



**NIAID
BIODEFENSE**
Preparing Through Research

NIAID Biodefense Research Agenda for CDC Category A Agents



February 2002



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

NIAID Biodefense Research Agenda for CDC Category A Agents



February 2002



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

National Institute of Allergy and Infectious Diseases

NIH Publication No. 03-5308

February 2002
<http://biodefense.niaid.nih.gov>

TABLE OF CONTENTS

v	PREFACE
1	INTRODUCTION
2	AREAS OF RESEARCH EMPHASIS
5	GENERAL RECOMMENDATIONS
8	ANTHRAX
14	SMALLPOX
20	PLAGUE
24	BOTULISM
28	TULAREMIA
32	VIRAL HEMORRHAGIC FEVERS
A-1	APPENDIX A

PREFACE

On February 4 and 5, 2002, the National Institute of Allergy and Infectious Diseases (NIAID) convened the Blue Ribbon Panel on Bioterrorism and Its Implications for Biomedical Research. This panel of experts was brought together by NIAID to provide objective expertise on the Institute's future Biodefense research agenda, which is articulated in this document. Specifically, the panel was asked to provide the following guidance to NIAID:

- Assess the current research sponsored by NIAID related to the development of effective measures to counter the health consequences of bioterrorism.
- Identify goals for the highest priority areas for immediate, intermediate, and long-term research related to Biodefense.
- Make recommendations on the role of NIAID in achieving these priorities.

The panel included researchers from academic centers and private industry as well as representatives from government, civilian agencies, and the military. (See Appendix A.) Participants were selected for their scientific expertise on the infectious agents considered to be the major bioterrorist threats, their scientific leadership, and their broad research experience. An opening plenary session described the state of the science for each microbe identified by the Centers for Disease Control and Prevention (CDC) as a Category A Agent of Bioterrorism.* Panel members then divided into breakout groups to discuss each organism in more detail.

The panel commended NIAID for developing a Biodefense research agenda and was supportive of the planned goals. The content of the research agenda that follows reflects the panel's recommendations. Specific goals for each pathogen are articulated within each chapter and include priorities for immediate as well as intermediate/long-term research. The introduction includes general recommendations that are applicable for all areas of NIAID Biodefense research.

* The Centers for Disease Control and Prevention (CDC) Category A Agents of Bioterrorism include anthrax, smallpox, plague, botulism, tularemia, and viral hemorrhagic fevers. (<http://www.bt.cdc.gov/Agent/Agentlist.asp#categoryadiseases>)

INTRODUCTION

As concern grows about the use of biological agents in acts of terrorism or war, Federal health agencies are evaluating and accelerating measures to protect the public from the health consequences of such an attack. In 1996, when NIAID made public its *Research Agenda for Emerging Diseases*, intentional introduction of infectious agents was not discussed explicitly. However, recent events have reminded us that bioterrorism can be a major contributor in disease emergence.

Our ability to detect and prevent infections related to bioterrorist incidents depends to a large degree on the state of biomedical science. Basic and applied research supported by the National Institutes of Health (NIH) complements the efforts of other Federal agencies by developing the essential tools—diagnostics, therapeutics, and vaccines—that are needed by physicians, nurses, epidemiologists, and other public health workers to prevent and control a disease outbreak. NIAID is the primary NIH Institute that supports and conducts research on the diagnosis, prevention, and treatment of infections caused by a wide variety of emerging pathogens, including agents that could be intentionally introduced.

The Institute has developed a *Strategic Plan for Biodefense Research at the National Institute of Allergy and Infectious Diseases (NIAID)*, which outlines plans for addressing research needs in the broad area of bioterrorism and emerging and reemerging infectious diseases. (See Appendix B.) This *Biodefense Research Agenda* supplements the strategic plan and articulates the goals for research on anthrax, smallpox, plague, botulism, tularemia, and viral hemorrhagic fevers. The research agenda focuses on the need for basic research on the biology of the microbe, the host response, and basic and applied research aimed at the development of diagnostics, therapeutics, and vaccines against these agents. In addition, the agenda addresses the research resources, facilities, and scientific manpower needed to conduct both basic and applied research on these agents.

AREAS OF RESEARCH EMPHASIS

The following areas have been identified as priorities for Biodefense research.

Biology of the Microbe

Research into the basic biology and disease-causing mechanisms of pathogens underpins efforts to develop interventions against agents of bioterrorism. NIAID supports research to better understand the microbial components that define a pathogen's life cycle, as well as the events or processes that are critical to initiating infection or influencing the severity of disease. This knowledge is crucial to the development of preventative and therapeutic strategies. An important tool in understanding the basic biology of pathogenic microbes, including potential agents of bioterrorism, is the ability to rapidly obtain genome-sequencing information about these agents. The application of genomics research, coupled with other biochemical and microbiological information, is expected to facilitate the discovery of new targets for diagnostics, drugs, and vaccines. Comparative genomics (comparing the sequences of different strains of particular organisms) will be a particularly important component of future research, helping us to understand virulence and pathogenicity factors.

Host Response

Research into both innate and adaptive immune responses is critical in the development of interventions against agents of bioterrorism. We must define pathogen-triggered protective and deleterious immune responses to inform the development of products aimed at controlling and preventing disease. The identification of innate immune receptors and the functional responses that they trigger will enable targeted activation of the innate immune response and induction of specific adaptive immunity. Only one adjuvant (alum) is currently approved for general vaccine use. Therefore, we need new immunostimulatory agents. To develop new tools for disease control, we need a more detailed understanding of signaling pathways, of compartmentalization of immune responses, and of pathogen-specific immunoregulation. Little is known about the ways that immune responses can vary among different individuals according to age, general health status, treatment with immunosuppressive drugs, and genetic makeup. An enhanced understanding of these variables and their impact on immunity is critical for the design and development of effective vaccines and immunotherapeutics.

Vaccines

Vaccines are the most effective method of protecting the public against infectious diseases. New and improved vaccines against agents of bioterrorism must be suitable for civilian populations of varying ages and health status. In addition, vaccines developed to counter civilian bioterrorist attacks must be safe, easy to administer, and capable of an immediate protective and/or transmission-blocking immune response. Scientists must develop and characterize adjuvants that can enhance these desirable characteristics. Basic research on the host immune response, including correlates of protection and the basic biology of the microbe, will enhance vaccine development efforts. The initial focus at NIAID has been on the development of vaccines against smallpox and anthrax. However, the development of candidate vaccines for other category A agents remains a priority. A critical component of efforts to achieve these goals is enhanced linkages and partnerships with industry.

Therapeutics

In the event of a bioterrorism incident, we will need effective therapeutics to address the immediate health needs of the public. Antimicrobial agents for treating many infectious diseases currently exist. However, we need a broader, more robust arsenal of anti-infective agents to treat the broad civilian population and to intervene against drug-resistant variants that may emerge. Detailed knowledge of the pathways that are essential for replication and pathogenesis will enhance drug development. However, many diseases caused by category A agents do not provide adequate economic incentives for the development of therapeutic or preventive measures by industry. Therefore, collaboration between industry and government will be needed to produce the next generation of anti-infectives. Another priority is the development of monoclonal antibodies with activity against toxins or other critical virulence factors.

Diagnostics

A successful response to a bioterrorist threat requires diagnostics that can identify the pathogen involved. However, the initial clinical signs and symptoms of many agents considered biotreats are nonspecific and resemble those of common infections. The ability to rapidly identify the introduction of a bioterrorism organism

or toxin will require diagnostic tools that are highly sensitive, specific, inexpensive, easy to use, and located in primary care settings. Ideally, these tests also could be used to evaluate the possible spectrum of antimicrobial resistance. We have the theoretical means to design and develop such assays. For example, microchip-based platforms containing thousands of microbial signature profiles could be developed. A centralized database could be constructed to collect this information and allow the rapid identification of unusual patterns or clustering anywhere in the country. Functional genomic tools to identify multiple organisms simultaneously will be important, as will enhanced linkages and partnerships with industry.

Research Resources

Basic research and the development of new vaccines, therapeutics, and diagnostics depend on the availability of research resources. Among the resources needed to conduct Biodefense research are genetic, genomics, and proteomics information; appropriate *in vitro* and animal models; validated assays to measure immune and other host responses; and standardized reagents. In addition, research with potentially deadly category A agents must be conducted in appropriate containment facilities and must follow procedures that eliminate the threat to laboratory and clinical personnel and adjacent communities. Access to biosafety level (BSL) 3/4 facilities, particularly those with the capacity for animal model and clinical research, is limited and must be expanded. Expertise in a wide variety of areas, including structural biology, ecology, medicinal chemistry, bioinformatics, the development and humanization of antibodies, diagnostic validation, and therapeutic and vaccine candidate production, is needed. NIAID understands that a “goal oriented approach” to developing countermeasures against bioterrorism agents will require new collaborations between basic scientists, process development scientists, and engineers to quickly transition vaccine and therapeutic candidates into clinical testing. In addition, enhanced relationships among NIH, academia, and industry will be necessary to produce clinical material that meets good clinical practice requirements. NIAID’s research agenda will include the training of a new cohort of investigators, the physical infrastructure within which to conduct this research and production, and the technologies, animal models, and reagents necessary to pursue this line of research and clinical testing.

GENERAL RECOMMENDATIONS

The following recommendations apply to all areas of NIAID counter-bioterrorism research.

