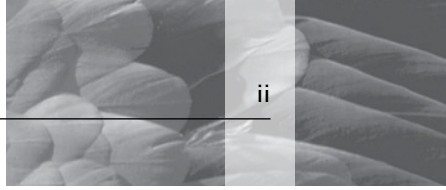
NIAID Influenza Research:
2009 Progress Report

NIAID

National Institute of Allergy and Infectious Diseases

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



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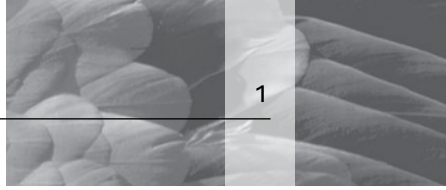


Introduction

In 2006, the National Institute of Allergy and Infectious Diseases (NIAID) convened a 35-member "Blue Ribbon Panel on Influenza Research" to provide guidance for NIAID influenza research activities in the coming years. Panel members were chosen from academic research institutions, U.S. government and international agencies, vaccine and pharmaceutical manufacturers, and nongovernmental organizations to create a panel with broad experience and a wide variety of viewpoints and expertise.

To prepare for the panel's work, NIAID first held an internal meeting of senior scientific staff at which the Institute's influenza research activities were reviewed and specific scientific gaps and opportunities were identified. A report from this meeting was provided to the panel, which then met in Bethesda, Maryland, in September 2006.

At the meeting, panel members were asked to recommend approaches to an innovative research agenda that would guide the NIAID influenza research program into the future. They also were asked to identify barriers that must be overcome, partnerships that must be forged, and resources that must be provided to speed research progress. Participants were urged to think broadly and for the long term, paying particular attention to how new technological advances in research could be applied for maximum effect.

A report summarizing the results of the meeting was published on the NIAID Web site.[1] The report outlined five broad guiding principles to guide influenza research and a more detailed list of research recommendations encompassing eight subject areas.

This report describes the progress that NIAID has made in influenza research since the panel's 2006 meeting. The Guiding Principles section discusses in general terms how NIAID has worked to follow the guiding principles. The Summary of Progress section summarizes scientific advances made through research conducted or supported by NIAID and ongoing NIAID activities relevant to each of the eight categories. (This report does not cover progress made through work conducted elsewhere and not supported by NIAID.) The Appendix provides more details, in the form of short paragraphs, about selected published findings and ongoing programs, initiatives, and other research activities.

[1]National Institute of Allergy and Infectious Diseases. *The report of the Blue Ribbon Panel on Influenza Research*. Bethesda, MD: National Institutes of Health; 2007. Available at: http://www3.niaid.nih.gov/topics/Flu/PDF/InfluenzaBlueRibbonPanel2006.pdf. Accessed March 23, 2009.

Guiding Principles

During the deliberations of the Blue Ribbon Panel on Influenza Research, several themes arose repeatedly. The panel's report captured five guiding principles that NIAID should follow as it pursues its influenza research program. Examples of how NIAID has worked to implement each principle are discussed below.

1. Promote Innovative Multidisciplinary Research

The panel noted that a multidisciplinary approach that integrates diverse fields, investigators, and perspectives into a well-coordinated influenza research program would speed basic discovery and the translation of research findings into effective interventions.

One program that addresses this need directly is the Centers of Excellence for Influenza Research and Surveillance (CEIRS) (see page 6). CEIRS is an integrated network of six centers designed to bring together multidisciplinary teams of researchers to study host immune responses, pathogenesis, and the factors that control the emergence and transmission of influenza viruses among animal reservoirs. The overall goal of this network is to provide information and public health tools needed to control the impact of epidemic influenza and the threat of pandemic influenza. CEIRS also provides scientific support for the research and public health response to newly emerging influenza viruses with pandemic potential.

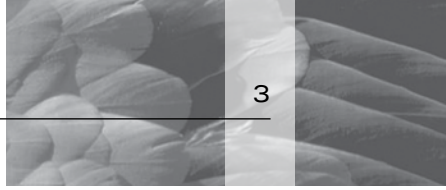
In addition, NIAID supports several initiatives to increase the scope of multidisciplinary research in influenza. Several of these initiatives encompass influenza within the broader context of other pathogens.

- NIAID's Cooperative Centers for Translational Research on Human Immunology and Biodefense program is a cooperative research enterprise that strongly promotes innovative interdisciplinary research on human immunity for NIAID priority A – C biodefense pathogens, including influenza.
- The Systems Approach to Innate Immunity and Inflammation program encompasses a multidisciplinary team of investigators with expertise in innate and adaptive immunity, biochemistry, proteomics, genomics, computational biology, and computer science to produce a comprehensive picture of molecular interactions that regulate innate and adaptive immune responses to infection.
- The Modeling Immunity for Biodefense program develops computational models of host immune responses to infection or vaccination. Each contract under this program includes a team of computational biologists, immunologists, and computer scientists to refine and validate the models.

2. Integrate Seasonal and Pandemic Influenza Research Activities

The panel emphasized that research on seasonal and pandemic influenza must be well integrated. Both types of influenza are caused by influenza viruses that can easily be transmitted among humans; they differ mostly in virulence and the degree of immunity present in the human population. A better understanding of seasonal influenza will help development of vaccines and other countermeasures that could be used against the next influenza pandemic.

The intertwined nature of seasonal and pandemic influenza research is reflected in several NIAID initiatives and programs. For example, the Southeast Asia Infectious Disease Clinical Research Network (SEAICRN) (see page 7) is a network of



NIAID-funded clinical research sites in Southeast Asia. Researchers at these sites study patients infected with either severe seasonal influenza or H5N1 influenza to understand pathogenic mechanisms and test treatments.

The origin of pandemic viruses cannot be understood without determining how seasonal viruses arise and spread. Therefore, ongoing efforts to understand influenza virus evolution and geographic transmission are directed toward the three 20th century pandemic viruses and recent seasonal epidemics.

The most consequential area of overlap is in the effort to develop new and optimized vaccines, diagnostics, and treatments. Although the need for these interventions becomes acute during a pandemic, it is impractical to build a system of countermeasures for use in a pandemic unless the system is also routinely used to combat seasonal influenza. Increased understanding and preparedness for one inherently furthers understanding and preparedness for the other.

3. Balance Investigator-Initiated Basic Research With Targeted Activities

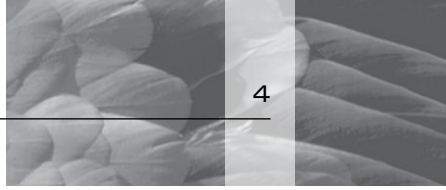
Basic research projects initiated by individual investigators form the foundation of the NIAID research program in influenza. Although NIAID maintains a substantial portfolio of investigator-initiated influenza-related grants and intramural projects, some NIAID programs simultaneously embrace both investigator-initiated and targeted approaches to pursue basic influenza research. In the CEIRS program, for example, the principal investigators at the various centers initiate most of the research projects; however, the established network allows NIAID to effectively coordinate activities. Moreover, multiple NIAID programs that supply reagents and data to scientists help to accelerate basic discovery. The Influenza Genome Sequencing Project, for example, supplies much-needed whole genome sequence data to promote basic discovery about influenza virus evolution (see page 11).

Product development activities sponsored by NIAID, which are intended to increase availability of pandemic countermeasures, are—out of necessity—more targeted. However, NIAID strives to partner with collaborators in these efforts. Early-stage influenza product development efforts, for example, usually include NIAID influenza program staff and personnel from the supported institutions working jointly in program planning and administration. In the intramural program, Cooperative Research and Development Agreements (CRADAs) promote partnerships with industry to conduct product development activities. On occasion, NIAID undertakes late-stage product development activities, such as large randomized clinical trials of pandemic[2] influenza vaccines that otherwise might not be conducted. In these situations, NIAID maximizes available resources, such as the NIAID Vaccine and Treatment Evaluation Units, so that the work is conducted as efficiently as possible.

4. Maximize Applicability of Influenza Research Results

The panel noted that knowledge about influenza and product platforms developed for influenza may be easily transferable to other infectious diseases. Therefore, they suggested that influenza research activities be structured to collect data with broad applicability and to create technology platforms and infrastructure that can be used

[2]Technically, pandemic vaccines that are currently under development are "pre-pandemic," in the sense that no pandemic has been declared. However, for the purposes of this document, nonseasonal influenza vaccines will generally be referred to as "pandemic."



for other infectious diseases. The broader applications of influenza research are especially relevant to basic research on innate immune system activation, where influenza infection serves as a model for similar approaches to other infections. For example, NIAID initiatives that target systems biology and computer modeling of immune responses shed light on the host adaptive immune response to influenza and other pathogens, and also contribute to the understanding of the innate immune response to infection.

Likewise, new vaccine platforms, vaccine adjuvants, and diagnostic technologies under development for influenza can pave the way for the application of similar technologies to other infectious diseases. For example, mechanistic studies of how adjuvants increase innate and mucosal influenza immune responses will provide information on how to exploit these adjuvants to vaccinate against other diseases. Moreover, new diagnostic platforms that can distinguish multiple influenza subtypes can be adapted to differentiate viruses that cause other respiratory infections.

5. Enhance Research Coordination and Collaboration

Optimal coordination of influenza research activities among various federal agencies, private industry, and international health organizations was cited as a high priority by the panel and NIAID. NIAID has subsequently taken several steps to improve coordination of influenza research. For the past 2 years, NIAID has held a series of face-to-face and telephone conferences with key influenza staff at CDC. NIAID also participates in weekly interagency meetings hosted by HHS to coordinate research, development, and regulatory and public education activities. NIAID's interactions with the HHS Biomedical Advanced Research and Development Authority increase coordination of research with private industry to bring new influenza drugs, vaccines, and diagnostics to market.

The panel also emphasized that research collaborations are essential to making rapid progress. Multiple NIAID actions support this goal. For example, the Influenza Genome Sequencing Project actively solicits collaborators to supply viral samples. Internationally, NIAID partnered with the World Health Organization (WHO), Oxford University, Wellcome Trust UK, and others to create the SEAICRN in Indonesia, Thailand, and Vietnam. The Bioinformatics Integration Support Contract (BISC) is building a comprehensive data management infrastructure for data sharing and collaboration across the NIAID-funded research community. Expansion of the NIAID-funded BioHealthBase Bioinformatics Research Center will provide a forum for the exchange of information across the entire scientific community. The Immune Epitope Database and Analysis Resource is collaborating with BISC and BioHealthBase to build tools that will allow scientists to easily integrate data from these resources and disseminate knowledge to the research community. The CEIRS program is a major collaborative effort across many research centers in multiple countries. For example, a 2008 CEIRS meeting held in Hyderabad, India, brought participants together from academic research centers and agencies including the U.S. Department of Agriculture, CDC, the U.S. Agency for International Development, and the World Organization for Animal Health. Finally, to foster and encourage broad communication inside and outside the U.S. government, NIAID has cohosted several workshops—including the 2007 Immune Correlates of Protection Against Influenza A Viruses, with FDA and WHO; and a 2008 public workshop, Adjuvants and Adjuvanted Preventative and Therapeutic Vaccines for Infectious Disease Indications with FDA. The latter covered vaccine adjuvants in general, including influenza vaccine adjuvants.

Summary of Progress

The panel made 25 recommendations that were organized into eight categories:

A. Influenza at the Animal–Human Interface
B. Influenza in Individuals
C. Influenza in Human Populations
D. Animal Models
E. Vaccines
F. Therapies
G. Assay Technologies
H. Resources

This section summarizes progress that NIAID has made in addressing these recommendations since the 2006 Blue Ribbon Panel meeting. Within each category, the panel's recommendations are listed, followed by a short synopsis of research findings and ongoing research activities. For detailed descriptions of scientific advances and ongoing progress of NIAID influenza research, see Appendices A – H.

A. Influenza at the Animal–Human Interface

Recommendations

1. *Elucidate patterns of influenza virus diversity and evolution*
2. *Define patterns of influenza virus transmission within and between human and animal populations*
3. *Examine interactions of diverse influenza viruses with various hosts*

Precisely how animal and human influenza virus reservoirs interact to generate seasonal drift variants and viruses with pandemic potential is not well understood. Although most infections of humans with avian H5N1 influenza viruses are the result of contact with infected chickens, other wild and domestic species likely participate in the propagation of H5N1 to new areas. The panel recommended that NIAID learn more about how influenza viruses circulate within and between animal reservoirs and about the evolutionary pressures that lead to the emergence and spread of new viral subtypes—especially the factors that favor transmission from animals to humans.

Sampling and surveillance of wild and domestic birds have provided new insights into patterns of virus evolution, including the origin of H5N1 viruses that emerged in 2003. For example, sequence analysis suggested that H5N1 viruses that emerged in poultry in Vietnam in 2003 and similar isolates from Indonesia originated in southern China and possibly were spread through the poultry trade. Genomic analyses of avian isolates and samples of seasonal influenza viruses from widely dispersed human populations are providing a wealth of data on the temporal and geographic patterns of viral transmission and emergence. One such study, for example, suggests that avian influenza viruses in wild birds form transient "genome constellations" that are continually reshuffled by reassortment (the exchange of genes between viruses). When one of

these transient viruses passes into a mammalian host, however, the rate of reassortment slows dramatically. Another study is helping researchers understand the genetic factors that led to the 1957 and 1968 influenza pandemics. Finally, NIAID researchers have developed improved laboratory detection and characterization methods for influenza viruses. These methods have increased the rate at which field samples can be processed and analyzed. (For more information, see Appendix A, pages 14 – 16.)

B. Influenza in Individuals

Recommendations

1. *Increase understanding of influenza pathology and pathogenesis in humans*
2. *Fully describe human cellular immune responses to influenza infection*
3. *Describe innate and mucosal immune mechanisms in influenza*
4. *Map genomics and proteomics of human responses to influenza viruses*
5. *Strengthen infrastructure for clinical influenza research*

Many fundamental aspects of human clinical and immune responses to influenza infection have not been fully described. These include innate and adaptive responses and the role they play in pathogenesis; host genetic factors that may affect susceptibility to severe influenza outcomes; and the innate immune mechanisms that prevent or slow infection or, conversely, exacerbate illness.

