphatases: Therapy and Biodefense, Medical College of Wisconsin

Novel Vaccines for Smallpox, University of Pennsylvania

Therapeutics for Pox, Filo and Other Viral Pathogens, Auburn University

FY04 Awards:

Development of Protease Inhibitor to Treat Smallpox, Transtech Pharma, Inc.

Human Monoclonal Antibodies for Bioterrorism Defense, Vaccinex, Inc.

Human T-cell Responses to Vaccinia Vaccine, Thomas Jefferson University

Immunologic Mechanisms of Atopic Dermatitis, Boston Children's Hospital

Immunomodulation of Vaccinia-induced Antimicrobial Peptides, National Jewish Medical and Research Center

Novel Inhibitors of Poxvirus Replication, State University of New York at Buffalo

Novel Inhibitors of the Replication of Poxviruses, K.U. Leuven Universiteitshal

Novel Orthopox Small Molecule Anti-Virals, Transtech Pharma, Inc.

Poxvirus Adverse Effects on Cardiac Cells & the Heart, North Carolina State University, Raleigh

Production and Testing of a Modified Vaccinia Ankara (MVA) Vaccine, Acambis, Inc.

Production and Testing of a Modified Vaccinia Ankara (MVA) Vaccine, Bavarian Nordic

Small Molecule Inhibitors of Smallpox Virus Replication, Siga Technologies, Inc.

Statistics and Data Management Center, RHO Federal Systems

Study of Monkeypox Virus in Rodents, St. Louis University:

Structure-based Drug Design for Smallpox Therapy, Burnham Instititute

Vaccinia Proteome Affinity Reagents From Phage Display, Immport Therapeutics, Inc.

Vaccinia DNA Replication, Medical College of Wisconsin

Vaccinia Virus Genetics and Morphogenesis, University of Florida

Variola Virus G1L: an Antiviral Drug Target, Oregon State University

Vaccinia Virus-specific T-cell Phenotypes, Vanderbilt University

FY05 Awards:

Analogs of HPMPA for Treatment of Smallpox, Veterans Medical Research Foundation, San Diego

Biochemical Mechanism of Poxvirus Replication, Rockefeller University

Development of an Alphavirus Replicon Vaccine against Smallpox, AlphaVax Human Vaccines, Inc.

Development of a Post-exposure Vaccine for Smallpox, Arizona State University

Disabling Vaccinia IFNr: A New Smallpox Vaccine, Arizona State University

Functional Characterization of the Poxviral RING Protein, Oregon Health & Science University

Humoral Immunity to Vaccinia Virus, La Jolla Institute for Allergy/Immunology

Innate Immunomodulating Genes of Smallpox Vaccines, University of California Davis

Lung-Targeted Poxvirus Antivirals, Veterans Medical Research Foundation - San Diego

Novel Smallpox Vaccine Derived from VV/VAR Immunome, EpiVax, Inc.

Pox Proteomics, Oregon State University

Poxvirus Regulation of NF-kB: Mechanisms for Virulence, University of Illinois Urbana-Champaign

Rapid Diagnosis of Monkeypox and Smallpox Infections, Najit Technologies, Inc.

Smallpox Subunit Vaccine in the C-PERL Expression System, Chesapeake Perl

Smallpox Vaccine and Vaccinia Complement Control Protein, University of Pennsylvania

SNPs in Handling of Smallpox Antivirals & Other Drugs, University of California, San Diego

Vaccinia Encoded Kinases and Phosphatases, Medical College of Wisconsin

Vaccinia Virus Antibody Kinetics and Residual Immunity, Washington University

Inhibitors of Poxvirus Enzymes as Novel Drugs, LifePharms, Inc.

FY06 Awards:

Bacterial Commensal Vector Delivery/Smallpox Vaccine, Siga Technologies, Inc.

CD8+ T Cell Specificity in Mouse Models of Smallpox Vaccination and Challenge, Australian National University

Imaging Vaccina Viral-Host Pathogenesis, University of Michigan

Inhibitors of Poxvirus Motility and Release, Emory University

Leukotrienes in Innate and Adaptive Antiviral Immunity, Pennsylvania State University Hershey Medical Center

Mucosal Modified Vaccinia Ankara-Based Plaque Vaccines, InViragen, Inc.

