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10

Encephalitic Development in Alphaviral Infection

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1. Introduction

Viruses are currently the most common cause of encephalitis. With more than 20 viruses known to cause human encephalitis, arboviruses, arthropod-borne viruses, represent a significant number of emerging infectious diseases both in the United States and world-wide. 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). WEEV and EEEV are endemic to the United States and South America while VEEV circulates in Central and South America; however, spread of epidemic outbreaks has resulted in disease in North America. No specific therapy or vaccine is currently available against these viruses. In this review, we summarize the development and progression of alphavirus encephalitis in both human populations and murine models of alphavirus infection. We concentrate on the host response that characterizes the development of central nervous system (CNS) disease following alphaviral infection. In addition, we focus on immune factors that influence successful resolution of infection.

Alphaviruses represent a significant public health threat as both emerging infectious diseases and possible agents of bioterrorism. Natural, endemic infections of alphavirus disease are currently absent in certain areas of the globe where the mosquito vector capable of transmitting alphaviruses exists making potential virus introduction into such unaffected areas a significant public health concern (Calisher, 1994; Hawley & Eitzen, 2001; Weaver & Barrett, 2004; Zacks & Paessler, 2010). Additionally, many of the encephalitic alphaviruses are highly stable as an aerosol, cause significant mortality or incapacitating disease, and grow rapidly in easily utilized, readily available cell culture systems. This combination of factors makes this group of viruses of significant interest for biodefense in the United States (Hawley & Eitzen, 2001; Weaver, 2005).

One of three of the original serological groups of arboviruses, the group A complex became the *Alphavirus* genus within the *Togaviridae* family representing enveloped, plus-strand RNA viruses (Porterfield, 1975; Porterfield, 1986). Pathogenic alphaviruses can be roughly grouped further by a combination of phylogenetics, geographical circulation, and disease manifestation into two groups. Viruses circulating in the Old World (Europe, Asia, and Africa) typically cause arthralgia, malaise or a rash. (Griffin, 2007; Zacks & Paessler, 2010). New World alphaviruses' infections result in a flu-like syndrome that may progress to neurological involvement. New World encephalitic alphaviruses include EEE, WEE, and

VEE complex viruses. Of these the primary causes of neurological involvement and lethal encephalitis are WEE, EEE, and VEE viruses. More rarely, Highlands J virus, a member of the WEE antigenic complex, and some Old World alphaviruses may be neuroinvasive (Zacks & Paessler, 2010).

Neurovirulence of the viruses associates with efficient and rapid spread of virus throughout the neurons of the central nervous system (CNS) resulting in pathogenesis and death of neuronal cells; however, the host response also represents significant determinant of pathogenesis and mortality (Griffin, 2007; Ryman & Klimstra, 2008). This chapter will briefly describe basic viral dissemination and spread in natural cycles, both enzootic and epizootic, followed by a more in-depth overview of the primary encephalitic alphavirus, EEE, WEE, and VEE encompassing the description of disease in animal and man with an emphasis on the host response.

2. Overview of encephalitic alphaviruses

2.1 History and Medical Importance

Records of fatal encephalitis in horses in the northeastern United States date to the 19th century and reports of *peste loca* in South America exist back further though etiology of these infections is uncertain (Groot, 1971; Hanson, 1957). Throughout the early 20th century, sporadic outbreaks of encephalitis in horses continued to occur along the Atlantic seaboard of the United States as well as in regions of South America (Groot, 1971; Sabattini, et al., 1985). In 1930, WEEV became the first of the encephalitic alphaviruses isolated from the brain of an infected equine, and isolation of EEEV closely followed(Meyer, et al., 1931; Tenbroeck, et al., 1935; Tenbroeck & Merrill, 1933). In 1936, an outbreak of equine disease in Venezuela similar to that found in conjunction with EEE and WEE presented isolates not neutralized by either EEE or WEE antisera. VEEV was subsequently discovered as a causative pathogen of equine disease (Kubes & Rios, 1939a; 1939b). Isolation of virus from human cases of encephalitis associated with epizootic outbreaks followed for all three of the viruses (Casals, et al., 1943; Feemster, 1938; Howitt, 1938).

The largely summer occurrence of epidemic disease and disappearance in fall and winter months suggested a mosquito vector and Kelser subsequently demonstrated transmission of WEE virus by mosquitoes (Kelser, 1937). Since that time the EEE, WEE, and VEE viruses have been isolated from mosquitoes, horses, humans, and other vertebrate species, namely birds and rodents (Griffin, 2007). A more detailed history of each virus will be presenting in the sections below.

2.2 Molecular biology of the *Alphavirus* genus

The virions of the encephalitic alphaviruses contain a single-stranded, positive sense RNA genome composed of 5′ non structural proteins (NsP) and 3′ structural proteins totalling 12 kilobases. The first two-thirds of the genome encode non-structural proteins NsP1, NsP2, NsP3 and NsP4 (Frolov, 2004; Griffin, 2007). The structural proteins are encoded in the 3′ third of the genome and translated from a 26S subgenomic promoter resulting in production of the five protein products, the capsid (C), and envelope proteins 1-3 (E1-E3) and 6K (Griffin, 2007). The structural proteins are considered the primary targets for adaptive, antigen specific immunity, both humoral and cell-mediated, and are also capable of interfering with host defense mechanisms (Aguilar, et al., 2008a; Simmons, et al., 2009). A more detailed description of specific viruses will be included in the sections below.