Immediate Research

- Develop regional Centers of Excellence for Bioterrorism and Emerging Diseases Research.
- Expand the capacity to conduct phase I, II, and III evaluations of candidate vaccines and treatments for agents of bioterrorism.
- Expand nonhuman primate capability to evaluate new therapeutic and vaccine products.
- Attract new scientific disciplines to Biodefense research, and expand the research training of a new cohort of investigators.
- Expand extramural and intramural research and clinical infrastructure, including construction and renovation of BSL-3/4 laboratories.
- Expand the availability of animal models for preclinical research.
- Develop rapid, inexpensive, and broad-based clinical diagnostics approaches using genomics and proteomics.
- Encourage structural genomics and proteomics research for the targeted development of drugs, vaccines, and diagnostics.
- Encourage industry participation to ensure the availability of rapid, sensitive, and licensed diagnostics to hospital clinical laboratories.
- Expand partnership opportunities with other agencies and governments.
- Develop a centralized repository for reagents and clinical specimens for agents of bioterrorism.
- Develop procedures and cGMP facilities capable of producing monoclonal antibodies, vaccines, and other immunotherapies for phase I and II clinical studies.

- Enhance adjuvant discovery and rational design of *Toll* system mediators.
- Identify and characterize innate and adaptive immune responses that occur after exposure to agents of bioterrorism and enhance basic research on mucosal immunology.
- 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.

Intermediate and Long-Term Research

- Examine human genetic variation in response to therapy, in drug susceptibility, and in infectivity.
- Define the human microbiome in states of health and disease.
- Develop predictive immunology tools that will allow for the design of interventions for currently unknown biological weapons.
- Analyze unique responses at the genome level to infection with agents of bioterrorism.
- Analyze gene expression of agents of bioterrorism *in vivo*.
- Standardize and validate protocols that involve studies using animal models.
- Expand clinical capacity for phase I safety studies.
- Develop, test, and analyze novel vaccination and protective strategies in all populations (e.g., the very young, the elderly, and immunocompromised individuals).
- Enhance currently available international capability by expanding existing clinical trial networks (e.g., the International Collaborations in Infectious Disease Research Program).
- Address human resource needs required for supporting research through training grants, fellowships, travel grants, and faculty development.

- Define the genetic basis of host susceptibility to infection with agents of bioterrorism.
- Identify pathogen-induced immunoregulatory and immunosuppressive effects that influence the host's ability to mount an effective immune response and respond to postexposure vaccination or immunotherapy.

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.

Biology of the Microbe

Inhalational anthrax develops after spores are deposited in alveolar spaces and subsequently ingested by pulmonary alveolar macrophages. Surviving spores are then transported to the mediastinal lymph nodes, where they may germinate up to 60 days or longer. After germination, replicating bacteria release toxins that result in disease. Major virulence factors include an antiphagocytic outer capsule and at least two well-characterized toxins. The two toxins, called edema factor (EF) and lethal factor (LF), can destroy cells or inhibit their normal functioning. A third component, called protective antigen (PA), when associated with both EF and LF, enables EF and LF to bind to a specific receptor on mammalian cells. After this complex is internalized, the bacteria's toxic effects are activated. Researchers recently engineered mutant recombinant PAs (rPAs) that bind to the native receptor. These mutant rPAs also can displace wild-type PA by blocking and interrupting the delivery of LF and EF into cells. Recent studies also have identified the region of the mammalian cell receptor to which PA binds and have determined the structure of the LF binding site. Soluble fragments of the receptor containing the toxin-binding site can function as decoys to protect cells from damage by LF. Other recent studies have characterized the site where LF binds to

MAPKK (mitogen-activated protein kinase kinase), a vital intracellular enzyme whose disruption by LF causes cell death.

Sequencing of the chromosomal genome of *B. anthracis* is nearly completed. The genes for LF, EF, and PA are contained on plasmids that already have been sequenced. NIAID is expanding sequencing efforts with a comprehensive genomic analysis of *B. anthracis* and related bacilli. Researchers will use sequence data derived from selected strains, isolates, and related species to assess the degree of genetic variation and diversity. This genetic information will provide a framework in which to evaluate the basis for differences in pathogenicity and virulence that have been noted between strains. Other uses for the genomic data include supporting basic research to identify specific molecular markers and targets for strain identification and molecular genotyping; developing sequence-based detection technologies; and designing effective vaccines, therapies, and diagnostic tools. In addition, the data will enhance the detection of genetic polymorphisms that correlate with phenotypes, such as drug resistance, morbidity, and infectivity, as well as key events or processes that influence the germination of spores *in vivo*. A comprehensive bioinformatics resource will support and maintain microbial genomic databases and the development of associated software and bioinformatics tools. These approaches will serve as a prototype for other microorganisms with potential to be used as agents of bioterrorism.

Host Response

Individuals who contract anthrax produce IgG1 and IgG3 anti-PA specific antibodies. Vaccination with the UK human vaccine generates primarily IgG1 antibodies, but IgG3, IgG2, and IgG4 antibodies also are detectable. Protection in animal models correlates with anti-PA antibody production. Recent evidence suggests that spore components also induce antibody production in rabbits, monkeys, and guinea pigs that have been vaccinated with an unencapsulated, toxigenic live vaccine strain. Sera from these vaccinated animals exhibit two antispore activities associated with antibody function (i.e., inhibition of spore germination and stimulation of spore phagocytosis by macrophages). Antibiotic therapy started after initial infection does not interfere with the immune response to *B. anthracis*. These findings indicate that it may be feasible to design a postexposure vaccination strategy to protect against the persistence of small numbers of spores in the lungs after antibiotic therapy.

Vaccines

Anthrax vaccine adsorbed (AVA), prepared from a cell-free filtrate of a nonencapsulated attenuated strain of *B. anthracis*, is the only licensed anthrax vaccine. PA of the anthrax toxin is the main component responsible for generating immunity. The Department of Defense (DoD) is using AVA in its mandatory immunization program to protect U.S. military personnel in high-threat areas. CDC recommends civilian use of AVA only for high-risk populations, such as veterinarians and laboratory workers. The schedule for administering this vaccine—six doses over the course of 18 months—is too protracted for use in response to a bioterrorism incident. Therefore, efforts are underway to devise an abbreviated, yet effective, postexposure immunization schedule.

Ensuring the safety of very young, elderly, and immunocompromised individuals requires a different vaccine regimen than that used for the military. NIAID has been collaborating with DoD to support the development of next-generation anthrax vaccines that may be more appropriate than AVA for use in civilians and military personnel. A vaccine based on a rPA appears to be the most promising candidate.

Although the correlates of immunity have not yet been defined, several animal studies have shown that neutralizing antibodies to PA elicit significant protective immunity against inhalational spore challenge. *In vitro* studies have shown that antibodies to PA also recognize spore coat proteins, stimulate the uptake of spores by phagocytes, and inhibit spore germination.

Therapeutics

NIAID is exploring the use of alternative antimicrobials or antitoxin therapies for anthrax. Together with the Food and Drug Administration (FDA), CDC, and DoD, NIAID is working to prioritize and accelerate testing of additional antimicrobial candidates for inhalational anthrax. NIAID-supported investigators recently published the results of two studies on the mechanisms by which anthrax toxins destroy cells. In one study, scientists identified the site at which PA binds on mammalian cells as well as a compound that can interfere with binding of the toxin. In the other study, researchers determined the structure of the LF component of the anthrax toxin. The information gained through these studies will likely hasten the development of new drugs to treat anthrax.

For the treatment of inhalational anthrax in humans, FDA has approved both penicillin G procaine and doxycycline. Ciprofloxacin also has shown efficacy in animal studies and recently received a label indication for inhalational anthrax. Current CDC recommendations for treating inhalational anthrax include initial intravenous therapy with ciprofloxacin or doxycycline and one or two additional antibiotics; patients may be switched to oral antibiotics when clinically appropriate. Treatment should continue for 60 days. Cephalosporins are not an effective treatment because *B. anthracis* isolates produce a cephalosporinase. However, recent cases of inhalational anthrax indicate that the combination of a fluoroquinolone with at least one other active drug may improve efficacy.

Diagnostics

Rapid diagnostic tests, such as enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) for PA, are available at national and state reference laboratories. However, these tests are not licensed. They are used primarily as confirmation of a clinical diagnosis because of the time required to transport samples and complete testing. Culture remains the standard confirmatory test, but definitive identification of *B. anthracis* requires additional testing beyond a standard blood culture. In the recent intentional exposures to anthrax, organisms could not be cultured from samples obtained after the initiation of antibiotic treatment. Several transportable, rapid assays are being developed for use in humans and veterinary practice.

Research Resources

Several pressing resource needs must be addressed to ensure a robust research program on anthrax. Although most animal species are susceptible to anthrax, mice are generally used in experimental studies because of their availability and cost. Nonhuman primates, rabbits, and guinea pigs are the animal models used in inhalational spore challenge studies. All animal evaluations that involve infectious strains of anthrax will require adequate and secure BSL-3 facilities. In addition, any clinical studies that involve infected patients will require appropriate facilities. Attracting new and experienced investigators into anthrax research also will require appropriate support in terms of facilities, reagents, and training.

Goals

The following goals are priorities for Biodefense research on anthrax.

Immediate Research Goals

- Establish capacity for the development and production of pilot lots of candidate anthrax vaccines.
- Conduct phase I and II trials with rPA anthrax vaccine candidates and alternative adjuvant formulations.
- Expand clinical capability to accelerate phase I and II testing of candidate anthrax vaccines.
- Establish a centralized immunology laboratory to develop and validate tests required for the licensure of anthrax vaccines.
- Screen existing FDA-approved antimicrobials and immunomodulators for efficacy against anthrax.
- Encourage exploration of new targets for antimicrobial therapies, including strategies to prevent germination of spores, to synthesize or neutralize toxins, and to interfere with the attachment and entry of toxins into host target cells.
- Conduct comparative genomic sequencing of selected *Bacillus* strains to detect subtle differences in pathogenesis and virulence associated with antigens or other factors.
- Develop and evaluate *in vivo* transmission and spore germination models.
- Identify and characterize new virulence and pathogenicity factors.
- Identify targets within innate and adaptive pathways that can be used to modulate infection.
- Identify and characterize innate and adaptive immune responses that occur after initial exposure to anthrax, including responses associated with spore germination.

