Neuroviral Infections
RNA Viruses and Retroviruses

Edited by
Sunit K. Singh and Daniel Růžek

Neurovirology is an interdisciplinary field representing a melding of virology, clinical neuroscience, molecular pathogenesis, diagnostic virology, molecular biology, and immunology. Neuroviral Infections: RNA Viruses and Retroviruses presents an up-to-date overview on general principles of infections and major neuroviral infections caused by RNA viruses and retroviruses. It is designed for virologists, specialists in infectious diseases, teachers of virology, and postgraduate students of medicine, virology, neuroscience, and immunology.

Rooted firmly in basic principles, this book guides readers through 19 chapters, each dedicated to a major RNA virus, retrovirus, and virus family. Each chapter details the organization of the viral genome and its pattern of gene expression, explains molecular mechanisms of pathogenesis, and examines interactive host dynamics—encompassing principles, transmission cycle, and vector species.
Neuroviral Infections
RNA Viruses and Retroviruses
# Contents

**Preface** ......................................................................................................................................................... ix  
**Acknowledgement** ...................................................................................................................................... xi  
**Editors** ........................................................................................................................................................ xiii  
**Contributors** ................................................................................................................................................ xv

## SECTION I  RNA Viruses

**Chapter 1**  Alphavirus Neurovirulence ........................................................................................................ 3  
*Katherine Taylor and Slobodan Paessler*

**Chapter 2**  Neurological Chikungunya: Lessons from Recent Epidemics, Animal Models, and Other Alphavirus Family Members .......... 21  
*Vincent G. Thon-Hon, Shiril Kumar, Duksha Ramful, Stephanie Robin, Marie Christine Jaffar-Bandjee, and Philippe Gasque*

**Chapter 3**  Arenaviruses and Neurovirology .................................................................................................... 39  
*Larry Lutwick and Jana Preis*

**Chapter 4**  Bunyaviruses ................................................................................................................................. 67  
*Patrik Kilian, Vlasta Danielová, and Daniel Růžek*

**Chapter 5**  Human Coronaviruses: Respiratory Pathogens Revisited as Infectious Neuroinvasive, Neurotropic, and Neuroviral Agents ............................................................................................................. 93  
*Marc Desforges, Dominique J. Favreau, Élodie Brison, Jessica Desjardins, Mathieu Meessen-Pinard, Hélène Jacomy, and Pierre J. Talbot*

**Chapter 6**  Nonpolio Enteroviruses, Polioviruses, and Human CNS Infections ......................................................................................................................... 123  
*Anda Baicus and Cristian Baicus*
Chapter 7  Neurovirulence of the West Nile Virus................................. 149
          Kim-Long Yeo and Mah-Lee Ng

Chapter 8  Murray Valley Encephalitis Virus...................................... 167
          Natalie A. Prow, Roy A. Hall, and Mario Lobigs

Chapter 9  Japanese Encephalitis Virus and Human CNS Infection......... 193
          Kallol Dutta, Arshed Nazmi, and Anirban Basu

Chapter 10 Tick-Borne Encephalitis.................................................... 211
           Daniel Růžek, Bartosz Bilski, and Göran Günther

Chapter 11 St. Louis Encephalitis....................................................... 239
           Luis Adrian Diaz, Lorena I. Spinsanti, and Marta S. Contigiani

Chapter 12 Powassan Virus ................................................................. 261
           Laura D. Kramer, Alan P. Dupuis II, and Norma P. Tavakoli

Chapter 13 Neurological Dengue.......................................................... 289
           Aravinthan Varatharaj

Chapter 14 Influenza Virus and CNS Infections .................................. 325
           Jun Zeng, Gefei Wang, and Kang-Sheng Li

Chapter 15 Human Paramyxoviruses and Infections of the Central Nervous System .................................................. 341
           Michael R. Wilson, Martin Ludlow, and W. Paul Duprex

Chapter 16 Rabies Virus Neurovirulence .......................................... 373
           Claire L. Jeffries, Ashley C. Banyard, Derek M. Healy,
           Daniel L. Horton, Nicholas Johnson, and Anthony R. Fooks

Chapter 17 Rubella Virus Infections .................................................. 395
           Jennifer M. Best, Susan Reef, and Liliane Grangeot-Keros
SECTION II  Retroviruses

Chapter 18  Human T-Lymphotropic Virus ......................................................... 431

Motohiro Yukitake and Hideo Hara

Chapter 19  Human Immunodeficiency Virus Neuropathogenesis .................. 457

Ritu Mishra and Sunit K. Singh
Preface

Neurovirology is an interdisciplinary field that represents a melding of virology, clinical neuroscience, molecular biology, and immunology. Apart from clinical neuroscience, neurovirology includes molecular virology, biochemical virology, diagnostic virology, and molecular pathogenesis and is inextricably bound to the field of immunology. Neurovirology became an established field within the past 30 years. Since then, there has been a tremendous explosion of information related to viral infections of the central nervous system, and, also, several new viruses have been discovered. The aim of this book is to present an up-to-date overview on major RNA viruses and retrovirus-mediated neuroviral infections to virologists, specialists in infectious diseases, teachers of virology, and postgraduate students of medicine, virology, neurosciences, or immunology. We hope that it will serve as a useful resource for all others interested in the field of viral infections of the central nervous system.

An inclusive and comprehensive book such as this is clearly beyond the capacity of an individual's effort. Therefore, we are fortunate and honored to have a large panel of internationally renowned virologists as chapter contributors, whose detailed knowledge on viral neuroinfections have greatly enriched this book.

We conceptualized this book in two sections. Section I includes the major RNA virus chapters and Section II concludes with the specific information pertinent to individual major retroviruses and their diseases. Each chapter consists of a review on the classification, epidemiology, clinical features, and diagnostic and therapeutic approaches of one or a group of related viruses.

The professionalism and dedication of executive editor Barbara Norwitz and senior project coordinator Jill Jurgensen at CRC Press greatly contributed to the final presentation of the book. Our appreciations extend to our families for their understanding and support during the compilation of this book.

Sunit K. Singh
Daniel Růžek
Acknowledgment

This book is dedicated to a magnanimous group of virologists, whose willingness to share their in-depth knowledge and expertise has made this extensive overview on viral neuroinfections possible.
Editors

**Dr. Sunit Kumar Singh** completed his bachelor’s degree program from GB Pant University of Agriculture and Technology, Pantnagar, India, and master’s degree program from the CIFE, Mumbai, India. After receiving his master’s degree, Dr. Singh joined the Department of Paediatric Rheumatology, Immunology, and Infectious Diseases, Children’s Hospital, University of Wuerzburg, Wuerzburg, Germany, as a biologist. Dr. Singh completed his PhD degree from the University of Wuerzburg in the area of molecular infection biology. Dr. Singh has completed his postdoctoral trainings at the Department of Internal Medicine, Yale University, School of Medicine, New Haven, Connecticut, USA, and the Department of Neurology, University of California Davis Medical Center, Sacramento, California, USA, in the areas of vector-borne infectious diseases and neuroinflammation, respectively.

He has also worked as visiting scientist at the Department of Pathology, Albert Einstein College of Medicine, New York, USA, Department of Microbiology, College of Veterinary Medicine, Chonbuk National University, Republic of Korea; and the Department of Arbovirology, Institute of Parasitology, Ceske Budejovice, Czech Republic. Presently, he is serving as a scientist and leading a research group in the area of neurovirology and inflammation biology at the prestigious Centre for Cellular and Molecular Biology, Hyderabad, India. His main areas of research interest are Neurovirology and Immunology.