CEIRS: Enhancing Influenza Research and Surveillance

In April 2007, NIAID established six Centers of Excellence for Influenza Research and Surveillance (CEIRS). The program builds on an NIAID-funded program begun at St. Jude Children's Research Hospital in Memphis, Tennessee, in 1999. The main goals of CEIRS are to expand the NIAID influenza virus surveillance program, both internationally and in the United States, and to conduct research in key areas, including the following studies:

- How influenza viruses cause disease
- How the human immune system responds to infection
- The prevalence of avian influenza in animals that routinely come into close contact with people
- How influenza viruses evolve, adapt, and are transmitted
- The immunological factors that determine whether an influenza virus causes only mild illness or death

Additionally, some CEIRS will continually monitor international and domestic cases of animal and human influenza to rapidly detect and characterize viruses that may have pandemic potential and to create pandemic vaccine candidates. Ultimately, the CEIRS network will lay the groundwork for new and improved control measures for emerging and reemerging influenza viruses.

During the first year of the program, the CEIRS scientists published approximately 50 peer-reviewed scientific journal articles and collected more than 50,000 samples from several species—including wild birds, domestic poultry, swine, marine mammals, and humans. More than 1,000 influenza viruses were identified in these samples. In addition, 483 of these viral genomes have been fully sequenced and deposited into public databases. For more information, see http://www3.niaid.nih.gov/research/resources/ceirs/.

Recent NIAID research has advanced basic scientific understanding of viral and host contributions to influenza disease. Several studies have examined the role of immune cells in protection against influenza infection across drifted strains. For example, one study found that B cells may be important to the development of cross-reactive immunity. Other studies have examined how immune responses contribute to severe disease outcomes and how host and virus factors contribute to secondary bacterial infection during severe influenza infection. A recent study, for example, showed that interferon gamma production during influenza infection may increase host susceptibility to secondary bacterial infection. Multiple approaches—including computer modeling, *in vitro* laboratory techniques, and clinical studies—have further characterized the immune response to influenza virus. For example, the CEIRS program has begun several new clinical research projects in influenza to examine immune responses and transmissibility of the virus among human populations. (For more information, see Appendix B, pages 17 – 21.)

C. Influenza in Human Populations

Recommendation

1. Expand understanding of influenza viruses in different human populations

Improved understanding of influenza epidemiology will inform efforts to control both seasonal and pandemic influenza. Topics that require further research include the

NIAID Collaboratively Establishes a Network for Clinical Research Overseas

Established in 2005, the Southeast Asia Infectious Disease Clinical Research Network (SEAICRN) is a multilateral, collaborative partnership of hospitals and institutions in Indonesia, Thailand, the United Kingdom, the United States, and Vietnam. International partners include NIAID, Oxford University, and Wellcome Trust UK. WHO participates as an observer and liaison with host country governments. The principal sources of financial support are NIAID and Wellcome Trust UK. SEAICRN was created to advance scientific understanding and clinical treatment of human influenza through integrated, collaborative clinical research. In November 2008, the SEAICRN steering committee voted to expand its work beyond influenza to include other emerging infectious diseases that are substantial public health problems in host countries.

A primary focus of SEAICRN is the clinical study of patients with severe seasonal influenza or H5N1 avian influenza, with the aim of improving patient care. SEAICRN will conduct protocol-based, multi-institutional studies in accord with international standards to inform health policies and clinical practice and to enhance the clinical research capacity of institutions within the network. Several clinical protocols are already underway, including a protocol investigating standard versus high-dose oseltamivir in infected patients, a pharmacology study of oseltamivir administered with probenecid, and a study of host genetic susceptibility to H5N1 infection. SEAICRN also is building a clinical database of avian influenza cases. An important long-term goal is to improve clinical research infrastructure and capacity at multiple levels—that is, the entire network of participating countries and individual network sites. For more information, see http://www.seaclinicalresearch.org/.

relative importance of aerosol, droplet, and fomite transmission; the contribution of specific subpopulations, such as schoolchildren, to epidemic spread; factors that contribute to the emergence of seasonal influenza viruses; and the effect of increases in the number of immunosuppressed and elderly individuals on the spread of virus within a community.

Recent studies in animals have begun to shed light on how specific sequence variations can affect the primary mode of influenza transmission among humans. The rapidly expanding database of viral genomes collected over a broad geographic and temporal range will improve the understanding of the effects of sequence variation on transmission and the factors that control influenza evolution as the virus moves throughout the country and the world. In addition, advances in understanding the effects of vaccination in specific populations point to specific needs in vaccine development and suggest new paradigms for influenza immunization policies. One study underscores the importance of optimizing seasonal influenza vaccines for the elderly. In another study, large-scale sequence analyses demonstrated that seasonal influenza viruses emerge annually from the tropics, rather than persisting from year to year in the temperate regions and that genetic reassortment contributes significantly to seasonal influenza virus evolution, both within and across seasons. Two vaccine efficacy studies in children and young adults showed that by building "herd immunity," live-attenuated seasonal influenza vaccine can provide indirect protection of people who were not vaccinated and may also provide better cross-protection against drifted strains. Studies to determine the best immunization approaches for protecting different populations from influenza and related studies to understand the mechanisms of viral transmission are ongoing. (For more information, see Appendix C, pages 22 – 24.)

D. Animal Models

Recommendations

1. *Enhance the depth of understanding of commonly used models*
2. *Increase the range of options for animal models*
3. *Improve the availability of appropriate animals for use in influenza research*

Animal models are essential tools for all aspects of influenza research, from basic immunology and pathogenesis studies to testing and developing vaccines, antiviral drugs, and other countermeasures. Many models have been developed over the years to serve distinct research needs in such areas as transmission, pathogenesis, immunity, and vaccine development. However, the Blue Ribbon Panel urged the development of new models and a more detailed understanding of immune mechanisms in existing models.

Recent NIAID-supported studies have examined the immune response to, and the transmissibility and pathogenesis of, a variety of influenza A subtypes in multiple animal models, including mice and ferrets. The guinea pig model developed in early 2006 has proved to be very useful for the evaluation of influenza virus transmission. Other new animal models, such as "humanized" mice, have been developed and are available for distribution to influenza researchers. Other studies are focusing on improving the standardization of animal models for influenza. In addition, new reagents are being developed and made available to researchers. For example, monoclonal antibodies (mAbs) have been used to study the immune response to influenza in ferrets. (For more information, see Appendix D, pages 25 and 26.)

E. Vaccines

Recommendations

1. *Define correlates of immune protection against influenza*
2. *Facilitate studies that include challenge of human volunteers with live virus*
3. *Improve adjuvants and other dose-optimization technologies for influenza vaccines*
4. *Define evaluation criteria for vaccine efficacy testing*

Development of new influenza vaccines and more efficient and scalable manufacturing technologies are important research priorities. Current vaccines must be reformulated annually to match the strains most likely to circulate in the coming influenza season. Live-attenuated vaccines may offer enhanced protection against genetically drifted viruses, but these are not yet used in a large proportion of the population. Inactivated virus vaccines work well in adults and children, but in general, the elderly do not respond to such vaccines as vigorously as younger adults. In addition, most influenza vaccines are cultured in fertilized chicken eggs, a manufacturing technology that would be difficult to scale up rapidly to respond to a pandemic.

NIAID has made significant progress in vaccine research in recent years, spurred on by the emergence and spread of H5N1 influenza in Asia and the growing awareness of the damage the next influenza pandemic could do to our highly mobile society and the global economy. New attention has focused on defining all components of the immune response that contribute to immunity. In cooperation with FDA and WHO, NIAID organized a conference on the correlates of influenza immunity in late 2007. Promising new technologies for influenza vaccine platforms and production methods—including cell culture-based, gene-based, and protein-based technologies, some of which are currently used in licensed vaccines for other diseases—are actively being developed. Novel vaccine adjuvants to extend the vaccine supply and broaden protection against drifted strains also are being pursued, as are studies comparing the efficacy of different types and dosages of seasonal influenza vaccines in diverse human populations. Studies to understand the structural changes that H5N1 hemagglutinin (HA) antigens might undergo as the virus adapts to human transmission are underway; results may guide the preemptive development of vaccines before the emergence of human-adapted H5N1 strains. In addition, research to develop vaccines that induce protective immunity to parts of the influenza virus that do not change from year to year is underway in hopes of developing a so-called “universal vaccine” that would not need to be updated annually. Substantial progress also has been made on development and clinical testing of different types of human vaccines, such as DNA and live-attenuated and inactivated vaccines against H5N1 and other influenza viruses with pandemic potential. Based on NIAID-sponsored trials, FDA in April 2007 approved an inactivated H5N1 vaccine for pandemic use. Lastly, results of a small study of the so-called “prime-boost strategy” support the feasibility of this approach in a pre-pandemic situation. Similar studies on a larger scale are currently underway. (For more information, see Appendix E, pages 27 – 33.)

F. Therapies

Recommendations

1. *Expand studies of currently licensed antiviral drugs*
2. *Develop new drugs and new drug targets for influenza*
3. *Investigate therapies for late-stage, severe influenza*

Antiviral drugs and other therapies to reduce the severity of influenza outcomes could be important tools for reducing the toll of both seasonal and pandemic influenza. Moreover, antiviral drugs can sometimes prevent infection if taken before or shortly after exposure. Until an effective vaccine can be developed and manufactured in large quantity, antiviral drugs could play a significant role in limiting the effects of a future pandemic.

NIAID conducts and supports an array of activities to develop new drugs and other influenza therapies. For example, preclinical and clinical evaluations of antibodies suggest that passive immunization could be an effective strategy for mitigating an influenza pandemic. The use of high-throughput screening to develop new antiviral drug targets and the development of new influenza antivirals and immune modulators are leading to proof-of-concept studies and preclinical evaluations in animal models, and clinical evaluations in human volunteers. Several new influenza neuraminidase (NA) inhibitors recently have been developed and are being evaluated in clinical trials. Researchers recently solved the three-dimensional structure of the antiviral binding site of the influenza A M2 proton channel. This advance will help researchers better understand the mechanisms of and develop strategies to avoid antiviral resistance. Activators of innate immune response to influenza are being developed for prophylaxis and treatment, and studies are being conducted in animal models and in humans to optimize dose levels and test combinations of existing antiviral drugs. The discovery of antibodies that bind a region of the HA protein that does not change from year to year has created new possibilities both for a vaccine that could last for many influenza seasons and a broad antibody-based influenza treatment. Finally, a new method for rapidly producing mAbs against influenza has potential to speed the development of diagnostic and therapeutic applications. (For more information, see Appendix F, pages 34 – 38.)

G. Assay Technologies

Recommendations

1. *Develop new point-of-care diagnostic assays*
2. *Improve and standardize assays for cellular immune responses to influenza*
3. *Improve methods for assaying immune responses in the respiratory tract*

Because many viral and bacterial illnesses cause symptoms that are similar to those of influenza, rapid and accurate diagnostic assays are essential tools for determining who needs treatment with antiviral drugs and for controlling influenza transmission through quarantine and prophylactic antiviral treatment.

NIAID recently has helped to develop new technologies to detect the presence of viruses in clinical samples. Microarray-based diagnostics can detect the presence of very small concentrations of specific nucleotide sequences. These diagnostics are now being converted into clinically useful products to identify the specific virus that is causing a patient's influenza-like symptoms. A different strategy to accomplish the same goal, based on examining patterns of gene expression of immune cells in a

patient's blood, also shows promise. Researchers have made substantial progress in developing faster and more reliable techniques to screen human and animal samples for influenza antibodies and other evidence of infection by a wide array of influenza viruses. (For more information, see Appendix G, pages 39 – 41.)

H. Resources

Recommendations

1. *Expand the range of materials available to the research community*
2. *Increase services for researchers*
3. *Improve mechanisms for the exchange of information among researchers*

NIAID recently has expanded the services and resources it offers to influenza researchers. These research resources are designed to speed translation of basic research findings into safe and effective drugs, vaccines, and diagnostics and to strengthen the infrastructure of basic and clinical research. For example, the NIAID Influenza Genome Sequencing Project (see sidebar) has led to many publications on the evolution and emergence of seasonal influenza viruses. Moreover, NIAID has expanded its Web-accessible BioHealthBase database to allow researchers to simultaneously analyze influenza genomic data from GenBank® with proteomic, immune epitope, and clinical data from a variety of sources. Biologic and computational tools and reagents are available for use in different animal models for surveillance activities and for characterizing immune responses to influenza infection. Resources to help researchers determine the three-dimensional structures of influenza virus proteins are also available. (For more information, see Appendix H, pages 42 – 44.)

Thousands of New Influenza Virus Sequences Made Publicly Available

In 2004, NIAID launched the Influenza Genome Sequencing Project, a collaborative effort to vastly expand the number of available influenza genome sequences. All researchers with collections of influenza viruses from various seasons and different parts of the world can contribute to the project. Sequencing of samples is largely conducted at the NIAID Microbial Sequencing Center at the J. Craig Venter Institute in Rockville, Maryland. All sequences are then placed in GenBank®, a publicly available genome database, so that researchers worldwide can use them to better understand how influenza viruses evolve, spread, and cause disease.

Thus far, more than 3,000 whole influenza genomes have been sequenced from samples from dozens of different contributors. These new data are already providing novel insights and have resulted in numerous publications since 2005. For example, researchers analyzed more than 300 whole genome sequences of influenza viruses collected across the United States during the 2006–2007 influenza season. These sequences have shown that the pattern of influenza virus evolution and spread is surprisingly complex. Several different variants circulated simultaneously and frequently recombined with one another, and one strain even reacquired susceptibility to the adamantane class of anti-influenza drugs (Nelson MI, Edelman L, Spiro DJ, et al. Molecular epidemiology of A/H3N2 and A/H1N1 influenza virus during a single epidemic season in the United States. *PLoS Pathog.* 2008;4:e1000133). As more whole genome sequences become available, scientists will be able to learn much more about how influenza changes from season to season and from one geographical region to the next.

Future Directions

Although influenza research has been an active scientific field for many decades, research conducted in the past few years has accelerated the delivery of tangible benefits to the American public and to individuals throughout the world. In the past decade, the continuing sporadic occurrence of H5N1 influenza infection among humans has focused the world's attention on the damage that a highly pathogenic and transmissible pandemic virus could inflict on public health and the modern global economy. As a result, international and domestic pandemic planning efforts have been intensified and funding for research on new and improved influenza vaccines, diagnostics, and therapeutics has increased substantially.

The research efforts described in this Progress Report illustrate how we, as a society, are beginning to reap the benefits of such an investment. The dividends include a better understanding of basic scientific concepts, including the dominant mechanisms of influenza evolution, pathogenesis, and the human immune response. These insights have led to new strategies for improving the tools needed to control influenza; NIAID has already begun to pursue such strategies.