Intermediate and Long-Term Research Goals

- Determine the surrogates of immunity for rPA vaccines using nonhuman primate challenge models.
- Identify new therapeutics by translating basic research findings into targets for intervention, for the development and optimization of monoclonal and polyclonal antibodies, and for new immunotherapeutics.
- Synthesize active lead compounds in sufficient quantities for preclinical pharmacokinetics and studies of animal model efficacy, mechanism of action, and toxicology.
- Use genomic-sequencing information to conduct comparative genomic analysis and to identify genetic variations and potential targets for vaccines and treatments against anthrax.
- Develop robust, sensitive, and specific diagnostics for the detection of anthrax antigens in clinical specimens.
- Explore the use of microbial signatures in gene expression profiles of host cells.
- Develop technologies for *in situ* studies of anthrax.
- Continue to improve anthrax vaccines by focusing basic research on the identification of potential targets, the development and optimization of vaccine delivery systems, the optimization of B- and T-cell protective responses, the induction of long-term immunologic memory, and the development of adjuvants based on innate immunity.

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 2 to 3 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 3 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.

Biology of the Microbe

The variola virus has not been well studied because of the hazards associated with potential exposure. However, vaccinia virus, which is used as a smallpox vaccine and is closely related to variola, is well studied. The few comparative studies of the two viruses have shown that the major differences are in the host ranges: whereas vaccinia infects several hosts, variola infects only humans naturally and cynomolgus monkeys under artificial laboratory conditions. The two viruses can be distinguished by the appearance of lesions on chick embryo chorioallantoic membranes and by tissue culture growth characteristics. The viruses share antigens and generate cross-neutralizing antibodies, a characteristic that has been exploited in the use of the vaccinia vaccine to prevent smallpox. The two viruses can be distinguished by PCR, ELISA, radioimmunoassays, and

monoclonal antibodies. Vaccinia is now being investigated extensively as a vector for the delivery of other vaccine genes.

Two forms of infectious orthopoxvirus are produced in infected cells: intracellular mature virus (IMV) that remain in the infected cell and extracellular enveloped virus (EEV) that are released from the cell late in infection. The EEV form of the virus contains an additional lipid envelope and cellular and viral proteins, thus making EEV immunologically different from IMV. In addition, the EEV and IMV forms enter cells by different mechanisms, use different cell receptors, and have different sensitivities to antibodies and complement. Immune evasion by poxviruses is accomplished through mechanisms related to the release of proteins that bind chemokines, EEV resistance to neutralizing antibodies, and EEV resistance to complement destruction through acquisition of host complement control proteins.

Variola and vaccinia belong to the *Orthopoxvirus* genus of poxviruses. These double-stranded DNA viruses replicate in the cytoplasm, unlike other DNA viruses that depend on host nuclear DNA replication enzymes. Several strains of variola and vaccinia have been genomically sequenced. The genes for structural, membrane, and core proteins appear to be highly conserved among orthopoxviruses. Genes responsible for growth in human cells also have been identified. NIAID will actively pursue further research in these areas.

Host Response

Sparse data are available on the duration of protection that vaccinia immunization confers against natural smallpox infection. However, it is known that natural infection confers life-long protection. After a smallpox outbreak in Europe, the case-fatality rate was 52 percent in unvaccinated people and 1.4 percent in people who were vaccinated up to 10 years previously. However, the case-fatality rate rose to 11.4 percent in people who were vaccinated more than 20 years previously. Individuals who recover from natural infection with variola or are immunized with vaccinia develop neutralizing antibodies. These antibodies are long-lived and can be detected up to 20 years after vaccination. Because the science of cellular-mediated immunity was in its infancy at the time that smallpox vaccination was discontinued, few data are available about the type and duration of both CD4 and CD8 T-cell responses or their correlation with immunity. Viral core and envelope proteins induce antibody responses in humans, but no specific

B-cell, CD4, or CD8 T-cell epitopes have been mapped. Delayed type hypersensitivity, a measure of T cell-mediated immunity, begins to develop as early as 2 days after vaccinia vaccination.

Vaccines

Smallpox vaccine consists of a laboratory strain of vaccinia virus and has proven to be highly effective in preventing infection. It is believed that in unvaccinated people exposed to smallpox, the vaccine can lessen the severity of, or even prevent, illness if given within 4 days of exposure.

Vaccination to prevent smallpox has not been required in the United States since 1972. People vaccinated before 1972 very likely have diminished immunity to smallpox; people born in the United States after 1972 have not been routinely vaccinated. Currently, smallpox vaccination is recommended and available only for individuals who are at risk of imminent exposure, such as laboratory personnel who work with vaccinia and other orthopoxviruses related to the smallpox virus. The smallpox vaccine is highly reactogenic and often causes fever as well as large local reactions. In addition, the vaccine is associated with several complications, some of which are life threatening, particularly in people with immune deficiencies.

A bioterrorist attack involving smallpox, or the accidental release of the virus from a clandestine laboratory, would require using stores of the existing smallpox vaccine. Approximately 15 million doses of the FDA-approved Dryvax vaccine have been stored since production stopped in 1983. This supply probably would not be enough to respond to a national smallpox epidemic. As an immediate response to this vulnerability, NIAID initiated a study to determine the feasibility of diluting Dryvax vaccine to make more doses available from the existing stores. In this study, investigators examined the skin and immune system responses of normal, unimmunized adult volunteers who were given a 1:10 dilution or a 1:100 dilution of Dryvax vaccine. Researchers compared the responses to those of other volunteers who had received the full-strength vaccine. The results demonstrate that the full-strength vaccine has maintained its potency. In addition, 70 percent of the people who received a single dose of the 10-percent vaccine developed a sore followed by a scab at the injection site and antibodies in their blood, both signs that the vaccine had conferred protection.

A larger study to compare 1:5 and 1:10 dilutions with the full-strength Dryvax vaccine is now underway.

Therapeutics

In collaboration with DoD, NIH has screened more than 500 compounds against smallpox and related viruses. Compounds were selected for antiviral testing from the following categories: FDA-approved drugs that are effective against other viruses, antiviral compounds that are in clinical development for other viruses, known experimental antiviral compounds, and new chemical entities. In addition, scientists are evaluating some drugs approved by FDA for nonviral indications. *In vitro* evaluation has shown that several drugs tested in these screening studies, including a number of cidofovir derivatives, are active against both vaccinia and cowpox.

Diagnostics

Most smallpox cases present with a characteristic rash that is denser on the extremities and face than on the torso. Confirmatory tests include electron microscopy of vesicular or pustular fluid or virus culture, PCR analysis of peripheral blood mononuclear cells (PBMCs) or vesicle fluid, or restriction fragment length polymorphisms (RFLPs).

Research Resources

NIAID supports evaluation of experimental antiviral compounds in a number of localized and systemic mouse models of vaccinia and cowpox. DoD also supports antiviral evaluations in similar rodent systems. Both resources increase the total capacity for mouse model evaluation and allow for independent confirmation of positive results. NIAID also supports a mouse/ectromelia model and a vaccinia model in rabbits. Compounds that are effective in these small-animal models are prioritized for evaluation in the monkeypox primate model by DoD investigators. Several pressing resource needs must still be addressed to ensure a robust research program on vaccinia/cowpox models. Attracting new and experienced investigators into research on surrogate models of smallpox also will require appropriate support in terms of facilities, reagents, and training.

Goals

The following goals are priorities for Biodefense research on smallpox.

Immediate Research Goals

- Expand the existing supply of live and attenuated vaccines, with particular emphasis on vaccines with reduced reactogenicity.
- Initiate and expand clinical trials of existing smallpox vaccines.
- Determine the correlates of immunity for smallpox vaccines through the detailed evaluation of immune responses to Dryvax vaccine.
- Conduct phase I and II trials with new candidate smallpox vaccines, with particular emphasis on the cell culture vaccines currently under development.
- Develop a centralized immunology laboratory to validate assays required for the licensure of smallpox vaccines.
- Fully characterize cidofovir's activity against poxviruses and ensure that an adequate supply of the drug is available to treat complications from vaccination.
- Develop animal models for studying smallpox pathogenesis.
- Expand *in vitro* and *in vivo* screening capability for oral antivirals, immunotherapies, and replacements for vaccinia immune globulin.
- Validate current diagnostics for orthopoxvirus infections and other vesicular rashes (i.e., chickenpox).
- Conduct comparative genomic sequencing of additional pox virus strains to detect potential differences in pathogenesis and virulence.
- Identify and characterize host factors and viral proteins that are involved in the production and maintenance of the two forms of infectious orthopoxviruses, IMV and EEV.

Intermediate and Long-Term Research Goals

- Develop detailed innate and adaptive immunologic profiles of responses to orthopox vaccines.
- Understand and prevent complications of smallpox vaccination, such as eczema vaccinatum and vaccinia gangrenosa.
- Develop animal model capability and provide the required standardization and validation for the development of vaccines and therapeutics against smallpox.
- Develop and optimize human monoclonal and polyclonal antibodies as potential therapies against smallpox.
- Expand basic research on host-virus interactions, viral replication, and immune evasion mechanisms.
- Investigate the effect of infection on cytokine production.

