National Institute of Allergy and Infectious Diseases

BIODEFENSE AND EMERGING INFECTIOUS DISEASES WORKSHOP
WEST NILE: RESEARCH ADVANCES IN IMMUNOLOGY, VIROLOGY, AND GENETICS

June 13-14, 2007

Sheraton Rockville Hotel
Rockville, Maryland

Abstract

The Division of Allergy, Immunology and Transplantation and the Division of Microbiology and Infectious Diseases, of the National Institute of Allergy and Infectious Diseases (NIAID) convened a workshop on June 13-14, 2007 to discuss research advances in immunology, virology, and genetics of West Nile virus; identify gaps in the science; and foster opportunities for collaborative projects to advance both basic research and clinical approaches for WNV interventions. A panel was assembled*, including immunologists, epidemiologists, virologists, geneticists, structural biologists, and physicians. The topics presented included:

- Clinical manifestations of West Nile virus
- Host immunity and virus-host interactions
- Virus Neutralization and Immune Epitope Discovery
- Epidemiology, Ecology, and Vector-Virus Transmission of West Nile virus in the Americas

Introduction

West Nile virus (WNV) is a member of the Flavivirus family, which is composed of single-stranded RNA viruses, many of which, including WNV, are transmitted by mosquitoes. WNV infects a variety of vertebrate hosts including birds, horses, and humans. WNV is an emerging infectious disease is endemic in many parts of the world and was first detected in North America in 1999 during an outbreak of encephalitis in New York City. Since then, WNV has spread across North America and southward into Latin American and the Caribbean. Though about 80% of human WNV infections are asymptomatic, 20% of the infections result in West Nile fever. A subgroup of the virally infected will go on to develop neurological illness, which is most prevalent in elderly and immunocompromised individuals. The number of cases of WNV infection has decreased in recent years, but future outbreaks and long-term sequela remains unclear. A more comprehensive understanding of the human immune response, host-pathogen interactions, and virus lifecycle/transmission is needed for the development of appropriate clinical interventions and vaccination strategies against WNV.

Clinical Manifestations

Clinically, the spectrum of human disease associated with WNV infection has expanded in recent years. Approximately 80% of human infections with WNV are clinically silent. Patients who do become symptomatic develop West Nile fever, which is characterized by fever, chills, headache, and fatigue. Most people recover completely from West Nile fever, but recent data suggests that some people experience persistent difficulties after WNV infection. Dr. James Sejvar of the Centers for Disease Control, presented clinical case reports describing long-term neurological and non-neurological clinical pathology associated with WNV infection. Non-neurological conditions include myocarditis, ocular disease, hepatitis, and pancreatitis. Neurological pathology is associated with West Nile Neuroinvasive Disease (WNND), which is seen in about 1% of all infected individuals. The severe illness associated with neuroinvasive WNV can manifest as meningitis, encephalitis, and/or movement disorders (poliomyelitis-like syndrome). The elderly and immunocompromised are at greatest risk of developing WNND.

In a longitudinal clinical cohort study by Drs. Mark Loeb and Jonathan Bramson, patients infected with WNV were observed serially over a four year period from 2003-2007. Data were collected from 157 persons infected with WNV that either caused a non-neuroinvasive infection, meningitis, or encephalitis. Measurements were made that included: measurements of physical and mental health, depression, anxiety, and fatigue. Encephalitic patients exhibited increased long-term morbidity compared to patients who did not have neurological disease. Although the majority of WNV-infected people are either asymptomatic or fully recover from the infection, the spectrum of clinical illness and the potential for persistent, or even delayed-onset sequelae are clinical issues that need to be studied in more detail.

Host Immunity and Host-Pathogen Interactions

Host immune responses to viral infection are complex, involving numerous cells, cellular components, and molecular pathways. Gaining a clearer understanding of the key immune mediators required for the generation and maintenance of protective immunity to infection will provide direction for pharmacological intervention. A significant part of this meeting was devoted to exploring the host immune response to WNV and how host genetics may impact viral virulence.

Drs. Loeb and Bramson hypothesized that the ability of WNV to become neuroinvasive is a consequence of host genetic factors that result in increased WNV replication and subsequent pathology. In their cohort study, described above, they assessed associations between immune response genotypes and susceptibility to neuroinvasive disease by characterizing the relationship between gene polymorphisms, protein function, and WNV infection. Cytokine profiles from actively infected patients are being analyzed to identify possible differences between individuals with and without WNND. Candidate genes and cytokines, that are being examined, are those that influence cellular immune response: interferon regulatory elements, tumor necrosis factor, and other interferon stimulated genes.