2.3 Transmission cycles

Alphaviruses circulate in enzootic and epizootic cycles between hematophagous arthropods and vertebrate animals. The nature of the vertebrate host determines the maintenance of virus in epizootic or enzootic cycles. As such, vertebrate hosts can be loosely divided into two groups: the natural vertebrate host acting as the primary source of mosquito infection in which the virus replicates, but no symptomatic disease presents and vertebrates that develop symptomatic disease where ability to transmit the virus back to mosquito populations varies based on strain and infected host species. For the encephalitic alphaviruses, the primary host is typically murine or avian (Calisher, 1994). In epizootic or epidemic cycles, infected mosquitoes transmit the virus to a secondary host, usually man or equine. Large outbreaks of disease occur when infected secondary hosts act to amplify viral replication and transmission generating a high titer viremia permitting further infection of mosquito, and, subsequently, host populations. The ability of the secondary host to amplify the virus furthering epidemic spread varies based on virus and host, and is detailed specifically below (Weaver, 2005; Weaver & Barrett, 2004; Zacks & Paessler, 2010). Epizootic outbreaks in equines frequently signal the beginning of epidemic spread of virus followed by subsequent disease in human populations.

2.4 Virus spread

Due to the nature of infection and prevalence of asymptomatic infections, much of what is known about the virus spread has been determined utilizing murine models. As the mosquito feeds on the experimental host, virus is deposited in infected saliva extravascularly (Turell, et al., 1995). Virus initially replicates at the site of inoculation, typically skeletal muscle or immune cells, and dissemination to dendritic cells or Langerhans cells of the skin closely follows (Grimley & Friedman, 1970; Johnston, et al., 2000; Liu, et al., 1970; Murphy & Whitfield, 1970). As these infected cell populations migrate to the draining lymph nodes (DLN) for the site of inoculation, the virus spreads through the blood to other skeletal muscles and through lymph and blood to lymphatic tissue. As the virus continues replication, spread occurs to secondary sites of replication which include the brain and spinal cord neurons (Griffin, 2007). While tropism of other cells of the CNS apart from neurons is uncertain, alphaviruses may be capable of replication in the meningeal cells, ependymal cells, and other glial cell populations (Ehrengruber, et al., 2003; Mims, et al., 1973; Schoneboom, et al., 1999a).

2.5 Pathogenic sequence of alphaviral infection in humans

Upon virus infection the disease progression in humans typically follows a biphasic disease process characterized by a mild, flu-like illness potentially associated with replication at the primary site of infection, viremia, and initiation of the immune response (Calisher, 1994). Length of acute disease varies with virus, but patients typically improve slightly following initial illness then develop a second febrile episode congruent with dissemination of virus to target tissues and development of an adaptive immune response (Brown, 1993; CFSPH, 2008; Zacks & Paessler, 2010). During this second phase, a subset of patients progress to neurological involvement associated with mortality. Typical lesions in fatal cases appear as severe inflammation of the gray matter with neuronal degeneration, infiltration of inflammatory cells, gliosis, perivascular cuffing and hemorrhages (Griffin, 2007). Despite the close phylogenetic relation of VEE, WEE and EEE complex viruses, the genotypes vary significantly in disease, mortality rates, severity of sequelae, and location and pattern of

lesions in the brain as detailed below for individual viruses (Luers, et al., 2005; Steele & Twenhafel, 2010; Weaver & Barrett, 2004).

2.6 Host response

Again, as with viral transmission most of what is known regarding the host response has been determined in small animal and non-human primate (NHP) models. The host response has been primarily characterized by the early production of type I interferon (IFN) and the later production of virus-specific neutralizing antibody (Adler & Rabinowitz, 1973; Grieder & Vogel, 1999; Houston, et al., 1977; Konopka, et al., 2009; Schoneboom, et al., 2000d). This set of viruses is sensitive to the effects of the type I IFNs, IFN-α and IFN-β, and, as a result, have developed evasive mechanisms of interference with type I IFN signaling. Thus, the ability to resist type I IFN is considered a factor for the level of virulence of WEE, EEE, and VEE virus strains, and mutations altering type I IFN resistance result in attenuation of the virus. Early control of viral replication is dependent on type I IFN production and the absence of type I IFN signaling is typically fatal in murine models (Aguilar, et al., 2008a; Aguilar, et al., 2005; Frolov, 2004; Grieder & Vogel, 1999; Jahrling, et al., 1976; Spotts, et al., 1998). Conversely, prophylactic treatment with type I IFN or artificial induction of type I IFN prior to infection provides protection or extended time to death in murine models (Julander, et al., 2008a; Julander, et al., 2007; Julander, et al., 2008b). Viral load in target organs and viremia typically correlates with amount of IFN produced (Ryman, 2008).