There are several awards to his credit, including the Skinner Memorial Award, Travel Grant Award, NIH-Fogarty Fellowship, and Young Scientist Award. Dr. Singh is associated with several international journals of repute as associate editor and editorial board member.

**Dr. Daniel Růžek** is a research scientist at the Institute of Parasitology, Academy of Sciences of the Czech Republic, and an assistant professor at the Department of Medical Biology, Faculty of Science, University of South Bohemia. He received his PhD in the field of molecular and cellular biology and genetics from the Academy of Sciences of the Czech Republic and the University of South Bohemia. He had postdoctoral training at the Department of Virology and Immunology, Texas Biomedical Research Institute (formerly Southwest Foundation for Biomedical Research), San Antonio, Texas, USA. His primary field is virology with research emphasis on vector-borne viruses, especially tick-borne encephalitis virus, Omsk hemorrhagic fever virus, dengue virus, West Nile virus, and so forth. In 2009, he was awarded with a prestigious international Sinnecker–Kunz Award for young researchers.
Contributors

Anda Baicus
Microbiology Department,  
“Cantacuzino”  
University of Medicine and Pharmacy  
“Carol Davila”  
Viral Enteric Infections Laboratory  
National Institute of Research and  
Development for Microbiology and  
Immunology  
Bucharest, Romania

Cristian Baicus  
Department of Internal Medicine  
Colentina University Hospital  
University of Medicine and Pharmacy  
“Carol Davila”  
Bucharest, Romania

Ashley C. Banyard  
Wildlife Zoonoses and Vector Borne  
Disease Research Group  
Department of Virology  
Animal Health and Veterinary  
Laboratories Agency  
Surrey, United Kingdom

Anirban Basu  
Cellular and Molecular Neuroscience  
Division  
National Brain Research Centre  
Haryana, India

Jennifer M. Best  
King’s College London  
London, United Kingdom

Bartosz Bilski  
University of Medical Sciences Poznań  
Poznań, Poland

Élodie Brison  
Laboratory of Neuroimmunovirology  
Institut National de la Recherche  
Scientifique  
Université du Québec  
Laval, Québec, Canada

Marta S. Contigiani  
Laboratorio de Arbovirus  
Instituto de Virología “Dr. J. M.  
Vanella”  
Universidad Nacional de Córdoba  
Córdoba, Argentina

Vlasta Danielová  
Centre of Epidemiology and  
Microbiology  
National Institute of Public Health  
Prague, Czech Republic

Marc Desforges  
Laboratory of Neuroimmunovirology  
Institut National de la Recherche  
Scientifique  
Université du Québec  
Laval, Québec, Canada

Jessica Desjardins  
Laboratory of Neuroimmunovirology  
Institut National de la Recherche  
Scientifique  
Université du Québec  
Laval, Québec, Canada
Luis Adrián Diaz
Laboratorio de Arbovirus
Instituto de Virología “Dr. J. M. Vanella”
Universidad Nacional de Córdoba
and
Instituto de Investigaciones Biológicas y Tecnológicas
Consejo Nacional de Investigaciones Científicas y Técnicas
Córdoba, Argentina

W. Paul Duprex
Departments of Microbiology and Neurology
and
National Emerging Infectious Disease Laboratories
Boston University
Boston, Massachusetts

Alan P. Dupuis II
Department of Zoonotic Diseases
New York State Department of Health
Albany, New York

Kallol Dutta
Cellular and Molecular Neuroscience Division
National Brain Research Centre
Manesar, Haryana, India

Dominique J. Favreau
Laboratory of Neuroimmunovirology
Institut National de la Recherche Scientifique
Université du Québec
Laval, Québec, Canada

Anthony R. Fooks
Wildlife Zoonoses and Vector Borne Disease Research Group
Department of Virology
Animal Health and Veterinary Laboratories Agency
Surrey, United Kingdom

and
University of Liverpool
National Consortium for Zoonosis Research
Neston, United Kingdom

Philippe Gasque
Immunopathology and Infectious Disease Research Group
University of la Reunion
St. Denis, Reunion, France

Liliane Grangeot-Keros
Virology Department
Antoine Béclère Hospital
National Reference Laboratory for Rubella
Clamart, France

Göran Günther
Department of Medical Sciences
Uppsala University
Uppsala, Sweden

Roy A. Hall
Australian Infectious Diseases Research Centre
University of Queensland
St Lucia, Australia

Hideo Hara
Department of Internal Medicine
Saga University
Saga, Japan

Derek M. Healy
Wildlife Zoonoses and Vector Borne Disease Research Group
Department of Virology
Animal Health and Veterinary Laboratories Agency
Surrey, United Kingdom
Contributors

Daniel L. Horton
Wildlife Zoonoses and Vector Borne Disease Research Group
Department of Virology
Animal Health and Veterinary Laboratories Agency
Surrey, United Kingdom

Hélène Jacomy
Laboratory of Neuroimmunovirology
Institut National de la Recherche Scientifique
Université du Québec
Laval, Québec, Canada

Marie Christine Jaffar-Bandjee
Immunopathology and Infectious Disease Research Group
University of la Reunion
St. Denis, Reunion, France

Claire L. Jeffries
Wildlife Zoonoses and Vector Borne Disease Research Group
Department of Virology
Animal Health and Veterinary Laboratories Agency
Surrey, United Kingdom

Nicholas Johnson
Wildlife Zoonoses and Vector Borne Disease Research Group
Department of Virology
Animal Health and Veterinary Laboratories Agency
Surrey, United Kingdom

Patrik Kilian
Institute of Parasitology
Biology Centre of the Academy of Sciences of the Czech Republic
and
Faculty of Science
University of South Bohemia
České Budějovice, Czech Republic

Laura D. Kramer
Department of Zoonotic Diseases
New York State Department of Health and
Department of Biomedical Sciences
State University of New York
Albany, New York

Shiril Kumar
Immunopathology and Infectious Disease Research Group
University of la Reunion
St. Denis, Reunion, France

Kang-Sheng Li
Department of Microbiology and Immunology
Shantou University Medical College
Shantou, Guangdong, China

Mario Lobigs
Australian Infectious Diseases Research Centre
University of Queensland
St Lucia, Australia

Martin Ludlow
Departments of Microbiology and Neurology
and
National Emerging Infectious Disease Laboratories
Boston University
Boston, Massachusetts

Larry Lutwick
Veterans Affairs New York Harbor Health Care Center
and
State University of New York Downstate Medical School
Brooklyn, New York
Mathieu Meessen-Pinard
Laboratory of Neuroimmunovirology
Institut National de la Recherche Scientifique
Université du Québec
Laval, Québec, Canada

Ritu Mishra
Laboratory of Neurovirology and Inflammation Biology
Centre for Cellular and Molecular Biology
Council of Scientific and Industrial Research
Hyderabad, India

Arshed Nazmi
Cellular and Molecular Neuroscience Division
National Brain Research Centre
Manesar, Haryana, India

Mah-Lee Ng
Department of Microbiology
National University of Singapore
Singapore

Slobodan Paessler
Department of Pathology
University of Texas
Galveston, Texas

Jana Preis
Veterans Affairs New York Harbor Health Care Center
and
State University of New York Down State Medical School
Brooklyn, New York

Natalie A. Prow
Australian Infectious Diseases Research Centre
University of Queensland
St Lucia, Australia

Duksha Ramful
Immunopathology and Infectious Disease Research Group
University of la Reunion
St. Denis, Reunion, France

