Infectious diseases are significant causes of human mortality, morbidity, and economic loss. Although effective antimicrobial agents are available for treating bacterial infections, as are limited agents for viral, fungal, and parasitic infections, many diseases are still very difficult to treat effectively and may present serious health concerns. The emergence of drug resistance among common pathogens continues to render ineffective many previously frontline therapies, and the use of many drugs is often limited by toxicity concerns. Emergence of diseases caused by new or drug-resistant pathogens demands more effective drugs. However, current advances in chemistry, bioinformatics, and structural biology should make it possible to discover or design novel anti-infective agents that target specific functions required for pathogen growth and pathogenesis (http://www3.niaid.nih.gov/about/organization/dmid/overview.htm).

One major research goal of NIAID is to facilitate the discovery and evaluation of clinically effective drugs for a host of infectious diseases by supporting research at three levels: basic research and drug discovery; preclinical evaluation; and clinical evaluation (http://www.niaid.nih.gov/dmid/meetings/anti_infective_mtg_2004.pdf), as follows:

**Basic Research**
- Continue strong support of basic research, including focused emphasis on mechanisms of antimicrobial resistance and microbial membrane biophysics.
- Continue support for basic discovery research, including identification of targets and development of assays and diagnostic tools for more rapid, earlier detection of antimicrobial resistance.
- Expand support for preclinical toxicology (e.g., in vitro toxicology, animal toxicology) and drug metabolism studies.
- Continue strong support of genomic research, including analysis, proteomics capabilities, and protein structure.
- Support the involvement of medicinal chemists and molecular biophysicists in research on anti-infective drugs.

**Translational Research**
- Establish a prioritization process for allocating resources. Criteria could include public health priorities; feasibility of scientific and clinical research paths to product licensure; feasibility of product production; and feasibility of product distribution.
- Support resources for developing models for pharmacokinetic and/or pharmacodynamic analysis and development of nonmurine animal models.
- Provide resources for medicinal chemistry and formulation methodologies. Provide support for developing new statistical tools for analyzing clinical trial data.
- Support the conduct of early-phase clinical trials, including pharmacokinetic studies in special populations, in low incidence diseases, or difficult indications.
- Provide support for assessing the impact of the use of diagnostics on drug resistance.
- Support development of improved methodologies, including statistical tools to allow more efficient use of clinical trial resources.
- Establish collaborations with FDA and the pharmaceutical industry to evaluate possible alternative end points for prospective clinical trials.
- Promote the evaluation of drugs not developed as anti-infectives for use as anti-infectives and discontinued candidates for potential niche indications.

**26.1 Recent Programmatic Accomplishments/Developments**

**26.1.1 Antiviral Drug Development**

NIAID is continuing to support both in vitro and in vivo antiviral screening programs, preclinical evaluation of antiviral lead compounds, and clinical evaluation of antiviral drugs for medically important, emerging/re-emerging, and rare viral diseases.
26.1.1.1 The Collaborative Antiviral Testing Group

The Collaborative Antiviral Testing Group (CATG) (http://www.niaid.nih.gov/dmid/viral) continues to support a number of contracts that perform in vitro screening and in vivo testing in animal models and that conduct preliminary studies of efficacy, pharmacology, toxicology, and drug delivery:

In Vitro Screening Systems. Currently, CATG supports the following antiviral in vitro screening systems:

- Orthopoxviruses: vaccinia, cowpox
- Herpesviruses: HSV-1, HSV-2, VZV, EBV, CMV, HHV-6, HHV-8
- BK virus
- Papillomaviruses
- Hepatitis B virus
- Hepatitis C virus
- Respiratory viruses: influenza A, influenza B, respiratory syncytial virus (RSV), parainfluenza, rhinoviruses, measles, human coronaviruses, SARS coronavirus (SARS-CoV)
- Biodefense viral hemorrhagic fevers and encephalitides: dengue virus, yellow fever virus, West Nile virus, Venezuelan equine encephalitis virus (a Togavirus), Pichinde virus (an Arenavirus), Punta Toro virus (a Bunyavirus)