Interestingly, other models of CNS pathogenesis with no viral etiology demonstrate a role for type I IFN in an anti-inflammatory capacity capable of modulating the early, acute response and altering the adaptive immune response in the CNS (Galligan, et al., 2010; Plosker, 2011). The overwhelming pro-inflammatory cytokine response associated with initiation of the immune response to the encephalitic alphaviruses has been loosely linked to neuropathogenesis apart from viral load and replication in neuronal cell populations (Dikii, et al., 1976; Koterski, et al., 2007; Mokhtarian, et al., 1996; Phillpotts, et al., 2003; Ryman, 2008; Schoneboom, et al., 2000b; Valerol, et al., 2008). Thus, the pleiotropic nature of type I IFN may induce a more forgiving environment in the CNS, reducing levels of proinflammatory cytokines, altering the adaptive response, and buffering the microenviroment of the CNS during alphaviral infection (Plosker, 2011). However, other effects of type I IFN in the CNS pathologies of alphavirus infection remain to be explored.

Resolution of infection and disease has focused on the neutralizing antibody response to infection. Antibody is undoubtedly significant in recovery from peripheral infection and in blocking neuroinvasion (Bedenice, et al., 2009; Burke, et al., 1977; Calisher, et al., 1986a; DeMeio, et al., 1979). However, its effectiveness once neuroinvasion occurs is not well understood. Peripheral passive transfer of antibody has little effect following neuroinvasion; however, production of antibody from B-cells resident in the CNS may impact course of neurological disease though little is known about the presence or role of B-cells in the CNS microenviroment (Yun, et al., 2009). More recently, T-cells, particularly CD4+ T-cells, have been implicated in resolution of CNS infection with these neurotropic viruses (Brooke, et al., 2010; Paessler, et al., 2007; Yun, et al., 2009). Additionally, the early production of TNF-alpha, IL-1, and IL-6 indicates a T-helper 1 (Th1) bias to the adaptive immune response following alphavirus infection (Valerol, et al., 2008). The Th1 response is characterized by predominately IFN-γ producing CD4+ T-cells (Brooke, et al., 2010; Yun, et al., 2009). As a corollary, IFN-γ secretion associated with a Th1 response may be partially protective following entry of virus to the CNS (Paessler, et al., 2007).

3. Western equine encephalitis

3.1 History and significance

Initially isolated from the brain of an infected equine in 1930 in California, WEE virus was the first of the encephalitic alphaviruses to be discovered(Meyer, et al., 1931). However, the virus was not recognized as a cause of human illness till isolation from the brain of an infected child in 1938 (Howitt, 1938). According to the United States Center for Disease Control (CDC) and the Center for Food Security and Public Health (CFSPH), 640 confirmed or probable human cases of WEE occurred between 1964 and 2009 in the United States alone (CDC, 2009b; CFSPH, 2008). Overall case fatality rates are estimated at 3-7%, but mortality rates depend on both age and other factors such as virus strain, dose, and route of infection (Calisher, 1994; CFSPH, 2008; Hanson, et al., 1967; Ryzhikov, et al., 1991; Steele, et al., 1998; Steele & Twenhafel, 2010; Zacks & Paessler, 2010). Accidental, artificial exposure of aerosolized virus in laboratory settings has been documented with the few cases demonstrating a 40% fatality rate (Fothergill, et al., 1939; Gold & Hampil, 1942; Hanson, et al., 1967; Helwig, 1940). As with all the encephalitic alphaviruses, higher mortality rates and incidence of neurological complications are associated with pediatric patients. For WEE, an estimated 90% of children under one year of age show severe CNS signs; additionally, an estimated 15-30% of pediatric and adult patients that survive neurological involvement are left with severe, permanent sequelae (Zacks & Paessler, 2010). Children older than one year of age show a steep increase in numbers of asymptomatic infections (Bruyn & Lennette, 1953; Cohen, et al., 1953). Transplacental transmission has been documented with all know instances surviving acute encephalitis, but developing subsequent neurological sequelae (Bruyn & Lennette, 1953; CFSPH, 2008; Copps & Giddings, 1959; Romero & Newland, 2006; Shinefield & Townsend, 1953). Thus, while overall mortality rates due to WEE infection are low, sequelae are significant and costly particularly in pediatric patients (CFSPH, 2008). A steady decline in cases since the middle of the 20th century has been partially attributed to changes in the natural habitat for the common mosquito vector due to altered irrigation practices and mosquito control programs though the true cause of decline remains in question (Calisher, 1994; Forrester, et al., 2008).

3.2 Transmission cycles and geographic range

WEE acts over a broad geographic range throughout North and South America as an equine pathogen; however, despite the isolation of WEE strains throughout the Americas, human disease appears less frequently and with decreased severity in South America (Calisher, 1994; Sulkin, 1946; Tsai, 1991). Neither equines or humans act as amplifying hosts. Strains of WEE virus have been isolated from humans, horses, wild birds, and vertebrates from western Canada to Argentina (Calisher, et al., 1985; Reisen, 1988). Specifically, infection of bobwhite quails, house finches, sparrows, and domestic chickens has been demonstrated (Hardy, 1987; O'Brien, et al.,; Watts & Williams, 1972; Williams, et al., 1971). The virus can also infect some small mammals including opossum, squirrels, bats, rabbits, and hares. Like horses and humans, a number of these species exhibit a tangential infection and do not act as amplifying hosts (CFSPH, 2008; Fastier, 1952; Hardy, 1987; Kiorpes & Yuill, 1975; Reisen, et al., 2004; Ubico & McLean, 1995; Yuill & Hanson, 1964) Isolations of WEE viruses have been reported in the Veracruz region of Mexico and South America and the genetic analyses of isolates from Brazil and northern Argentina revealed a level of nucleotide identity >90% in the E2/6K/E1 coding region when compared to isolates from the US. This data suggests a monophyletic nature of the WEEV lineage, with an overall slow evolution (Weaver, 2005; Weaver & Barrett, 2004; Zacks & Paessler, 2010)