Orthopoxvirus Pathogenesis and Vaccines, Institute for Cancer Research

Small Molecule Inhibitors of Smallpox Virus Replication, Siga Technologies, Inc.

TNFR Members in T-Cell Immunity to Vaccinia, La Jolla Institute for Allergy/Immunology

To Model Cross-Reacting T-Memory of Virus-Infected Mice, New York University School of Medicine

Vaccinia: T-Cell Immunity and Homing to Skin, University of Washington

Viral PYRIN-Only Proteins as Suppressors of the Host Immune Response, West Virginia University

PLAGUE

FY03 Awards:

Antigen Presentation and Pulmonary Immunity to Plague, Colorado State University

Multi-Gene Plague Vaccine with Expanded Protection, The University of Massachusetts Medical School

Novel Vaccine Candidates for *Y. pestis* from Genomics, Chiron S.P.A

Polymer-Based *Yersinia Pestis* Point-of-Case Diagnostics, Nomadics, Inc.

Rational Design of Inhibitors of *Yersinia pestis* EF-Tu, Molsoft, LLC

FY04 Awards:

Alternative Endpoints for Plague Challenge Models, Duke University

Attenuated Live and Recombinant *Yersinia pestis* Vaccines, Washington University

Cellular Analysis of the Yersinia Protein Kinase A, University of Miami

Developing Novel Antibiotics against *Yersinia pestis*, NovoBiotic Pharmaceuticals

Development of Protollin Plague Vaccine, ID Biomedical Corporation of Washington

Development, Testing and Evaluation of Candidate Vaccines against Plague, Avecia, Ltd

Differential Gene Expression in *Yersinia pestis*, Wadsworth Center

Function & Immunogenicity of *Yersinia pestis* Fimbriae, University of Pennsylvania

Genetic Clustering & Virulence of *Y. pestis* Strains, University of Maryland Baltimore

Mucosal Vaccines for Plague, Montana State University

Polymer-Based *Yersinia Pestis* Point-of-Case Diagnostics, Nomadics

A Rapid, Sensitive and Fully Automated *Y. pestis* Test, Cellex, Inc.

Rational Design of Inhibitors of *Yersinia pestis* EF-Tu, Molsoft, LLC

The Role of LPS and Toll-like Receptors in Plague, University of Massachusetts Medical School

Surface Proteins in Pneumonic Plague, University of Kentucky

Targeting of Yop Proteins by *Yersinia enterocolitica*, University of Chicago

Yersinia pestis* Biofilms on *C. elegans, University of Alabama at Birmingham

FY05 Awards:

Antigen Capture Assays for Rapid Detection of *Y. Pestis*, New York Medical College

Autotransporter Proteins and Virulence of *Y. pestis*, Washington University

Identification of New Antigens for a Plague Vaccine, The University of Texas Medical Branch at Galveston

Inhaled Aminoglycoside Formula for Plague and Tularemia, Nanotherapeutics, Inc.

Lung Injury and Shock Pathogenesis in *Y. pestis* Sepsis, St. Louis University

Modulation of Host Signaling Functions by Yersinia Yops, State University of New York at Stony Brook

The Role of Host B Lymphocytes in Yersinia Pathogenesis, University of Illinois at Urbana-Champaign

Signature SNPs to Distinguish *Yersinia pestis* Strains, Perlegen Sciences, Inc.

Validation of an RSK-inhibitor as a Novel Therapeutic for Yersinia, Luna Innovations, Inc.

YopT: A Yersinia Virulence Factor, University of California, San Diego

FY06 Awards:

Bacterial Cell Killing Topoisomerase I—DNA Lesion, New York Medical College

Developing Novel Antibiotics against *Yersinia pestis*, NovoBiotic Pharmaceuticals, LLC

Host Range Diversity of Bacteriophage for *Y. pestis*, AvidBiotics Corporation

A Novel Treatment in a Bioterrorism Model of Pneumonic Plague Targets A1 ARs, Endacea, Inc.

Poly-N-Acetyl Glucosamine as a Vaccine for Bacterial Pathogens, Brigham and Women's Hospital

Protein Translocation by the Type III Secretion System, University of California, San Diego

YopM and Protective Innate Defenses against Plague, University of Kentucky

BOTULISM

FY03 Awards:

Alphavirus Replicon Vaccines against Botulinum Neurotoxins, AlphaVax Human Vaccines, Inc.