For example, new, powerful, and cost-efficient tools for the study of genomics—such as rapid, high-throughput genetic sequencing and gene microarray technologies—are changing the study of all infectious diseases, including influenza. A group of NIAID-funded researchers in China and the United States recently used these techniques and all available viral sequence data to gain new perspectives on the origins of H5N1 influenza. They found that H5N1 influenza probably crossed from wild to domestic birds only once, in 1996. The virus then spread to other domestic birds (geese, chickens, and ducks) in China's mixed domestic poultry markets, where it became adapted to many species through reassortment of viral genetic material within these domestic bird populations. In doing so, the barriers to H5N1 transmission between species were reduced, making it possible for H5N1 to infect not only domestic birds but also many mammalian species, including humans. Improved understanding of the dynamics of H5N1 transmission among and between species could in turn lead to new strategies for detecting and controlling influenza viruses with pandemic potential.

In pursuing research to develop new responses to seasonal and pandemic influenza, NIAID has shifted emphasis from the traditional "one bug-one drug" approach to a more flexible, broad-spectrum strategy intended to efficiently leverage limited research resources against an expanding range of biological threats. For example, much of the Institute's diagnostics research aims to develop a new generation of rapid, accurate tools in which one test can be used in the field and at the bedside to detect a wide variety of pathogens. NIAID-supported scientists are also working to develop vaccine platforms that can easily be adapted to protect against multiple microbes by simply shuttling in and out the genes for different antigens.

This broad-spectrum strategy—combined with NIAID's multidisciplinary approach that engages industrial, academic, governmental, nongovernmental, and international partners—holds great promise for the rapid development of new ways to prevent, detect, and treat seasonal and pandemic influenza. By continuing to support talented scientists and maintaining a balance between basic studies and targeted research, NIAID is helping to create innovative tools to prevent, diagnose, and treat seasonal influenza and to combat influenza viruses that may cause a global pandemic.

Appendices: Details of Selected Scientific Advances and Ongoing Activities

Appendix A. Influenza at the Animal–Human Interface

Recommendations

1. *Elucidate patterns of influenza virus diversity and evolution*
2. *Define patterns of influenza virus transmission within and between human and animal populations*
3. *Examine interactions of diverse influenza viruses with various hosts*

Scientific Advances

Investigators identify possible source of 2003 H5N1 outbreak. NIAID-supported investigators identified the location of the source for the 2003 outbreak of H5N1 influenza. The researchers sequenced the genomes of viruses that were isolated from samples collected from live poultry markets in southern China from 2001 to 2004. Analysis of the sequences suggests a direct transmission link for H5N1 viruses between Yunnan (China) and Vietnam and between Hunan (China) and Indonesia during 2002 and 2003. Poultry trade between China and Vietnam may be responsible for virus introduction into Vietnam; the transmission route from China to Indonesia remains unclear.

Wang J, Vijaykrishna D, Duan L, et al. Identification of the progenitors of Indonesia and Vietnam avian influenza A (H5N1) viruses from southern China. *J Virol.* 2008;7:3405-3414.

H2 viruses found in swine pose a pandemic risk. NIAID-supported investigators isolated and characterized H2N3 influenza A viruses collected from diseased swine on two farms in the United States. These H2N3 viruses, which were related to both avian and swine viruses, caused disease in experimentally infected swine and mice without prior adaptation. In addition, the swine H2N3 virus was highly transmissible in both swine and ferrets. Although H2 influenza viruses have not circulated in the human population since 1968, H2 viruses pose a pandemic risk. The findings suggest that swine H2N3 viruses should be closely monitored.

Ma W, Vincent AL, Gramer MR, et al. Identification of H2N3 influenza a viruses from swine in the United States. *Proc Natl Acad Sci USA.* 2007;104:20949-20954.

Waterfowl hunters have an increased risk of exposure to avian influenza. A recent study partially supported by NIAID and based on samples taken from wild bird species showed that waterfowl hunters were eight times more likely to have contact with avian influenza-infected wildlife than persons with casual or occupational exposures. This finding could have important implications for prevention and surveillance policies.

Siembieda J, Johnson CK, Boyce W, Sandrock C, Cardona C. Risk for avian influenza virus exposure at human-wildlife interface. *Emerg Infect Dis.* 2008;147:1151-1153.

Scientists use reverse transcription polymerase chain reaction (RT-PCR) to detect avian influenza virus in wild birds. In collaboration with investigators at the University of Alaska, NIAID intramural scientists assisted with a study of influenza in wild birds in the Minto Flats State Game Refuge, a waterfowl breeding ground in Alaska where Asian/Pacific and North American migratory flyways converge. The study compared virus detection by real-time RT-PCR, a method that detects specific viral genetic sequences present in a sample, with standard viral isolation techniques.

Twenty-seven percent of samples screened by RT-PCR were positive for at least one influenza A virus; viral isolation screening methods found a smaller percentage of positive samples. The researchers note that virus isolation should not be abandoned because it allows further molecular and pathogenic characterization of viruses.

Runstadler JA, Happ GM, Slemons RD, Sheng ZM, Gundlach N, Petrula M, et al. Using RRT-PCR analysis and virus isolation to determine the prevalence of avian influenza virus infections in ducks at Minto Flats State Game Refuge, Alaska, during August 2005. *Arch Virol.* 2007;152:1901-1910.

Study finds that influenza virus genomes in wild birds are continually reshuffled by reassortment. A team of NIAID intramural investigators and extramural collaborators analyzed the largest dataset of avian influenza virus genomes compiled to date, comprising 167 full genomes from 14 wild bird species and containing 29 HA and NA subtype combinations. Up to 26 percent of birds sampled showed evidence of infection by more than one subtype. The team documented a remarkably high rate of genome reassortment and showed that the general pattern of viral genes flow from Eurasia to North America. The team proposes that influenza virus genomes in wild birds are continually reshuffled by reassortment, which differs from the spread of a limited number of stable genome groupings seen during the evolution of mammalian-adapted influenza A viruses.

Dugan VG, Chen R, Spiro DJ, Sengamalay N, Zaborsky J, Dugan EG, et al. The evolutionary genetics and emergence of avian influenza viruses in wild birds. *PLoS Pathog.* 2008;4:e1000076.

A new clade of H5N1 viruses emerged in Vietnam in 2007. Before 2007, H5N1 viruses isolated from poultry and humans in Vietnam were consistently found to be clade 1 viruses that were susceptible to the antiviral drug oseltamivir but resistant to amantadine. Researchers at the Southeast Asia Infectious Disease Clinical Research Network showed that a new H5N1 clade—2.3.4—replaced clade 1 viruses in both poultry and humans in Vietnam in 2007. These viruses were susceptible to amantadine but partially resistant to oseltamivir. Combination antiviral therapy with oseltamivir and amantadine for human cases in Vietnam is recommended.

Le MTQ, Wertheim HFL, Nguyen HD, Taylor W, Hoang PVM, Vuong CD, et al. Influenza A H5N1 clade 2.3.4 virus with a different antiviral susceptibility profile replaced clade 1 virus in humans in northern Vietnam. *PLoS ONE.* 2008;3:e3339.

Scientists recover 1918 influenza virus sample in Alaska. NIAID scientists described the recovery of samples of the 1918 pandemic strain in fixed autopsy tissues and in the body of a woman buried in the Alaskan permafrost. The article places recovered sequences in the context of decades of research into the cause of pandemic influenza, and details the events that allowed them to recover and sequence the virus. The genetic material of the virus is so fragile that it should not have survived for days, let alone decades. This remarkable good fortune enabled scientists to open a window onto a past pandemic—and perhaps gain a foothold for preventing a future one.

Taubenberger J, Hultin J, Morens D. Discovery and characterization of the 1918 pandemic influenza virus in historical context. *Antivir Ther.* 2007;12:581–591.

Ongoing Activities

Determining factors that led to the emergence of 1957 and 1968 pandemic influenza viruses. As part of the Center for Research on Influenza Pathogenesis at Mt. Sinai University Medical School in New York, investigators at Erasmus Medical

Center are conducting a study to understand the emergence of pandemic influenza A viruses during the last two pandemics (H2N2 in 1957 and H3N2 in 1968) in the context of the genetic and phenotypic variability of avian, human, and porcine influenza A viruses. They are also conducting extensive analyses to determine the ancestral strains of the viruses responsible for the 1957 and 1968 pandemics and will attempt to generate ancestral viruses through reverse genetics.

Tracing transmission of animal A/H5N1 viruses to humans. Several studies are now underway at various CEIRS to understand the mechanisms and risk factors that control transmission of animal influenza viruses to the human population:

- Study of children from Chinese families that frequent live bird markets
- Prospective study of U.S. bird banders—people who capture, tag, and release birds during scientific studies—for evidence of avian influenza infection
- Surveillance of human populations in the United States that are at high risk for being exposed to avian influenza viruses through close contact with wild birds
- Collection of samples, during suspected outbreaks in Thailand, from animals and people who live within a 10 kilometer radius of the outbreak center and who have close contact to potentially infected animals
- Surveillance in Tanzania of domestic poultry and people with exposure to poultry

Seeking to determine the transmissibility of influenza A/H5N1 within and between species. The research team at the Influenza Pathogenesis & Immunology Research Center at the University of Georgia CEIRS is conducting a study to determine how efficiently influenza isolates can be transmitted within and between members of various species and the mechanisms of immunity and disease pathogenesis associated with infection. This group is also evaluating small interfering RNA molecules that can be used as disease intervention and prevention strategies against H5N1 influenza viruses.

Initiating intramural studies of influenza viral genomics and evolution. A collaborative research program is under way among NIAID intramural scientists to study the complex ecology, biology, and evolution of influenza A viruses in different animal and human hosts. Several research efforts are either planned or underway:

- Study of the evolutionary dynamics of avian influenza viruses—including H5N1 strains and swine influenza viruses—to understand the mechanisms of host switching.
- Development of robust molecular subtyping tools for influenza surveillance, including rapid, real-time universal HA subtyping methods.
- Characterization of the molecular basis for the emergence of the 16 distinct HA subtypes of influenza A and estimation of when these subtypes diverged from a common ancestor.
- Analysis of historical influenza viruses from the decades before and after 1918 to understand the formation, development, and early evolution of the 1918 pandemic influenza virus. Because no human influenza isolates are available before 1933, these viruses will be characterized by identification of influenza RNA fragments preserved in formalin-fixed, paraffin-embedded autopsy tissues.

Appendix B. Influenza in Individuals

Recommendations

1. *Increase understanding of influenza pathology and pathogenesis in humans*
2. *Fully describe human cellular immune responses to influenza infection*
3. *Describe innate and mucosal immune mechanisms in influenza*
4. *Map genomics and proteomics of human responses to influenza viruses*
5. *Strengthen infrastructure for clinical influenza research*

Scientific Advances

How do B cells that respond to a particular influenza virus subtype confer resistance to other influenza strains? Exposure to one subtype of influenza virus sometimes protects people from subsequent infection from other subtypes, a phenomenon known as heterosubtypic immunity. This cross-reactive immunity had been thought to be mediated by memory T cells directed against viral peptides that are common to many viral subtypes; the role of B cells has been thought to be minimal. Recently, however, NIAID-funded researchers found that B cells may also be important in cross-reactive immunity. Specifically, the researchers found that unlike normal mice, mice unable to mount an effective B-cell response were highly susceptible to exposure to H1N1 influenza, even though they had earlier been exposed to H3N2 influenza. Further investigation may provide insights that could lead to better influenza vaccines.

Rangel-Moreno J. Carragher DM, Misra RS, Kusser K, Hartson L, Moquin A, et al. B cells promote resistance to heterosubtypic strains of influenza via multiple mechanisms. *J Immunol.* 2008;180:454-463

Researchers identify interferon-gamma (IFN-γ) production as a risk factor for secondary bacterial infection following influenza infection. Increased susceptibility to secondary bacterial infections is common during recovery from influenza. This phenomenon suggests that influenza infection causes a transient, general defect in respiratory immunity. NIAID-funded researchers at Albany Medical College found that mice infected with influenza were more susceptible to pneumococcal infection than mice that were not exposed. The researchers also found that mice infected with influenza did not clear bacteria from their lungs as efficiently as animals with no exposure to influenza, which cleared the bacteria within 4 hours. Direct respiratory inoculation of healthy animals with IFN-γ mimicked the enhanced pneumococcal infection evident in influenza-infected mice, whereas influenza-infected mice with a defect in IFN-γ production were no more susceptible to secondary pneumococcal infection than normal mice. Moreover, treatment with IFN-γ reduced the expression of a key binding protein on the surfaces of macrophages, a key type of immune cells. These findings may explain how influenza infection increases susceptibility to secondary bacterial infections.

Sun K, Metzger DW. Inhibition of pulmonary antibacterial defense by interferon-γ during recovery from influenza infection. *Nat Med.* 2008;14:558-564.

Reactivation of memory T cells by bone marrow-derived dendritic cells is required for protection against influenza A. Transferring memory T cells from mice that have recovered from influenza to normal, unimmunized mice protects the recipients from lethal influenza virus infection. NIAID-funded investigators recently found that memory T cells could protect mice with normal bone marrow-derived dendritic cells—cells that ingest pathogens and present antigens to T cells. However, transplanted memory

T cells alone did not protect animals deficient in a gene called relB, which is required for proper development and function of bone marrow-derived dendritic cells. The investigators concluded that reactivation of memory T cells depends critically on presentation of influenza peptides by bone marrow-derived dendritic cells near the site of infection.

Castiglioni, P, Hall DS, Jacovetty EL, Ingulli E, Zanetti M. Protection against Influenza A virus by memory CD8 T cells requires reactivation by bone marrow-derived dendritic cells. *J Immunol.* 2008;180:4956-4964.

Protective influenza-specific CD8 T-cell responses require interactions with dendritic cells in the lungs. When influenza virus invades the airways, immune cells called dendritic cells engulf infected cells and migrate to the lymph nodes. The dendritic cells may contact other immune cells that help them activate, proliferate, and migrate back to the lung to kill infected cells. However, NIAID-funded researchers have found that a considerable number of dendritic cells that have not made a trip to the lymph node are recruited to the lung and provide an additional antigen-specific, cell contact-dependent interaction that is necessary for an effective immune response to influenza. These findings may provide a clue that could be used to limit T-cell activation to avoid the pathology associated with an overly robust immune response.

McGill J, Van Rooijen N, Legge KL. Protective influenza-specific CD8 T cell responses require interactions with dendritic cells in the lungs. *J Exp Med.* 2008;205:1635-1646.