WEE is maintained in an enzootic cycle between a number of passerine birds and its most common mosquito vector, *Culex;* however, the virus has also been isolated from mites (Hammon & Reeves, 1946; Hammon, et al., 1941; Hammon, et al., 1943; Reeves, et al., 1947; Zacks & Paessler, 2010). The avian host is likely responsible for the distribution of virus strains from North to South America, and explains the widespread geographic range of the virus (Reisen 2010, Calisher 1994). Transmission to humans and horses occurs via bridging mosquito vector species whose habitat and nature results in feeding on these specific vertebrate hosts (Calisher, 1994; Zacks & Paessler, 2010). Bridging vectors include *Ochlerotatus melanimon* in California, *Aedes dorsalis* in Utah and New Mexcio and *Ae. Campestris* in New Mexico (Clark, et al., 1986; Reisen, et al., 1998; Zacks & Paessler, 2010). In temperate climates, the transmission cycle may be maintained through the year, but in less mild climates WEE may either overwinter in unidentified hosts or be reintroduced annually through passerine bird. Isolates of WEE have been obtained from some amphibious species, garter snakes and leopard frogs, indicating that WEE may be able to overwinter in such hosts (Burton, et al., 1966; Gebhardt, et al., 1966; Gebhardt, et al., 1964). Transovarial transmission of the virus in mosquitoes has also been proposed as an overwintering mechanism. However, current literature on the subject is inconclusive (Reeves, et al., 1954; Thomas & Eklund, 1960; Watts & Eldridge, 1975).

3.3 Disease manifestations in humans

WEEV infections tend to have more in common with EEEV infections than with VEEV though infection is associated with less severe disease and lower mortality than EEE. As with both other viruses, WEE infections predominately are either asymptomatic or cause mild disease. Disease appears after a short incubation period of two to seven days and associates with general flu like symptoms and often times an altered mental status (CFSPH, 2008; Hoke, 2005; Zacks & Paessler, 2010). Typical recovery begins after approximately ten days of symptomatic illness (Calisher, 1994). When lethal disease occurs, mortality happens within seven days of symptomatic disease . A minority of infected individual will develop encephalitis and associated neurological symptoms: neck stiffness, confusion, tonic-clonic seizures, somnolence, coma and death (CFSPH, 2008; Steele & Twenhafel, 2010; Zacks & Paessler, 2010). Abnormal reflex responses in adults depend largely on stage of illness and typically consist of weakness and hyporeflexia. Disease progression is slower in adults than in infants which develop sudden onset of illness with fever and seizures. Both adolescents and older children have a prodromal phase typified by severe headaches and fever of two to three days followed by CNS manifestations. Unsurprisingly given the higher mortality rates, children display far more severe neurological symptoms with muscular rigidity, involuntary movements, and paralysis (Calisher, 1994; Steele & Twenhafel, 2010).

Cerebral spinal fluid (CSF) pressure may be mildly elevated with normal glucose, protein and elevated leukocyte counts (<500/mm^3) (Calisher, 1994; Rozdilsky, et al., 1968). While typical CSF white blood cell counts are in this range, they have been reported to be as high as 2000/mm^3, (Romero & Newland, 2003; 2006). Early infection is characterized by polymorphonuclear cells with mononuclear cells dominating later in infection (Calisher, 1994). A computed tomography scan (CT) of one case of neonatal WEE revealed multiple, symmetrical, calcifications in both hemispheres in both the insular cortex and the thalamus as well as dilatation of the temporal ventricles and compression of the peritroncal and sylvian cisterns. Electroencephalogram (EEG) showed diffuse cerebral suffering mimicking pathologies similar to HSV-1 encephalitis (Delfraro, et al., 2011; Romero & Newland, 2003; Somekh, et al., 1991).

Gross pathologies are limited to the CNS in lethal infections and do not appear in the periphery (Calisher, 1994; Steele & Twenhafel, 2010). The brain is typically edematous, but pathologies are primarily characterized by focal hemorrhages. These hemorrhages are observed in the white and gray matter throughout the brain though the basal nuclei, thalamus, and brain stem are the most often severely affected. Lesions also appear in the spinal cord (Steele & Twenhafel, 2010; Zacks & Paessler, 2010).
Histopathologically, the brains of infected individuals demonstrate wide spread perivascular cuffs of lymphocytes and neutrophils. Additionally, smaller areas of focal necrosis with and without cellular infiltrate are found throughout the brain in the striatum, globus pallidus, susbtantia nigra, cerebral cortex, thalamus, and pons. A subset of lethally infected patients displayed predominant concentrations of brain lesions in the subcortical white matter (Anderson, 1984; Rozdilsky, et al., 1968).

3.4 Disease manifestations in experimental models

WEE has not been extensively studied in animal models. While limited and often not precisely representative of human infection, the span of studies in small animal models and non-human primates (NHP) resulted in a general idea of the pathologies and host response to WEE. Animal models used include mice, hamsters, cynomolgus macaques, gerbils, guinea pigs and rabbits (Hayles, 1972; Hayles, et al., 1970; Hayles, et al., 1972; Holbrook & Gowen, 2008; Hurst, 1934; Reed, et al., 2005).