Development of Botulinum Neurotoxin Immunotherapy, University of California, San Francisco

Fast-Track Production of a Heptavalent Botulinum Vaccine, DynPort Vaccine Company, LLC

Focusing Immunity vs. Botulinum Toxin with Cytokine DNA, Thomas Jefferson University

Immunotherapeutics to Prevent & Treat BoNT Intoxication, University of California, Los Angeles

FY04 Awards:

A Broad, Long Acting Inhibitor of Botulinum Neurotoxin, BioRexis Pharmaceutical Corporation

Development of Genetic Tools for *Clostridium botulinum*, University of Wisconsin, Madison

Identification of Botulinum Toxin Membrane Targets, University of Georgia

Intracellular Inhibitors of Botulinum Neurotoxins, Vanderbilt University

Vitrification-stabilized Multivalent Botulinum Vaccines, DynPort Vaccine Company, LLC

FY05 Awards:

Botulinum Neurotoxin Substrate Specificity, Vanderbilt University

Development and Production of Antibodies that Protect Against Botulinum Toxin Type A, Xoma, LLC

Fast-Track Production of a Heptavalent Botulinum Vaccine, DynPort Vaccine Company, LLC

High-Throughput Drug Screen against Botulinum Neurotoxin, Vanderbilt University

High-Throughput Identification of BoNT Inhibitors, Scripps Research Institute

High Throughput Screens for Botulinum Toxin Therapeutics, Veritas, Inc.

Human Monoclonal Antibodies against Botulinum Toxins, Scripps Research Institute

Neutralizing Botulinum Neurotoxin with Human Antibodies, SRI International

Target-Guided Botulinum Neurotoxin Inhibitor Discovery, University of California, Los Angeles

FY06 Awards:

Botulinum Toxin Plantibodies, Planet Biotechnology, Inc.

Development of a Mucosally Administered Trivalent Serotype A,B, E Botulinum Vaccine, DOR BioPharma, Inc.

Human Monoclonal Antibodies that Bind Botulinum Toxins, Thomas Jefferson University

Optimization of Small Molecule Botulinum Neurotoxin Inhibitors, Hawaii Biotech, Inc.

TULAREMIA

FY03 Awards:

Development of a Mucosal Vaccine against *F. tularensis*, University of Alabama, Birmingham

Immuno-Prophylaxis-Therapy & Diagnosis of Tularemia, Boston Medical Center

Novel Antibacterial Agents for Treatment of Tularemia, Affinium Pharmaceuticals, Inc.

Scanning the *F. tularensis* Proteome for Vaccine Antigens, University of California, Irvine

FY04 Awards:

Antigenic Variation in *F. tularensis*, The University of North Carolina at Chapel Hill

Francisella Genomics, Baylor College of Medicine

***Francisella tularensis* Pathogenicity Island**, University of Victoria

Genetic susceptibility to *F. tularensis*, Duke University

Genome Biology of *Francisella tularensis* Populations, University of Nebraska, Lincoln

A Genome-Derived, Epitope-Driven Tularemia Vaccine, Epivac, Inc.

Effect of Aging on Immunity to Tularemia, The University of Texas Health Science Center at Houston

Molecular Basis of Francisella Virulence and Immunity, The University of North Carolina at Chapel Hill

Mouse Model of Oral Infection with Virulent Francisella, National Research Council of Canada

Protein Expression in Strains of *F. tularensis*, University of Tennessee Health Sciences Center

FY05 Awards:

Comparative Genomics of Francisella, Baylor College of Medicine

Development of Live *Francisella tularensis* Vaccines, Umea University

Development of a New Tularemia Vaccine, University of Rochester

Inhaled Aminoglycoside Formula for Plague and Tularemia, Nanotherapeutics, Inc

Innate Immunity to *Francisella tularensis*, University of Kansas Medical Center

Proteomics of *Francisella tularensis* infection/immunity, National Research Council of Canada

Tularemia: Pathogenesis and Host Response, The University of Texas at San Antonio

Tularemia Vaccine Development Team, DynPort Vaccine Company, LLC

Tularemia Vaccine Development Team, University of New Mexico

VSV as a Vector for Cytokine-assisted Tularemia Vaccines, University of Tennessee Health Science Center

FY06 Awards:

The Alpha Subunits of *Francisella tularensis* RNA Polymerase, Children's Hospital Boston

Development of Novel Proteins as a Defense against Potential Biothreat Agents, Alpha Universe, LLC

Identification of secreted proteins important for tularemia pathogenesis, University of Rochester

Innate Immune Stimulation as a Pathogen Countermeasure, Juvaris BioTherapeutics, Inc.