In Vivo Animal Models. Currently, CATG also supports the following in vivo animal disease models:

- Orthopoxviruses: murine models of vaccinia, cowpox, and ectromelia
- Herpesviruses: murine models of herpes simplex virus (HSV)-1, HSV-2; guinea pig HSV-1, HSV-2; murine cytomegalovirus (CMV); guinea pig CMV; human CMV in SCID-hu mice
- Hepatitis viruses: woodchuck hepatitis in woodchucks; hepatitis B virus (HBV) transgenic mice
- Respiratory viruses: murine model of SARS-CoV; murine models of influenza A and influenza B; cotton rat models of RSV, measles, bovine parainfluenza type3 (PIV3), and human metapneumovirus (hMPV)
- Papillomaviruses: Shope papilloma in rabbits; human papillomavirus 6 (HPV6) or HPV11 in SCID-hu mice
- Hamster scrapie; in hamster-prion transgenic mice
- Biodefense: Pichinde virus in hamsters; Banzi virus in mice; Punta Toro virus in mice and hamsters; Semliki Forest virus in mice; West Nile virus in mice and hamsters; Venezuelan equine encephalitis in mice; Western equine encephalitis in hamsters; yellow fever virus in hamsters

26.1.1.2 The Collaborative Antiviral Study Group

The Collaborative Antiviral Study Group (CASG) (http://www.niaid.nih.gov/daids/PDATguide/casg.htm) — which consists of a multi-institute infrastructure comprising more than 90 sites in the United States and internationally—is continuing its support for clinical studies on antiviral compounds. Clinical studies on therapies for the following viral infections are under way: cytomegalovirus; herpes simplex virus; BK virus; West Nile virus; and influenza. Specific activities include:

- A protocol has been developed to use cidofovir as a contingency to treat smallpox in the event of an outbreak. More information can be found at the CASG Web site: http://www.peds.uab.edu/casg/.
- In 2005, CASG initiated a chart review in selected pediatric practices that used oseltamivir in infants with influenza to gather safety data to help inform prospective users. Data have been collected and partially analyzed on 120 subjects up to October 2006. By November 2006, data were collected and analyzed on 150 to 200 subjects.
- In July 2006, CASG, in collaboration with Hoffman-LaRoche, Inc., developed a protocol for a safety and pharmacokinetic/pharmacodynamic (PK/PD) prospective study of oseltamivir for the treatment of children under the age of 2 with documented influenza. CASG opened a study in October 2006 in as many as 25 centers across the United States.
- Recently, CASG initiated a study to evaluate the safety and tolerability of cidofovir as a treatment for BK virus renal nephropathy in renal transplant patients.

26.1.1.3 Development of Therapeutic Agents for Selected Viral Diseases

In 2006, NIAID awarded four new contracts to biotechnology companies to develop antiviral therapeutics against biodefense viral pathogens:

- To Alnylam Pharmaceuticals, Inc. (Cambridge, MA) to perform preclinical development and Investigational New Drug (IND)-enabling studies on RNA interference (RNAi)-based therapeutic agents designed to treat hemorrhagic fever caused by Ebola virus.
- To Macrogenics, Inc. (Rockville, MD) to perform pivotal nonclinical animal efficacy and IND-enabling studies, Phase I human safety trials, and biologic license application (BLA)-enabling studies on a therapeutic monoclonal antibody to treat West Nile virus infection.
- To SIGA Technologies, Inc. (Corvallis, OR) to perform Phase I human safety trials and NDA-enabling studies.
on a small-molecule compound, ST-246, as a preexposure and postexposure therapeutic against smallpox virus.

- To NexBio, Inc. (San Diego, CA) to perform Phase I human safety and Phase II clinical trials and other NDA-enabling studies on a recombinant therapeutic enzyme, Fludase, as a preexposure/treatment therapeutic against influenza virus.