Susan Reef
Centers for Disease Control and Prevention
Atlanta, Georgia

Stephanie Robin
Immunopathology and Infectious Disease Research Group
University of la Reunion
St. Denis, Reunion, France

Daniel Růžek
Institute of Parasitology
Biology Centre of the Academy of Sciences of the Czech Republic
České Budějovice, Czech Republic

Sunit K. Singh
Laboratory of Neurovirology and Inflammation Biology
Centre for Cellular and Molecular Biology
Council of Scientific and Industrial Research
Hyderabad, India

Lorena I. Spinsanti
Laboratorio de Arbovirus
Instituto de Virología “Dr. J.M. Vanella”
Universidad Nacional de Córdoba
Córdoba, Argentina

Pierre J. Talbot
Laboratory of Neuroimmunovirology
Institut National de la Recherche Scientifique
Université du Québec
Laval, Québec, Canada
Contributors

Norma P. Tavakoli
Department of Genetics
New York State Department of Health and
Department of Biomedical Sciences
State University of New York
Albany, New York

Katherine Taylor
Department of Pathology
University of Texas
Galveston, Texas

Vincent G. Thon-Hon
Immunopathology and Infectious Disease Research Group
University of la Reunion
St. Denis, Reunion, France

Aravinthan Varatharaj
Brain Infections Group
Department of Clinical Infection, Microbiology and Immunology
University of Liverpool
Liverpool, United Kingdom

Gefei Wang
Department of Microbiology and Immunology
Shantou University Medical College
Shantou, Guangdong, China

Michael R. Wilson
Departments of Microbiology and Neurology
and
National Emerging Infectious Disease Laboratories
Boston University
Boston, Massachusetts

Kim-Long Yeo
Department of Microbiology
National University of Singapore
and
NUS Graduate School for Integrative Sciences and Engineering
Centre for Life Sciences
Singapore

Motohiro Yukitake
Division of Neurology
Department of Internal Medicine
Saga University
Saga, Japan

Jun Zeng
Department of Microbiology and Immunology
Shantou University Medical College
Shantou, Guangdong, China
Section I

RNA Viruses
1 Alphavirus Neurovirulence

Katherine Taylor and Slobodan Paessler

CONTENTS
1.1 Introduction ......................................................................................................3
1.2 Alphavirus ........................................................................................................4
1.3 Interference with Antiviral Transcription ........................................................6
1.4 Changes in Cellular Tropism ............................................................................8
1.5 Host-Antiviral Response ..................................................................................9
1.6 Interferon .........................................................................................................10
1.7 Innate Immune Response ................................................................................11
1.8 Adaptive Immune Response ..........................................................................12
1.9 Host Response and Vaccine Development .....................................................13
1.10 Conclusion ......................................................................................................16
References ............................................................................................................16

1.1 INTRODUCTION

Due to their parasitic, obligate, intracellular nature, evolution favors viruses with the ability to evade the barriers and impediments the host organism utilizes to limit their ability to replicate or cause cellular dysfunction. Avoidance of host defense mechanisms and successful replication often leads to virulence, the ability to cause fatal disease. Neurovirulent viruses alter the highly sensitive nature and critical functioning of the central nervous system (CNS) leading to fatal encephalitis or, in the event of recovery, severe neurological sequelae. With 20 viruses known to cause human encephalitis, arboviruses (arthropod-borne viruses) represent a significant public health threat as emerging infectious diseases both in the United States and worldwide. The focus of this review, arboviruses in the Alphavirus genus in the family Togaviridae, contains three viruses capable of causing human encephalitis: Venezuelan equine encephalitis virus (VEEV), eastern equine encephalitis virus (EEEV), and western equine encephalitis virus (WEEV). No specific therapy or vaccine is currently available against these viruses.

For the encephalitic alphaviruses, virulence reflects the severity of neurological disease and is determined by the efficiency of host and viral factors. Peripheral replication, viremia, neuroinvasiveness, and neurotropism combined and interacting together lead to neurovirulence and subsequent disease (Griffin 2007). Each individual component is influenced by the overall molecular character of the infecting
virus as well as the subsequent response, genetic background, age, and sex of the infected host (Griffin 2007). The balance of the characteristics of the host and virus ultimately determines outcome to infection, and minor changes in either can drastically impact disease etiology, leading to lethality, persistence, or abortive infection. Thus, each stage or step of viral pathogenesis can be considered a struggle by the virus against host defense to obtain the evolutionary ideal of a relative equilibrium between the host and the virus.

In this context, two experimental paradigms are utilized to determine the components of neurovirulence: (1) the use of mutated viruses to define the molecular changes capable of altering pathogenesis and (2) the study of host variables utilizing analysis of viral pathogenesis in hosts where key immunological components are missing or altered (Fleming 1988). In considering these two experimental approaches, it is important to remember that minor variability and alterations in the viral genome can result in significant changes in neuropathogenesis. Pathogenic alphaviruses can be roughly grouped by a combination of phylogenetics, geographical circulation, and disease manifestation into two groups: Old World and New World viruses. Old World viruses differ significantly from New World viruses in tropism and disease manifestations in humans, causing arthralgia, malaise, or rash, whereas New World alphaviruses result in a flu-like syndrome that may progress to encephalitis. Two Old World viruses, Sindbis and Semliki Forest, have been used extensively in small animal models as prototypical encephalitic alphaviruses through the use of either neuroadapted or the rare naturally encephalitic strains, respectively. Although valuable knowledge regarding neuropathogenesis has been derived from these models, experimental approaches utilizing Old World alphaviruses to represent naturally encephalitic strains need to be confirmed using New World alphaviruses (Atasheva et al. 2008; Charles et al. 2001; Garmashova et al. 2007a,b; Kolokoltsov et al. 2006a,b; Yin et al. 2009). Thus, this review will first seek to understand how changes in viral replication of the primary encephalitic New World viruses, EEE, WEE, and VEE alter pathogenesis followed by analysis of how the host response potentially contributes to the disease.

1.2 ALPHAVIRUS

The encephalitic alphaviruses spread in a biphasic manner through the mammalian host. Viral-host interactions and the viability of the virus as a neurological agent require entry and successful replication at the site of infection. Evidence from experimental models indicates that as the mosquito feeds on the experimental host, virus is deposited from infected saliva extravascularly into the tissues (Turell et al. 1995). Virus then replicates at the site of inoculation, typically skeletal muscle or immune cells such as Langerhan’s cells of the skin (Grimley and Friedman 1970; Johnston et al. 2000; Liu et al. 1970; Murphy and Whitfield 1970). As the infected immune cells carry the virus to the draining lymph nodes, the virus is delivered to the vascular system, where it spreads to other target tissues, initiating the secondary phase of infection (Griffin 2007). Griffin (2007), in Fields Virology, defines peripheral replication and viremia as key components of neurovirulence. In the initial, peripheral phase, efficient replication at the site of inoculation enhances neuroinvasiveness
Alphavirus Neurovirulence

through viremia although sustained, high levels of blood-borne virus may not be required for neuroinvasion.

Following replication in lymphoid and/or myeloid tissues, the virus enters the CNS, resulting in a second phase of replication in the brain and spinal cord neurons. Entry to the CNS is critical to neurovirulence, but the precise mechanism of entry remains unknown. However, literature supports a model of nasal mucosa infection leading to the infection of the olfactory neurons and neuronal spread to through the CNS (Charles et al. 2001; Steele et al. 1998).