Molecular pathogenesis of *Francisella tularensis*, University of Louisville

Perpetuation of *Francisella tularensis*, Tufts University

Regulation of Gene Expression in Francisella, Medical College of Wisconsin

Virulence Gene Control in *Francisella tularensis*, Children's Hospital Boston

VIRAL HEMORRHAGIC FEVERS

FY03 Awards, General VHF:

Cell-Based Assays against Venezuelan Equine Encephalitis Virus, Yellow Fever Virus, Pichinde Virus, Punta Toro Virus, West Nile Virus, Dengue Virus, Utah State University

FY04 Awards, General VHF:

A Bioinformatic Approach to Inferring Protein Contracts, Montana State University, Bozeman

Structure-Function of Junin Virus Envelope Glycoproteins, University of Montana

Virus Assembly and Transmission, Purdue University

FY03 Awards, Arenaviruses:

Novel Anti-Viral Agents for Treating Lassa Fever, The University of Texas Southwestern Medical Center at Dallas

FY04 Awards, Arenaviruses:
Antiviral Drugs for Category A Arenavirus, Siga Technologies, Inc.

MOP/LAS Chimeric Vaccine against Lassa Fever, University of Maryland Biotechnology Institute

Therapeutics to Prevent/Treat Lassa Fever Virus, Scripps Research Institute

FY06 Awards, Arenaviruses:
Antiviral Drugs for Lassa Fever Virus, Siga Technologies, Inc.

Arenavirus Receptor-Structure/Function Studies, Scripps Research Institute

Cellular Receptors for Pathogenic New World Arenaviruses, Scripps Research Institute

FY03 Awards, Bunyaviruses:
Cell Entry Inhibitors for Sin Nombre Virus, The University of New Mexico, Albuquerque

Development of Rapid Diagnostic Tests for Hantaviruses, Chiron Corporation

New Broad-Spectrum Antivirals: Target Bunyaviruses, Hepadnavirus Testing, Inc

FY04 Awards, Bunyaviruses:
Analysis of Sin Nombre Virus Inhibition in Lung Cells, Oregon Health & Science University

Hantavirus Vaccines Based on Non-Replicating Adenoviruses, Oregon Health & Science University

Investigation of Bunyamwera Bunyavirus Gene Expression, University of Alabama at Birmingham

Molecular Tools for Bunyavirus Antiviral Screening, Apath, LLC

New Broad-Spectrum Antivirals: Target Bunyaviruses, Hepadnavirus Testing, Inc

Rift Valley Fever Virus MP-12 Vaccine Completion, The University of Texas Medical Branch at Galveston

FY05 Awards, Bunyaviruses:
Animal Models of Hantavirus Cardiopulmonary Disease, The University of Texas Medical Branch at Galveston

Crimean Congo Hemorrhagic Fever Virus Glycoproteins, University of Pennsylvania

Hantavirus Pathogenesis and Prevention, Southern Research Institute

Hantavirus RNA Encapsidation, The University of New Mexico, Albuquerque

Rationally Designed Rift Valley Fever Virus Vaccine, The University of Texas Medical Branch at Galveston

FY04 Awards, Filoviruses:
Ebola Virus VP40-Host Interactions *in Vivo*, **University of Pennsylvania**

New Adjuvant Technologies for a Marburg Virus Vaccine, **Southern Research Institute**

FY05 Awards, Filoviruses:
Development of a Vaccine for Ebola Virus in Plant System, Arizona State University

Elucidating the Entry Mechanism of Ebola Viruses, University of Illinois at Chicago

Ebola VP35 Interferon-Antagonist: Mechanism and Significance, Mount Sinai School of Medicine

Gene Expression Profiling in Filovirus-Infected Cells, University of Washington

Monoclonal Antibodies in Biodefense: Ebola Viruses, Mapp Biopharmaceuticals, Inc

Poly-ICLC Prophylaxis Treatment of Ebola Virus Infection, Oncovir, Inc

Therapeutics for Ebola Virus, Apath, LLC

FY06 Awards, Filoviruses:
Ebola Virus Glycoproteins: Structural Analysis, Scripps Research Institute

Filovirus/Cellular Receptor Interactions, University of Iowa

Identification of Ebola Virus Entry Inhibitors, MicroBiotiX, Inc.