### 26.1.2 Antibacterial and Antitoxin Drug Development

NIAID is continuing its support of *in vitro* and *in vivo* antibacterial screening programs, preclinical evaluation of antibacterial lead compounds, and clinical evaluation of antibacterial drugs for medically important, emerging/re-emerging, and rare bacterial and toxin-caused diseases.

- **Monoclonal Antibody Therapeutic for Botulinum Neurotoxin Serotype A.** In 2006, NIAID awarded a 3-year contract to XOMA LLC to formulate, finish, and release a mixture of the botulinum neurotoxin A monoclonal antibodies, to perform long-term stability studies and investigational new drug-enabling nonclinical safety studies, and to develop analytical assays that support future Phase I clinical trials.

- **The Bacteriology and Mycology Study Group (BAMSG) and Bacteriology and Mycology Biostatistical and Operations Unit (BAMBU).** The BAMSG is managed under a contract awarded to the University of Alabama at Birmingham and supports clinical studies to evaluate interventions for serious fungal diseases, as well as health care-associated resistant bacterial infections. The BAMBU is providing biostatistical and administrative support for the clinical studies. The BAMBU contract is managed by Rho Federal Systems Division, Inc. Under the BAMSG resource, a reserve fund has been established to support orphan studies that cannot be funded through industrial sponsors.

### 26.1.3 Antiparasitic Drug Development

Identification, validation, and evaluation of new antimalarial therapies remain NIAID priority activities. Highlights of specific activities are summarized below.

- **The Tropical Diseases Research Units (TDRU)** program is to support translational research leading to the discovery and preclinical development of new drugs or vector control methods to reduce or eliminate morbidity and mortality resulting from parasitic infection. One of the three awards made under this program focuses on development of novel antimalarial drugs.

- NIAID is continuing its support for investigator-initiated research on preclinical development and evaluation of novel compounds and has released a new initiative (RFA) seeking applications from public-private partnerships that are engaged in developing therapeutic or diagnostic products directed against neglected diseases, including malaria. NIAID is also supporting preclinical and clinical studies of combination therapies for malaria, especially those including artesunate. In December 2005, NIAID and the Medicines for Malaria Venture (MMV) jointly convened a meeting, including participants from FDA, CDC, and international drug regulatory agencies, to establish consensus regarding the appropriate design of Phase III clinical trials for new antimalarial drug combinations.

- Identifying, validating, and evaluating new vector control compounds and strategies remain NIAID priority activities. Under the partnership initiatives (RFAs), these projects will explore the role of strategies to control larva and mosquitoes in reducing the transmission of malaria and will develop new and safe insecticides targeting mosquito activities, including those aimed at mitigating resistance to insecticides.

### 26.1.4 Research Resources

NIAID is also maintaining several contracts that provide a broad range of services to support the nonclinical and clinical development of new drugs.

- **Services for the Preclinical Development of Therapeutic Agents.** In 2006, SRI International was awarded a contract from NIAID to provide a suite of services for preclinical development of therapeutic agents. This resource is intended to rapidly and efficiently close gaps in the preclinical development of promising new therapeutic agents that emerge from academia, the private sector, and other areas. This will allow for the commercial development of new therapeutics against potential agents of bioterrorism, drug-resistant pathogens, emerging and re-emerging infectious diseases, and diseases prevalent in resource-limited countries.