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 1 to 6 days after exposure and lead rapidly to death. If untreated, pneumonic plague has a mortality rate that approaches 100 percent. Antibiotics are effective against plague, but an effective vaccine is not widely available.

Biology of the Microbe

Although *Y. pestis* is very efficient at invading host epithelial cells, the molecular mechanisms that contribute to its invasiveness are not understood. Various iron transport mechanisms as well as the interaction of at least three quorum-sensing mechanisms appear to be involved.

Because the genome of *Y. pestis* has been completely sequenced, it should be possible to accelerate efforts to characterize key events in pathogenesis that will help identify suitable vaccine candidates, diagnostic reagents, and key targets for drug intervention. The *Y. pestis* outer surface membrane proteins (Yomps), of which there are several, appear to be important virulence factors and play a major role in pathogenesis. *Y. pestis* has a set of virulence-associated proteins that are plasmid encoded. Ambient temperature and Ca⁺⁺ levels regulate the expression and secretion of these proteins through the so-called low-Ca⁺⁺ response (LCR) mechanism. Further characterization of plasmid-encoded proteins and their role in pathogenesis could provide the basis for an effective subunit vaccine.

To cause infection, *Y. pestis* and other pathogenic bacteria need to remove iron—an essential trace nutrient—from host iron- and/or heme-chelating proteins. *Y. pestis* has three partially characterized iron transport systems that play an

important role in iron transport and removal. One of these systems is siderophore-dependent and involves the synthesis of yersiniabactin (Ybt). Since the Ybt system is essential for iron acquisition during the early stages of plague, it may be an excellent target for early intervention and treatment.

Host Response

Two *Y. pestis* proteins, F1 and V, are being evaluated as vaccine candidates in animal models. The F1 protein is considered the principle protective antigen in the Plague Vaccine USP. Recent protection studies in mice show that injection with recombinant F1, V, or F1+V protein adsorbed to an aluminum hydroxide adjuvant provided protection against bubonic and pneumonic forms of the disease; similar information is not available for humans. The F1+V vaccine provides significant protection as early as 14 days after immunization. Antibody titers to F1 and V peak at 5 to 12 weeks after immunization and last for at least 1 year. Passive transfer of either V protein-specific polyclonal antiserum or a monoclonal anti-V antibody protects animals against lethal challenge with *Y. pestis*. In addition, *Yersinia* outer proteins may be antigenic. Antibodies to some of these proteins have been identified in sera from convalescent plague patients and in sera from experimentally infected mice.

Vaccines

A killed vaccine has been licensed for use in the United States for individuals considered to be at high risk for developing bubonic plague, including laboratory workers and military personnel in areas where plague is endemic. Vaccination before exposure was effective in preventing or reducing disease. However, the vaccine did not prevent or reduce disease from pneumonic plague. The vaccine has not been produced since 1999 and is no longer available. Clearly, we need to develop a new vaccine for use in a preexposure setting or in a postexposure setting in conjunction with antibiotic therapy to protect against pneumonic plague. The V antigen has been identified as a possible vaccine candidate. However, other virulence-associated antigens must be defined to devise effective multivalent vaccines to combat the threat of new, genetically engineered resistant strains.

Therapeutics

Treatment of plague with antibiotics generally is effective, but success strongly depends on how quickly drug therapy is started, the dose of inhaled bacteria, and

the level of supportive care for the patient. If treatment is delayed more than 24 hours after symptoms begin, mortality remains high. A number of drugs, including streptomycin, gentamicin, tetracycline, and doxycycline, are recommended for use in the treatment of pneumonic plague on the basis of their efficacy in animal models of infection. However, the potential use of engineered, multi-drug-resistant strains as agents of bioterrorism emphasizes the need to develop alternative treatment strategies and antimicrobials with novel mechanisms of action.

Diagnostics

A rapid diagnostic test for plague is not widely available, but confirmatory tests are available at some state laboratories, CDC, and military facilities. Antibody detection assays are not useful for diagnosis because antibodies take several days or weeks to develop. The relatively rare occurrence of pneumonic plague in the United States has resulted in a low index of suspicion of naturally occurring cases and a lack of early-warning sentinel systems to detect aerosol exposure.

Research Resources

Several resource needs must be addressed to ensure a robust research program on *Y. pestis*. Because most animal species are susceptible to plague, several species have been used in experimental studies. Currently, the mouse is the most commonly used animal model of disease. Adequate and secure BSL-2/3 facilities will be required for all animal evaluations that involve infectious strains of *Y. pestis*. In addition, any clinical studies that involve infected patients will require appropriate facilities. Attracting new and experienced investigators into *Y. pestis* research also will require appropriate support in terms of facilities, reagents, and training.

Goals

The following goals are priorities for Biodefense research on plague.

Immediate Research Goals

- Accelerate the search for candidate *Y. pestis* vaccines.
- Establish capacity for the development, refinement, production, and testing of pilot lots of candidate *Y. pestis* vaccines.
- 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).

- Screen existing FDA-approved antimicrobials and immunomodulators for efficacy against *Y. pestis*.
- Develop rapid, inexpensive, and broad-based clinical diagnostics approaches for plague.
- Develop standards for validation and comparison of potential plague diagnostics.

Intermediate and Long-Term Research Goals

- Characterize natural plague foci worldwide by examining factors such as microbial ecology and defining infectious reservoirs and vectors.
- Develop microarrays for functional genomics studies of plague.
- Identify and characterize innate and adaptive immune responses that occur after initial exposure to plague.
- Identify inappropriate and immunopathogenic responses to infection with plague and strategies for the minimization of such responses.
- Determine correlates of immunity for *Y. pestis*.
- Examine host-pathogen interactions and host response to infection with plague.
- Identify novel diagnostic and prognostic signatures based on molecular and spectral patterns after infections with plague.

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 easily produced and released 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 illnesses 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.

Biology of the Microbe

C. botulinum does not normally infect humans. However, humans are exposed to the toxin after eating food contaminated with the organism. Botulinum toxin's mechanism of action is well understood. The toxin consists of a heavy chain and a light chain joined by a single disulfide bond that is essential for neurotoxicity. Both the sequence and three-dimensional structure of the toxin have been determined. The structure consists of three functional domains: a catalytic subunit, a translocation domain, and a binding domain. The toxin binds irreversibly to an unidentified receptor on presynaptic membranes of peripheral cholinergic synapses, mainly at neuromuscular junctions. After internalization of the toxin and translocation into the cytosol, a Zn⁺⁺-containing endopeptidase on the light chain blocks acetylcholine release from motor neurons. The release is blocked by preventing fusion of acetylcholine-containing vesicles with the terminal membrane. The seven botulinum toxins exhibit somewhat different protease activities, cleaving three SNARE proteins (synaptobrevin/ VAMP, SNAP-25, and syntaxin) at different sites. The molecular basis of this proteolytic specificity is not fully understood. The SNARE proteins are essential in the trafficking of synaptic vesicles to the presynaptic membrane.

Host Response

The botulinum neurotoxins that are produced by *C. botulinum* consist of seven immunologic serotypes, A–G. Therapy for human botulism includes passive immunization with equine antitoxin. The licensed antitoxin, which consists of neutralizing antibodies to the A, B, and E toxins, is available from CDC. Recent studies in mice identified antigenic epitopes within toxin A that activate B- and T-cell responses. Comparison of the amino acid sequences of toxins A, B, and E showed considerable homology among the three proteins in some of the defined antigenic regions. This finding suggests that it may be possible to induce cross-protective immunity.

Vaccines

No approved vaccine to prevent botulism exists. However, an investigational botulinum toxoid is available in limited quantities to induce immunity in laboratory workers. Since immunity develops over several months, the toxoid would not be useful for postexposure prophylaxis. Development of a vaccine strategy should take into account the therapeutic use of Botox™ for a number of human conditions (e.g., strabismus, spasticity, and cervical dystonia). Vaccination of the larger community would negate the effectiveness of Botox™ treatment. Therefore, it is likely that a vaccine would be used only in high-risk groups.

Therapeutics

Treatment of botulism consists of supportive care and the use of antitoxins. Timely administration of the available antitoxin minimizes subsequent nerve damage and disease severity, but it does not appear to alter existing paralysis. An investigational equine heptavalent antitoxin (A–G reactive), which covers all seven toxins, is being developed by DoD. The safety of this antitoxin has not been established. Although available data are limited, they do not indicate that the antitoxin poses safety concerns for special populations, such as pregnant women, older adults, and young children. Antibiotics are not effective against botulinum toxin, although clindamycin has been reported to have activity against one of the toxins.

Diagnostics

Botulism is frequently misdiagnosed. Clinical diagnosis must be confirmed with a mouse bioassay, which is based on neutralization by specific antitoxins. This assay takes up to 2 days to complete and is available in only a few laboratories. A rapid and sensitive diagnostic for botulism is essential for treatment because samples must be obtained before antitoxin treatment is begun.

Research Resources

Unlike several other agents of bioterrorism, botulinum toxin is not an infectious agent. Although highly lethal, it does not require specialized facilities to ensure containment. However, BSL-3 procedures conducted in BSL-2 facilities are recommended for work with botulinum toxin. Because most animal species are susceptible to botulism, several species have been used in experimental studies. Currently, the mouse is the most commonly used animal model of disease. Attracting new and experienced investigators to botulinum toxin research will require appropriate support in terms of reagents and training.

Goals

The following goals are priorities for Biodefense research on botulism.