FY03 Awards, Hemorrhagic Flavivruses:
Dengue Epitope Vaccine,Tetravalent & MHCII-Targeted, Johns Hopkins University

Preclinical Development of a Recombinant Dengue Vaccine, Hawaii Biotech, Inc

FY04 Awards, Hemorrhagic Flavivruses:

Antibody Prophylaxis and Therapy of Flavivirus Infection, L2 Diagnostics, LLC

Clinical Development of a Dengue Vaccine, Hawaii Biotech, Inc.

Dengue & West Nile Viral Protease Inhibitors, Georgetown University

Dengue Reporter Virus Particle Neutralization Assay, Integral Molecular

Identifying Inhibitors of West Nile and Dengue Viruses, Wadsworth Center

Interactions Between Dengue 3 and Human Dendritic Cells, The University of North Carolina at Chapel Hill

Molecular Genetics of Dengue Resistance in Mosquitoes, University of Notre Dame

Role of Cis and Trans Factors in Dengue Virus Translation, University of California, Berkeley

A Tetravalent Dengue Recombinanat Protein Vaccine, Protein Potential, LLC

FY05 Awards, Hemorrhagic Flavivruses:

Imino Sugars for Flavivirus Infections of Bioterror, Drexel University College of Medicine

Immune Targets During Natural Dengue Infection, Ponce School of Medicine

Immunoregulation of Flavivirus Infection, Scripps Research Institute

Study of Murine Interferon Responses to Dengue Virus, La Jolla Institute for Allergy/Immunology

Vector-Driven Selection in Dengue Virus, New Mexico State University

A VEE Replicon-Based Vaccine for Dengue Virus, The University of North Carolina at Chapel Hill

West Nile Virus Life Cycle in Flavivirus-Resistant Cells, Wadsworth Center

FY06 Awards, Hemorrhagic Flavivruses:

Antivirals Targeting Flavivirus Envelope Proteins, L2 Diagnostics, LLC

Apoptosis as an Anti-Viral Response in Mosquito Midgut, Kansas State University

Broad-Spectrum Therapeutic Human Antibodies for Dengue Virus Infections, L2 Diagnostics, LLC

Dengue Virus Determinants of Virulence and Transmission, Southwest Foundation for Biomedical Research

DNA Vaccine Delivery System for Biodefense with Dengue, Cyto Pulse Sciences, Inc.

Functional Analysis of Flavivirus Genetic Resistance, Georgia State University

Identification and Analysis of Flavivirus Protease and RNA Helicase Inhibitors, Georgetown University

Measuring Entomological Risk for Dengue, University of California, Davis

Peptide Inhibitors of Dengue Virus Infectivity, Tulane University

Structure Based Design of Dengue Virus Fusion Inhibitors, Hawaii Biotech, Inc.

GENERAL CATEGORY A AWARDS

FY03 Awards:

Antibacterial Peptidoglycan Recognition Proteins, Indiana University - Purdue University Indianapolis

Antibodies for BioDefense using Directed Evolution, Diversa Corporation

Biodefense Proteomics Collaboratory, The University of Texas Medical Branch at Galveston

Characterization of Proteomes of Category A Pathogens, Diversa Corporation

Chemokine Adjuvants for Biothreat Pathogen Vaccines, ChemoCentryx, Inc

Comprehensive Pathogen Diagnostics with rSBH System, Callida Genomics

Design and Production of Virus-like Particles, Emory University

Development of Immunotherapeutics for Biodefense, University of Pittsburgh

Granulysin Derived Immunotherapeutics for Biodefense, Stanford University

Hydrophobic Polyamine Amides as Anti-Endotoxin Agents, The University of Kansas, Lawrence

Improving Absorption and Targeting of Antiviral Drugs, TSRL, Inc

Lethal Mutagenesis as an Antiviral Strategy, Pennsylvania State University

Live Vector Vaccines against Agents of Bioterror, University of Maryland Baltimore

Multiplex PCR Detection of CDC A Bioterrorism Agents, Medical College of Wisconsin

Novel Adjuvants/Delivery Systems for Biodefense Vaccines, Chiron Corporation

Novel Adjuvants for Biodefense Vaccines, Tulane University

A Novel Platform to Discover Biodefense Therapeutics, Elitra Pharmaceuticals, Inc.