3.4.1 Mouse model

Like in humans, age and strain of mouse infected, route of inoculations, and virulence of virus strain all influence the susceptibility of the host (Aguilar, 1970; Bianchi, et al., 1993; Logue, et al., 2009; Monath, et al., 1978; Nagata, et al., 2006). Peripheral routes of infection result in approximately 50% mortality with intracerebral (IC) or intranasal (IN) challenge resulting in more uniform, dose-dependent mortality depending on strain of virus and background of murine model (Hardy, et al., 1997; Julander, et al., 2007; Liu, et al., 1970).
In suckling mice, subcutaneous (SC) inoculation results in rapid, acute disease with uniform mortality by 48 hours post-infection (PI). Interestingly, in infant animals, mice developed pathologies largely in mesodermally derived tissues, and not in the CNS or other peripheral organs such as the lung, liver, or heart. Histologically, these mice displayed necrotic degeneration of the bone marrow. Despite showing no clinical signs of illness, young adult mice (three to four weeks) develop widespread viral encephomeningitis associated with focal necrosis and perivascular cuffs similar to human disease. Additional pathological changes were noted in the lung, liver, kidney and brown fat though no clinical evidence of disease was present throughout disease course indicating the resilience of the host in the presence of significant CNS pathologies. (Aguilar, 1970). This age group of mice also develop myocardial infection and necrosis as well as peripheral lesions in the heart (Monath, et al., 1978). Thus, tissue tropism and susceptibility to WEE vary with age of the animal.
Comparison of WEEV isolates McMillan (McM) vs. Imperial 181(IMP) in outbred CD1 mice, demonstrated changes time to death and mortality rates varying by route of infection and strain of virus used though both strains are neuroinvasive. More virulent McM causes 100% mortality within five days following SC injection. In contrast, IMP causes no death in infected animals. A similar trend was noted for aerosol, IN and intravenous routes (IV). Thus, while both strains are neuroinvasive, only McM is also neurovirulent. McM when

administered IN also causes extensive damage to the neurons of the CNS. The alterations in pathogenicity between the two strains are possible attributed to either slower replication of the attenuated strain or changes in the response to the innate immune system (Logue, et al., 2009). Accumulated data from EEE and VEE indicate that alterations in response of the host and susceptibility of the virus to the immune response are a strong determinant of outcome.

3.4.2 Hamster and guinea pig models

Virulent strains of WEE administered by multiple routes demonstrated lethal disease in hamster models. Hamsters have been postulated as a working model for WEE disease due to high mortality observed and lesions of encephalitis found following infection. Characteristic pathologies associate with lethality include tachypnea, conjunctivitis, incoordination, and seizures (Zlotnik, et al., 1972). Both IC and peripheral infection leads to high viral load in the brain and severe neurological disease characterized by neuronal necrosis and infiltration of the meninges and Virchow-Robins space by lymphocytes (Holbrook & Gowen, 2008; Julander, et al., 2007; Zlotnik, et al., 1972). These pathologies are diffuse throughout the brain with olfactory regions begin particularly damaged. Despite the wide-spread nature of CNS pathologies, groups of neurons from the cerebral cortex and in areas of the mid-brain are typically infected with WEE indicative of bystander activation and killing of nearby cells resulting in disseminated pathologies (Julander, et al., 2007). Astrocytes do not appear to be susceptible to infection *in vivo* (Julander, et al., 2007). Guinea pigs demonstrate a severe disease similar to hamster models with death occurring in less than ten days following infection (Bianchi, et al., 1997; Nalca, et al., 2003a).

3.4.3 Nonhuman primate model

WEE causes lethal encephalitis in non-human primates: cynomolgus macaques and rhesus monkeys (Macaca mulatta) (Hurst, 1936; London, et al., 1982; Reed, et al., 2005; Wyckoff & Tesar, 1939). IC and IN challenge with WEE results in severe lethal encephalitis in NHP with IC infection resulting in uniform fatalities and IN or aerosol challenge resulting in severe CNS infection with some associated mortality. Peripheral routes of challenge including endermally, intramuscularly (IM) or IV do not result in consistent CNS infection (Hurst, 1936; Wyckoff & Tesar, 1939).

Following aerosol inoculation, cynomolgus macaques develop a fever between four and five days and show symptomatic disease including reduced appetite, decreased behavior, and tremors. Leukocytosis, serum glucose levels, and fever associated with severity of disease though none accurately predict survival vs. mortality. Viral antigen was only detected in the CNS and not the periphery of lethally infected animals possibly attributed to the later time point of collection following clearance of virus from the periphery by neutralizing, serum antibody. Antigen appeared in the CNS in the neurons, microglia and Purkinje cells. However, virus was focused in areas of inflammation and demyelination. Interestingly, virus was not detectable in the blood or by throat swab during the course of infection suggestive of entry of WEE to the CNS through the olfactory nerves (Reed, et al., 2005). Thus, aerosol inoculation of WEEV in NHPs appears to be limited to the brain.