Influenza protein PB1-F2 enhances the pathogenesis of viral and secondary bacterial pneumonia. Secondary bacterial pneumonia often caused death during the 1918 influenza A virus pandemic, but little is known about the factors that made that year's virus so devastating. Scientists from St. Jude Children's Research Hospital recently teamed with NIAID intramural investigators to examine how the influenza virus protein PB1-F2 modulates influenza-associated damage. They showed that an influenza virus engineered to produce the PB1-F2 protein found in the 1918 pandemic strain was more virulent in mice than unmodified virus, in that it induced more damage to the lungs, and led to more severe secondary bacterial pneumonia. These findings may help to explain both the unparalleled virulence of the 1918 strain and the high incidence of fatal pneumonia during the pandemic.

McAuley JL, Hornung F, Boyd KL, Smith AM, McKeon R, Bennink J, et al. Expression of the 1918 influenza A virus PB1-F2 enhances the pathogenesis of viral and secondary bacterial pneumonia. *Cell Host Microbe.* 2007;2:240-249.

Influenza A virus infection activates the sympathetic nervous system. The sympathetic nervous system regulates the "fight-or-flight" stress response. NIAID intramural investigators demonstrated that the immune response to influenza in mice is dramatically different in the absence of the sympathetic nervous system. Mice that lacked a functioning sympathetic nervous system showed increased CD8 and CD4 T-cell responses to influenza antigens and showed moderate but consistent resistance to lethal influenza infection. These data point strongly to a major contribution of the sympathetic nervous system to the immune response to influenza A virus, including a possible role in enhancing damage caused by the immune system's response to influenza infection.

Grebe KM, Hickman HD, Irvine KR, Takeda K, Bennink JR, Yewdell JW. Sympathetic nervous system control of anti-influenza CD8+ T cell responses. *Proc Natl Acad Sci.* In press.

Secondary bacterial pneumonia was an important cause of death in the 1918 influenza pandemic. NIAID investigators studied lung tissue sections from 58 autopsies of victims from the 1918 influenza pandemic and reviewed pathologic and bacteriologic data from 118 published autopsy series describing 8,398 individual autopsies of pandemic victims. The autopsy lung tissues examined uniformly exhibited severe changes indicative of secondary bacterial pneumonia. Bacteriologic and histopathologic results from published autopsy series also frequently implicated secondary bacterial pneumonia caused by common upper respiratory flora. As with subsequent pandemics, the majority of deaths during the 1918–1919 pandemic likely resulted from secondary bacterial pneumonia. These results suggest that in addition to countermeasures, such as influenza vaccines and antivirals, pandemic planners should consider stockpiling materials need to prevent, diagnose, and treat secondary bacterial pneumonias, including antibiotics and bacterial vaccines.

Morens DM, Taubenberger JK, Fauci AS. Predominant role of bacterial pneumonia as a cause of death in influenza pandemics: Implications for pandemic influenza preparedness. *J Infect Dis.* 2008;198:962-970.

Study reviews and compares the documented histopathology of fatal influenza virus pneumonias during the past 120 years. An NIAID study found that the description of the spectrum of pathologic changes in autopsied lung tissue from 1918 influenza pandemic fatalities is not significantly different from the histopathology observed in other less lethal pandemics or even in deaths associated with seasonal influenza outbreaks. Coincident or secondary bacterial pneumonias are extremely common in severe influenza. What separates 1918 influenza pandemic cases from other cases of influenza infection is the significantly higher case fatality rate and unusual age distribution of deaths.

Taubenberger JK, Morens DM. The pathology of influenza virus infections. *Ann Rev Pathol.* 2008;3:499-522.

Protective antibodies to the 1918 influenza are discovered in pandemic survivors nine decades later. The 1918 influenza pandemic is among the most devastating infectious disease events known, causing more than 50 million fatalities. NIAID-supported scientists recently obtained neutralizing antibodies from survivors of the 1918 influenza pandemic. Administration of these virus-neutralizing antibodies protected mice from what would otherwise have been a lethal dose of the 1918 influenza virus. Remarkably, the elderly survivors of the 1918 influenza pandemic have sustained highly effective virus-neutralizing antibodies for 90 years, the longest period of immunological memory ever documented.

Yu X, Tsibane T, McGraw PA, House FS, Keefer CJ, Hicar MD, et al. Neutralizing antibodies derived from the B cells of 1918 influenza pandemic survivors. *Nature.* 2008;455:532-536.

RNAi screen identifies host genes needed for influenza virus replication. NIAID-funded researchers recently applied an innovative technique to identify human genes that are required for the replication of influenza viruses. Researchers used a genome-wide Drosophila RNA interference screen to identify 110 host genes that are important for efficient influenza replication. The scientists confirmed that some of these human genes play key roles in the replication of highly pathogenic influenza viruses. This discovery has significant implications for the identification of human host factors that are involved in influenza replication. Scientists may be able to exploit these host factors to rapidly develop novel therapeutics and vaccines for influenza.

Hao L, Sakurai A, Watanabe T, Sorensen E, Nidom CA, Newton MA, et al. Drosophila RNAi screen identifies host genes important for influenza virus replication. *Nature.* 2008;454:890-893.

Ongoing Activities

Conducting translational research in human immunology. The NIAID-sponsored Cooperative Centers for Translational Research on Human Immunology and Biodefense program supports basic, clinical, and applied research on human immune responses to influenza and the development of improved vaccines against influenza. These centers carry out the following types of research:

- Examination of influenza virus interactions with human dendritic cells, which are critical components of the innate immune response
- Identification of T-cell responses to H1N1, H3N2, and H5N1 viruses
- Characterization of memory T-cell responses to influenza
- Assessment of T-cell cross-reactivity to newly emerging influenza subtypes

Assessing immune function in immune-compromised populations. NIAID's Immune Function and Biodefense in Children, Elderly, and Immunocompromised Populations contracts examine innate and adaptive immune responses to infection or vaccination in human populations with some form of immune deficiency, including stem cell transplant patients, patients receiving cancer chemotherapy, children with juvenile rheumatoid arthritis, and people taking immunosuppressive therapies. Studies include characterization of influenza immunity in the elderly; use of animal models to develop improved influenza vaccines for the elderly; and examination of immune responses to influenza vaccination in pregnant women and people with immune systems suppressed by treatments for transplant rejection, autoimmune disorders, and cancer.

Studying the use of immune mechanisms to control viral infections. The Immune Mechanisms of Viral Control exploratory grant program supports research into the host response to infection by or vaccination against NIAID Category A – C viruses, including influenza. The program began in September 2007 and funded 32 grants covering a diverse selection of viruses and immunological issues. Eighteen of the awards focused on the host response to influenza infection or vaccination. Mouse, nonhuman primate, and human samples are included. The research topics fall into the following three broad study categories:

- Innate immune response: antigen processing and presentation, interferon processing pathways, and roles of innate immune cells in controlling infection
- Adaptive immune response: T-cell memory responses and T-cell responses in the elderly population
- Vaccination: comparison of the immune response to live-attenuated influenza vaccines, inactivated vaccine, and natural infection

The second phase of this program will establish a network of synergistic research teams that will study basic immunological parameters of virus infection, mechanisms of virus-induced inflammation, and protective vaccination.

Using a systems biology approach to study immune responses to infections. NIAID awarded a 5-year contract in September 2007 to support a systems biology approach to studying immunity. The Scripps Research Institute will lead the program in collaboration with the Institute for Systems Biology, Stanford University, and the Australian National University. This research will build on a highly successful cooperative agreement that NIAID awarded to Scripps Research Institute in 2003. Researchers will develop and use mouse models to study immune responses to infection with NIAID Category A, B, and C priority pathogens, including influenza virus. The animal studies are complemented by detailed genomics, proteomics, computational biology,

and bioinformatics that focus on transcriptional regulation and signaling mechanisms. The project provides tools—such as antibodies, protein expression vectors, data analysis tools, and genetically engineered mice—to characterize immune responses to influenza infection. Investigators will analyze a subset of newly discovered immune regulatory genes in human correlation studies. The collaboration will contribute to a comprehensive understanding of molecular interactions that regulate innate and adaptive immune responses to infection.

Developing computer models of host immune responses. The Modeling Immunity for Biodefense program is developing computational models of host immune responses to infection or vaccination. Each contract includes a team of computational biologists and immunologists that are working to refine and validate the models. Two contracts supported under this program examine various aspects of anti-influenza immunity, including analysis of innate and adaptive immune responses to different influenza subtypes and a comprehensive examination of adaptive immune responses to influenza vaccination in healthy adults and mouse model systems. Another contract focuses on the development of computational models to test potential vaccine adjuvants and their effects on innate and adaptive immune responses to infectious agents.

Studying factors required for immune memory to influenza infections. An NIAID-supported project combines cellular and molecular approaches to study factors required for long-lasting T-cell memory to influenza virus in animal models, including

- Understanding the development of CD4 and CD8 memory and effector T cells
- Investigating which T-cell subpopulations migrate to the lung during infection, the timing of migration in and out of the lung, and survival properties of responding T cells
- Analyzing factors that influence circulation, homing, and tissue distribution of memory T cells
- Studying the role of antigen, costimulation, adhesion molecules, and cytokines in the generation and maintenance of protective T-cell memory responses to influenza

Analyzing human immune responses to influenza vaccination and infection. NIAID-supported investigators at Emory University are analyzing human immune responses to influenza vaccination and infection. Specifically, researchers are evaluating the nature of the innate immune response to both inactivated and live-attenuated influenza vaccines. Researchers will also quantitatively analyze the memory B-cell response to influenza vaccination and infection and measure the number and specificity of CD4 and CD8 T cells that are generated in response to vaccination.

Focusing on influenza viral pathogenesis. NIAID intramural researchers are conducting studies that focus on understanding the viral and host contributions to disease caused by past and potential pandemic influenza strains, such as the 1918 influenza virus and H5N1 avian influenza virus and seasonal influenza strains. The following studies are already underway:

- Collaboration among CDC and University of Washington scientists to investigate how sialic acid molecules on host cells affect virulence and pathogenesis and to examine the role of the influenza virus ribonucleoprotein complex in host adaptation and pathogenesis
- Clinical studies to evaluate influenza in immunocompromised people
- Research to understand the interaction between the nervous and immune systems in regulating anti-influenza immunity

Appendix C. Influenza in Human Populations

Recommendation

1. Expand understanding of influenza viruses in different human populations

Scientific Advances

Study reconsiders the mortality benefits of influenza vaccine in the elderly. Influenza vaccination policies in most high-income countries attempt to reduce the mortality burden of influenza by vaccinating seniors, who account for the majority of influenza-related deaths. NIAID researchers and colleagues analyzed previous observational studies and concluded that the vaccine's value in preventing influenza deaths among seniors may have been exaggerated. They suggest that a much stronger evidence base is needed to measure the mortality benefit that elderly people derive from influenza vaccination. The researchers suggest methods to more accurately assess mortality benefits of influenza vaccination and call for intensive study of alternative approaches to reduce influenza-related deaths among seniors, such as higher dose vaccines, better adjuvants, and vaccination of more people in all age groups to reduce viral transmission to seniors.

Simonsen L, Taylor RJ, Viboud C, Miller MA, Jackson LA. Mortality benefits of influenza vaccination in elderly people: an ongoing controversy. *Lancet Infect Dis.* 2007;10:658-666.

High doses of licensed seasonal influenza vaccine produce strong responses in the elderly. NIAID and NIAID-supported researchers partnered with industry to demonstrate that elderly volunteers responded with substantially stronger antibody responses to inactivated influenza vaccine when they received a vaccine dose four times higher than normal The results indicate that high-dose vaccine is well-tolerated and may provide increased protection against seasonal influenza in the elderly, a population that suffers a disproportionate level of hospitalization and death due to seasonal influenza.

Couch R, Winokur P, Brady R, Belshe R, Chen WH, Cate TR, et al. Safety and immunogenicity of a high dosage trivalent influenza vaccine among elderly subjects. *Vaccine.* 2007;25:7656-7663.

The emergence of virus strains from one influenza season to the next likely begins in the tropics. An international team of researchers, including NIAID intramural scientists and NIH-funded collaborators, analyzed full genetic sequences of 1,302 influenza A viruses collected over 12 years in New Zealand and New York State. Researchers quantified the degree of genetic diversity among the strains' subtypes, gene segments, and geographic locations and found patterns indicating that virus strains do not persist from one influenza season to the next in temperate regions. Instead, the researchers deduced that new influenza viruses emerge annually from tropical regions.

Rambaut A, Pybus OG, Nelson MI, Viboud C, Taubenberger JK, Holmes EC. The genomic and epidemiological dynamics of human influenza A virus. *Nature.* 2008;453:615-619.

Random events shape the evolution of human seasonal influenza A virus. Understanding the evolutionary dynamics of influenza A virus is central to its surveillance and control. While immune-driven antigenic drift is a key determinant of viral evolution across influenza seasons, the evolutionary processes that determine virus diversity within a season are less clear. NIAID intramural investigators and extramural collaborators analyzed more than 400 human H3N2 influenza virus genomes and

demonstrated that genetic diversity within a single season is substantial and largely generated by seasonal importation of many different genetic variants that cocirculate and frequently reassort. The results indicate that random processes of viral migration and reassortment play a vital role in shaping short-term evolutionary dynamics.

Nelson MI, Simonsen L, Viboud C, Miller MA, Taylor J, George KS. Stochastic processes are key determinants of the short-term evolution of influenza A virus. *PLoS Pathog.* 2006;2:e125.

Reassortment played an integral part in the abrupt antigenic evolution of the H1N1 influenza A virus. Using a dataset of 71 complete genome sequences sampled between 1918 and 2006, a team of NIAID extramural and intramural scientists showed that reassortment has played an important role in the genomic evolution of A/H1N1 since 1918. They found that an A/H1N1 isolate from the 1947 epidemic acquired novel PB2 and HA genes through reassortment of H1N1 viruses; this may explain the abrupt antigenic evolution of the 1947 virus and subsequent vaccine failure. Similarly, the 1951 influenza epidemic may also have been associated with reassortment A/H1N1 viruses.

Nelson MI, Viboud C, Simonsen L, Bennett RT, Griesemer SB, St George K. Multiple reassortment events in the evolutionary history of H1N1 influenza A virus since 1918. *PLoS Pathog.* 2008;4:e1000012.

Homologous recombination plays a small role in viral evolution. If proven to occur, homologous recombination would facilitate two evolutionary processes in the influenza virus: the purging of deleterious mutations and the rapid generation of new genotypes, potentially including new antigenic and drug-resistant variants. To determine the extent of homologous recombination in human influenza A virus, NIAID intramural and extramural investigators used a dataset of 13,852 human influenza A virus sequences. Of these, only two contained recombinant regions that indicated the occurrence of homologous recombination. Thus, homologous recombination plays, at most, a very minor role in the evolution of human influenza A virus.