The initiation of inflammation leads to damage to the vascular integrity of the blood–brain barrier (BBB), enabling further entry of the virus to the compromised CNS (Schafer et al. 2009, 2011). The multifocal nature of the infection in the brain by 5 or 6 days postinfection indicates damage to the BBB and free crossing of viral particles across the typically sealed barrier (Charles et al. 2001; Jackson et al. 1991; Roy et al. 2009; Vogel et al. 2005; Zlotnik et al. 1972). Damage to the vascular integrity of the BBB as a result of inflammation has additional support from a range of studies examining viral entry to the CNS (Cook and Griffin 2003; Lossinsky and Shivers 2004; Wang et al. 2004).

Disease symptoms in patients mimic the biphasic nature of both the immune response and viral replication. In patients, as the initial immune response and viral propagation exponentially grow, a fever develops. As the secondary spread of virus begins, patients may develop another fever that coincides with development of neurological symptoms. This phase results in neuropathology and, in some cases, fatal encephalitis. Regardless, in the majority of infected individuals, the host response appears to be sufficient to prevent CNS damage and results in asymptomatic infections. This serves to emphasize how little is known regarding the susceptibility of particular individuals to neurovirulent viruses. For instance, in WEE epidemics, only 1 of 1000 infected individuals actually develop clinical encephalitis with fatality resulting in only 3% of these cases (Rennels 1984). Similar statistics are observed for VEE and EEE, although mortality in these infections is significantly higher. Nevertheless, the question remains why the viruses that are apparently intrinsically of low virulence cause severe disease in some patients and what factors account for occasional severe neurovirulence in some individuals? This is reflected in experimental situations where variability between inbred strains of mice results in alterations in outcomes (Ludwig et al. 2001). In human infections, there is undoubtedly an age-associated susceptibility to the encephalitic alphaviruses, with neurovirulence and associated mortality increasing at the extremes of the age spectrum in pediatric and elderly patients; however, the underlying causes for increased susceptibility in these populations remain poorly defined. The immature CNS may explain the susceptibility in pediatric patients, as WEE is more cytopathic in immature human neuronal cells than mature cells. Additionally, mature neuronal cells are more sensitive to type I IFN requiring less to reduce viral cytopathology and replication (Castorena et al. 2008).

The ability of the immunocompetent host to prevent neuroinvasion is uncertain and viral invasion without associated symptomatic illness has been reported in the literature in experimental models. Indicative of the importance of immune host control at the site of initial replication, intracerebral or intranasal inoculation of
many alphaviruses causes fatal disease in experimental models, whereas subcutaneous or intraperitoneal infection results in either asymptomatic disease or a clinical syndrome without lethality or severe neuropathology (Griffin 2007). Although the development of encephalitis and neurological disease in animal models is readily apparent as gauged by seizures or measurable paralysis, the utility of these models in evaluating the human host response is unclear as the disease development may be significantly different. Gaining a clearer understanding of the effects viral infection has on the host is a major component of drug development and vaccine platforms (Holbrook and Gowen 2008).

The two-wave model of infection is supported by a series of studies using VEE viral replicon particles (VRP), which are capable of targeting and infecting the same cells as VEE but unable to propagate beyond the first infected cell, to model early events in neuroinvasion. Direct intracranial infection of VRP resulted in VEE-like encephalitis in mice and was associated with a robust and rapid innate immune response that resulted in compromised BBB integrity. These initial results established a model for the identification of host and viral factors that contribute to the invasion of the brain (Schafer et al. 2009). More recent studies show that replication of the VRP in the nasal mucosa induced the opening of the BBB allowing peripherally administered VRP to enter the brain (Schafer et al. 2009, 2011). Subsequent inhibition of the initial opening of the BBB resulted in a delay in viral neuroinvasion and pathogenesis. Thus, initial entry into the CNS through the olfactory pathways initiates viral replication in the brain, inducing the opening of the BBB and allowing for a secondary wave of virus from the periphery to enter the brain (Schafer et al. 2011). Further supporting initial replication and entry through the olfactory epithelium and neurons, the administration of the toxin tunamycin damages the ultrastructure of the BBB. However, entry to the CNS still appears to occur via the olfactory system despite the prior damage BBB induced by pretreatment with the toxin. An increased viral load in the brain with both virulent and attenuated strains of virus is observed in mice treated with tunamycin to induce artificial BBB breakdown (Steele et al. 2006).

1.3 INTERFERENCE WITH ANTIVIRAL TRANSCRIPTION

All three encephalitic alphaviruses are highly susceptible to the effects of type I IFN (IFN-α and IFN-β), and as such, resistance to type I IFN signaling and production is associated with increasing neurovirulence of the virus (Aguilar et al. 2008a; Armstrong et al. 1971; Jahrling et al. 1976; Jordan 1973; Julander et al. 2007). Closely related virulent and avirulent strains of virus and indeed virulent New World and less virulent Old World alphaviruses demonstrate significant differences in their ability to interfere with gene transcription associated with viral attenuation linked to enhanced susceptibility to the effects of type I IFN. Comparison of IFN-sensitive avirulent strains and IFN-resistant virulent strains has led to comprehensive knowledge of the genes responsible for controlling IFN resistances and subsequent neurovirulence. Research supports the underlying cause of differences in disease presentation and incidence in humans between North and South American isolates of EEE results from alterations to IFN sensitivity. Comparison of replication
of NA and SA strains in IFN pretreated Vero cells showed a suppressive effect only on the replication of the less virulent SA strains. However, no differences in induction of IFN in vivo were observed (Aguilar et al. 2005). Similar results were found for VEE with attenuation in enzoonotic strains limiting the ability of the virus to interfere with the type I IFN pathways, and this may partially explains the absence of disease symptoms following infection with enzoonotic strains (Anishchenko et al. 2004; Jahrling et al. 1976; Simmons et al. 2009). Conversely, epizootic and epidemic strains are able to limit the host production of type I interferon.

Comparison of both naturally and experimentally attenuated viruses to virulent strains has generated valuable knowledge regarding the basis for IFN resistance and subsequent neurovirulence. Priming neurons with IFN before infection with either VEEV or SINV further demonstrates VEEV’s resistance to an established antiviral state compared with SINV as VEEV continues to replicate and produce progeny virion in primed cells in contrast to more sensitive SINV. VEEV resistance was attributed to partial blockade of phosphorylation of IFN signaling pathway molecules, STAT1 and STAT2, mediated by expression of nonstructural proteins. VEEV also inhibits interferon signaling genes (ISG) through structural protein expression (Yin et al. 2009).

Furthermore, comparison of an IFN-sensitive SA EEE strain to a resistant NA EEE strain identified both structural and nonstructural genes as important in IFN sensitivity (Aguilar et al. 2008a,b). Additional data derived from the genetic manipulation of virulent viruses has contributed to the understanding of the mechanisms underlying virulence associated with IFN resistance. Artificial attenuation of virulent EEE results in a marked increased in sensitivity to type I IFN and is associated with decreased virulence of the virus (Aguilar et al. 2008b). Attenuations in WEE and VEE reflect similar results (Anishchenko et al. 2004; Aronson et al. 2000; White et al. 2001). The significant differences in the an antihost response for New World alphaviruses compared with the Old World likely play a role in the increased neurovirulence of the former with the ability to down-regulate the cellular antiviral machinery increases neurovirulence. The recombination events leading to the formation of WEE from SINV and EEV-like ancestors allowed WEEV to acquire capsid protein function to inhibit the transcription of antiviral factors and thereby effectively evade the antiviral effects of type I IFN. Thus, the acquisition of type I IFN evasion led to the emergence of WEEV as a pathogenic virus (Garmashova et al. 2007b; Hahn et al. 1988).