Genetic factors can also contribute to resistance to WNV-induced disease. Resistant strains of mice exhibit reduced viral replication and delayed cell-to-cell virus transmission compared to susceptible animals. A single Mendelian dominant allele was discovered that proffered this immune phenotype (Flv^r). Fine mapping of the Flv gene, performed by Dr. Margo Brinton,

identified oligoadenylate synthetase (OAS) genes as important mediators of WNV resistance. OAS is responsible (at least in part) for the direct antiviral effects of IFN-alpha (IFN-α) and IFN-beta (IFN-β). In the presence of interferon and double stranded ribonucleic acid (dsRNA), OAS catalyzes the formation of 2'-5' oligoadenylates from ATP. 2'-5' oligoadenylate activates RNaseL, an enzyme that cleaves viral and cellular RNA. In resistant mice there is a lower viral titer, which data suggests is due to the activity of RNAseL. In comparison susceptible mice have a higher viral titer and produce greater amounts of IFN-β. The differences found by Dr. Brinton in the induction of IFN-β, interferon regulatory factors, and interferon stimulated genes suggest differential gene activity in mice can modulate susceptibility to WNV.

Adaptive immunity provides the vertebrate immune system with the ability to recognize and remember specific pathogens. General immune senescence is common in the aging population and is marked by a decline in protective immune responses and increase morbidity and mortality from infectious diseases. Immune senescence appears to begin soon after puberty. Proportional to thymic size, thymic activity is most active before puberty. After puberty, the size and activity of the thymus are dramatically reduce and the organ is primarily replaced with fat, a phenomenon known as thymic involution, which may result in decreased efficiency of T cell responses to pathogen infections. Age-related differences in immune response are significant for WNV infected individuals. Persons over 70 years of age are at a 40-50 fold increased risk of developing meningoencephalitis as compared to people under the age of 40.

Dr. Jack Gorski is studying the effects of puberty and thymic involution on the generation of immune responses to novel pathogens. Dr. Gorski hypothesizes that adult immune memory is composed of the T cells that were productively engaged from infectious exposures through puberty, and that a person's immune repertoire is learned from the common pathogens associated with an individual's environment. Once established, adult memory responses to novel pathogens is based on pre-existing T cells that exhibit cross-reactivity to the novel pathogen. To test this hypothesis, Dr. Gorski is establishing a clinical cohort composed of two age groups: middle aged (35-55), and aging (>65 years old) people. Each cohort will donate blood at least three times a year. These samples will be used to examine immune reactivity to WNV, to determine the complexity of the responsive immune cell repertoire, and in those individuals with good immune responses, determine if possible sources of cross-reactive primary stimulations can be identified.

Studies from Dr. Janko Nikolich-Žugich are aimed at determining the key contributors to age-related immune senescence and how they can be modulated to enhance immunity. The approaches being used examine the sum of age- and virus-induced changes in anti-WNV immune responses to identify mechanisms of age-related vulnerability to WNV and other novel pathogens. Aged mice (18-24 months old) are more susceptible to death from WNV infection and have greater viral titers in the brain, kidney, liver, and spleen after receiving a sub-lethal dose of WNV. Although it seems that the innate immune response of aged mice is intact, the T cell response of aged mice is deficient in production of key immune components: interferon-gamma (IFN-γ); and the lytic granule components, granzyme and perforin. These factors seem to be the most acutely altered in the aged mouse adaptive immune response.

The innate immune response also plays a role in immunity to WNV, but may also contribute to disease progression. Macrophage migration inhibitory factor (MIF) is a pro-inflammatory

cytokine known to activate macrophages and T cells. MIF arrests macrophage movement and inhibits activation-induced apoptosis in macrophages and neutrophils, and thus sustains an inflammatory immune response. Patients suffering from acute WNV infection present with increased MIF levels in plasma and cerebrospinal fluid. Dr. Richard Bucala, exploring this arm of the innate immune response to WNV, suggests that MIF dysregulation in WNV infection may be partially responsible for the pathology associated with infection. High levels of MIF increase blood-brain barrier permeability and may increase neuroinvasion by WNV. To ameliorate the effects of MIF, Dr. Bucala has developed a small molecule that inhibits MIF interaction with its receptor. This inhibition has proved effective in protecting mice from death when infected with WNV. Dr. Bucala also studies the role of inflammatory mediators produced by macrophages, such as tumor necrosis factor-alpha (TNF-α) and other cytokines and chemokines, upon disruption of the blood-brain and development of WNND.