- **The In Vitro and Animal Models for Emerging Infectious Diseases and Biodefense Program.** This program continues to provide a wide range of resources for *in vitro* and *in vivo* nonclinical testing of new therapies and vaccines. These contracts provide resources for developing and validating small laboratory animal and non-human primate infection models for licensure of therapeutics.
Specific projects include the following:

- **Anthrax**: (i) Screening of existing FDA-approved antimicrobials and immunomodulators for efficacy against inhalational anthrax; (ii) evaluating whether immunization with recombinant Protective Antigen (rPA) vaccines can reduce the course of antibiotic therapy; and (iii) developing therapeutic animal models in rabbits and non-human primates.
- **Plague**: (i) Screening of existing FDA-approved antimicrobials for efficacy; and (ii) developing alternative non-human primate and mouse models.
- **Smallpox**: (i) Performing therapeutic efficacy studies in non-human primates; and (ii) evaluating toxicology of specific antiviral agents.
- **Tularemia**: Developing alternative non-human primate and mouse models.
- **Botulinum neurotoxin**: Developing small laboratory animal assay and therapeutic efficacy model.
- **Ricin**: Determining toxicokinetics in small animals.
- **SARS**: Developing small laboratory animal and non-human primate models.
- **Avian influenza**: Developing ferret model and testing its efficacy; evaluating toxicology.
- **Antimicrobials**: Performing in vitro screening.

### 26.2 Recent Research Programs in Drug Development

Several awards were made in 2006 to drug development–related programs:

- **NIAID’s Cooperative Research for the Development of Vaccines, Adjuvants, Therapeutic Immunotherapeutics, and Diagnostics for Biodefense and SARS Program**. This program supports discovery/design and development of vaccines, therapeutics, adjuvants, and diagnostics for biodefense. The program will help translate research from the target identification stage through target validation to early product development. In 2006, 27 awards were made, several of which focused on therapeutic development.

- **NIAID’s Small Business Advanced Technology Program**. This program is designed to encourage SBIR/STTR applications to develop therapeutics, vaccines, adjuvants/immunostimulants, diagnostics, and selected resources for biodefense. Several grants related to therapeutic development were made in 2006. A small-molecule inhibitor, ST-294, against viral hemorrhagic fever caused by New World arenaviruses was advanced through preclinical development through this program.

### 26.3 Recent NIAID-Supported Scientific Advances

- **New Developments in Yellow Fever Virus Research.** A hamster model for evaluating compounds against yellow fever virus (YFV) was developed and characterized (1). Challenge with yellow fever virus resulted in 50% to 80% mortality in female hamsters with virus detected in many organs, including the liver, kidney, and spleen. Treatment of hamsters with interferon, viramidine or ribavirin, initiated 4 hours prior to YFV infection, resulted in significant improvement in survival and liver enzyme levels.

- **Derivatives of Cidofovir Inhibit Polyomavirus BK Replication In Vitro.** Polyomavirus BK is a significant pathogen in transplant recipients, but no effective antiviral therapy is available. Esterification of cidofovir with hexadecyloxypropyl, octadecyloxyethyl, and oleyloxyethyl groups resulted in significantly enhanced activity against BK virus replication in vitro (2). These lipid esters of cidofovir are orally bioavailable and are not nephrotoxic; thus, they are promising new antivirals for BK virus infection in transplant recipients.

- **Development of New Cell-Based Screen May Help Identify New Antivirals.** Identification of new antiviral lead compounds depends on robust primary assays for high-throughput screening (HTS) of large compound libraries. In a recent study, researchers developed a cell-based screen in 384-well plates to identify potential antiviral agents against influenza by measuring the cytopathic effect (CPE) induced by influenza virus (A/Udorn/72, H3N2) infection in Madin Darby canine kidney (MDCK) cells using the luminescent-based CellTiter Glo system (3). This assay is translatable for screening against other influenza strains, such as avian flu, and may facilitate identification of antivirals for other viruses that induce CPE, such as West Nile or dengue.