The ability of EEEV and VEEV to interfere with cellular transcription and induce subsequent cytopathic effect is controlled by a N-terminal 35-amino acid long peptide fragment of the capsid protein. One domain is critical in balancing the presence of protein in the cytoplasm and nucleus, and the downstream peptide may contain nuclear localization signals. These domains determine the intracellular distribution of the VEEV capsid and are essential for protein function in the inhibition of transcription. The cytopathologic effects are reduced and infection is attenuated in vivo without effecting viral replication by replacing the N-terminal fragment of the VEEV capsid with the Old World alphavirus, SINV (Garmashova et al. 2007a). The pathogenic effect of the capsid protein appears to work via inhibition of multiple receptor-mediated nuclear import pathways leading to down-regulation of the
cellular antiviral machinery. Again, the capsid protein of SINV had no effect on nuclear import (Atasheva et al. 2008). Interestingly, the Old World alphaviruses, SINV and SFV, are both able to interfere with cellular transcription. However, different virus-specific proteins are utilized to cause this effect. For the Old World, alphaviruses transcriptional shutoff depends on nsP2, whereas for the New World alphaviruses, it depends on VEEV and EEEV (Garmashova et al. 2007a,b).

Thus, changes to the E2 envelope glycoprotein are significant attenuators of the neurovirulent virus. Early studies comparing TC83 structural proteins to virulent parent strain VEE-TRD found that the two strains differed at 12 nucleotides with no alterations in the nonstructural proteins or the open reading frame coding the viral polyprotein. Only nine of these changes occurred in the dominant population of the RNAs from plaque-purified viruses. Significantly, six of the nine mutations appeared in the E2 surface glycoprotein, and all five of the nucleotide changes producing non-conservative amino acid substitutions were located here (Johnson et al. 1986). Two mutations in E1 were found: one silent and one that did not alter the character of the protein. An additional nucleotide difference was found in the noncoding region preceding the 5′ end of the 26S mRNA. This early publication determined that both E2 and the noncoding regions were candidates for the molecular determinants of VEE neurovirulence. In the early 1990s, the proof of attenuation of VEEV through serial cell-culture passage that resulted in TC83 is encoded in the 5′ noncoding region and the E2 envelope glycoprotein was confirmed. Studies showed E2-120 appears to be the major structural determinant of attenuation, but genetic markers composed of genome nucleotide position 3 in the 5′ noncoding region were also significant to the attenuated phenotype (Kinney et al. 1993). The biological effect of the attenuating mutation in the 5′ untranslated region during murine infection was ultimately traced to increased sensitivity to IFN-α and IFN-β (White et al. 2001).

1.4 CHANGES IN CELLULAR TROPISM

The molecular changes responsible for changes in cellular tropism between the encephalitic alphaviruses are poorly defined; however, viral tropism for the cells of the periphery varies among the three encephalitic alphaviruses. The altered tropism for the cells of the CNS (neurons, oligodendria, microglia, and astrocytes) does not appear to account for the changes in neurovirulence between the closely related strains, as both virulent and avirulent strains are capable of productive infection of neurons. However, the efficiency of replication in neurons differs dramatically without necessarily effecting replication in the cells of the periphery. The molecular basis for enhanced replication appears to be targeted to specific changes in the coding and noncoding regions of the genome, ultimately leading to enhanced neurovirulence (Griffin 2007).

However, altered tropism for other cells may impact the level of peripheral replication, viremia, and subsequent neuroinvasion and virulence. In the case of EEEV and VEEV, such changes are reflected in altered cell tropism. Indeed, the infection of immune cells by viruses may have a significant effect on a pathogenesis as evidenced by differences in tropism between EEE and VEE. In the case of EEEV and VEEV, such changes are reflected in altered cell tropism. Although both viruses
cause severe morbidity and mortality in equines and humans, VEEV infects the DCs and macrophages of the lymphoid tissues, whereas EEEV replicates poorly in lymphoid tissues and preferentially infects muscle and fat cells. Both viruses replicate efficiently in mesenchymal cell lineages. The inability of EEEV to replicate in myeloid cell lineages is due to interferon-independent inhibition of EEEV translation. VEEV-infected mice display higher levels of serum IFN and result in IFN up-regulation in more animals compared with EEEV infection. Interestingly, the altered tropism of EEEV may help the virus to evade systemic IFN induction in vivo enhancing EEEV neurovirulence and contributing to differences in disease etiology (Gardner et al. 2008).

1.5 HOST-ANTIVIRAL RESPONSE

Viral replication at the site of inoculation, level of viremia, and subsequent spread to secondary sites are controlled by viral clearance after the initiation of the host response. Given the close relation and ability of the innate immune response to modulate the later adaptive immune response needed to effectively clear the virus, an early, robust, and properly directed innate immune response is essential. In the biphasic model of alphavirus infection, as the primary phase of viral replication is initiated, the host begins responding to the pathogenic changes at the site of inoculation by inducing robust production of cytokines, particularly IFN (Griffin 2007). Cytokine production also results in recruitment of other immune effectors capable of generating positive feedback producing an antiviral environment in the host. The infection of immune cells serves to propagate the virus but also results in the infiltration of antigen-presenting cells to the nearest lymph node, introducing antigen to naive B and T cells in the lymph nodes and initiating the adaptive immune response. Given the primary tropism of the encephalitic alphaviruses for dendritic cells, it would be unsurprising if alterations in antigen presentation and subsequent modification of an efficient adaptive immune response occurred; however, little work has been done to determine the effect of such tropism in these infections. These early mechanisms are also required to limit peripheral replication, viremia, and spread to secondary sites of infection before the development of an effective adaptive immune response.

Immunocompetent mice infected with VEEV develop a typical biphasic illness with an early lymphoid phase, characterized by ruffling of fur and progression to hunching, and a later fatal CNS phase, characterized by progression from ataxia to severe paralysis. In the lymphoid phase, peripheral serum viremia and replication in organs is resolved concurrent with production of IgM at 3 days postinfection, which steadily increases to the time of death as well as with rapid, robust production of type I IFN by 18–24 h postinfection that rapidly waned after 24-h postinfection. However, mice still developed fulminate encephalitis, and the mean time to death is 7.8 days (Charles et al. 2001).

In contrast, severely immunocompromised SCID mice fail to develop early signs of disease and symptoms develop at 6–7 days postinfection marked by aggression and agitation with death occurring at 8.9 days postinfection. Animals fail to develop hallmark hind-limb paralysis and become ataxic and progressively less responsive,
indicating alterations of the neurological disease in the immunocompromised host. Organ tropism differed in these animals with persistent viral replication at or near peak titer appearing in peripheral organs until the death of the animal without the resolution seen by 120 h after infection in sera and lymphoid tissues in competent animals. Unsurprisingly, patterns of antibody and type I IFN production vary from the immunocompetent host with a complete lack of neutralizing antibody production and lower levels of IFN that increased slowly and were below the limit of detection by 48 h postinfection. The time of death is delayed in these mice, with an average survival time of 8.9 days (Charles et al. 2001).

Given the alteration in clinical symptoms, the pathologies in the brain present as a severe spongiform encephalopathy different from that of the immunocompetent hosts’ fulminate encephalitis, indicative of a significant immunological component to CNS pathology. Charles et al. (2011) does not attribute the proximal cause of death to brain lesions in either case. Thus, the establishment of a functional host response in the periphery requires a competent immune system but is unable to prevent death. The changes in peripheral cellular tropism occur more rapidly than can be explained by the adaptive response and likely involve innate nonspecific response to viral pathogens, particularly the lack of IFN in SCID mice, preventing the establishment of the antiviral state (Charles et al. 2001).