Novel TLR Ligand Mimetics as Adjuvants and Therapeutics, Duke University

Pathogen-Sensing with Novel PAEs, Georgia Institute of Technology:

Pathogen-Specific Drug Targets for Weaponized Bacteria, Rockefeller University

Pulmonary Innate Immune Activation for Bioterror Defense, Coley Pharmaceutical Group, Inc

Rapid Turn-Around Multiplex Testing: Bioweapon Agents, EraGen Biosciences, Inc.

RNA Interference as a Weapon against Bioterrorism, CBR Institute for Biomedical Research

FY04 Awards:

Activation of Innate Antiviral Defenses by LTbetaR, La Jolla Institute for Allergy/Immunology

Adjuvants and Toll-Like Receptors in Vaccine Development, University of Massachusetts Medical School

Agents of Bioterrorism: Pathogenesis and Host Defense, State University of New York at Stony Brook

Antigen Delivery Using Novel Fc Receptor Ligands, University of Chicago

Biodefense Therapeutics from Uncultured Microorganisms, Northeastern University

Bioinformatics Strategies: Biodefense Vaccine Research, Dartmouth College

Detection of Category A Pathogens by Gold Nanoparticles, Northwestern University

Development of DHPS as a Bioterrorism Therapeutic Target, St. Jude Children's Research Hospital:

Direct Regulation of CD8 T Cells by Interferon Gamma, University of Wisconsin, Madison

Discovery of Anti-Bioweapon Agents in BAC Librarie, eMetagen, LLC

Improving Absorption and Targeting of Antiviral Drugs, TSRL, Inc

Interaction of Enteroinvasive Pathogens with Neutrophils, New York University School of Medicine

Mechanism and Inhibition for LuxS: a Biodefense Target, Washington State University

Modeling of Multivalent Vaccination for Variable Viruses, Rice University

Molecular Basis for Inhibition of Edema Factor, Tufts University

Multiplexed Detection of Bioterror Agents, Weill Medical College of Cornell University

Novel Methods for Discovery of Antimicrobials, Northeastern University

Novel Therapeutics for Biodefense, Paratek Pharmaceuticals, Inc.

Rapid Turn-Around Multiplex Testing: Bioweapon Agents, EraGen Biosciences, Inc.

Regulation of IFN-g-Induced Innate Immunity by LRG-47, Duke University:

Respiratory Immunity against Agents of Bioterrorism, Wake Forest University Health Sciences Center

SMART Virus Vectors with Built-In Safety Mechanism, University of California, Davis

Training and Career Development for Biodefense and Emerging Diseases, Harvard University School of Public Health; The University of Texas Medical Branch at Galveston; University of Virginia, Charlottesville; Wadsworth Center

Vaccine Development: Purinergic Agonists as Adjuvants, Weill Medical College of Cornell University

Virulence Factors and Cell Death, Burnham University

FY05 Awards:

Acoustic Detection of Viruses, Bacteria and Toxins, Akubio, Ltd.

Adjuvants for Agile Vaccine Development, ImmPORT Therapeutics, Inc.

Biodefense and Emerging Infectious Diseases (BEID) , University of Florida

Biodefense Training in Host-Pathogen Interactions, The University of Chicago

Broad Spectrum Agents against Cat A Bacterial Pathogens, MaxThera, Inc.