Boni MF, Zhou Y, Taubenberger JK, Holmes EC. Homologous recombination is very rare or absent in human influenza A virus. *J Virol.* 2008;82:4807-4811.

T-cell responses offer better measure of influenza vaccine efficacy in older adults than serum antibodies. Influenza is a major cause of morbidity and mortality in older adults. A recent NIAID-supported clinical trial evaluated the relationship between the development of influenza illness and the vigor of the immune response to influenza vaccination in older adults, with and without congestive heart failure. Among the study's 90 volunteers aged 60 years and older, levels of anti-influenza antibodies did not predict participants' risk of contracting influenza over the next year. However, the degree of T-cell activation from patient samples *in vitro* correlated well with a reduced risk of contracting influenza. These results suggest that strong cellular immune responses to influenza correlate with protection against influenza and that serum antibody response may not be an ideal measure of vaccine efficacy in older adults.

McElhaney JE, Xie D, Hager WD, Barry MB, Wang Y, Kleppinger A. T cell responses are better correlates of vaccine protection in the elderly. *J Immunol.* 2006;176:6333-6339.

Live-attenuated influenza vaccine provides greater cross-protection against genetically drifted influenza viruses compared with inactivated vaccine. Children have high rates of respiratory illness and are crucial in the transmission of influenza throughout the general population. NIAID-supported studies evaluated the effectiveness of live-attenuated and inactivated influenza vaccines in children and

young adults in 2003, a year in which the epidemic started earlier than usual and the predominant virus was not well-matched to the vaccine. One study compared rates of illness in communities where children and young adults were given inactivated vaccine to rates in communities that received live-attenuated vaccine. The other examined the influenza-associated illness rates in a single community that received live-attenuated vaccine. The results showed that not only does live-attenuated vaccine provide significant indirect protection of children and adults by limiting virus transmission, it also can protect against a viral strain that is not well-matched to the vaccine. The study's researchers suggested that because live-attenuated vaccines may provide greater cross-protection against genetically drifted influenza viruses, these vaccines may prove useful in preparing for an influenza pandemic for which it is impossible to predict with certainty which strain will circulate.

Piedra PA, Gaglani MJ, Kozinetz CA, Herschler GB, Fewlass C, Harvey D, et al. Trivalent live attenuated intranasal influenza vaccine administered during the 2003-2004 influenza type A (H3N2) outbreak provided immediate, direct, and indirect protection in children. *Pediatrics.* 2007;120:553-564.

Halloran ME, Piedra PA, Longini IM Jr, Gaglani MJ, Schmotzer B, Fewlass C, et al. Efficacy of trivalent, cold-adapted, influenza virus vaccine against influenza A (Fujian), a drift variant, during 2003-2004. *Vaccine.* 2007;25:4038-4045.

Ongoing Activities

Using a surveillance system to monitor seasonal human influenza activity. NIAID-funded investigators are currently obtaining influenza-positive clinical samples from adults and children who seek care from university health services or collaborating pediatric practices for research focusing on human immune responses during and after infection. In addition, researchers are using this system to study the effectiveness of annual immunization programs using both inactivated and live-attenuated influenza virus vaccines.

Examining whether influenza vaccination among children can limit viral transmission among adults. Hutterites are members of a religious community in Alberta, Saskatchewan, and Manitoba, Canada. They practice communal farming on small colonies that are isolated from towns and cities. Canadian researchers, supported in part by NIAID, are conducting a clinical trial to determine whether immunizing children in these Hutterite colonies with inactivated influenza vaccine can prevent influenza and its complications in other colony members. Furthermore, the study will assess the indirect benefit to Hutterites at high risk of influenza complications. These homogeneous communities, where influenza transmission is facilitated by a communal lifestyle but where limited reintroduction of the virus occurs, provide a valuable opportunity to test the hypothesis that vaccination of children can limit transmission among adults.

Appendix D. Animal Models

Recommendations

1. *Enhance the depth of understanding of commonly used models*
2. *Increase the range of options for animal models*
3. *Improve the availability of appropriate animals for use in influenza research*

Scientific Advances

Scientists develop new methods to learn more about influenza with the ferret model. Ferrets infected with influenza develop symptoms similar to those of humans, including sneezing, fever, and weight loss. However, the ferret model of influenza infection is limited by the lack of ferret-specific reagents needed to measure many immune responses. NIAID-funded scientists recently developed methods to detect ferret interferon gamma (IFN-γ), a key cytokine in the regulation of innate and adaptive immune response to viral infection. The new method substantially increases what scientists can learn about influenza with the ferret model.

Ochi, A, Danesha A, Seneviratnea C, Bannera D, Devriesc ME, Rowe T, et al. Cloning, expression and immunoassay detection of ferret IFN-gamma. *Dev Comp Immunol.* 2008;32:890-897.

Studies identify benefits of innate immune responses in ferrets infected with lethal influenza H5N1 virus. Studies of ferrets infected with lethal influenza H5N1 virus revealed that high IFN responses may decrease severity of disease caused by the virus. The studies also showed that inhibiting the chemokine CXCR3 signaling pathway reduced disease symptoms and delayed mortality. These studies demonstrate that cytokines can have both positive and negative effects on disease outcome.

Cameron CM, Cameron MJ, Bermejo-Martin JF, Ran L, Xu L, Turner PV, Ran R, et al. Gene expression analysis of host innate immune responses during lethal H5N1 infection in ferrets. *J Virol.* 2008;82:11308-11317.

NIAID addresses study gap of H7 influenza viruses in mice and ferrets. Although avian influenza viruses of the H7 subtype have caused human disease and death, very limited information is available on the immune response and pathogenesis of H7 viruses in animal models. To address this gap, NIAID researchers selected 10 H7 viruses, based on their phylogenetic relationships and geographical locations, for possible vaccine development. The virulence and immunogenicity of these 10 viruses were studied in mice and ferrets to evaluate the extent to which the viruses are related antigenically and the cross-reactivity of antibodies they induce. The mouse and ferret models will be used for further preclinical evaluation of vaccines against H7 subtype viruses.

Joseph T, McAuliffe J, Lu B, Jin H, Kemble G, Subbarao K, et al. Evaluation of replication and pathogenicity of avian influenza a H7 subtype viruses in a mouse model. *J Virol.* 2007;81:10558-10566.

Tests of H6 virus vaccines in mice and ferrets yield promising candidate for vaccine development. An H6 influenza virus, identified earlier as a potential progenitor of the H5N1 viruses that emerged in Hong Kong in 1997, continues to circulate in birds in Asia. Other H6 viruses are prevalent in birds in North America and Asia. In collaboration with industry scientists, NIAID scientists evaluated the ability of 14 H6 viruses to replicate and induce a cross-reactive immune response in mice and ferrets.

This work established the utility of mice and ferrets for testing H6 virus vaccines and revealed a promising candidate for vaccine development.

Gillim-Ross L, Santos C, Chen Z, Aspelund A, Yang C-F, Ye D, et al. Avian influenza H6 viruses productively infect and cause illness in mice and ferrets. *J Virol.* 2008;82:10854-10863.

Ferret models offer insight into the transmissibility of H5N1 virus isolates. To gain insight into the pandemic potential of H5N1 influenza viruses, NIAID-funded researchers recently used ferrets to study the transmissibility of H5N1 virus isolates with different degrees of pathogenicity and different receptor binding specificities via direct contact. The results showed that H5N1 viruses with more human-like receptor binding affinity nonetheless retain other molecular determinants that restrict their spread among mammalian species.

Yen H-L, Lipatov AS, Ilyushina NA, Govorkova EA, Franks J, Yilmaz N, et al. Inefficient transmission of H5N1 influenza viruses in a ferret contact model. *J Virol.* 2007;81:6890-6898.

New guinea pig model reveals that oseltamivir-resistance mutations reduce aerosol transmission of influenza viruses. NIAID-supported investigators have developed a guinea pig model to study the transmission of the influenza virus. Recombinant human influenza A/H3N2 viruses with and without mutations encoding oseltamivir-resistance had similar growth kinetics and infectivity in guinea pigs and was transmitted efficiently by direct contact. However, with regard to aerosol transmission, researchers showed that oseltamivir-resistant viruses are transmitted poorly or not at all while the oseltamivir-sensitive virus is transmitted efficiently via aerosol. These results suggest that oseltamivir-resistance mutations reduce aerosol transmission of influenza viruses.

Bouvier NM, Lowen AC, Palese P. Oseltamivir-resistant influenza a viruses transmit efficiently among guinea pigs by direct contact but not by aerosol. *J Virol.* 2008;82:10052-10058.

Ongoing Activities

Developing a humanized mouse model to evaluate and improve novel influenza vaccine candidates. A humanized mouse model is being developed within the NIAID-funded Cooperative Centers for Translational Research on Human Immunology and Biodefense program to serve as a surrogate system in which to evaluate and improve novel influenza vaccine candidates. Components of the human immune system—including B cells, T cells, and dendritic cells—are engrafted into mice to allow study and manipulation of human immune responses in mice.

Producing novel animal models to study immunity to infection. Researchers supported by the Systems Approach to Innate Immunity and Inflammation contract produced mutant mouse lines with defects in various immune response genes. Sixty-seven genes have been identified. Of these, 45 mutations affect the immune system, and 13 are involved in toll-like receptor signaling pathways of the innate immune responses to bacterial and viral infections, including influenza. Many of the mutant mice are now available to researchers through existing mouse repositories.

Standardizing ferret models to more efficiently evaluate candidate influenza vaccines and therapies. NIAID awarded two contracts in fiscal year 2007 to further develop a standardized ferret animal model. An important scientific resource, standardized ferret models should allow more efficient evaluation of candidate vaccines and therapies for both seasonal and highly pathogenic influenza viruses. An initial vaccine evaluation in the standardized ferret model is currently underway.

Appendix E. Vaccines

Recommendations

1. *Define correlates of immune protection against influenza*
2. *Facilitate studies that include challenge of human volunteers with live virus*
3. *Improve adjuvants and other dose-optimization technologies for influenza vaccines*
4. *Define evaluation criteria for vaccine efficacy testing*

Scientific Advances

Half-dose inactivated influenza vaccine found to be almost as immunogenic as full-dose in persons aged 16 – 49 years. NIAID scientists collaborated with researchers from CDC, the U.S. Department of Defense, and academic institutions in a study that compared the immunogenicity of half-dose inactivated influenza vaccine to full-dose among people aged 18 – 64 years. The results showed that among people aged 18 – 49 years, half-dose vaccine produced an antibody response that was virtually indistinguishable from full-dose. Among people aged 50 to 64 years, the antibody response to half-dose vaccine was somewhat lower. The authors conclude that vaccinating the younger age group with only half a vaccine dose may be an effective way to stretch vaccine supply in a pandemic.

Engler RJ, Nelson MR, Klote MM, VanRaden MJ, Huang CY, Cox NJ, et al. Half- vs full-dose trivalent inactivated influenza vaccine (2004-2005). *Arch Int Med.* 2008;168:2405-2414.

Experimental influenza vaccine highlights the critical role that type 1 interferon signaling plays in influenza immunity. Immune signaling molecules called type 1 interferons play a critical role in the development of protective immunity against viruses. NIAID-supported researchers added a gene from the influenza virus to a weakened Venezuelan Equine Encephalitis (VEE) virus to create an experimental influenza vaccine. They gave the vaccine to mice that lacked the gene for type 1 interferon. The researchers found that unlike mice that produce type 1 interferon, mice without type 1 interferon did not produce anti-influenza antibodies at mucosal sites when vaccinated. These results, which require further investigation, are important because mucosal antibodies provide critical protection in the nasal passages, lungs, and gastrointestinal tract.

Thompson JM, Whitmore AC, Staats HF, Johnston R. The contribution of type I interferon signaling to immunity induced by alphavirus replicon vaccines. *Vaccine.* 2008;26:4998-5003.

Clinical trial finds trivalent influenza vaccine to be somewhat more effective than live-attenuated influenza vaccine in healthy adults. Two types of influenza vaccines are licensed in the United States: inactivated, trivalent influenza vaccine (TIV), based on killed virus; and live-attenuated influenza vaccine (LAIV), based on a weakened virus. An NIAID-sponsored clinical trial evaluated the absolute and relative efficacies of both vaccines in preventing laboratory-confirmed influenza infection. More than 2,000 adult volunteers were vaccinated and followed throughout the 2005 – 2006 influenza season. The study found that both vaccines prevented laboratory-confirmed symptomatic influenza illness in the study population. However, TIV showed somewhat

greater efficacy than LAIV during a year in which antigenic differences existed between circulating influenza virus strains and the vaccine.

Ohmit SE, Victor JC, Teich ER, Truscon RK, Rotthoff JR, Newton DW, et al. Prevention of symptomatic seasonal influenza in 2005-2006 by inactivated and live attenuated vaccines. *J Infect Dis.* 2008;198:312-317.

High dose inactivated influenza vaccine may increase immune response to antigenically different influenza strains. NIAID-supported researchers demonstrated that antibodies produced after vaccination with a high dose of H1N1 influenza vaccine are broadly cross-reactive. Specifically, the antibodies recognized not only the H1N1 influenza virus used to make the vaccine but also three different H1N1 viruses that evolved over a period of 13 years. These results suggest that higher doses of influenza vaccines may increase the level of protection, especially in years when the strains selected for the vaccine do not closely match the viruses that circulate in the community.

Keitel WA, Atmar RL, Nino D, Cate TR, Couch RB. Increasing doses of an inactivated influenza A/H1N1 vaccine induce increasing levels of cross-reacting antibody to subsequent, antigenically different, variants. *J Infect Dis.* 2008;198:1016-1018.

Trial of novel candidate seasonal influenza vaccine produces positive preliminary results in older adults. Under an NIAID-sponsored contract, researchers evaluated the safety and immunogenicity of a trivalent baculovirus-expressed influenza vaccine in 399 adults aged 65 years and older. The effectiveness of the new vaccine, containing 135 µg of each of three HA antigens, was compared to a licensed influenza vaccine that contained 15 µg of each antigen. The purpose of this trial was to evaluate the tolerability of higher doses of a trivalent formulation of baculovirus-expressed HA vaccine in the elderly population. The results of the trial showed that the new vaccine—produced through a non-egg based method—was safe, well-tolerated, and immunogenic in elderly adults.