Dr. Erol Fikrig also studies the effects of innate immunity on WNV infection. He showed that toll-like receptor-3 (TLR-3) is activated in cells after WNV infection. TLR-3 is responsive to dsRNA and engagement of the receptor activates an inflammatory pathway. This pathway activates nuclear factor-kappa B (NF-κB) and results in the production of inflammatory cytokines. TLR-3 deficient mice are more resistant to lethal viral infection, due to a delay in the breakdown of the blood-brain barrier. Perhaps by a similar mechanism, Dr. David Gorenstein demonstrated that by blocking NF-κB through delivery of NF-κB reactive liposome nanoparticles, mice given lethal doses of WNV are able to survive the infection. Liposome-protected mice exhibit decreased viremia and decreased viral penetrance into the brain. Toll-like receptor-7 (TLR-7) deficient mice, unlike TLR-3 deficient mice, show an increased susceptibility to WNV. The natural ligand for TLR-7 is single-stranded RNA; TLR-7 also is reactive to imidazoquinolines (ribonucleoside analogs with anti-viral activity). Data from Dr. Fikrig show that TLR-7 deficient mice exhibit increased viral loads in the brain and the periphery and that selected cytokines are altered. This observation suggests that TLR-7 and the activation pathway associated with TLR-7 engagement are important in protective immune responses to WNV.

Virus Neutralization and Immune Epitope Discovery

The envelope protein of WNV is one of three structural proteins of the virus and the major virus surface protein. It also plays a central role in viral infectivity and is the primary target of virus neutralizing antibodies. Thus, understanding the structural biology of the virus becomes critical in identifying targets for virus neutralization, detection, attenuation, and vaccination strategies.

An important aspect of antibody-mediated virus neutralization and successful vaccine strategies to WNV is the activation of virus-specific B and T cells. The portions of the virus that are recognized by B and T cells are called epitopes. Antibody-mediated neutralization of WNV appears to be dependent on several parameters including the epitope location, binding affinity of the antibody, mechanism by which the antibody inhibits viral function, and the number of sites on the virus that are occupied by neutralizing antibodies. Dr. David Beasley conducted analysis of WNV virulence across different strains of WNV to identify factors that influence viremia and neuroinvasion. His research shows that glycosylation of the envelope protein is a major determinant of virulence. In mutational analysis experiments, loss of envelope protein glycosylation resulted in attenuation of the virus.

Another key determinant of WNV virulence, as well as antigenicity, is the lateral ridge of domain III of the envelope protein. This region is conserved in most WNV strains; and laboratories studying neutralizing antibody binding find that the lateral ridge of domain III of the envelope protein is the most effective neutralization site on the virus. The lateral ridge of domain III is composed of four discontinuous regions of the domain III protein. Dr. David Gorenstein used Nuclear Magnetic Resonance (NMR) spectroscopic methods to solve the solution structure of domain III. The potent neutralizing antibody, E16, binds to the domain III lateral ridge; the four critical residues of the E16 epitope are S306, K307, T330, and T332. Dr. Beasley finds that amino acid substitution in this region can limit WNV neuroinvasion and decrease virus viability.

The ability of an antibody to neutralize WNV is a function of the strength of antibody binding and the accessibility of the binding sites. The envelope of WNV is composed of 180 envelope protein monomers that assemble as sets of three antiparallel homodimers. During WNV maturation, the envelope protein assumes three different conformations. The conformation of a mature WNV is an icosahedron, where the domain III lateral ridge is at the surface. In the mature virion, binding of E16 to the domain III lateral ridge is somewhat limited by steric hindrance. Only 120 of the possible 180 binding sites can be occupied. Studies from Dr. Theodore Piersen model and experimentally test the number of antibodies required to neutralize a virion. Since the E16 antibody is a high affinity binder, neutralization can be achieved with lower occupancy of the binding sites. E16-mediated neutralization can be achieved by occupying as few as 30 of the 180 binding sites on the virus.