Cell-Free Biosensor for the Detection of Pathogens, Integral Molecular

Choosing Drug Doses for Biodefence Pathogens, Ordway University

CODA Assembly of Mutant Genes, Coda Genomics, LLC

Computational and Structural Biology in Biodefense, The University of Texas Medical Branch at Galveston

Determinants of RNA Virus Evolution. Medical College of Ohio

Development of a Helicase-Based Rapid DNA Diagnostic System for Biodefense, Biohelix Corporation

Electrochemical Multispecific Molecular Detection System, Fractal Systems, Inc

Enhancing the Potency of Viral Vectors with C3d, Wake Forest University Health Sciences

A Helminth-Derived Immune Potentiator for Biodefense, New York Blood Center

HRF, an NFkB Antagonist Targeting Multiple Pathogenes, St. Luke's-Roosevelt Institute for Health Sciences

Immunology/Infectious Diseases Training Grant, University of Vermont & St. Agric College:

Immunology of Infectious Diseases, University of Pittsburgh

Molecular Basis of Viral Pathogenesis, Scripps Research Institute

Multivariate Pathogen Diagnostic Products, Isis Pharmaceuticals

Multiplexed POC Diagnostic System for Bio-Threat Agents, Meso Scale Diagnostics, LLC

Non-Antibiotic Resistance Markers for Bacteria, Colorado State University, Fort Collins

Novel Antibiotics from Unculturable Actinomycetes, NovoBiotic Pharmaceuticals, LLC

Novel Approaches to Accessing Secondary Metabolites, NovoBiotic Pharmaceuticals, LLC

Novel Vaccine Adjuvants to Counter Bioterrorist Threats, Flinders University of South Australia

Oral Antiviral Prodrugs for Biodefense Initiative, TSRL, Inc.

PL 3-Kinase Gamma and T Cell-Mediated Antiviral Responses, University of Minnesota - Twin Cities

Pulmonary Responses to Bioweapon Category A Pathogens, University of New Mexico, Albuquerque

Rapid Clinical Surge-Testing for Biothreat Agents, Genomic Profiling Systems

Recombinant Antigen Multiagent Diagnostic Assays, Tulane University

Regulation of Toll-Like Receptor Signaling by RP105, Children's Hospital Medical Center (Cincinnati)

Risk Factors for Eczema Vaccinatum in Atopic Dermatitis, Northern California Institute for Research and Education

RNA Processing in Non-Segmented Minus-Strand RNA Viruses, Harvard University Medical School

Selective Instructions for Memory Precursor T Cells, La Jolla Institute for Allergy/Immunology

Sensing Biowarfare Agents by Surface Enhanced Raman, MicroBiotiX, Inc

Targeting Cofactor Biosynthesis in Biodefense Pathogens, Burnham Institute

Type 1 Interferon-Regulated T Helper Development, The University of Texas Southwestern Medical Center at Dallas

Upstream ORFs: A General Strategy for Virus Attenuation, Oregon State University

Viral Migration and Predicting Viral Changes, University of California, Irvine

Virulence-Conferring Siderophore Biosynthesis Inhibitors, Weill Medical College of Cornell University

FY06 Awards:

Accelerating Metabolic Discovery Using Characterization Data, American Type Culture Collection

Acoustic Detection of Viruses, Bacteria and Toxins, Akubio, Ltd

Bioemulsifying Vaccine Delivery System for Immunomodulation, Tufts University

Genomic Approaches to Host-Pathogen Interactions, Massachusetts General Hospital

Immune Modulation by Bacterial Autolysins, National Jewish Medical and Research Center

Immune Responses to Biodefense Vaccines Early in Life, University of Maryland, Baltimore

A Microfabrication Device for Rapid, Portable, Viral Genome Analysis, University of Michigan

Microfluidic Refractometric Biosensor, Physical Optics Corporation

Mouse Models for Understanding Host Responses to Intracellular Pathogens, University of California, Berkeley

New Broad-Acting Cell Wall Antibiotics for Biodefense, MaxThera, Inc.

Novel Bacterial Cell Wall Biosynthesis Inhibitors for Biodefense Therapeutics, Rx3 Pharmaceuticals, Inc.

Novel Inhibitors to DHPS to Probe Catalytic Mechanism & Therapeutic Potential, St. Jude Children's Research Hospital

Retrocyclin Reinforcement of Pulmonary Defenses Against Viral Aerosols, University of California, Los Angeles

Siderophore Biosynthesis Inhibitors as New Antibiotics for Biodefense, Sloan-Kettering Institute for Cancer Research

Surface Protein Dynamics in Live Bacterial Pathogens, Stanford University

Viral Mediated Type I Interferon Induction, University of California, Los Angeles

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

National Institute of Allergy and Infectious Diseases

NIH Publication No. 06-5883
January 2007
http://biodefense.niaid.nih.gov