Immediate Research Goals

- Process, produce, and conduct phase I and II trials with the heptavalent equine antitoxin.
- Scale up production and phase I testing of three human monoclonal antibodies to toxin A.
- Develop and test human monoclonal antibodies to toxins B, E, C, F, G, and D.
- Produce and conduct phase I and II trials of recombinant toxin B vaccine fragment C.
- Develop recombinant fragment C vaccine against toxins A, F, E, C, G, and D.
- Develop rapid and inexpensive diagnostics for botulism toxins and their genes for use in multiple settings.
- Develop effective cell culture systems to study toxin binding, internalization, and protease activity.

Intermediate and Long-Term Research Goals

- Identify toxin receptors on both mucosal transport cells (digestive and respiratory tracts) and on human cholinergic cells.
- Clone, overexpress, and determine the structure of toxin receptors.
- Develop specific pharmacologic agents as toxin-binding inhibitors for mucosal and nerve cells.
- Characterize the molecular basis of proteolytic specificity of botulinum toxins.
- Conduct basic research on the mechanisms of action of the seven toxin serotypes, including receptor-binding specificity and proteolytic substrate specificity.
- Develop small molecule inhibitors of toxin protease activity.
- Develop systems for the delivery of small molecule inhibitors to cholinergic nerve cells.

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 non-spore-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 or contaminated food, soil, or water to humans. Natural infection occurs after inhalation of airborne particles.

Tularemia can take one of several forms, depending on the route of exposure. The disease resulting from the inhalation of airborne *F. tularensis* is the most likely intentional exposure. The inhalation form is an acute, nonspecific illness beginning 3 to 5 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 to 60 percent. A live attenuated tularemia vaccine developed by DoD has been administered under an IND (investigational new drug) application to thousands of volunteers. To date, use of this vaccine has been limited to laboratory and other high-risk personnel.

Biology of the Microbe

The fundamental mechanisms involved in virulence and pathogenesis are not known. The cell wall of *F. tularensis* is unusually high in fatty acids. Loss of the capsule may lead to loss of virulence but not viability; however, the capsule is neither toxic nor immunogenic. Infection with *F. tularensis* involves the reticuloendothelial system and results in bacterial replication in the lungs, liver, and spleen. After respiratory exposure, *F. tularensis* infects phagocytic cells, including pulmonary macrophages. In the liver, *F. tularensis* has been shown to invade and replicate in hepatocytes. Destruction of infected hepatocytes results in the release of bacteria and subsequent uptake by phagocytes. When lysis of hepatocytes was prevented by the administration of a monoclonal antibody, bacteria continued to replicate in the hepatocytes, leading to rapid lethality.

Host Response

Immunity against *F. tularensis* has been studied by evaluating the response of humans and mice to live vaccine strain (LVS). In humans, LVS vaccination or *F. tularensis* infection induces the proliferation of bacterium-specific T cells and the production of antibodies, including IgM, IgG, and IgA isotypes. The T-cell response is directed towards a variety of bacterial proteins, including membrane and heat-shock proteins; however, no specific epitopes have been identified. Memory T-cell responses are long-lived and have been documented 10 to 30 years after vaccination. In the mouse, immunity is primarily cellular. *In vivo* studies demonstrate that either CD4 or CD8 T cells can mediate resolution of LVS infections. Antibodies appear to contribute little, if any, to protective immunity. Passive antibody transfer provides only limited protection against LVS and no protection against wild type *F. tularensis* infection in mice.

Vaccines

A live attenuated vaccine derived from an avirulent strain of *F. tularensis* is available for laboratory workers. However, the vaccine is not effective for postexposure immunization because of the incomplete protection it affords and the short incubation time of inhaled tularemia. Although the correlates of immune protection are not fully understood, vaccination appears to induce T-cell specific responses to membrane proteins and to heat shock proteins chaperone-60 (Cpn60) and DnaK. Long-term studies of survivors infected with tularemia indicate long-lasting anamnestic responses of T cells to heat shock proteins from *F. tularensis*.

Therapeutics

Tularemia has been successfully treated with aminoglycosides, macrolides, chloramphenicol, and fluoroquinolones. Recent studies in mice have shown that aerosolized liposome-encapsulated ciprofloxacin is more effective in treating infection than free aerosolized drug. Knowledge of optimal therapeutic approaches for tularemia is incomplete and limited because very few investigators are working on this disease.

Diagnostics

Rapid diagnostics for tularemia are not widely available, and normal microbiological tests are unlikely to result in identification. Culture of *F. tularensis* from patient specimens is the definitive test, but it takes up to several days. PCR, antigen detection assays, enzyme-linked immunoassays, and other specialized tests that can be used to identify and type tularemia are available at some research and reference laboratories.

Research Resources

Several resource needs must be addressed to ensure a robust research program on *F. tularensis*. Because most animal species are susceptible to *F. tularensis*, several species have been used in experimental studies. At present, the mouse is the most commonly used animal model of disease. Animal evaluations that involve infectious strains of *F. tularensis* will require adequate and secure BSL-3/4 facilities. In addition, any clinical studies that involve infected patients will require appropriate facilities. Attracting new and experienced investigators into *F. tularensis* research also will require appropriate support in terms of facilities, reagents, and training.

Goals

The following goals are priorities for Biodefense research on tularemia.

Immediate Research Goals

- 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.
- Develop a bacterial repository of *Francisella* species.
- Characterize responses of *F. tularensis* to available chemotherapeutics *in vitro* and in animal models of infection and disease.
- Develop new techniques to improve conditions for the culture of the microbe and for rapid determination of drug sensitivity profiles.
- Attract scientific researchers with expertise in a diversity of fields (e.g., immunology, microbiology, and lipid biochemistry) to the study of tularemia.
- Develop rapid, inexpensive, and broad-based clinical diagnostics approaches for tularemia.

Intermediate and Long-Term Research Goals

- Identify and characterize innate and adaptive immune responses that occur after initial exposure to *F. tularensis*.
- Identify correlates of immunity for *F. tularensis*.
- Develop antimicrobials and immunotherapies with novel mechanisms of action for treatment of tularemia.
- Synthesize active chemical lead compounds in sufficient quantities for preclinical pharmacokinetic and pharmacodynamic analysis and studies of efficacy in animal models, mechanism of action, and toxicology.
- Identify new *F. tularensis* vaccine candidates that can prevent or modulate infection, both before and after exposure.
- Conduct phase I and II trials of *F. tularensis* vaccine candidates and alternative adjunctive vaccine formulations.
- Establish capacity for development, refinement, and production of candidate *F. tularensis* vaccines.

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 Crimean-Congo hemorrhagic fever, Rift Valley fever, and Hantavirus infection
- *Filoviruses*, comprising Ebola and Marburg hemorrhagic fevers
- *Hemorrhagic flaviviruses*, including yellow fever, 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 rodents 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, and it often can be quite high. Some of these viruses also cause significant morbidity and mortality in domestic animals that are economically important.

Biology of the Microbe

The natural life cycle of these viruses is multifaceted. For many viruses, the life cycle includes one or more reservoir or amplifying hosts and often an arthropod (tick or mosquito) vector. However, for some viruses, such as Ebola, the natural reservoirs and/or vectors have not been identified. Infections with viruses that cause VHF generally occur in areas where the reservoir hosts or vectors live. Contact with body fluids, urine, or feces from rodents that serve as a reservoir can lead to infection. Infection also can occur when people come into contact with

infected animals, such as monkeys. For example, laboratory workers became infected with Marburg hemorrhagic fever after handling monkeys infected with the Marburg virus.

The viruses that cause hemorrhagic fevers are all enveloped RNA viruses. Some of the viruses have been extensively studied. The Ebola and Marburg viruses replicate in the cytoplasm of infected cells, and viruses bud from the cell surface. Viral proteins include a type I transmembrane glycoprotein (GP), a nucleocapsid protein, a matrix protein, an RNA-dependent RNA polymerase, and three other structural proteins. Ebola virus also codes for a nonstructural soluble GP. Soluble GP is found in the serum of infected people and may bind antibodies against the transmembrane GP. The transmembrane GP, which is located on the surface of the virions and infected cells, may play a role in viral entry via receptor-mediated endocytosis. Similar envelope and nucleocapsid proteins, as well as viral polymerases, have been identified for the bunyaviruses.

The arenaviruses contain ribosomes acquired from host cells; the sandy (Latin, *arenosos*) appearance of the ribosomes within the viruses gave rise to their name. Replication of arenaviruses includes the transcription of viral proteins from genomic sense mRNA, a process termed “ambisense.”

Host Response

Arenaviruses induce both T- and B-cell responses that seem to provide life-long protection from secondary infection in humans. Neutralizing antibodies appear to provide good protection against New World arenavirus infections such as Junin. Although Old World arenaviruses, such as Lassa fever, induce high-titer IgG and IgM production, these responses do not always correlate with protection.

Virus-specific T and B cells characterize the immune response to Hantavirus, which is a bunyavirus. For example, researchers used the Hantaan strain to identify at least 13 CD8 T-cell epitopes presented by the HLA-B51 molecule in humans. Studies of B-cell responses to the Puumala strain show that the majority of early IgG antibodies are directed against the nucleocapsid protein; however, other proteins also are recognized.

Relatively little is known about the human immune response to Ebola, the most well-characterized filovirus. Cell-mediated immunity seems to play an important role because surviving patients exhibit stronger T-cell responses than do nonsurvivors. A recent study of primary immune responses in asymptomatic Ebola-infected individuals identified evidence of T-cell activation and production of Ebola-specific antibodies of the IgG1 and IgG3 subclasses. Recent studies in guinea pigs, mice, and monkeys demonstrate that DNA vaccines encoding Ebola GP or nucleoprotein (NP) induce protective immunity. However, a similar DNA vaccine was not effective in primates infected with the Marburg virus. Studies to determine the protective effects of anti-Ebola hyperimmune sera in humans and animals have been inconclusive. For example, the transfer of neutralizing monoclonal antibodies completely protected mice from lethal Ebola infection, even if administered after a virus challenge. However, other studies showed the existence of infectivity-enhancing antibodies that are directed to the viral GP.