Unfortunately, little is known about the specific mechanisms of host defense apart from an early, antiviral role for type I IFN and a correlation of neutralizing antibody production with peripheral clearance.

### 1.6 INTERFERON

All three of the encephalitic alphaviruses are highly susceptible to the effects of type I IFN and, as mentioned previously, have developed effective evasion tactics to avoid the antiviral effect. In fact, in the absence of type I IFN signaling in receptor knock-out mice, even attenuated strains of VEE typically unassociated with illness can cause complete mortality (White et al. 2001). However, prophylactic and, in some cases, early therapeutic treatment with type I IFN or compounds capable of inducing type I IFN provides protection, indicating the importance of the early generation of an immune environment conducive to host protection.

Beginning with the discovery that EEE replication was suppressed in the presence of type I IFN, Wagner (1961, 1963) showed peak viral production and high levels of cytopathogenicity in chick embryos and L-cells correlated with a high-level production of IFN. In vivo serum levels of type I IFN are low compared with those of VEE-infected mice and likely reflect the inability of EEE to infect cells of the myeloid lineages because the ability of EEE to antagonize type I IFN induction is cell-dependent (Burke et al. 2009). By artificially increasing the serum type I IFN before infection by administration of a TLR3 agonist, poly-IC, Aguilar et al. (2005) demonstrated a dose-dependent IFN-mediated protection of mice to EEE infection. Similar results are seen for WEE infections, where pretreatment of hamsters with either a consensus type IFN-α or a stimulator of type I IFN signaling, Ampligen, resulted in the complete survival of the animal. The antiviral effect of type I IFN levels was reflected in decreased clinical symptoms and weight loss associated with a significantly lower viral load in the brain at
4 days postinfection (Julander et al. 2007). Complete survival and depression of clinical symptoms is also associated with transiently expressed, artificially high levels of IFN-α in mouse models of WEE infection. Therapeutic treatment up to 7 days before infection provides complete protection. Early prophylactic elevation of IFN-α at 6 h postinfection results in increased survival rates but fails to provide complete protection (Wu et al. 2007a,b). Unsurprisingly, VEE responds similarly to the early induction of type I IFN with artificial induction of signaling through administration of a TLR3 agonist or prophylactic administration of pegylated IFN-α, resulting in delayed time to death and increased survival in mouse models (Julander et al. 2008b). Although the early administration of IFN demonstrates some prophylactic effects in decreasing time to death or disease symptoms, in the case of intranasal or aerosol exposure, the rapid entry of the virus to the CNS may limit or alter the effectiveness of early innate immune mechanisms such as IFN or other unexplored factors. The substantial difference in the effect of therapeutic and prophylactic administration of type I IFN or inducers of IFN production indicates the importance of modulating the specific immune response in the CNS to create a distinct antipathogenic environment after viral spread and replication.

The CNS of immunologically normal mice is still invaded in the presence of very high circulating levels of IFN, indicating that the cells that comprise the CNS may be less sensitive to the presence of IFN or have slower kinetics for the establishment of an effective antiviral state (Charles et al. 2001).

Due to the greater stability of IFN-α, most studies use its modified forms, and to the authors’ knowledge, no studies examining the effects of IFN-β have been performed to date. Interestingly, IFN-β treatment of the CNS disorder, multiple sclerosis, helps control the disease in some patients and indicates that mechanisms other than the antiviral effect of the type I interferons may be important in control of CNS damage (Galligan et al. 2010; Plosker 2011).

1.7 INNATE IMMUNE RESPONSE

Pretreatment and, in some cases, therapeutic administration (up to 12 h pi) of cationic-liposome DNA complexes (CDLCs) in mice infected with WEE results in significant protection. This protection was associated with changes in the host immune response due to CDLC administration. Treated mice had significantly increased serum IFN-γ, TNF-α, and IL-12, indicative of a strong TH1-biased antiviral activation of the immune system. In infected animals large increases in IFN-γ, TNF-α, IL-12, MCP-1, and IL-10 in the brain were observed by 72 h pi, as expected, with neuroinvasion and viral replication in the CNS (Schafer et al. 2009). Similar cytokine profiles are found in the brain homogenates of C3H/HeN mice lethally infected with the vaccine strain of VEEV, TC83. In these animals, IL-1α, IL-1β, IL-6, IL-12, MCP-1, IFN-γ, TNF-α, MIP1-α, and RANTES were significantly elevated over time with peak cytokine levels at six to seven days postinfection. Depression in cytokine levels occurs immediately before death around 8 to 10 days postinfection. Interestingly, treatment with IFN-α B/D or TLR3 agonist significantly improved cytokine levels and mean day to death, indicating a connection between mortality and the early host response (Julander et al. 2008a,b). Thus, robust, nonspecific activation of the innate immune response, while necessary to influence the phenotype of the adaptive
immune response, requires careful modulation in the CNS to elicit significant protective immunity against rapidly lethal strains of encephalitic alphaviruses (Schafer et al. 2009).

1.8 ADAPTIVE IMMUNE RESPONSE

The second phase of the immune response to alphavirus infection is characterized by the waning of type I IFN and the development of a robust cell-mediated immunity that theoretically limits CNS damage and clears the virus from circulation and sites of replication.

Of the factors in the cell-mediated immune response responsible of resolving infection, the role of B-cells and antibody production is well-defined. The development of antigen specific B-cells capable of antibody production play a key role in reducing peripheral replication and removing virus from the blood stream. Thus, an efficient antibody response is integral to preventing or limiting neurovirulence from the earliest phase. However, the time lag between peripheral infection and antigen-specific antibody production permits spread and replication at secondary sites of infection as mentioned previously, and additional cell-mediated mechanisms may be required to resolve infections once they reach the CNS (Griffin 2007).

In the lymphoid phase of murine infection (CB17), high-titer serum viremia is associated with the production of VEE-specific IgM antibody at 3 days postinfection, with titers increasing until the time of death. Animals failed to develop VEEV-specific IgG or IgA. However, fulminate encephalitis still develops and animals survive only an average of 6.8 days postinfection (Charles et al. 2001). Further reconstitution of SCID mice and depletion of immunologically normal mice identified production of VEE-specific IgM antibody, produced in the absence of T-cell help, as the significant factor determining immune mediated clearance in the periphery. Given the paralysis and death of these animals following infection, it appears that peripheral production of neutralizing antibody does not play a significant role in preventing lethal encephalitis once virus reaches the CNS (Charles et al. 2001).

Once the virus becomes neuroinvasive, the utility of peripherally produced antibody is uncertain, and T cells, particularly CD4+ T cells, are integral in clearance of CNS infection. Indicative of the role of T cells in resolving viral invasion of the CNS, α/β T-cell receptor knockout mice deficient in both CD4+ and CD8+ T cells develop lethal VEE encephalitis following vaccination protective to the wild-type counterpart. Specifically, reconstitution of the CD4+ T cells, but not CD8+ T cells, from vaccinated wild-type donors resulted in recovery following vaccination and challenge in α/β T-cell receptor knockout mice, indicating an integral role for CD4+ T cells in preventing lethal encephalitis (Paessler et al. 2007; Yun et al. 2009). In the absence of a competent T-cell compartment, α/β T-cell receptor knockout mice also have impaired antibody production in the absence of CD4+ T-cell help. However, passive transfer of HIAF antibody failed to induce a protective response in vaccinated animals indicating either a direct role for CD4+ T cells in the CNS or an indirect, B-cell, antibody-independent mechanism of action (Yun et al. 2009). Studies using B-cell-deficient uMT mice infected with an attenuated strain of VEEV resulted in the development of severe, but ultimately, asymptomatic encephalitis. An
antibody-independent Th1-biased response characterized by CD4+ T-cell production of IFN-γ was implicated in the control of viral replication and survival of the animals (Brooke et al. 2010).