Treanor JJ, Schiff GM, Couch RB, Cate TR, Brady RC, Hay CM, et al. Dose-related safety and immunogenicity of a trivalent baculovirus-expressed influenza-virus hemagglutinin vaccine in elderly adults. *J Infect Dis.* 2006;193:1223-1228.

Researchers focus on key stimulator of influenza-specific cytotoxic T cells to improve age-related changes in human immune responses. Older adults have diminishing response to influenza vaccination. Researchers believe that the weaker immune response to influenza vaccine observed in seniors is likely the result of poor stimulation of cellular immunity. A recent NIAID-supported clinical study that described age-related reduction in a key stimulator of influenza-specific cytotoxic T cells directed against the virus, may help point the way toward more effective influenza vaccines for seniors.

Xie D, McElhaney J. Lower GrB+ CD62Lhigh CD8 TCM effector lymphocyte response to influenza virus in older adults is associated with increased CD28null CD8 T lymphocytes. *Mech Aging Dev.* 2007;128:392-400.

Pilot study finds that candidate seasonal influenza vaccine for blood cancer patients is safe, tolerable, and antibody inducing against influenza A. Influenza can be a life-threatening infection in patients whose immune systems have been weakened by blood cancer. Many such patients do not mount a vigorous immune response to vaccination and thus remain susceptible to infection. A recent NIAID-supported pilot

study evaluated the safety and tolerability of increasing concentrations of a candidate baculovirus-expressed influenza vaccine in volunteers with non-Hodgkin lymphoma. Results indicated the recombinant vaccine was safe, well-tolerated, and induced antibody responses against influenza A. Further studies are needed to determine the value of using higher doses of influenza vaccine in cancer patients.

Safdar A, Rodriguez MA, Fayad LE, Rodriguez GH, Pro B, Wang M, et al. Dose-related safety and immunogenicity of baculovirus-expressed trivalent influenza vaccine: a double-blind, controlled trial in adult patients with non-Hodgkin B cell lymphoma. *J Infect Dis.* 2006;194:1394-1397.

Presence of MF59 adjuvant elicits greater immune response than aluminum hydroxide (AlOH). Adjuvants—vaccine additives designed to stimulate a stronger immune response—may play a critical role in increasing the effective supply of a pandemic influenza vaccine. NIAID-sponsored researchers conducted a randomized, double-blind study that compared inactivated H5N1 subunit vaccines with and without two different adjuvants—AlOH and MF59. In this study of nearly 400 healthy adult volunteers, the presence of AlOH did not increase immune response, but MF59 did: A 15 µg dose of vaccine plus MF59 produced a more vigorous response than a 45 µg dose of vaccine alone.

Bernstein DI, Edwards KM, Dekker CL, Belshe R, Talbot HKB, Graham IL, et al. Effects of adjuvants on the safety and immunogenicity of an avian influenza H5N1 vaccine in adults. *J Infect Dis.* 2008;197:667-675.

Zangwill KM, Treanor JJ, Campbell JD, Noah DL, Ryea J. Evaluation of the safety and immunogenicity of a booster (third) dose of inactivated subvirion H5N1 influenza vaccine in humans. *J Infect Dis.* 2008;197:580–583.

Live H5N1 avian influenza virus vaccines protect animal models against lethal infection. A study conducted by NIAID researchers and private-sector colleagues found that experimental vaccines based on live-attenuated (weakened) strains of the H5N1 avian influenza virus were well-tolerated and protected mice and ferrets from infection with naturally occurring (wild-type), genetically drifted H5N1 influenza viruses.

Suguitan AL Jr, McAuliffe J, Mills KL, Jin H, Duke G, Lu B, et al. Live, attenuated influenza A H5N1 candidate vaccines provide broad cross-protection in mice and ferrets. *PLoS Med.* 2006;3:e360.

Priming the immune response with a pre-pandemic vaccine may decrease the impact of an H5N1 pandemic. WHO reported human infection with H5N1 influenza in Hong Kong in 1997. A vaccine based on that strain was developed and tested in healthy adults in 1998. In 2006, some of the same volunteers from that study were vaccinated with an H5N1 vaccine based on an H5N1 virus isolated in 2004. These revaccinated volunteers produced more vigorous antibody responses than volunteers who had never been vaccinated against H5N1 influenza. The results indicate that priming the immune response with a pre-pandemic vaccine, followed by a second dose of vaccine made from a more recently isolated strain, may be an effective strategy for decreasing the impact of an H5N1 pandemic.

Goji NA, Nolan C, Hill H, Wolff M, Noah DL, Williams TB, et al. Immune responses of healthy subjects to a single dose of intramuscular inactivated influenza A/Vietnam/1203/2004 (H5N1) vaccine after priming with an antigenic variant. *J Infect Dis.* 2008;198:635-641.

Influenza A H7N3 virus vaccine protects mice and ferrets. The appearance of human infections caused by avian influenza A H7 subtype viruses indicates that these viruses may have pandemic potential. A live-attenuated H7N3 virus vaccine was generated by reverse genetics using the HA and NA genes of a low pathogenicity H7N3 virus isolated from a chicken and the genes encoding the six internal proteins of the cold-adapted master donor virus. Intranasal immunization with one dose of the vaccine protected mice and ferrets when challenged with highly pathogenic H7 avian influenza viruses of the same and drifted strains. The vaccine virus showed comparable levels of attenuation, immunogenicity, and efficacy in mice and ferret models. These data support the evaluation of this vaccine in clinical trials.

Joseph T, McAuliffe J, Lu B, Vogel L, Swayne D, Jin H, et al. A live attenuated cold-adapted influenza A H7N3 virus vaccine provides protection against homologous and heterologous H7 viruses in mice and ferrets. *Virology.* 2008;378:123-132.

Approach may allow vaccines and treatments to be evaluated before a human-adapted pandemic virus emerges. Structure-based modification of HA specificity can guide the development of preemptive vaccines and therapeutic mAbs that can be evaluated before the emergence of human-adapted H5N1 strains. Influenza virus entry is mediated by the receptor-binding domain (RBD) of its spike, HA. Adaptation of avian viruses to humans is associated with HA specificity for alpha 2,6- rather than alpha 2,3-linked sialic acid receptors. NIAID Vaccine Research Center (VRC) scientists defined H5N1 mutations that alter the specificity of the RBD and used these mutants to develop vaccines and mAbs that neutralized new H5N1 variants. This approach might allow evaluation of vaccines and treatments before a human-adapted pandemic virus emerges.

Yang ZY, Wei CJ, Kong WP, Wu L, Xu L, Smith DF, et al. Immunization by avian H5 influenza hemagglutinin mutants with altered receptor binding specificity. *Science.* 2007;317:825-828.

DNA vaccine protects mice from 1918 pandemic virus. NIAID scientists demonstrated a vaccine that can protect mice against the highly lethal 1918 pandemic influenza virus, and defined the vaccine's mechanism of action. The vaccine was based on DNA encoding the HA gene of the 1918 virus. It elicited potent CD4 and CD8 cellular responses in the mice and neutralizing antibodies. Vaccinated mice were also completely protected against lethal challenge. Because T-cell depletion had no effect on the result and transfer of purified antibodies from immunized mice protected naive mice, researchers concluded that humoral immunity directed at the viral HA can protect against the 1918 pandemic virus.

Kong WP, Hood C, Yang ZY, Wei CJ, Xu L, García-Sastre A, et al. Protective immunity to lethal challenge of the 1918 pandemic influenza virus by vaccination. *Proc Natl Acad Sci USA.* 2006;103:15987-15991.

Ongoing Activities

Supporting clinical trials of pandemic influenza vaccines. As part of the federal government's efforts to prepare for an influenza pandemic, NIAID has supported many clinical trials to evaluate the safety and immunogenicity of various inactivated vaccine formulations made from influenza viruses with pandemic potential. The vaccines have been evaluated at varying doses in diverse populations, including the elderly, children, and young adults. NIAID-funded investigators have also evaluated vaccines with and without adjuvants, administered by different routes, or produced using non-egg based

methods. Results of many of these trials have been published since 2006. Several more trials are ongoing or being prepared for publication, including the following selected ongoing studies:

- Dose-optimization studies to assess the safety and immunogenicity of inactivated H5N1 and H9N2 vaccines when combined with adjuvants AlOH and MF59. (Overall, vaccines with and without these adjuvants have been well-tolerated. Multiple studies demonstrated that adding MF59 resulted in clear increases in immunogenicity compared to no adjuvant; no significant advantage has been observed with the addition of AlOH.)
- Studies to evaluate intramuscular versus intradermal administration of H5N1 vaccines.
- H5N1 vaccine trials in special populations, such as children and the elderly.
- Dose-optimizing trial to assess the safety of an inactivated H7N7 vaccine.
- A Phase I/II clinical trial of a whole virus H5N1 vaccine manufactured using cell culture technology.
- Phase II clinical trials to assess the safety and immunogenicity of prepriming and boosting with inactivated vaccines prepared with antigenically distinct H5N1 viruses with and without adjuvant.

Discovering and developing adjuvants. NIAID supports multiple adjuvant development projects at various stages along the research pathway, including the following selected highlights:

- Early development studies to investigate the effectiveness of adjuvant candidates designed to stimulate the innate immune response to influenza.
- Development of VEE virus replicon particles as adjuvants capable of stimulating systemic and mucosal immunity to influenza.
- Preclinical testing of an adjuvant that uses immunostimulatory genetic sequences linked to the influenza nucleoprotein gene, for use in a DNA vaccine preparation.
- Clinical evaluation of an inactivated H5N1 virus vaccine with and without MF59 adjuvant. (This study builds on a previously published study but tests an alternative vaccine formulation: in-vial as opposed to mix-at-point-of-use.)
- Two planned clinical trials, conducted in collaboration with the HHS Biomedical Advanced Research and Development Authority, to determine the safety and effectiveness of the sanofi pasteur H5N1 influenza vaccine antigen combined with either the Novartis MF59 adjuvant or the GlaxoSmithKline AS03 adjuvant

Expanding vaccine treatment and evaluation units (VTEUs). In 2007, NIAID awarded eight contracts to strengthen and expand its nationwide network of institutions conducting clinical trials of promising candidate vaccines and therapies for infectious diseases, including influenza. The expansion will allow the VTEUs to carry out more clinical trials in larger populations and to safely test vaccines in specific vulnerable populations, such as infants and the elderly. An important strength of the VTEUs is their ability to rapidly enroll large numbers of volunteers into clinical trials and vaccinate them safely, efficiently, and quickly to yield results. This rapid-response capability is especially important for testing vaccines designed to counteract emerging public health concerns, such as pandemic influenza. In addition, the NIAID VTEUs and Viral

Respiratory Pathogens Research Unit have the capacity to conduct clinical studies that include challenge in humans with live viral pathogens, such as influenza. NIAID is currently developing influenza viruses suitable for use in human challenge studies.

Accelerating the development of new influenza vaccine technologies. NIAID supports multiple projects to accelerate the development of new kinds of influenza vaccines. These include vaccines based on the following:

- Live viruses or bacteria engineered to produce influenza antigens
- Influenza proteins produced in cell culture systems
- Synthetic influenza peptides
- DNA sequences encoding influenza proteins; when injected, host cells use the sequences to make influenza proteins that stimulate immunity

Several influenza vaccine candidates under development are designed to stimulate immunity to influenza antigens that are common among many viral subtypes and do not drift substantially from year to year. These so-called "universal" vaccines would provide protection against influenza that lasts for many influenza seasons and might provide protection against an emerging pandemic virus. Other vaccine candidates rely on combinations of more than one strategy. For example, researchers at the NIAID VRC have developed several candidates based on a "prime-boost strategy." For seasonal influenza, VRC scientists plan to assess a prime-boost strategy by using a trivalent seasonal DNA vaccine to prime the immune response, then boosting that response with standard inactivated seasonal vaccine. Such a vaccine combination might induce a more effective immune response in older persons than inactivated influenza vaccine alone. In Phase I clinical trials, researchers have begun to assess the use of H5 DNA as a priming vaccine to improve overall immunogenicity and to reduce the necessary dose of inactivated virus vaccine. In addition to these new strategies, NIAID is working on novel delivery systems, such as an inactivated nasal powder vaccine and microneedles for intradermal delivery.

Supporting the Immune Epitope Discovery program. NIAID supports the Large-Scale Immune Epitope Discovery program. The main goals of the program are to identify and validate epitopes (structures that provoke an immune response) from NIAID Category A, B, and C priority pathogens, including influenza. Influenza studies include the following:

- Identification and validation of human T-cell epitopes for influenza A and B viruses
- Detection of CD4 T cells from healthy humans that recognize epitopes from H5N1 influenza virus
- Development of computational tools to predict T-cell epitopes from influenza A
- Characterization of viral T-cell epitopes that have been processed by influenza-infected cells

Supporting license applications for pre-pandemic inactivated H5N1 vaccines. On April 17, 2007, FDA approved a Biologics License Application that was submitted by sanofi pasteur for a two-dose inactivated H5N1 influenza vaccine for use in persons aged 18 – 64 years who are at increased risk of exposure to the H5N1 influenza virus subtype contained in the vaccine. NIAID conducted the clinical trial that supported the vaccine's license application through its VTEU network.

Developing and conducting clinical evaluations of live-attenuated vaccines against pandemic influenza. Live-attenuated influenza vaccines for seasonal influenza may have advantages over other vaccine types for use in an influenza pandemic. These advantages include rapid induction of both mucosal and systemic antibodies and cellular immune responses and broad cross-protection against drifted strains. Broad cross-reactivity might be particularly useful in the period before a well-matched vaccine against a newly emerged pandemic strain could be generated, manufactured, and distributed.

Under a Cooperative Research and Development Agreement with MedImmune, NIAID investigators are generating and evaluating, in preclinical studies and clinical trials, candidate live-attenuated vaccines against a wide range of influenza A subtypes that have pandemic potential, including viruses with H1, H2, H5, H7, and H9 HA antigens. Results of clinical studies in humans conducted to date have shown the replication capacity and immunogenicity of the different attenuated virus against each subtype of influenza A.

Appendix F. Therapies

Recommendations

1. *Expand studies of currently licensed antiviral drugs*
2. *Develop new drugs and new drug targets for influenza*
3. *Investigate therapies for late-stage, severe influenza*

Scientific Advances

Human antibodies protect mice from avian influenza. NIAID researchers and an international team of scientists generated mAbs from immune cells of four Vietnamese survivors of H5N1 avian influenza and then used the antibodies to successfully treat H5N1-infected mice and to protect uninfected mice from lethal infection. The antibodies' therapeutic effect was similar to that of antibodies found in survivors of the 1918 influenza pandemic (see page 19). This work showed that fully human mAbs with potent H5N1 influenza virus-neutralizing ability can be rapidly generated from the blood of convalescent patients. The scientists plan to conduct further preclinical studies of the antibodies in ferrets and, if successful, evaluate them in clinical trials.