Flaviviruses, such as dengue, elicit strong immune responses that are directed at numerous viral proteins as defined by studies in both humans and mice. Human B- and T-cell epitopes have been mapped for dengue. Murine B- and T-cell epitopes have been defined for several viruses, including dengue and yellow fever.

Vaccines

No vaccines exist for VHF s except for a licensed yellow-fever vaccine. Candidate experimental vaccines against Argentine hemorrhagic fever and Rift Valley fever have advanced to early human trials. Because of the rapid onset of disease with many VHF s, these vaccines may not be useful for postexposure prophylaxis. In addition, the presence of different serotypes of some viruses, such as Hantavirus and dengue, may require development of multivalent vaccines.

Recent progress has been made in the development of a potential vaccine for Ebola virus. Priming with a DNA vaccine, including the genes for the transmembrane GP and nucleocapsid protein, followed by a boost with a recombinant adenovirus that contains the same genes, protected cynomolgus monkeys against a highly pathogenic challenge. A strong cellular response was identified in these animals and two correlates of immune protection have been identified, which will help guide human studies.

Therapeutics

Generally, there are no effective treatments for VHFs other than supportive care. Lassa fever has been successfully treated with the antiviral ribavirin, and plasma from recovering patients has been used to treat Argentine hemorrhagic fever. Ribavirin was shown to be effective against Hantavirus that causes renal disease in China. An open-label observational study in the United States of ribavirin as a treatment for Hantavirus pulmonary syndrome was inconclusive. Several potential therapeutics showed promise against Ebola infection in mouse models but were not effective when tested in cynomolgus monkeys.

Diagnostics

Clinical diagnosis of VHFs may be difficult because initial symptoms are nonspecific. However, ELISA and PCR tests, as well as viral culture, can be used to confirm some infections within days of disease onset. These tests have been developed for Ebola, Marburg, Lassa, dengue, Rift Valley fever, and Hantaviruses.

Research Resources

Animal models exist for some of the VHFs. For example, Ebola infection of nonhuman primates, including African green monkeys, baboons, rhesus monkeys, and cynomolgus monkeys, leads to disease that is similar to human disease. Ebola that has been adapted to grow in mice and guinea pigs provides a small-animal model. Animal evaluations that involve infectious strains of most VHFs require BSL-3/4 facilities. In addition, appropriate facilities will be required for any clinical studies that involve infected patients.

Goals

The following goals are priorities for Biodefense research on VHFs.

Immediate Research Goals

- Develop animal models that mimic human disease for studying VHF pathogenesis in humans.
- Expand the determination of the correlates of immunity for VHF vaccines by using appropriate models of natural infection.
- Advance the development of existing VHF vaccine candidates (e.g., Rift Valley fever and Junin fever).

- Establish capacity for the development, refinement, and production of pilot lots of candidate vaccines for VHFs.
- Develop a centralized immunology laboratory for the validation of tests required for licensure of priority VHF vaccines.
- Screen antibodies to evaluate their possible use as immune therapy for VHFs.
- Obtain clinical samples from patients with VHFs to help validate potential diagnostics and aid the development of new vaccine therapies.
- Expand the *in vitro* and *in vivo* screening capability for antivirals against VHFs.
- Encourage the exploration of new targets for antiviral therapies against VHFs.
- Complete the genomic sequencing of representative members and strains of the VHFs, and compare them to detect differences in pathogenesis and virulence.

Intermediate and Long-Term Research Goals

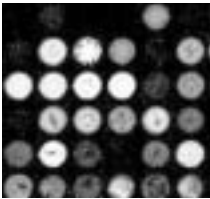
- Perform comparative studies of VHFs to determine shared mechanisms of pathogenesis.
- Expand the genomic analysis of VHFs, and conduct structural studies to aid the understanding of function.
- Explore the development of genetic systems to understand VHF replication and pathogenesis.
- Establish partnerships as appropriate to expand the study of the ecology of VHFs in their natural environment.
- Expand the knowledge of host and vector factors and viral proteins that contribute to the pathogenesis and transmission of VHFs.
- Encourage the structure-based design of new antivirals for multiple VHFs.
- Identify targets within the innate and adaptive pathways that may be used to modulate infection.
- Expand the preclinical toxicology capability and clinical capacity needed for the development of antivirals against VHF.

- Synthesize lead compounds in sufficient quantities for preclinical pharmacokinetics and studies of animal model efficacy, mechanism of action, and toxicology.
- Examine new technologies for the development of safe vaccines.
- Conduct phase I and II trials with new candidate VHF vaccines.
- Initiate human clinical trials of an Ebola vaccine.
- Develop and optimize human monoclonal and polyclonal antibodies as therapies for priority VHFs.
- Evaluate the basis of immune potentiation observed in some VHFs.

A p p e n d i x A



NIAID Blue Ribbon Panel on Bioterrorism
and Its Implications for Biomedical Research
February 4–5, 2002



Participant List



PARTICIPANT LIST

Stephen S. Arnon, M.D.

Infant Botulism Treatment and Prevention Program
 California Department of Health Services
 State of California
 Room 506
 2151 Berkeley Way
 Berkeley, CA 94704-1011
 Phone: (510) 540-2646
 Fax: (510) 540-3205
 Email: sarnon@dhs.ca.gov

Abdu F. Azad, Ph.D., M.P.H.

Microbiology and Immunology
 University of Maryland School of Medicine
 655 West Baltimore Street
 Baltimore, MD 21201
 Phone: (410) 706-3335
 Fax: (410) 706-0282
 Email: aazad@umaryland.edu

Phil Baker, Ph.D.

Program Officer, Lyme Disease Program
 Division of Microbiology and Infectious Diseases
 National Institute of Allergy and Infectious Diseases
 National Institutes of Health
 Room 3114
 6700-B Rockledge Drive
 Bethesda, MD 20892
 Phone: (301) 435-2855
 Fax: (301) 402-2508
 Email: pbaker@niaid.nih.gov

Jorge Benach, Ph.D.

Molecular Genetics and Microbiology
 SUNY at Stony Brook
 5120 Stony Brook
 Stony Brook, NY 11794-5120
 Phone: (631) 632-4225
 Fax: (631) 632-4294
 Email: jbenach@notes.cc.sunysb.edu

Bruce Beutler, M.D.

Immunology
 The Scripps Research Institute
 Imm-31
 10550 North Torrey Pines Road
 La Jolla, CA 92037
 Phone: (858) 784-8610
 Fax: (858) 784-8444
 Email: bruce@scripps.edu

Michael J. Buchmeier, Ph.D.

Neuropharmacology
 Division of Virology, MC CVN-8
 The Scripps Research Institute
 10550 North Torrey Pines Road
 La Jolla, CA 92037
 Phone: (858) 784-7056
 Fax: (858) 784-7369
 Email: buchm@scripps.edu

R. Mark Bueller, Ph.D.

Departments of Molecular, Microbiology and
 Immunology
 Medical School
 Saint Louis University
 1402 South Grand Boulevard
 St. Louis, MO 63104
 Phone: (314) 577-8451
 Fax: (314) 773-3403
 Email: bullerrm@slu.edu

Arturo Casadevall, M.D., Ph.D.

Medicine/Infectious Diseases
 Albert Einstein College of Medicine
 Golding Building
 1300 Morris Park Avenue
 Bronx, NY 10461
 Phone: (718) 430-3665
 Fax: (718) 430-8968
 Email: casadeva@aecom.yu.edu

Gail H. Cassell, Ph.D.

Vice President
Eli Lilly and Company
Drop Code 0438
Lilly Corporate Center
Indianapolis, IN 46285
Phone: (317) 276-7374
Fax: (317) 276-1743
Email: cassell_gail_h@lilly.com

Robert Chanock, M.D.

Chief, Laboratory of Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 7, Room 100
9000 Rockville Pike
Bethesda, MD 20892
Phone: (301) 496-4205
Email: rc106v@nih.gov

Frank Chisari, M.D.

Departments of Molecular and Experimental
Medicine
Division of Experimental Pathology
The Scripps Research Institute
10550 North Torrey Pines Road
La Jolla, CA 92037
Phone: (858) 784-8228
Fax: (858) 784-2160
Email: fchisari@scripps.edu

Purnell Choppin, M.D.

President Emeritus
Howard Hughes Medical Institute
4000 Jones Bridge Road
Chevy Chase, MD 20815
Phone: (301) 215-8554
Fax: (301) 215-8566
Email: choppin@hhmi.org

John Collier, Ph.D.

Department of Microbiology and Molecular Genetics
Harvard Medical School
Alpert 355
200 Longwood Avenue
Boston, MA 02115
Phone: (617) 432-1930
Fax: (617) 432-0115
Email: jcollier@hms.harvard.edu

Robert B. Couch, M.D.

Director, Distinguished Service Professor
Center for Infection and Immunity Research
Baylor College of Medicine
Room BCMA-205A, MS BCM-280
One Baylor Plaza
Houston, TX 77030-3498
Phone: (713) 798-4474
Fax: (713) 798-7375
Email: rcouch@bcm.kmc.edu

Walla Dempsey, Ph.D.

Clinical Trials Program Officer
Virology Branch
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Room 3150
6700-B Rockledge Drive
Bethesda, MD 20892
Phone: (301) 496-7453
Fax: (301) 402-0659
Email: wdempsey@niaid.nih.gov

Dennis M. Dixon, Ph.D.