Gross findings were also limited to the CNS and consisted of meningeal congestion with histological lesions characterized by meningoencephalitis. Microscopic changes included alteration of the Virchow-Robins spaces of the brain and some spinal cord sections by infiltrating lymphocytes and monocytes, as well as foci of necrosis containing lymphocytes, microglia, and some neutrophils, discrete glial nodules and areas of neuronal demyelination

(Reed, et al., 2005; Wyckoff, 1939). These studies combined with clinical findings in man indicate that aerosolized WEEV is infectious through the olfactory neurons, and likely does not affect the peripheral organs.

3.5 Host response in experimental models

Due to the limitations of WEE studies and lack of clinical cases, little is known about the pathogenic and protective mechanisms mediating WEE in natural infections, and experimental models are responsible for most of the current knowledge of WEE pathogenesis and immunology. This section details the innate immune response to WEE and related therapeutics followed by knowledge of the adaptive immune response and effective vaccination measures to induce a useful memory response.

3.5.1 Innate immune response to WEE

The innate antiviral immune response is a crucial mechanism to control viral replication prior to induction of an adaptive immune response. In addition, the early immune response also molds the quality and phenotype of later immune responses and may be critical in control of pro-inflammatory pathogenic mechanisms following infection. Unfortunately, little is known about innate immune mechanisms important in alphavirus infection apart from a role for type I IFN Like other alphaviruses, WEE is sensitive to the effects of IFN-α and IFN-β. Recombination events that lead to the formation of the WEE from SIN- and EEE-like ancestors was essential for the virus to gain the ability to shut off transcription of anti-viral IFN factors, and likely lead to the emergence of WEE as a pathogenic virus (Garmashova, et al., 2007a; Garmashova, et al., 2007b; Weaver, et al., 1997). *In vitro* examination of human neuronal cells response to WEE indicates that virus cytopathology is reduced depending on maturation state of neurons in a manner independent of autocrine type I IFN activity and may explain the age related susceptibility to WEE infection. Additionally, mature neuronal cells were more sensitive with five to ten-fold less type I IFN needed to reduce viral cytopathology and replication. Type I IFN treatment is unable to completely eliminate WEEV cytopathogenicity in neuronal cells lines indicating factors apart for type I IFN may be important in control of viral replication (Castorena, et al., 2008).

Pre-treatment of hamsters IP with either a consensus type IFN-α , or with Ampligen®, a stimulator of Toll-like receptor 3 and subsequently type I IFN expression, resulted in complete survival compared to 100% lethality in controls (Julander 2007). Additionally, surviving animals treated with IFN were less symptomatic and lost less weight than controls. Both compounds acted as effective anti-virals significantly reducing the viral load in the brain at four days PI (Julander, et al., 2007).

A single dose of adenovirus-construct expressing transient INF-α (Ad5-mIFN-α) is capable of prophylactic and some therapeutic protection in mice against lethal, intranasal WEE challenge. The construct when administered 24 h, 48 h, or 7 d prior to infection provides 100% protection and when given 6 h PI provides 60% protection associated with delayed progression of disease. Clinical signs of infection were decreased in animals receiving the Ad5-mIFN-α. When animals received the vectored cytokine at 13 weeks prior to infection, survival rates were reduced to 38% and delayed time course of infection compared to sham treated controls. These animals lacked observable IFN in serum. The authors hypothesize that the discrepancy between serum IFN and protection could be due to continued higher levels of IFN in the brain providing protection following decrease of serum IFN (Wu, et al.,

2007b). Thus, when given prior to infection, IFN-α treatment provides rapid, lasting protection, and is indicative of the importance of a rapid, strong innate immune response.

3.5.2 Adaptive immune response

Much of what is known about the adaptive immune response to WEEV is derived utilizing models of pre-existing immunity, particularly regarding the antibody response. Currently, little is known about other mechanisms of cell mediated immunity.

3.5.2.1 Antibody response

Vaccination studies in experimental models provide the main basis of what is currently known about immunoprotection to CNS infection with WEE. Early studies in the 1970's on the efficacy of formalin inactivated WEE vaccine in humans demonstrated immunogenicity following a series of two vaccinations. Recipients developed high levels of serum neutralizing antibody 28 days following final vaccination. High levels of neutralizing antibody were present through day 360 with the exception of one recipient (Bartelloni, et al., 1971). Currently, a similar formalin inactivated vaccine is available under investigational new drug (IND) status for vaccination of at risk laboratory workers and personnel (Hoke, 2005). The poor immunogenicity of the inactivated vaccine however requires three immunizations and annual boosts to be effective (Hoke, 2005; Smith, et al., 1997)

Live-attenuated vaccine candidates generate rapid protection associated with high levels of neutralizing antibody in animal models (Atasheva, et al., 2009; Schoepp, et al., 2002). DNA vaccination is able to completely protect mice against WEE; however, as with the inactive vaccine, three immunizations are required to achieve high levels of protection (Nagata, et al., 2005). A single dose of adenovirus vectored WEE vaccine protects against intranasal challenge of WEE, but use of adenovirus vectors is associated with significant risks (Barabe, et al., 2007; Wu, et al., 2007a). Chimeric alphavirus vaccine candidates combining the non-structural proteins of one alphavirus with the structural proteins of WEE provide protection in mice from IN challenge with WEE. Vaccination studies indicate that high level, long-lasting neutralizing antibody is capable of protecting mice from a variety of routes of WEE infection (Atasheva, et al., 2009).