One means by which E16 is able to exert its neutralizing effects is shown by Dr. Michael Diamond. In general, neutralizing antibodies can either block attachment of the virus to host cells, block entry into the cells, or block fusion with the cellular membranes. It has been suggested that the domain III lateral ridge is not responsible for viral binding, but acts at a post-attachment step. Using E16-bound WNV, Dr. Diamond demonstrates that virus colocalizes with endosomes of the infected cells, but that E16 blocks the low pH-dependent structural transition of the virus. In other flaviviruses this structural transition exposes the fusion peptide, which then mediates virus-endosomal membrane fusion and viral replication. This appears to be the mechanism by which E16 mediates it neutralizing effects on WNV.

Use of E16 has been proposed for human monoclonal antibody therapy after WNV infection. Humanized E16 (hE16) was tested for efficacy in small animal models. Hamsters were inoculated subcutaneously with WNV; infected neurons were detectable at 5 days post infection. Dr. John Morrey administered hE16 to hamsters at various time points after WNV infection to determine the treatment window. Protection from WNV-induced mortality and poliomyelitis could be achieved if antibody was administered intraperitoneally as late as 5 days post infection. If the antibody was administered directly into the pontine of the mid-brain, it was effective 6 days post infection in animals without advanced neurological disease. Efficacy of hE16 was limited when treatment was delayed beyond 5 days. Dr. Diamond noted that although the domain III lateral ridge antibodies exhibited strong therapeutic activity in animals *in vivo*, less than 50% of convalescent patients had domain III lateral ridge antibodies in their serum. The

immunodominant epitope in the envelop protein is within the domain II fusion loop; antibodies to this region are present in high levels in human convalescent serum.

T cell epitope identification was performed using a variety of methods, including computational analysis of the WNV genome to predict epitopes for major histocompatibility complex (MHC) class I and class II molecules; enzyme-linked immunospot assay (ELISpot) to validate predicted epitopes; and elution of naturally processed peptides from MHC molecules followed by mass spectrometric identification of the eluted peptides. Computer algorithms were used by two investigators, Drs. Ole Lund and J. Thomas August. Dr. Lund predicted epitopes for MHC class I molecules across 12 MHC class I supertypes. Sixteen peptides were selected for each supertype and analyzed for MHC binding capacity. Conventionally, peptides exhibiting a binding affinity of <500nM for MHC class I binding are considered to be possible epitopes. Predictions were made for 192 peptides and of those peptides, 99 peptides bound to MHC molecules with an affinity greater than 500 nM, and an additional 53 bound with an affinity greater than 50 nM. Dr. August analyzed the 2476 WNV protein sequences collected in the NCBI Entrez database for sequence conservation across viral variants. Prediction algorithms using binding motif analysis for MHC class I and class II molecules predicted a total of 233 T cell epitopes. ELISpot analysis of HLA transgenic mice showed T cell interferon-gamma responses to over 100 class II epitopes. There were also 17 identified T cell:MHC class II epitopes that were 100% conserved in both WNV and Japanese Encephalitis Virus, another member of the flavivirus family. This may form the basis for the design of polyepitope and conserved sequence based vaccines applicable to all WNV genomic variants and to vaccines across the flavivirus family.

Revealing MHC class I binding peptides unique to WNV infected cells is an experimental area studied by Dr. William Hildebrand. Direct epitope identification utilizes infected cells that express soluble MHC class I molecules. Soluble MHC class I molecules follow the same intracellular pathway as membrane bound MHC class I molecules, except the molecules are secreted into the supernatant instead of being retained at the cell surface. The soluble MHC class I molecules and their associated peptides are collected, and the peptides are eluted and analyzed by mass spectrometry. In this way, Dr. Hildebrand has identified six high affinity WNV specific peptides. These peptides are recognized by T cells from WNV infected individuals and represent immunodominant cytotoxic T cell responses from these patients.

Epidemiology, Ecology, and Vector-Virus Transmission of West Nile virus in the Americas

WNV is transmitted by mosquitoes to a variety of vertebrate hosts, although the virus cycles primarily through mosquitoes and birds. Therefore, it is necessary to understand the virus-vector relationship, in addition to monitoring the human epidemic. A series of presentations focused on the monitoring strategy for WNV prevalence in mosquitoes, and infection of birds and humans.