Chief
Bacteriology and Mycology Branch
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Room 3115
6700-B Rockledge Drive
Bethesda, MD 20892-7630
Phone: (301) 435-2858
Fax: (301) 402-2508
Email: dmdixon@niaid.nih.gov

Eric Eisenstadt, Ph.D.

Defense Advanced Research Projects Agency
3701 North Fairfax Drive
Arlington, VA 22203-1714
Phone: (703) 696-2322
Fax: (703) 696-3999
Email: eeisenstadt@darpa.mil

Karen L. Elkins, Ph.D.

Laboratory of Mycobacteria
Division of Bacterial, Allergenic,
and Parasitic Products
Center for Biologics Evaluation and Research
HFM 431
1401 Rockville Pike
Rockville, MD 20852
Phone: (301) 496-0544
Fax: (301) 402-2776
Email: elkins@cber.fda.gov

Francis A. Ennis, M.D.

Center for Infectious Disease and Vaccine Research
University of Massachusetts Medical School
55 Lake Avenue North
Worcester, MA 01655
Phone: (508) 856-4182
Fax: (508) 856-4890
Email: francis.ennis@umassmed.edu

Anthony S. Fauci, M.D.

Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 31, Room 7A03C
31 Center Drive
Bethesda, MD 20892
Phone: (301) 496-2263
Fax: (301) 496-4409
Email: afauci@niaid.nih.gov

Claire Fraser, Ph.D.

The Institute for Genomic Research
9712 Medical Center Drive
Rockville, MD 20850
Phone: (301) 838-3504
Fax: (301) 838-0209
Email: cmfraser@tigr.org

Arthur M. Friedlander, M.D.

U.S. Army Medical Research Institute
of Infectious Diseases
Department of Defense
1425 Porter Street
Frederick, MD 21702
Phone: (301) 619-7341
Fax: (301) 619-2152
Email: arthur.friedlander@amedd.army.mil

Maria Giovanni, Ph.D.

Assistant Director for Microbial Genomics and
Related Technology Development
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Room 3139
6700-B Rockledge Drive
Bethesda, MD 20892
Phone: (301) 496-1884
Fax: (301) 480-4528
Email: mgiovanni@niaid.nih.gov

Irene Glowinski, Ph.D.

Chief
Office of Scientific Coordination
and Program Operations
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Room 3145
6700-B Rockledge Drive
Bethesda, MD 20892
Phone: (301) 435-8357
Fax: (301) 480-4528
Email: iglowinski@niaid.nih.gov

Jesse L. Goodman, M.D., M.P.H.

Center for Biologics Evaluation and Research
Food and Drug Administration
Suite 200N HFM-6
1401 Rockville Pike
Rockville, MD 20852
Phone: (301) 435-5680
Fax: (301) 827-0440
Email: goodmanj@cber.fda.gov

Harry B. Greenberg, M.D.

Microbiology and Immunology
Department of Research
Aviron
297 North Bernardo Avenue
Mountain View, CA 94043
Phone: (650) 919-3711
Fax: (650) 919-2426
Email: hgreenberg@aviron.com

Duane J. Gubler, Sc.D.

Division of Vector-Borne Infectious Diseases
National Center for Infectious Diseases
Centers for Disease Control and Prevention
Foothills Campus
P.O. Box 2087
Fort Collins, CO 80522
Phone: (970) 221-6428
Fax: (970) 266-3502
Email: dgubler@cdc.gov

Philip Hanna, Ph.D.

Department of Microbiology and Immunology
University of Michigan Medical School
5641 Medical Science II
Box 0620
Ann Arbor, MI 48109
Phone: (734) 615-3707
Fax: (734) 764-3562
Email: pchanna@umich.edu

Barton F. Haynes, M.D.

Department of Medicine
Duke University Medical Center
Box 3703, DUMC
Durham, NC 27710
Phone: (919) 681-1095
Fax: (919) 681-8992
Email: hayne002@mc.duke.edu

Carole A. Heilman, Ph.D.

Director
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Room 3142
6700-B Rockledge Drive
Bethesda, MD 20892
Phone: 301-496-1884
Fax: (301) 480-4528
Email: cheilman@niaid.nih.gov

Thomas V. Inglesby, M.D.

Deputy Director
Center for Civilian Biodefense Strategies
Johns Hopkins University
Suite 830
111 Market Place
Baltimore, MD 21202
Phone: (410) 223-1655
Fax: (410) 223-1665
Email: tvi@jhsph.edu

Peter B. Jahrling, Ph.D.

U.S. Army Medical Research Institute
of Infectious Diseases
1425 Porter Street
Fort Detrick, MD 21702-5011
Phone: (301) 619-2772
Fax: (301) 619-4625
Email: peter.jahrling@det.amedd.army.mil

Dennis L. Kasper, M.D.

Channing Laboratory, Department of Medicine
Brigham and Women's Hospital
181 Longwood Avenue
Boston, MA 02115
Phone: (617) 525-2280
Fax: (617) 525-0080
Email: dennis_kasper@hms.harvard.edu

Edwin Kilbourne, M.D.

Department of Microbiology and Immunology
New York Medical College
Basic Science Building
Vahalla, NY 10595
Phone: (914) 594-4193
Fax: (914) 594-4176
Email: ekilbourne@snet.net

John Y. Killen, M.D.

Associate Director for Research Ethics
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 10, Room 1C118
9000 Rockville Pike
Bethesda, MD 20892
Phone: (301) 496-2429
Email: jkillen@niaid.nih.gov

Thomas Kindt, Ph.D.

Director, Division of Intramural Research
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 10, Room 4A31
9000 Rockville Pike
Bethesda, MD 20892
Phone: (301) 496-3006
Email: tk9c@nih.gov

Richard D. Klausner, M.D.

National Academy of Sciences
2101 Constitution Avenue, NW
Washington, DC 20418
Phone: (202) 334-1238
Fax: (202) 334-1565
Email: coconnor@nas.edu

John La Montagne, Ph.D.

Deputy Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 31, Room 7A03B
31 Center Drive
Bethesda, MD 20892
Phone: (301) 496-9677
Fax: (301) 496-4409
Email: jlamontagn@niaid.nih.gov

Sarah Landry, M.F.A.

Health Science Communication Specialist
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Room 3140
6700-B Rockledge Drive
Bethesda, MD 20892
Phone: (301) 496-1884
Fax: (301) 480-4528
Email: slandry@niaid.nih.gov

Clifford Lane, M.D.

Clinical Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 10, Room 11B09
9000 Rockville Pike
Bethesda, MD 20892
Phone: (301) 496-7196
Email: c117d@nih.gov

Dennis Lang, Ph.D.

Enteric Diseases Program Officer
Enteric and Hepatic Diseases Branch
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Room 3110
6700-B Rockledge Drive
Bethesda, MD 20892
Phone: (301) 496-7051
Fax: (301) 402-1456
Email: dlang@niaid.nih.gov

Catherine A. Laughlin, Ph.D.

Chief
Virology Branch
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Room 3150
6700-B Rockledge Drive
Bethesda, MD 20892
Phone: (301) 496-7453
Fax: (301) 402-0659
Email: claughlin@niaid.nih.gov

Stanley M. Lemon, M.D.

School of Medicine
University of Texas Medical Branch
301 University Boulevard
Galveston, TX 77555-0133
Phone: (409) 772-4793
Fax: (409) 772-2684
Email: smlemon@utmb.edu

Stephen Leppla, Ph.D.

Department of Oral Infection and Immunity
Division of Intramural Research
National Institute of Dental and Craniofacial
Research
National Institutes of Health
Building 30, Room 304
30 Convent Drive
Bethesda, MD 20892
Phone: (301) 594-2865
Fax: (301) 402-1064
Email: leppla@nih.gov

Stuart B. Levy, M.D.

Departments of Molecular Biology, Microbiology,
and Medicine
Tufts University School of Medicine
136 Harrison Avenue
Boston, MA 02111
Phone: (617) 636-6764
Fax: (617) 636-0458
Email: stuart.levy@tufts.edu

Margaret Liu, M.D.

Global Health Program
Bill and Melinda Gates Foundation
P.O. Box 23350
Seattle, WA 98102
Phone: (206) 709-3100
Fax: (206) 709-3170
Email: margaretl@gatesfoundation.org

Adel Mahmoud, M.D., Ph.D.

President
Merck Vaccines
Merck & Company, Inc.
One Merck Drive
Whitehouse Station, NJ 08889
Phone: (908) 423-4316
Fax: (908) 735-1232
Email: adel_mahmoud@merck.com

Harold S. Margolis, M.D.

Senior Advisor
Smallpox Preparedness and Response
Office of the Director
Centers for Disease Control and Prevention
1600 Clifton Road
Atlanta, GA 30333
Phone: (404) 639-7000
Email: hsm1@cdc.gov

Grant McFadden, Ph.D.

University of Western Ontario
Microbiology and Immunology
Robarts Research Institute
1400 Western Road
London, Ontario, Canada N6G2V4
Phone: (519) 663-3184
Fax: (519) 663-3847
Email: mcfadden@rri.ca

John McGowan, Ph.D.

Director, Division of Extramural Research
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Room 2142
6700-B Rockledge Drive
Bethesda, MD 20892
Phone: (301) 496-7291
Email: jm80c@nih.gov

Pamela McInnes, D.D.S

Deputy Director
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Room 3142
6700-B Rockledge Drive
Bethesda, MD 20892
Phone: (301) 496-1884
Fax: (301) 480-4528
Email: pm23m@nih.gov

Michael Meagher, Ph.D.

Chemical Engineering
University of Nebraska-Lincoln
East Campus
355 FIB
Lincoln, NE 68583-0919
Phone: (402) 472-2342
Fax: (402) 472-1693
Email: mmeagher1@unl.edu

James Meegan, Ph.D.