- **Development of Alternative Approaches to Treating Influenza.** To provide an urgently needed alternative treatment modality for influenza, NIAID-supported investigators have generated a recombinant fusion protein composed of a sialidase catalytic domain derived from Acidaminomyces viscosus fused with a cell surface–anchoring sequence (4). The sialidase fusion protein is to be applied as an inhalant to remove the influenza viral receptors, sialic acids, from the airway epithelium. Thus, a specific sialidase fusion construct, DAS181, effectively cleaved sialic acid receptors used by both human and avian influenza viruses. The treatment provided a long-lasting effect and is nontoxic to the cells. DAS181 demonstrated potent antiviral and cell protective efficacies against a panel of laboratory strains and clinical isolates of influenza A and B. Mouse and ferret studies confirmed
significant \textit{in vivo} efficacy of the sialidase fusion in both prophylactic and treatment modes.

- **Potential Therapeutic for Orthopoxvirus and Herpesvirus Infections.** \textit{N}-Methanocarbathymidine [(N)-MCT] is a novel nucleoside analogue that was found active against some herpesviruses and orthopoxviruses \textit{in vitro} (5). The antiviral activity of this molecule was dependent on the type I thymidine kinase (TK) in herpes simplex virus and also appeared to be dependent on the type II TK expressed by cowpox and vaccinia viruses. (N)-MCT was also a good inhibitor of viral DNA synthesis in both viruses and was consistent with inhibition of the viral DNA polymerase once it had been activated by the viral TK homologues. The compound was nontoxic \textit{in vivo} and effectively reduced the mortality of mice infected with orthopoxviruses, as well as those infected with herpes simplex virus type 1 when treatment was initiated 24 hours after infection. These results indicated that (N)-MCT is active \textit{in vitro} and \textit{in vivo}, and its mechanism of action suggested that the molecule may be an effective therapeutic agent for orthopoxvirus and herpesvirus infections, thus warranting further development.

- **Elusive Drug Target in Mycobacterium tuberculosis Is Identified.** Isoniazid (INH) is one of the most effective drugs against tuberculosis (TB), but \textit{Mycobacterium tuberculosis} (Mtb), the bacterium that causes TB, has found ways to resist this drug. Over the past decade, several proteins have been suggested to be the target for INH, but researchers have not been able to define exactly which one is attacked by INH and is responsible for the drug’s action on the bacteria. To define whether one or more proteins are directly affected by the drug, it was important to change these targets one at a time to demonstrate which one is primarily attacked by INH. Using new molecular tools that were developed, a team of researchers introduced, for the first time, a small but defined change (mutation) in the protein InhA alone, which was thought to be the primary target for INH (6). This small change is the same that is seen in this protein when the bacteria become resistant to INH. The bacteria with the changed protein InhA could no longer be killed with therapeutic amounts of INH. Furthermore, the altered InhA protein from Mtb was also isolated, and it was determined that it lost the ability to effectively bind to isoniazid. To make sure that this changed protein was indeed the primary target of INH, the same techniques were applied to create Mtb bacteria with defined changes in one other protein that had been hypothesized to be a target of INH. This change, however, could not alter the way the bacteria responded to the drug. With this information, scientists can now study the exact interaction between INH and its target in the bacterial cell and can start designing new versions of the drug that are still effective even if the bacterium changes the makeup of the InhA protein—as it has done to become resistant to this drug (6). INH remains the most effective drug against TB. However, resistance against INH is increasing despite the use of multidrug regimens against this disease. Because it cannot be presumed that a drug has only one target, it is important to characterize what bacterial components are primarily responsible for its action. With this information, it is now possible to characterize the exact effect of the drug on the bacterial metabolism, to better understand how bacteria create resistance against INH, to construct new versions of the drug that are effective even against mutated target drugs, and also to generate appropriate companion drugs to INH that make it more difficult for Mtb to become resistant. The proof that InhA is the primary target for INH is a new milestone in TB research that has been the focus of intense investigation since the early 1990s.