ArboNET is a WNV surveillance network that comprises state and local health departments in the 48 contiguous United States. The participating jurisdictions perform bird monitoring either in wild birds or sentinel chickens, mosquito surveillance, horse and other non-human mammal surveillance, and human surveillance. Dr. Lyle Petersen provided statistical information from ArboNET. As of June 2007, based on serosurvey data and the number of persons reported with neuroinvasive disease, it is estimated that 1.5 million person have become infected with WNV in the United States, of whom approximately 400,000 have become ill. ArboNET has recorded

9849 cases of WNV as being neuroinvasive resulting in 962 deaths. Dr. Petersen showed that with increasing age there is a statistically greater risk of developing WNV neuroinvasive disease. Older people, however, are less likely to develop West Nile fever. Another mode of transmission discussed in this presentation was human to human transmission. Human blood supplies are routinely screened for WNV nucleic acid and the risk of transfusion transmission has been virtually eliminated. There is however, an increased risk of developing WNV neuroinvasive disease after organ transplants.

ArboNET has received reports of WNV infection in >60 mosquito species and >300 bird species. The enzootic cycle of WNV is maintained by virus transmission between mosquitoes, predominantly of the *Culex* species, and wild birds. Understanding the factors that determine the intensity of spill-over of this zoonotic pathogen from birds to humans is a prerequisite for predicting and preventing human outbreaks and epidemics. In presentations made by Drs. Shannon LaDeau, Laura Kramer, and Robert Tesh the effects of WNV on bird populations was explored, as well as the effects of viral strain variation on the vector and transmission of the virus. Additionally, analyses of urban and suburban areas were made to determine influences of site-specific differences on infection prevalence.

Dr. LaDeau amassed over two decades of avian population dynamics data for bird populations across the United States to assess the effects of WNV as an introduced pathogen on domestic bird populations. In order to assess the initial impact and ecological persistence of the introduced pathogen it was necessary to determine host abundances and population dynamics before pathogen introduction. Seven out of twenty common bird species exhibited population-level declines, which followed the spread of WNV across the North American continent in a spatio-temporal fashion. The American crow population declined significantly after WNV emergence, with some regional decreases in the crow populations rising to greater than 45%. Six other bird species, including the American robin and blue jay, also demonstrated statistically significant declines following WNV emergence. This decline could have significant consequences on the human population, since a decrease in preferred host might make humans subject to incidental WNV infections as mosquitoes use humans for blood meals.

The intensity of viral transmission is also affected by viral strain. A newly emergent, dominant genotype of WNV has been identified – North American Dominant Clade/WN02. This strain contains three nucleotide changes that are consistently different from the introduced genotype – East Coast/NY99. In examining the genotypes of WNV from 2001 to 2005, Dr. Kramer has found the NY99 genotype appears to have been completely displaced by the WN02 genotype in the United States. In examining the two viruses to determine the cause of the strain displacement, she found that WN02 is able to infect mosquitoes at a higher infection rate, more quickly replicate, and transmit from the mosquito to a new host faster than NY99 viruses. The WN02 was also found to cause a higher viral titer and increased mortality in house sparrows. These insights into adaptation of this emerging pathogen may aid in the prediction of viral pathology.

Viral transmission by the mosquito and their feeding behavior may be affected by other site-specific factors, such as population density and climate. As demonstrated in the studies by Dr. Kramer, mosquito feeding behavior and WNV prevalence is highest in urban and residential

areas and lowest in forested areas. Even in metropolitan areas such as Houston, Texas where, because of the mild climate, mosquitoes are active year-round, there is a periodicity to the WNV infections. Through intensive surveillance of WNV activity in the Houston metropolitan area since 2002, Dr. Tesh was able to demonstrate the presence of WNV in mosquitoes, even during the winter months when human infections were not reported. Mosquito numbers increase during the warmer summer months of June to September. Paralleling this increased mosquito presence is an increase in human and avian cases of West Nile fever and neuroinvasive disease. Thus, many factors contribute to human infection with WNV: environment and human population density; virus replication kinetics and transmissibility; avian host populations; and other drivers at a local scale. Understanding the interplay of all of these factors will be important in developing controls for WNV infections.

Summary

West Nile virus is common in Africa, West Asia, and the Middle East, but was not seen in the United States until a mysterious cluster of encephalitis was identified in New York in 1999. This emerging pathogen quickly spread across the United States and is now endemic with outbreaks occurring annually. How WNV was transported to the United States is still a matter of speculation, but exemplifies the importance of understanding how our immune systems will respond to novel pathogen challenge.