Virology Program Officer
Virology Branch
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Room 3137
6700-B Rockledge Drive
Bethesda, MD 20892
Phone: (301) 496-7453
Fax: (301) 402-0659
Email: jmeegan@niaid.nih.gov

Gregory Mertz, M.D.

Division of Infectious Diseases
Internal Medicine
University of New Mexico
915 Camino De Salud
Albuquerque, NM 87131-5271
Phone: (505) 272-5666
Fax: (505) 272-8441
Email: gmertz@salud.unm.edu

Marissa Miller, D.V.M.

Antimicrobial Resistance Program Officer
Bacteriology and Mycology Branch
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Room 3244
6700-B Rockledge Drive
Bethesda, MD 20892
Phone: (301) 402-8643
Fax: (301) 402-2508
Email: mamiller@niaid.nih.gov

Thomas Monath, M.D., FACP

Vice President
Department of Research and Medical Affairs
Acambis, Inc.
38 Sidney Street
Cambridge, MA 02139
Phone: (617) 494-1339
Fax: (617) 494-0924
Email: thomas.monath@acambis.com

David Morens, M.D.

Medical Epidemiologist
Virology Branch
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Room 3149
6700-B Rockledge Drive
Bethesda, MD 20892
Phone: (301) 496-7453
Fax: (301) 480-1594
Email: dmorens@niaid.nih.gov

Bernard Moss, M.D., Ph.D.

Laboratory of Viral Diseases
Division of Intramural Research
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 4, Room 229A, MSC 0445
4 Center Drive
Bethesda, MD 20892-0445
Phone: (301) 496-9869
Fax: (301) 480-1147
Email: bmoss@nih.gov

Richard W. Moyer, Ph.D.

Molecular Genetics and Microbiology
University of Florida
P.O. Box 100266
Gainesville, FL 32610
Phone: (352) 392-7077
Fax: (352) 846-2042
Email: rmoyer@mgm.ufl.edu

Barbara Mulach, Ph.D.

Health Science Communication Specialist
Office of the Director
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Room 3266
6700-B Rockledge Drive
Bethesda, MD 20892
Phone: (301) 496-1884
Fax: (301) 480-4528
Email: bmulach@niaid.nih.gov

Brian Murphy, M.D.

Chief, Laboratory of Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 7, Room 106
9000 Rockville Pike
Bethesda, MD 20892
Phone: (301) 496-4205
Email: bmurphy@niaid.nih.gov

James Musser, M.D., Ph.D.

Laboratory of Human Bacterial Pathogenesis
Division of Intramural Research
National Institute of Allergy and Infectious Diseases
National Institutes of Health
903 South 4B Street
Hamilton, MT 59840
Phone: (406) 363-9287
Fax: (406) 363-9394
Email: jmusser@niaid.nih.gov

Gary Nabel, M.D., Ph.D.

Director, Vaccine Research Center
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 40, Room 4502
9000 Rockville Pike
Bethesda, MD 20892
Phone: (301) 496-1852
Email: gnabel@niaid.nih.gov

Bradley A. Perkins, M.D.

Chief, Meningitis and Special Pathogens Branch
Division of Bacterial and Mycotic Diseases
Centers for Disease Control and Prevention
MSC C09
1600 Clifton Road
Atlanta, GA 30333
Phone: (404) 639-3158
Fax: (404) 639-3059
Email: bap4@cdc.gov

Robert D. Perry, Ph.D.

Departments of Microbiology, Immunology,
and Molecular Genetics
University of Kentucky
MS415 Medical Center
800 Rose Street
Lexington, KY 40536-0298
Phone: (859) 323-6341
Fax: (859) 257-8994
Email: rperry@pop.uky.edu

C.J. Peters, M.D.

Center for Biodefense
Departments of Pathology, Microbiology,
and Immunology
University of Texas Medical Branch
301 University Boulevard
Galveston, TX 77555-0609
Phone: (409) 772-0090
Fax: (409) 772-2500

Robert Purcell, M.D.

Co-Chief, Laboratory of Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Building 7, Room 202
9000 Rockville Pike
Bethesda, MD 20892
Phone: (301) 496-6227
Email: rpurcell@niaid.nih.gov

David A. Relman, M.D.

Department of Microbiology, Immunology,
and Medicine
Veterans Affairs
Stanford University
3801 Miranda Avenue
Palo Alto, CA 94304
Phone: (650) 852-3308
Fax: (650) 852-3291
Email: relman@stanford.edu

Bernard Roizman, Sc.D.

University of Chicago
910 East 58th Street
Chicago, IL 60637
Phone: (773) 702-1898
Fax: (773) 702-1631
Email: bernard@cummings.uchicago.edu

Martin Rosenberg, Ph.D.

Research and Development
Promega Corporation
2800 Woods Hollow Road
Madison, WI 53711-5399
Phone: (608) 274-4330
Fax: (608) 277-2601
Email: mrosenbe@promega.com

Daniel Rotrosen, M.D.

Director, Division of Allergy and Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Room 5142
6700-B Rockledge Drive
Bethesda, MD 20892
Phone: (301) 496-1886
Fax: (301) 402-2571
Email: drotrosen@niaid.nih.gov

Philip K. Russell, M.D.

Office of Public Health and Preparedness
Department of Health and Human Services
Suite G36G
200 Independence Avenue
Washington, DC 20201
Phone: (202) 260-0391
Fax: (202) 690-7412
Email: philip.russell@hhs.gov

Polly Sager, Ph.D.

Director, Office of Scientific Coordination
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Room 4149
6700-B Rockledge Drive
Bethesda, MD 20892
Phone: (301) 402-0755
Fax: (301) 480-4456
Email: psager@niaid.nih.gov

Jeffrey Schlom, Ph.D.

Center for Cancer Research
Laboratory of Tumor Immunology and Biology
National Cancer Institute
National Institutes of Health
Room 8B09, MSC 1750
10 Center Drive, Building 10
Bethesda, MD 20892-1750
Phone: (301) 496-4343
Fax: (301) 496-2756
Email: js141c@nih.gov

Connie Schmaljohn, Ph.D.

Department of Molecular Virology
Virology Division
U.S. Army Medical Research Institute
of Infectious Diseases
1301 Ditto Avenue
Fort Detrick, MD 21702
Phone: (301) 619-4103
Fax: (301) 619-2439
Email: connie.schmaljohn@det.ameddarmy.mil

Gary K. Schoolnik, M.D.

Department of Medicine
Stanford Medical School
Room 241
Beckman Center
Stanford, CA 94305
Phone: (650) 723-8158
Fax: (650) 723-1399
Email: schoolni@cmgm.stanford.edu

Lance Simpson, Ph.D.

Occupational and Environmental Health Sciences
Department of Medicine
Jefferson Medical College
Room 314-JAH
1020 Locust Street
Philadelphia, PA 19107
Phone: (215) 955-8381
Fax: (215) 955-2169
Email: lance.simpson@mail.tju.edu

Christine Sizemore, Ph.D.

Tuberculosis Program Officer
Respiratory Diseases Branch
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Room 3244
6700-B Rockledge Drive
Bethesda, MD 20892
Phone: (301) 496-5305
Fax: (301) 496-8030
Email: csizemore@niaid.nih.gov

Leonard A. Smith, Ph.D.

Chief
Department of Immunology and Molecular Biology
Division of Toxinology and Aerobiology
United States Army Medical Research Institute of
Infectious Diseases
1425 Porter Street
Fort Detrick, MD 21702-5011
Phone: (301) 619-4238
Fax: (301) 619-2348
Email: leonard.smith@amedd.army.mil

Susan C. Straley, Ph.D.

Department of Microbiology, Immunology and
Molecular Genetics
University of Kentucky
800 Rose Street
Lexington, KY 40536-0298
Phone: (859) 323-6538
Fax: (859) 257-8994
Email: scstra01@uky.edu

James H. Strauss, Ph.D.

Division of Biology 156-29
California Institute of Technology
1200 East California Boulevard
Pasadena, CA 91125
Phone: (626) 395-4903
Fax: (626) 796-4209
Email: straussj@caltech.edu

Robert B. Tesh, M.D.

Center for Tropical Diseases
Department of Pathology
University of Texas Medical Branch
301 University Boulevard
Galveston, TX 77555-0609
Phone: (409) 747-2431
Fax: (409) 747-2429
Email: rtesh@utmb.edu

Ed Tramont, M.D.

Director, Division of AIDS
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Room 4142
6700-B Rockledge Drive
Bethesda, MD 20892
Phone: (301) 496-0545
Email: etramont@niaid.nih.gov

Emil R. Unanue, M.D.

Department of Pathology and Immunology
Washington University School of Medicine
660 South Euclid Avenue
Box 8118
St. Louis, MO 63110
Phone: (314) 362-7440
Fax: (314) 362-4096
Email: unanue@pathbox.wustl.edu

Richard J. Whitley, M.D.

Department of Pediatrics
University of Alabama at Birmingham
Suite 616
1600 Seventh Avenue South
Birmingham, AL 35233
Phone: (205) 934-5316
Fax: (205) 934-8559
Email: rwhitley@peds.uab.edu

John A.T. Young, Ph.D.

Department of Oncology
McArdle Laboratory for Cancer Research
University of Wisconsin-Madison
1400 University Avenue
Madison, WI 53706
Phone: (608) 265-5151
Fax: (608) 262-2824
Email: young@oncology.wisc.edu



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

National Institute of Allergy and Infectious Diseases

NIH Publication No. 03-5308
February 2002
<http://biodefense.niaid.nih.gov>