- **Effective Antimicrobial Regimens for Use in Humans for Therapy for Bacillus anthracis Infections and Post-exposure Prophylaxis.** Expanded options for treatments directed against pathogens that can be used for bioterrorism are urgently needed. Treatment regimens directed against such pathogens can be identified only by using data derived from \textit{in vitro} and animal studies. It is crucial that these studies reliably predict the efficacy of proposed treatments in humans. The objective of this study was to identify a levofloxacin treatment regimen that will serve as an effective therapy for \textit{Bacillus anthracis} infections and postexposure prophylaxis. An \textit{in vitro} hollow-fiber infection model that replicates the pharmacokinetic profile of levofloxacin observed in humans (half-life [$t_{1/2}$, 7.5 hours]) or in animals such as the mouse or the rhesus monkey ($t_{1/2}$, ~2 hours) was used to evaluate a proposed indication for levofloxacin (500 mg once daily) for treating \textit{Bacillus anthracis} infections (7). The results obtained with the \textit{in vitro} model served as the basis for the doses and the dose schedules that were evaluated in the mouse inhalational anthrax model. The effects of levofloxacin and ciprofloxacin treatment were compared with those of no treatment (untreated controls). The main outcome measure in the \textit{in vitro} hollow-fiber infection model was a persistent reduction of culture density ($\geq 4 \log_{10}$ reduction) and prevention of the emergence of levofloxacin-resistant organisms. In the mouse inhalational anthrax model, the main outcome measure was survival. The results indicated that levofloxacin given once daily with simulated human pharmacokinetics effectively sterilized \textit{Bacillus anthracis} cultures. By using a simulated animal pharmacokinetic profile, a once-daily dosing regimen that provided a human-equivalent exposure failed to sterilize the cultures. Dosing regimens that “partially humanized” levofloxacin exposures within the constraints of animal pharmacokinetics reproduced the antimicrobial efficacy
seen with human pharmacokinetics. In a mouse inhalational anthrax model, once-daily dosing was significantly inferior (survival end point) to regimens of dosing every 12 hours or every 6 hours with identical total daily levofloxacin doses. These results demonstrated the predictive value of the in vitro hollow-fiber infection model with respect to the success or the failure of treatment regimens in animals. Furthermore, the model permits the evaluation of treatment regimens that “humanize” antibiotic exposures in animal models, enhancing the confidence with which animal models may be used to reliably predict the efficacies of proposed antibiotic treatments in humans in situations where human trials cannot be performed (e.g., the release of pathogens as agents of bioterrorism or emerging infectious diseases). A treatment regimen effective in rhesus monkeys was identified (7).

This study demonstrated the combinational use of in vitro hollow-fiber and animal models to evaluate the effectiveness of certain antibiotics for treating human infection for diseases where human trials cannot be performed, such as anthrax and plague. Such systemic pharmacokinetic and pharmacodynamic characterization of existing antibiotics will make it possible to identify active anti-infective agents and to design effective treatment regimens. The findings gained from this study will provide the public with options of more than one antibiotic if there is an urgent need to counteract a bioterror attack or other unexpected outbreak of an emerging infectious disease.

**RNA Interference as a Potential Antiviral Therapy Against West Nile Virus.** RNA interference (RNAi) is a recently discovered cellular mechanism in which small pieces of double-stranded RNA (small interfering RNAs, or siRNAs) suppress the expression of genes with sequence homology. A team of investigators has been able to harness RNA interference to protect laboratory animals against lethal infection with important human pathogens like the herpes simplex virus 2 (HSV-2), West Nile virus (WNV), and the Japanese encephalitis virus (JEV) (8). It was demonstrated that a single siRNA, targeting a conserved sequence present in both WNV and JEV, protected mice infected with WNV or JEV from lethal encephalitis when administered before or after infection. WNV and JEV, both mosquito-borne flaviviruses that cause acute encephalitis, are important re-emerging viruses that cause tens of thousands of infections in the world and significant morbidity and mortality. Currently, no drugs exist to treat WNV or JEV. This study supports the further development of siRNAs as a novel, broad-spectrum antiviral therapy against emerging flaviviruses.

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