A separate study showed that mAbs against the H5N1 HA protein protected mice from illness even when the antibodies were administered 3 days after exposure to the virus. This study provides further support that passive immunization can be useful for treatment and prophylaxis against pandemic influenza.

Simmons CP, Bernasconi NL, Suguitan AL, Mills K, Ward JM, Chau NV, et al. Prophylactic and therapeutic efficacy of human monoclonal antibodies against H5N1 influenza. *PLoS Med.* 2007;4:e178.

Hanson BJ, Boon AC, Lim AP, Webb A, Ooi EE, Webby RJ. Passive immunoprophylaxis and therapy with humanized monoclonal antibody specific for influenza A H5 hemagglutinin in mice. *Respir Res.* 2006;14:126.

Laboratory-made mAbs neutralize multiple strains of seasonal and pandemic influenza viruses. HA is a protein found on the outer surfaces of influenza virus particles. HA allows influenza viruses to fuse with host cell membranes and to inject their viral genetic material. After infection, the human immune system makes antibodies that can bind HA and prevent fusion. However, these antibodies typically bind a portion of the HA molecule that changes greatly between influenza seasons—so antibodies developed by a person in the current year will be ineffective against the virus next year.

In February 2009, two groups of NIAID-funded researchers reported the discovery of antibodies that prevent HA from allowing the virus to fuse with its target cell by binding to the relatively invariant "stalk" of the protein rather than the hypervariable "head." The antibodies were able to prevent cellular infection by a wide variety of influenza A subtypes, including H3N2, H5N1, and the H1N1 influenza strain that caused the 1918 pandemic. The work suggests that it may be possible to develop a vaccine that does not need to be updated from year to year because the vaccine would be able to target the newly identified invariable antibody binding site on HA. In addition, the antibodies themselves may inform the development of a new antiviral treatment. Both strategies are being pursued in preclinical testing.

Sui J, Hwang WC, Perez S, Wei G, Aird D, Chen LM, et al. Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. *Nat Struct Mol Biol.* 2009;16:265-273.

Ekiert DC, Bhabha G, Elsliger MA, Friesen RH, Jongeneelen M, Throsby M, et al. Antibody recognition of a highly conserved influenza virus epitope. *Science.* 2009; Feb 26.

Member of new class of antiviral proteins inhibits influenza virus entry. NIAID-supported researchers are collaborating with scientists at the National Cancer Institute to evaluate the safety and efficacy of three different proteins derived from extracts of marine algae. The proteins—called cyanovirin-N, scytovirin, and griffithsin—selectively bind to carbohydrates on the surface of the influenza virus and prevent viral entry into host cells. Investigators found that cyanovirin-N was active against naturally occurring H1N1 and H3N2 influenza viruses in cell culture but not against avian H5N1 influenza and influenza B viruses. They also found that cyanovirin-N binds to specific sugar residues on the viral HA; viruses that do not display these sugar residues are resistant to the compound. In studies on mice and ferrets, cyanovirin-N was recently shown to prevent H1N1 and H3N2 influenza A infections but not H5N1 infections.

Smee DF, Wandersee MK, Checketts MB, O'Keefe BR, Saucedo C, Boyd MR, et al. Influenza A (H1N1) virus resistance to cyanovirin-N arises naturally during adaptation to mice and by passage in cell culture in the presence of the inhibitor. *Antivir Chem Chemother.* 2007;18:317-327.

Smee DF, Bailey KW, Wong MH, O'Keefe BR, Gustafson KR, Mishin VP, et al. Treatment of influenza A (H1N1) virus infections in mice and ferrets with cyanovirin-N. *Antiviral Res.* 2008;80:266-271.

Influenza drug candidate targeting host replication machinery shows promise in animal models. NIAID-supported researchers evaluated a new influenza drug candidate called T-705, both alone and in combination with currently licensed antiviral drugs. The researchers found that T-705 is highly active in a mouse model of H5N1 influenza virus infection, even when given alone. In cell culture, T-705 inhibits replication of all influenza A and B viruses tested to date. The manufacturer is supporting preclinical development and ongoing Phase I and II clinical trials in the United States and Japan.

Sidwell RW, Barnard DL, Day CW, Smee DF, Bailey KW, Wong M-H, et al. Efficacy of orally administered T-705 on lethal avian influenza A (H5N1) virus infections in mice. *Antimicrob Agents Chemother.* 2007;51:845-851.

High-throughput screening system identifies potential antiviral targets. NIAID-supported researchers have recently developed a cell-based high-throughput screening system to identify distinct groups of compounds that inhibit or enhance the growth of the influenza virus. Results using the new system suggest that compounds that bind to sodium channels and protein kinase C may have potential as antiviral drugs. Additionally, compounds that increase viral growth in cell culture may help to speed the production of cell culture-grown influenza vaccines.

Hoffman HH, Palese P, Shaw ML. Modulation of influenza virus replication by alteration of sodium ion transport and protein kinase C activity. *Antiviral Res.* 2008;80:124-134.

Structure of M2 proton channel protein may explain influenza A resistance to rimantadine and amantadine. Two teams of NIAID-supported investigators solved the three-dimensional structures of the influenza A M2 proton channel protein bound to the anti-influenza drugs rimantadine and amantadine. Influenza viruses throughout the world have become highly resistant to both drugs. The structures, revealed by different techniques, are similar, but because they differ in key details concerning the drug binding site, investigators propose different mechanisms to explain drug resistance. Knowledge of the structures can be used to design compounds that inhibit the viral protein, even in drug-resistant strains.

Schnell JR, Chou JJ. Structure and mechanism of the M2 proton channel of influenza A virus. *Nature.* 2008;451:591-595.

Stouffer AL, Acharya R, Salom D, Levine AS, Di Costanzo L, Soto CS, et al. Structural basis for the function and inhibition of an influenza virus proton channel. *Nature.* 2008;451:596-599.

Novel injectable influenza drug reduces morbidity and mortality in mice. Currently licensed influenza drugs are either swallowed or inhaled, but neither route may be ideal for treatment of hospitalized patients with severe influenza. To assist in the development and evaluation of injectable forms of influenza drugs, NIAID-supported investigators at the University of Texas Medical Branch in Galveston demonstrated that injection of the antiviral drug candidate peramivir reduced morbidity and mortality in animals infected with highly pathogenic H5N1 influenza virus. HHS currently is supporting Phase II/III clinical trials of injectable peramivir. In a separate study, NIAID-supported CEIRS investigators at St. Jude Children's Research Hospital in Memphis determined that injectable doses of peramivir reduced morbidity and mortality in mice infected with highly pathogenic H5N1.

Yun NE, Linde NS, Zacks MA, Barr IG, Hurt AC, Smith JN, et al. Injectable peramivir mitigates disease and promotes survival in ferrets and mice infected with the highly virulent influenza virus, A/Vietnam/1203/04 (H5N1). *Virology.* 2008;374:198-209.

Boltz DA, Ilyushina NA, Arnold CS, Babu YS, Webster RG, Govorkova EA. Intramuscularly administered neuraminidase inhibitor peramivir is effective against lethal H5N1 influenza virus in mice. *Antiviral Res.* 2008;80:150-157.

Preclinical and clinical studies continue to develop a new class of protein therapeutics that inhibits influenza viral entry. NIAID is currently supporting the preclinical and clinical development of DAS 181, a new antiviral drug candidate that blocks the influenza virus from attaching to host cells by enzymatically removing sialic acid—the cellular receptor for influenza viruses. DAS 181 has been shown to protect mice from highly pathogenic H5N1 infection. The first clinical trial to study the safety of the drug is ongoing.

Belser JA, Lu X, Szretter KJ, Jin X, Aschenbrenner LM, Lee A, et al. DAS181, a novel sialidase fusion protein, protects mice from lethal avian influenza H5N1 virus infection. *J Infect Dis.* 2007;196:1493-1499.

Potential immunotherapy suppresses the host immune response in late-stage, severe influenza. One of the causes of disease and death in influenza H5N1 virus-infected patients may be a stronger-than-normal host immune response to the virus. NIAID-supported investigators are testing compounds related to sphingosine, a membrane component involved in immune system regulation, as potential therapies to dampen the host immune response. Candidate sphingosine analogs given to mice infected with influenza suppress the host immune response to influenza. These compounds given alone or in combination with antiviral drugs may prevent tissue damage that often results from influenza virus infection.

Marsolais D, Hahm B, Edelmann KH, Walsh KB, Guerrero M, Hatta Y, et al. Local not systemic modulation of dendritic cell S1P receptors in lung blunts virus-specific immune responses to influenza. *Mol Pharmacol.* 2008;74:896-903.

Patients with severe influenza can absorb oseltamivir administered through a feeding tube. No licensed influenza antiviral drugs are available that can be administered by injection, making it difficult for doctors to give antivirals to some patients with severe influenza. Researchers at the Southeast Asia Infectious Disease Clinical Research Network (SEAICRN) conducted a study of three patients hospitalized in Vietnam with severe influenza—two with H5N1 infection and one with H3N2 infection—and determined that the influenza antiviral oseltamivir administered through a nasogastric tube could be absorbed and metabolically converted to its active form in the blood. More studies are needed to determine the utility of this approach for treatment of severe influenza.

Taylor WR, Thinh BN, Anh GT, Horby P, Wertheim H, Lindegårdh N, et al. Oseltamivir is adequately absorbed following nasogastric administration to adult patients with severe H5N1 influenza. *PLoS ONE.* 2008;3:e3410.

Healthy volunteers tolerate high doses of oseltamivir. The anti-influenza drug oseltamivir has been stockpiled around the world for use in an influenza pandemic. However, researchers do not know whether high doses of oseltamivir will be more effective than standard doses for treatment of H5N1 influenza infection in humans. A recent study by researchers associated with SEAICRN showed that high doses of oseltamivir were well-tolerated. They also found that coadministering a drug called probenecid can slow excretion of the drug through the kidneys, thereby prolonging its effects and reducing overall doses required. Further study is needed, however, to establish appropriate dose regimens.

Wattanagoon Y, Stepniewska K, Lindegårdh N, Pukrittayakamee S, Silachamroon U, Piyaphanee W, et al. Pharmacokinetics of high dose oseltamivir in healthy volunteers. *Antimicrob Agents Chemother.* 2009;53:945-952.

Ongoing Activities

Evaluating influenza therapies. The NIAID SEAICRN is conducting several clinical studies of therapy for severe seasonal and H5N1 influenza. These include an ongoing clinical trial of high-dose versus standard-dose oseltamivir. Studies currently in the planning stages include a comparison of the metabolism of oseltamivir in combination with intravenous zanamivir in healthy Thai volunteers and a multicenter study of the efficacy of intravenous zanamivir to treat severe H5N1 influenza.

Investigating candidate therapies in cell culture and mouse models. The NIAID Collaborative Antiviral Testing Group (CATG) supports a number of contracts that conduct *in vitro* screening and *in vivo* testing of drug candidates in animal models and studies on preliminary efficacy, pharmacology, toxicology, and drug delivery. For example, recent CATG *in vitro* studies found that combining amantadine with either oseltamivir or ribavirin (all FDA-approved drugs) was more effective than using either drug alone; combining oseltamivir and ribavirin was not more effective. *In vivo* studies found that antiviral combinations more effectively prevent death in mice than single drugs, except when the virus is resistant to one of the drugs (Smee DF, Hurst BL, Wong MH, Bailey KW, Morrey JD. Effects of double combinations of amantadine, oseltamivir, and ribavirin on influenza A (H5N1) virus infections in cell culture and in mice. *Antimicrob Agents Chemother.* 2009 Mar 9). CATG researchers are currently developing a mouse model that will be used for *in vivo* testing of candidate drugs against highly pathogenic H5N1 influenza infection.

Evaluating the safety of oseltamivir in infants with influenza. NIAID-supported scientists recently completed a retrospective safety study that involved extensive chart reviews in selected pediatric practices that treated infants with oseltamivir. Information supported an Investigational New Drug application to conduct clinical trials to assess the use of oseltamivir in young infants. A Phase I/II pharmacokinetic/pharmacodynamic and safety evaluation of oseltamivir therapy in children younger than 24 months of age with confirmed influenza infection was recently initiated. This trial is expected to enroll 50 – 100 subjects and may be completed in late 2009.

Developing and conducting clinical trials of a long-acting influenza antiviral. NIAID has supported the development and clinical testing of a long-acting NA inhibitor, called CS-8958, to evaluate its potential to prevent and treat influenza infection. A safety trial testing a single dose of CS-8958 in healthy adults has been completed, and

a second trial in the elderly is ongoing. Moreover, a multinational Phase III study of the drug (not funded by NIAID) is underway in Asia.

Developing and testing a novel chimeric protein to treat influenza. NIAID is supporting, through grants and contracts, the preclinical and clinical development of Fludase, a novel chimeric protein that removes the influenza virus receptor from the surface of host cells, thus preventing viral entry/infection. Developed by NexBio, Fludase contains a cell surface-anchoring element and an enzyme that removes the host sialic acid, which serves as the influenza virus receptor. Fludase currently is being tested in a Phase I dose-escalation study.

Developing human influenza antibodies through passive immunization strategies to potentially treat H5N1 influenza. NIAID researchers are developing passive immunization strategies to protect against infection with H5N1 or other potential pandemic influenza strains. The first step requires the generation of antibodies against the virus, which can then be used to treat infected patients. To determine the optimal vaccine dose for antibody production, volunteers were immunized with different doses of an inactivated H5N1 vaccine. Researchers found similar antibody responses at all three doses and observed that three doses of vaccine induced the optimum antibody levels; a fourth vaccination did not significantly improve the immune response. Volunteers will be evaluated again after 1 year to determine the sustainability of antibody response. Plasma from immunized individuals is being processed for use in proof-of-concept studies in animals.

Treating influenza with innate immune system activators. Molecules that activate the innate immune system are being investigated as a potential treatment for influenza and other viral infections. In the 1970s, NIAID researchers developed a synthetic double-stranded RNA molecule known as Poly-ICLC (polyinosinic and polycytidylic acid, stabilized with poly-L-lysine and carboxymethylcellulose) to induce interferon, a host protein that can inhibit viral replication. Poly-ICLC acts as a TLR-3 agonist and induces natural antiviral cellular enzyme systems. In a lethal H1N1 mouse model, Poly-ICLC was 100-percent protective against mortality up to 14 days after the drug was given. Because Poly-ICLC activates host antiviral enzymes, its protective effects against other viruses were assessed in animal models. Poly-ICLC was found to protect non-human primates against Ebola and mice against SARS and mousepox, a murine model of human smallpox. Phase I safety and tolerability studies of Poly-ICLC are being conducted in human volunteers.