Early studies focused on antibody and complement. Yoshino, et al. showed both early and late forming antibodies demonstrated enhanced virus neutralizing activity when in the presence of complement (Yoshino, et al., 1971). Jahrling et al. demonstrated that immune elimination from immunized hamsters involved formation of virus/antibody aggregates cleared by the reticuloendothelial system. Virulent strains of WEE were cleared slowly from the circulation of non-immune hamsters, but were rapidly cleared when inoculated into immunized hamsters. Mixtures of virus and specific immune sera and from inoculated animals formed aggregates of virus and antibody. Further studies showing more efficient adsorption of WEE by macrophages in the presence of complement led the group to hypothesize that immune clearance of the virus in the intact hamster involves a complement dependent interaction of virus/antibody complexes with cells with Fc and complement receptors (Jahrling, et al., 1983). Thus, the role of antibody dependent cytotoxicity of infected cells remains unclear based on these early studies. However, antibody is well-established as an important factor in the clearance of virus.

Furth studies utilizing hyperimmune sera clarified the role of antibody production in the periphery. Hyperimmune sera from rabbits or equines was experimentally tested and appeared to be efficacious in certain scenarios, again indicating the importance of a robust

antibody response in limiting viral pathologies (De Boer, et al., 1955). Hyperimmune horse sera administered to rhesus monkeys at time of development of symptomatic disease had no effect on disease course following IN infection. However, prophylactic or early (within 24 h) administration provided passive protection, but not sterilizing immunity(Wyckoff & Tesar, 1939). Some mouse monoclonal antibodies were shown some of these antibodies were shown to provide protection from infection following passive transfer of antibody (Long, et al., 2000; Long, et al., 2001). Mice were protected from challenge with WEE when injected with antibodies against E1 and E2 in passive transfer studies (Hunt & Roehrig, 1985; Yamamoto, 1986). Administration of an *E. coli* expressed E2 protein into mice demonstrated that immunization of this structural component of E2 could partially protect mice from lethal challenge with WEE, and was associated with a strong cell mediated immune response (Das, et al., 2007). The E1 protein has also been successfully developed as a vaccine target. Delivery of both E1 and E2 proteins by an adenovirus delivery vector offer complete protection against lethal challenge from WEE. Mice given a single dose of vectored E1 alone were also completely protected (Swayze, et al., 2010). The B-cell antibody mediated response and the T-cell responses are intertwined and quite likely both play significant roles in the mediation of infection. However, their specific place following infection of the CNS remains.

3.5.2.2 Other cell-mediated immune responses

In an early paper published in the late 1980's on the Th immune response, stimulation of both primed T-helper cells and B-cells resulted in a virus specific proliferative responses against the immunizing virus. Proliferating T cells were primarily IL-2 secreting Thy-I positive, Lyt-l positive, Lyt-2 negative, and L3T4 positive. This study indicated a Th1 bias to the immune response as well as suggesting highly specific WEE T-cell epitopes mediated the generation humoral immunity and neutralizing antibody. (Mathews & Roehrig, 1989).
Prophylactic administration of cationic liposome-DNA complexes in CD-1 outbred mice challenged with WEEV-McM by either SC or aerosol routes results in significant protection. Protection is associated with increased serum and brain levels of IFN-gamma, TNF-alpha, and IL-12 as well as increases in MCP-1 and IL-10 in the brain after neuroinvasion. Thus, a strong-non specific activation of the innate immune system with a distinct Th1 bias elicits protection in the absence of pre-exisiting memory response (Logue, et al., 2010). Administration of an E1 adenovirus vectored antibody also induced a strong WEE specific T-cell response associated with increased secretion of IFN-γ (Swayze, et al., 2010). The current literature indicates that a predominate Th1 response characterizes the adaptive immune response; however, much work remains to determine specific phenotypes of T-cell populations and to further corroborate the direct and indirect contributions of both antibody and T-cells.

4. Eastern equine encephalitis

4.1 History and significance

EEE is considers the most severe of the arboviral encephalids in North America with mortality from encephalitis in epidemics ranging from 30-75% (CFSPH, 2008; Zacks & Paessler, 2010). Even in the absence of immediate mortality, CNS infections typically result in death following recovery from acute disease with initial mortality rates of 74% progressing to 90% by 9 years after infection with only 3% recovering completely. Intitial survival following CNS symptoms leaves most patients with disabling sequela and

neurological impairment. However, in the absence of neurological symptoms, infections typically resolve within one to two weeks (Morris, 1988).

In the US and Canada, a steady decline in number of cases since 1955 has occurred (Zacks & Paessler, 2010). From 1964 to 2009, an estimated 260 cases of EEE occurred in the United States with an average of four to five cases yearly though the number can vary markedly from year to year. Additionally, only 83 cases were reported from 1998 to 2009 (CDC, 2009a). As with the other encephalids, risk of symptomatic EEE infection with neurological complications is higher in children, particularly infants (<1 year of age), and the elderly (Calisher, 1994; CFSPH, 2008; Zacks & Paessler, 2010). Early reports indicated symptomatic cases are primarily pediatric with a large portion (42%) being younger than one year of age (Feemster, 1938; 1957; Feemster & Haymaker, 1958). However as with WEE, decline in EEE indicative of changes in mosquito control practices and living conditions has altered the phenotype of cases and later reports indicate overall pediatric cases likely account for 20% of reported cases (Deresiewicz, et al., 1997; Przelomski, et al., 1988; Romero & Newland, 2003). However, the presence of both vector and host populations throughout North America indicated that cases of asymptomatic infection may be underreported (Weaver, 2005).