Charles et al. (2001) demonstrated that treatment of CD-1 mice with T-cell-depleting factors before infection results in a disease such as that of SCID mice with absent clinical symptoms early in disease and no development of paralysis. However, infection in the absence of T cells had no effect on clearance of virus from the serum and did not prevent production of IgM (Charles et al. 2001). Reconstitution of SCID mice with T cells results in the reversion to fulminant encephalitis seen in wild-type mice, as does the inverse scenario, with the depletion of wild-type Cd-1 in mice leading to spongiosis and vaculation of the neuropil as seen in SCID mice. Analysis of inflammatory infiltrates in the brain indicated that T cells represent the majority of infiltrates and the predominant phenotype was CD8+ (Charles et al. 2001). Treatment of murine splenocytes with both B- and T-cell mitogens increases the susceptibility of VEE infection and indicates that the activation state of lymphocytes may be critical to lymphoid pathogenesis of VEEV (Charles et al. 2001).

Animal models of infection have provided some insight into the role of IFN-γ in protection. For VEE, the priming of the immune response via the vaccination of mice with a deficiency in the type II IFN (IFN-γ) receptor is only partially protective, unlike the complete protection seen in wild-type animals following vaccination, indicating that type 2 IFN signaling may play some role in preventing the development of encephalitis. However, unlike type I IFN signaling, type 2 IFN signaling is not absolutely required for effective protection (Paessler et al. 2007). Additionally, IFN-γ signaling does not appear to be significant in controlling EEE infection, as IFN-γ-receptor-deficient animals demonstrate equivalent levels of viremia and mortality rates similar to wild-type animals (Aguilar et al. 2008a).

1.9 HOST RESPONSE AND VACCINE DEVELOPMENT

Neurovirulence is considered the standard for vaccine candidates derived from virulent neurotropic viruses (Arya and Agarwal 2008; Fine et al. 2008). Intracranial injection of susceptible mice is routinely used for vaccine safety studies, and the mice are evaluated based on the occurrence of pathological processes in the neurological tissues. Intracranial viral infection causes damage to neuronal and glial cell populations in addition to inducing migration of potentially harmful immunologically active cells in to the perivascular space and brain parenchyma. However, in the case of live vaccine candidates, such as TC83, the current IND vaccine for VEE, these studies fail to take into account the effects of neuroinvasion in the absence of detectable pathologies. The ability of RNA viruses to persist in the CNS and the tropism of live vaccine candidates for the cells of the CNS must also be considered in developing viable vaccines. Arya et al. points out that for practical use of vaccines derived from neurotropic viruses, a close examination of the CNS for subtle lesions is required, particularly neurological developments after vaccination for other such vaccines as seen with poliovirus vaccine lots in the 1960s (Arya and Agarwal 2008; Cristi and Dalbuono 1967). To evaluate potential CNS damage, experimental animals should be observed for much longer periods.
to examine the occurrence and location of brain lesions after vaccination (Arya and Agarwal 2008).

The current key requirements for the development of a VEEV vaccine are (a) a high level of immunogenic response in mice and hamster, which are both sensitive to infection, and (b) a protective response in the NHP model when challenged with virulent virus (Rao et al. 2006). Unfortunately, the sole parameter for immunogenic response in small animal models is the production of neutralizing antibody that may not necessarily correlate with protection once infection reaches the CNS.

In addition to neurovirulence as a safety parameter, vaccine studies use neutralizing antibody production as a correlate for the efficacy of vaccination. In such cases, antibody is used as a measure of protection; however, the ability of peripherally produced antibody to provide protection and complete clearance of virus once the virus invades the peripheral nervous tissue or the olfactory tissue via olfactory nerve tracts remains poorly determined (Arya and Agarwal 2008; Fine et al. 2008).

Alterations in pathogenesis between closely related strains can be demonstrated from vaccination studies using attenuated strains of virus. These studies also derived important data regarding the host response and indicate that additional parameters to antibody production may be necessary when evaluating safety and efficacy of vaccine or therapeutic candidates.

A series of studies beginning in small animal models and progressing to NHPs compared the current live, attenuated IND vaccine strain, TC83, to candidate V3526, a live attenuated virus derived from an infectious clone of VEEV, TrD. Intracranial inoculation of both TC83 and V3526 results in the replication in the brain, with V3526 replicating at lower levels. Although neither strain was lethal in BALB/c mice, those infected with TC83 developed symptomatic illness, and the infection of C3H/HeN mice resulted in complete lethality, demonstrating the differential susceptibility of different inbred mouse strains as shown previously by Steele et al. (Ludwig et al. 2001; Steele et al. 1998). V3526, despite its ability to replicate in the CNS, was “avirulent” in both strains and did not cause symptomatic illness. Interestingly, pathological changes were found in the brains of mice infected with either strain, although, correlating with viral load, changes in V3526 inoculated animals were less severe and of shorter duration than TC83-inoculated animals (Ludwig et al. 2001).

Comparison of wild-type virus to attenuated, vaccine strain TC83 and V3526 showed similar results following aerosol exposure: while the attenuated virus does not necessarily cause symptomatic disease or mortality, they can be neuroinvasive and cause lesions throughout the CNS, which, however, are readily resolved. All three strains infected the brains and induced encephalitis. However, viral spread varied, with a gradient occurring from complete invasion of all regions of the brain with TrD, to sparing the caudal regions with TC83, to the involvement of only the neocortex and diencephalon with V3526. TrD infection resulted in uniform mortality with significant peripheral dissemination between mouse strains. Despite alterations in viral spread through the CNS, TC83 still induced 100% mortality in C3H/HeN animals but not BALB/c mice. Interestingly, significant differences between inbred mouse strains exist, and TC83, which is avirulent, so to speak, in BALB/c mice, causes complete lethality in C3H/HeN animals. V3526 caused no mortality in either strain. Neither attenuated strain extended beyond the infection of the
olfactory epithelium (Steele et al. 1998). Thus, viral replication and spread do not necessarily correlate with mortality as seen in the case of the more limited spread of TC83 to caudal regions compared with TRD and equivalent mortality in C3H/HeN mice.

Manifestations of disease were found in rhesus macaques inoculated intrathecally/intraspinally with both V3526 and TC83, although a greater percentage of TC83 infected animals developed clinically significant signs. All symptomatic disease resolved by 3 weeks postinfection. Interestingly, one of seven animals infected with attenuated V3526 showed extensive brain lesions similar to all four wild-type animals at D18. Six of seven infected macaques showed scattered lesions throughout the CNS as did four of seven TC83-infected animals at D18. All lesions were resolved by termination of the study at D181. Thus, clinical symptoms do not necessarily correlate with viral invasion, replication, and pathogenesis in the CNS (Atasheva et al. 2008; Fine et al. 2008).