While the majority of WNV cases are asymptomatic, the pathology associated with the clinically detectable cases of WNV infection are acutely observed in the aged and immunocompromised. With WNV becoming endemic in North America it is likely there will continue to be considerable morbidity and mortality associated with this viral infection. Mounting clinical data show long term effects well after the active viral infection has been cleared. This suggests that the viral infection may have a more profound effect on human health than initially anticipated. To date there is no specific treatment for WNV disease, and the preventative measures are limited to decreasing exposure to mosquitoes and coordinated mosquito control programs involving the application of larvacides to breeding areas, and spraying pesticides for adult mosquitoes. The impact of these programs on human disease is yet to be determined.

Of significant concern, are viral adaptation and the potential for strain displacement. NY99 was the emergent strain of the West Nile in the United States, but was quickly displaced by WN02. Viral replication kinetics of WN02 surpassed NY99. If a more virulent strain of West Nile were to displace WN02, understanding the correlates of immune protection to WNV infection and identifying targets for immune intervention will be clinically important.

The workshop participants agreed there was a need to examine human immune response at the earliest stages of disease. Consensus was that a prospective study of the natural history of infection should be performed. In this way some of the more difficult questions about WNV infection could be answered, such as: what is the nature of viral pathogenesis? What cells are the primary target of viral infection and viral replication? What is the mechanism of immune protection in healthy individuals: innate and adaptive immune responses?

One consideration for increasing the number of blood samples available to scientists was through establishment of collaborations with Blood Centers in areas of high WNV infection incidence. Once an investigator is associated with a Blood Center, if a new case of WNV infection is identified by nucleic acid-amplification testing the donor and investigator could be notified immediately, and the clinical course of infection could be monitored in that individual.

Between the investigators there was discussion of sharing resources. One resource that was mentioned was serum samples. The lack of adequate samples was a concern for many investigators, and the need for functional studies involving peripheral blood mononuclear cells was highlighted. Other resources that could be shared were peptides and viral stocks for *in vitro* experimentation. A representative from the Biodefense and Emerging Infections Research Resources Repository (BEIresources) (www.beiresources.org) presented some of the existing resources within the repository and noted that BEIresources could act as a distribution source for materials to be shared among the investigators. Information on registering for the repository was distributed to participants and is available at the website above.

NIAID supports basic research, and vaccine and therapeutic development to better understand infectious diseases. Biodefense and Emerging Infectious Disease workshops attempt to promote multidisciplinary interactions and collaboration between investigators. This workshop is part of the NIAID's continuing effort to facilitate the application of resources and technologies to better understand WNV pathogenesis and virus-host interactions, with the ultimate goal of developing practical products to respond to WNV, and other emerging and re-emerging pathogens.

* Dr. J. Thomas August, Johns Hopkins University School of Medicine; Dr. Robert Baker, BEI Resources, ATCC; Dr. David W. C. Beasley, University of Texas Medical Branch; Dr. Jonathan Bramson, McMaster University; Dr. Margo A. Brinton, Georgia State University; Dr. Richard Bucala, Yale University School of Medicine; Dr. Michael S. Diamond, Washington University School of Medicine; Dr. Erol Fikrig, Yale University School of Medicine; Dr. Timothy A. Gondré-Lewis, NIAID/NIH; Dr. David Gorenstein, University of Texas Medical Branch; Dr. Jack Gorski, Blood Center of Wisconsin; Dr. Sharone Green, University of Massachusetts Medical School; Dr. Charles J. Hackett, NIAID/NIH; Dr. William H. Hildebrand, The University of Oklahoma Health Sciences Center; Dr. Laura D. Kramer, State University of New York, Albany and New York State Department of Health; Dr. Shannon L. LaDeau, Smithsonian Migratory Bird Center and Ohio State University; Dr. Catherine Laughlin, NIAID/NIH; Dr. Mark Loeb, McMaster University; Dr. Ole Lund, The Technical University of Denmark; Dr. John D. Morrey, Utah State University; Dr. Janko Nikolich-Zugich, Oregon Health & Science University; Dr. Lyle R. Petersen, Centers for Disease Control and Prevention; Dr. Theodore Pierson, NIAID/NIH; Dr. Patricia Repik, NIAID/NIH; Dr. James J. Sejvar, Centers for Disease Control and Prevention; Dr. Robert B. Tesh, University of Texas Medical Branch; Dr. Laura C. Wilson, NIAID/NIH