Exploring other avenues for influenza therapy. NIAID supports research to develop promising therapeutic candidates at various stages of development ranging from the synthesis of new chemical compounds with antiviral activity to evaluation of the candidates in animals or clinical trials. Examples of these projects include the following:

- Development of a new class of small molecule inhibitors of influenza NA
- Development of bifunctional molecules made by joining zanamivir and sialic acid, each of which functions as competitive inhibitors of influenza NA and HA proteins
- Development of an *in vitro* pharmacokinetics/pharmacodynamics model to predict optimal dosage and schedule of combination chemotherapy
- Development of mAbs that cross-neutralize several subtypes of influenza A
- Demonstration that administration of a CCR2 antagonist to mice prior to infecting them with influenza viruses prevents illness and death

Appendix G. Assay Technologies

Recommendations

1. *Develop new point-of-care diagnostic assays*
2. *Improve and standardize assays for cellular immune responses to influenza*
3. *Improve methods for assaying immune responses in the respiratory tract*

Scientific Advances

New method rapidly produces human mAbs against influenza. NIAID-supported investigators developed a method to rapidly generate mAbs from volunteers who have been vaccinated against influenza. The method allows identification of cells that secrete an mAb that can neutralize the virus within a month of vaccination. The antibody-producing cells could be grown in a laboratory to generate large amounts of highly specific and potent antibodies for use as diagnostics or as therapeutic agents. The method has the potential to be used for many pathogens in addition to influenza.

Wrammert J, Smith K, Miller J, Langley T, Kokko K, Larsen C, et al. Rapid cloning of high-affinity human monoclonal antibodies against influenza virus. *Nature.* 2008;453:667-671.

Innovative influenza detection technique relies on microarrays. Two groups of NIAID-funded scientists have developed diagnostic devices that may substantially improve influenza diagnostics and surveillance. Both the "MChip" and the "GreeneChipResp" candidate diagnostics rely on microarrays, which are made by precisely positioning short, fluorescence-labeled segments of influenza DNA on a glass slide. After processing, samples are spread over the array to allow sequences in the sample to bind to complementary sequences on the slide, where they are detected by laser scanning. The MChip was created through a collaboration of academic researchers, NIAID, and CDC. Although not yet commercially available, these diagnostic tools promise to allow doctors to rapidly separate patients with influenza infection from those with other respiratory viruses, allowing improved infection control and treatment.

Moore CL, Smagala JA, Smith CB, Dawson ED, Cox NJ, Kuchta RD, et al. Evaluation of MChip with historic subtype H1N1 influenza A viruses, including the 1918 "Spanish Flu" strain. *J Clin Microbiol.* 2007;45:3807-3810.

Quan, PL, Palacios G, Jabado OJ, Conlan S, Hirschberg DL, Pozo F, et al. Detection of respiratory viruses and subtype identification of influenza A viruses by GreeneChipResp oligonucleotide microarray. *J Clin Microbiol.* 2007;45:2359-2364.

New screening assay improves detection of emerging influenza viruses with pandemic potential. Surveillance of wild and domestic birds is an important tool for detecting the emergence of influenza viruses with pandemic potential. The HA inhibition (HI) assay, widely used to screen human samples for antibodies against influenza A viruses, relies on red blood cells from turkeys, guinea pigs, humans, and chickens. However, a recent study by NIAID-supported investigators found that using horse red blood cells in the HI assay substantially increased its sensitivity, making it more useful in large-scale epidemiologic studies.

Kayali G, Setterquist SF, Capuano AW, Myers KP, Gill JS, Gray GC. Testing human sera for antibodies against avian influenza viruses: Horse RBC hemagglutination inhibition vs. microneutralization assays. *J Clin Virol.* 2008;43:73-78.

Assay distinguishes influenza from other types of acute infections. A recent NIAID-supported study showed that distinct patterns of gene expression in blood cells could be defined for each of the pathogens tested: influenza A, *Escherichia coli, Staphylococcus aureus,* and *Streptococcus pneumoniae.* These results suggest that the human immune response pattern may be unique to each pathogen or class of pathogen and that clinicians will one day be able to use these patterns to make timely and specific diagnoses of the infection type. Future work will test this hypothesis using blinded samples obtained from emergency room patients to correlate gene expression results with conventional diagnostic techniques.

Ramilo O, Allman W, Chung W, Mejias A, Ardura M, Glaser C, et al. Gene expression patterns in blood leukocytes discriminate patients with acute infections. *Blood.* 2007;109:2066-2077.

Rapid assays could detect avian influenza A viruses. NIAID researchers recently investigated whether rapid assays routinely used in clinical microbiology laboratories could detect avian influenza A virus types H5N1 and H9N2. An assay that relies on mAbs to detect viral antigens after 24 hours of incubation was more sensitive than the two antigen capture assays tested. The researchers also noted that throat swabs may be a better specimen for the detection of avian influenza in humans.

Fedorko DP, Nelson NA. Performance of rapid tests for detection of avian influenza A virus types H5N1 and H9N2. *J Clin Microbiol.* 2006;44:1596-1597.

Improved techniques enhance avian influenza surveillance. When conducting surveillance of wild bird populations, the ability to preserve specimens at ambient temperature and use molecular assays would reduce costs and speed results. In a feasibility study, NIAID investigators showed that influenza A virus in avian feces preserved with guanidine at ambient temperature could reliably be detected with polymerase chain reaction techniques. In a separate study, NIAID researchers developed a sensitive method to determine the HA subtype of avian influenza viruses directly from cloacal swab material.

Evers DL, Slemons RD, Taubenberger JK. Effect of preservative on recoverable RT-PCR amplicon length from influenza A virus in bird feces. *Avian Dis.* 2007;51:965-968.

Wang R, Soll L, Dugan V, Runstadler J, Happ G, Slemons RD, et al. Examining the hemagglutinin subtype diversity among wild duck-origin influenza A viruses using ethanol-fixed cloacal swabs and a novel RT-PCR method. *Virology.* 2008;375:182-189.

Low-cost, highly sensitive method focuses on microarrays to detect the influenza virus. NIAID-funded scientists have developed an innovative way for microarrays to signal the presence of influenza viruses. Microarrays rely on the binding of genetic sequences in a sample to their complementary sequences bound to the array. Detection of positive signals typically involves using a laser to cause the bound sequences to fluoresce—a technique that requires expensive equipment. The new method uses ultraviolet light to create a small polymer cap only over spots on the array that have bound their complementary target; these caps are then stained with a visible-light dye. Because of its low cost and high sensitivity, the technique may be useful in both influenza diagnostics and surveillance.

Kuck LR, Taylor AW. Photopolymerization as an innovative detection technique for low-density microarray. *Biotechniques.* 2008;45:179-186.

Ongoing Activities

Characterizing human immune responses to infection. The Cooperative Centers for Translational Research on Human Immunology and Biodefense funds the development of new assays to facilitate the study of human immunity and define correlates of human immune protection against viral diseases, including influenza.

Supporting a field-portable, fast, and inexpensive method to rapidly detect avian influenza. NIAID is supporting a research project by scientists at Los Alamos National Laboratory to develop a new technology to detect nucleic acid sequences from a variety of viruses, including various influenza subtypes, respiratory syncytial virus, SARS, and parainfluenza. The technology, which is in the early stages of development, would use a simple "dipstick" technique—after sample processing, a small, specially treated strip would be dipped into the sample. Changes in the appearance of the strip would reveal the results in a short period of time. If successful, the method promises to be field-portable, fast, and inexpensive.

Appendix H. Resources

Recommendations

1. *Expand the range of materials available to the research community*
2. *Increase services for researchers*
3. *Improve mechanisms for the exchange of information among researchers*

Scientific Advances

Analysis tool identifies possible cross-reactive epitopes among seasonal and pandemic influenza virus strains. Contractors with the NIAID-funded Immune Epitope Database and Analysis Resource (IEDB) performed a comprehensive analysis of all influenza A antibody and T-cell epitopes in the database. The analysis compiled existing knowledge about influenza A immune epitopes and highlighted knowledge gaps. An analysis tool on the IEDB Web site identified possible cross-reactive epitopes among seasonal and pandemic influenza virus strains.

Bui HH, Peters B, Assarsson E, Mbawuike I, Sette A. Antibody and T cell epitopes of influenza A virus, knowledge and opportunities. *Proc Natl Acad Sci USA.* 2007;104:246-251.

Ongoing Activities

Supporting efforts to develop tetramers for analysis of T-cell responses to influenza. The NIH Tetramer Facility at Emory University was established in 1999 by NIAID, with support from the National Cancer Institute, to provide custom synthesis and distribution of special reagents that can be used to detect antigen-specific T cells, including T cells induced by influenza infection or vaccination. These reagents—known as soluble major histocompatibility complex (MHC)-peptide tetramers—consist of four identical peptides linked together that can be used to specifically label T cells that bind to a specific peptide. Tetramers that bind to 70 MHC class I and 15 MHC class II types are available for detection of influenza-specific T cells in mouse, nonhuman primate, and human samples. For more information, go to http://research.yerkes.emory.edu/tetramer_core/index.html.

Identifying viral peptides to examine immunity to influenza. The Cooperative Centers for Translational Research on Human Immunology and Biodefense developed a list of influenza peptides needed for further characterization of immunity to influenza. The peptides, representing protein segments from H1N1 and H5N1 influenza A strains, were synthesized and freely distributed to investigators through the NIAID Biodefense and Emerging Infections Research Resources Repository (BEI), allowing researchers to identify and characterize T- and B-cell epitopes that are important in the immune response to influenza and in the development of improved influenza vaccines. For more information, go to http://www.beiresources.org/.

Keeping current the IEDB. The NIAID IEDB includes a publicly available database that contains comprehensive antibody and T-cell epitope information for a large number of infectious diseases, including influenza. To date, the database contains 1,595 influenza epitopes, including antibody, CD4 T-cell, and CD8 T-cell epitopes. The IEDB also hosts a suite of epitope prediction, visualization, and analysis tools for use by the research community. For more information, go to http://www.immuneEpitope.org.

Developing resources for nonhuman primate studies. The NIH Nonhuman Primate Reagent Resource—supported in part by NIAID— identifies, develops, characterizes, produces, and distributes a wide variety of reagents for monitoring or modulating nonhuman primate immune responses to many infectious and immune-related diseases, including influenza. Reagents include mAbs for assays and *in vivo* modulation of immune responses or depletion of targeted cell populations, cell lines, immunoglobulin reference standards, and nonhuman primate recombinant proteins. Many additional reagents are being developed. For more information, go to http://nhpreagents.bidmc.harvard.edu/NHP/default.aspx.

Providing tools for systems biology investigation of immunity to infection. The Systems Approach to Innate Immunity and Inflammation contract, which uses systems biology approaches to produce a detailed map of innate immune responses to infection, led to the generation of more than 70 mAbs to Drosophila, mouse, and human innate immune response genes. Many of these antibodies are being submitted to the NIAID BEI for public distribution. This project provides additional tools to characterize immune responses to influenza infection, such as protein expression vectors, data analysis tools, and genetically engineered mice. For more information, go to http://www.InnateImmunity-SystemsBiology.org.

Developing reagents to characterize innate immune responses to infection. A request for applications titled *Reagent Development for Toll-like and Other Innate Immune Receptors* was issued seeking proposals to develop new reagents for the study of pathogen pattern recognition receptors in the innate immune system. This program will also promote interactions among the awardees to generate and characterize new reagents that will be made available to the research community.

Supporting computational tools to analyze immune function. Contractors for the Modeling Immunity for Biodefense program have developed a series of data analysis and modeling tools for use by the research community:

- BioPP: Tool for Web publication and hosting of large-scale, annotated signaling pathway diagrams (http://tsb.mssm.edu/pathwayPublisher/broadcast)
- Multiscale Systems Immunology (MSI) simulation: Tool that models early immune response to vaccination or infection (http://www.scfbm.org/content/3/1/6)
- DEDiscover: Cross-platform tool for building and understanding differential equation models of antiviral immunity (https://cbim.urmc.rochester.edu/software/dediscover/)
- Dynamic Regulatory Events Miner (DREM): Tool that models dynamic protein–DNA regulatory networks (http://www.sb.cs.cmu.edu/drem/)
- Short Time-series Expression Miner (STEM): Tool that analyzes short-time-series gene-expression data (http://www.cs.cmu.edu/~jernst/stem/)

Improving the global exchange of information among scientists conducting influenza research and surveillance. NIAID expanded the database it funds through the BioHealthBase Bioinformatics Research Center. The expansion includes development of an integrated database that receives diverse data—including viral sequences; surveillance and phenotypic data; and information on host/virus interactions, clinical studies, genotyping, and antigenic characterization. The database also provides researchers with customized tools to analyze genomic sequence data. For more information, go to http://www.biohealthbase.org/GSearch/home.do?decorator=Influenza.

Providing influenza virus clones free of charge to the research community. In cooperation with Invitrogen, the NIAID-sponsored Pathogen Functional Genomics Resource Center provides influenza virus clones free of charge to the research community. The resource consists of 27 strains, including 23 avian subtypes, as well as two human subtypes provided by the J. Craig Venter Institute. For more information, go to http://pfgrc.jcvi.org/index.php/gateway_clones.

Determining the three-dimensional structure of influenza proteins. In 2007, NIAID awarded support to two Structural Genomics Centers for Infectious Diseases. These centers will generate high-quality, experimentally determined three-dimensional structures of proteins from NIAID Category A – C organisms, including influenza. The three-dimensional structure coordinates and protein constructs generated will be available to the scientific community. The NIAID-funded Proteomic Research Center at the Scripps Research Institute is determining the three-dimensional structures of functional domains of avian influenza A polymerase subunits.

Generating reagents for use by the research community. NIAID CEIRS generates a variety of influenza reagents that are deposited into the BEI. The reagents are distributed to the influenza community and are available through the BEI Web site: http://www.beiresources.org.

Generating reagents to study the immune response to influenza in ferrets. Under contract with NIAID, researchers are generating reagents needed to study the immune response to influenza in ferrets. This team has generated numerous gene primer sets and mAbs for detection of ferret-specific genes and proteins, which have been delivered to the BEI (http://www.beiresources.org/).