Studies evaluating the propagation-defective VEEV replicon particles in mice resulted in weight loss and inflammatory changes in the brain. However, changes are less severe than those caused by TC83, the current IND vaccine. Peripheral inoculation demonstrated minimal neurovirulence and lack of neuroinvasive potential (Kowalski et al. 2007). Despite the rapid and transient nature of CNS lesions following vaccination, little is known about the degree of magnitude required to induce potentially undetectable but significant pathogenic alterations to the delicate homeostasis of the CNS. Although peripheral inoculation routes with propagation defective particles are likely unable to reach the CNS, this may not be true for live, attenuated vaccine candidates that may in fact reach, replicate, or even persist in the CNS at undetectable levels. The biological relevance of low level replication in the CNS is uncertain, but the immune-privileged nature of the CNS makes any alteration of concern.

Intracranial infection with VRP results in a VEE-like encephalitis. The use of a VRP-mRNP tagging system to distinguish the response of infected cells from bystander cells showed the initiation of a robust and rapid innate inflammatory response in the CNS by both infected neurons and uninfected bystander cells that led to an adaptive immune response characterized by proliferation and activation of microglia and infiltration of inflammatory monocytes as well as CD4+ and CD8+ T lymphocytes. Thus, the ability of the naive CNS to induce a robust innate immune response and activate local professional antigen-presenting cells that can, in turn, activate primed cells may be crucial to the outcome of infection by determining the composition and dynamics of the adaptive immune response and ultimate noncytopathic or lethal attempts to clear the virus (Schafer et al. 2009).

A formalin-inactivated form of TC83, C-84, is currently used as a booster if vaccinated individuals fail to develop a response to TC83. The low efficacy and undesirable side effects have led to additional vaccination strategies and vaccine platforms. One such approach utilizes microspheres to encapsulate the vaccine and induce a more robust response. These spheres are composed of DL-lactide-co-glycolide (DL-PLG). Microencapsulating the vaccine increased the primary circulating IgG and resulted in a rapid increase in antibody activity when boosted with a second vaccination. Interestingly, circulating anti-VEE virus antibody response was lower, with
nonformalin-fixed virus utilizing this platform. Following systemic challenge with virulent VEE, the microencapsulated virus was more effective at inducing a protective immune response (Greenway et al. 1995).

1.10 CONCLUSION

Alphaviruses represent a significant public health threat. A better understanding of the mechanisms both virus and host use to control infection and prevent neuroinvasion are required for development of safe and effective vaccines and therapeutics.

REFERENCES


Gardner, C. L., Yin, J., Burke, C. W., Klimstra, W. B., and Ryman, K. D. (2009). Type I interferon induction is correlated with attenuation of a South American eastern equine encephalitis virus strain in mice. *Virology* 390(2), 338–47.


Alphavirus Neurovirulence


Gardner, C. L. , Yin, J. , Burke, C. W. , Klimstra, W. B. , and Ryman, K. D. (2009). Type I interferon induction is correlated with attenuation of a South American eastern equine encephalitis virus strain in mice. Virology 390(2), 338-347.


Neurological Chikungunya


Brummer-Korvenkontio, M., Kuusisto, P. 1981. Onko Suomenlääsiosastyn Pogostallaa (Has western Finland been spared the Pogosta)? Suom Lak 32, 2606□□□07.


Reunion. Epidemiol Infect 137, 534□□□541.


Arenaviruses and Neurovirology


Bunyaviruses


Kilian, P., E De, Danielov, V., Hyspa, V., and Grubhoffer, L. 2010. Nucleotide variability of Tahyna virus (Bunyaviridae, Orthobunyavirus) small (S) and medium (M) genomic segments in field strains differing in biological properties. Virus Res. 149: 119-123.


Human Coronaviruses Respiratory Pathogens Revisited as Infectious Neuroinvasive, Neurotropic, and Neurovirulent Agents


Virology 347(2), 410□□□421.


Nonpolio Enteroviruses, Polioviruses, and Human CNS Infections


Neural. 67, 162□□□169.


Neurovirulence of the West Nile Virus


Agrawal, A. G., and Petersen, L. R. 2003. Human immunoglobulin as a treatment for West Nile virus infection. J. Infect. Dis. 188, 1□□□1


Goldblum, N., Sterk, V. V., and Paderski, B. 1954. West Nile fever; the clinical features of the disease and the isolation of West Nile virus from the blood of nine human cases. Am. J. Hyg. 59, 89□□□3.


Shrestha, B., and Diamond, M. S. 2004. Role of CD8+ T cells in control of West Nile virus infection. J. Virol. 78, 8312-8321.


Murray Valley Encephalitis Virus


Hurrelbrink, R. J. , and McMinn, P. C. (2001). Attenuation of Murray Valley encephalitis virus by site-directed mutagenesis of the hinge and putative receptor-binding regions of the


Sturrock, K. (2009). The changing epidemiology of Murray Valley encephalitis virus and West Nile virus (Kunjin strain) in epizootic regions of Western Australia. PhD thesis. The University of Western Australia, Perth.


Japanese Encephalitis Virus and Human CNS Infection


Swarup, V., Ghosh, J., Das, S., and Basu, A. (2008). Tumor necrosis factor receptor-associated death domain mediated neuronal death contributes to the glial activation and


Tick-Borne Encephalitis


Grekov, M. 1957. Secretion of tick-borne encephalitis virus in goat milk. Veter. 5, 177□182.


Grekov, M., and Žeber, J. 1959. Isolation of the tick encephalitis virus from the blood and milk of domestic animals (sheep and cow) after infection by ticks of the family Ixodes ricinus L. Arch. Gesamte Virusforsch. 9, 360□364.


St. Louis Encephalitis


Froeschle, J. E., and Reeves, W. C. 1965. Serologic epidemiology of Western Equine and St. Louis Encephalitis virus infection in California. II. Analysis of inapparent infections in residents of an endemic area. Am. J. Epidemiol. 81; 445□□50.


Powassan Virus


Beasley, D. W., Suderman, M. T., Holbrook, M. R., and Barrett, A. D. 2001. Nucleotide sequencing and serological evidence that the recently recognized deer tick virus is a genotype of Powassan virus. Virus Res. 79, 81B.


burgdorferi in Neotoma mexicana and Ixodes spinipalpis from northern Colorado, an area where Lyme disease is nonendemic. J. Infect. Dis. 170, 636□□□643.


Neurological Dengue


Influenza Virus and CNS Infections


Ichiyama, T., Morishima, T., Isumi, H., Matsufuji, H., Matsubara, T., and Furukawa, S. (2004). Analysis of cytokine levels and NF-kappaB activation in peripheral blood mononuclear...


Dawson, J. R. 1933. Cellular inclusions in cerebral lesions of lethargic encephalitis. Am. J. Pathol. 9, 7E–3E.


Erlenhofer, C., Wurzer, W. J., Loffler, S., Schneider-Schaulies, S., Ter, M. V., and Schneider-Schaulies, J. 2001. CD150 (SLAM) is a receptor for measles virus but is not involved in viral contact-mediated proliferation inhibition. J. Virol. 75, 4499E–4505E.


neurovirulence in humans. J. Virol. 74, 5382-5384.


Rabies Virus Neurovirulence


Healy, D. M. 2011. Comparative Pathology of Lyssaviruses in a Murine Model, School of Medicine, Dentistry and Biomedical Sciences, Centre for Infection and Immunity. Queen’s University, Belfast, p. 261.


Rubella Virus Infections


AP-HP, Gynecology-Obstetric Unit, Antoine B□□cl□□re Hospital, Clamart, France; UMR-S0782, Paris-Sud University, Clamart, France.


**Human T-Lymphotrophic Virus**


Infect. Dis. 7, 266□□□281.

Human Immunodeficiency Virus Neuropathogenesis