Given the preceding discovery of WEE, EEE likely existed in natural cycles long before its first isolation in 1933 from the brain of an infected equine (Tenbroeck, 1933). Corroborating this, reports of outbreaks of equine disease similar to EEE exist as early as 1831 (Feemster, 1957). The virus was first isolated from human brain tissue in 1938, and since that time has been attributed to epizootics and as a cause of avian disease (Brown, et al., 1993; Webster & Wright, 1938; Zacks & Paessler, 2010). While EEE was suspected to cause epidemic human disease following isolation, an outbreak involving 38 infants, children and adults formally linked EEE to epidemic disease (Farber, 1940; Feemster & Haymaker, 1958).

4.2 Transmission cycles and geographic range

The virus is found primarily on the Atlantic seaboard, but infections have been noted in the upper Midwest United States and southeastern Canada (Calisher, 1994). The virus has been isolated from birds and other mammals such as equines, sheep, cattle, and deer as well as mosquitoes throughout these regions (Carman, et al., 1995; Gibbs, 1976; McGee, et al., 1992; Schmitt, et al., 2007). Cotton rats and house sparrows have been proposed as other hosts for the virus (Arrigo, et al., 2010). Annual or recurrence of virus in enzoonotic habitats represents typical transmission with extension to other ranges based on the primary avian host range (Calisher, 1994). Overwintering mechanisms have been poorly studied, but vertical transmission from mosquitoes or, as with WEE, reptiles may explain persistence during the winter months (Cupp, et al., 2004; LeDuc, et al., 1975; Morris & Srihongse, 1978). Alternatively, the virus may be reintroduced annually from passerine birds. Horses and humans, as with WEE, are considered dead end hosts, though equines may act as an amplifying host in regions where they are high concentrations of both equines and mosquitoes(CFSPH, 2008; Zacks & Paessler, 2010). *Cs. melanura* mosquitoes are the accepted primary enzootic vector transmitting EEE between passerine birds, but rarely transmit the virus to equines or humans (Andreadis, et al., 1998). The transmission of EEE to humans and equine occurs via other mosquito populations such as *Coquillettidia, Aedes*, and *Culex* due to their more common feeding on mammals (Andreadis, et al., 1998; Armstrong & Andreadis, ; Moncayo, et al., 2000; Vaidyanathan, et al., 1997).

4.3 Disease manifestations in humans

As with WEE, geographic location is indicative of severity of epidemic disease in humans with southern isolates of disease typically causing mild or subclinical symptoms in humans, though outbreaks of equine disease are still associated with the South American strains (Causey, 1968). EEE presents as either a systemic or encephalitic syndrome depending on a number of external factors, but age correlates with prevalence and severity of neurological symptoms (Calisher, 1994; Zacks & Paessler, 2010). Infections are characterized by general malaise, fever and chills. Presentation includes headache of increasing severity, irritability, restlessness drowsiness, anorexia,, vomiting, diarrhea, cyanosis, convulsion, and coma. Antibody is present at time of onset of symptomatic illness, but virus can rarely be isolated from blood or cerebral spinal fluid (CSF) at this time point (Hauser, 1948; Steele & Twenhafel, 2010; Zacks & Paessler, 2010). The incubation period is thought to be somewhat more protracted that WEE, but reports vary and range from five to ten days. Typical systemic illness lasts one to two weeks and recovery is typically complete with no CNS involvement (CFSPH, 2008; Zacks & Paessler, 2010). In the 33% of patients exhibiting neurological symptoms, lethality usually occurs between two to ten days after disease presentation (CFSPH, 2008; Steele & Twenhafel, 2010; Zacks & Paessler, 2010).

In infants, EEE typically results in abrupt encephalitic involvement while in older children and adults the disease is manifested after systemic infection that is often subclinical (Calisher, 1994; Farber, 1940; Morse, et al., 1992; Winter, 1956). Paralysis develops during the acute phase of illness with tremors and muscle twitching proceeding to continuous nuchal rigidity. Young children present with edema and increased CSF pressure with enhanced cellular presence of neutrophils. During encephalitis, fever, headache, vomiting, respiratory symptoms, leukocytosis, hematuria, seizures and coma may occur (Calisher, 1994). Cause of death is typically attributed to encephalitis, but some patients evidence myocardial damage and impairment of pulmonary functions, unique to EEE and differentiating pathogenesis from that of WEE or VEE (Deresiewicz, et al., 1997; Gutierrez & Prober, 1998; Lury & Castillo, 2004; Zacks & Paessler, 2010). Magnetic resonance imaging and CT images of infected brains demonstrate early changes in basal ganglia and thalami suggestive of edema, ischemia, and hypoperfusion following early, symptomatic disease (Deresiewicz, et al., 1997; Gutierrez & Prober, 1998; Lury & Castillo, 2004).

Gross pathological examination of organs reveals brain edema with necrosis, facial and generalized edema, vascular congestion and hemorrhage in the brain and visceral organs. Examination of brain lesions reveals neuronal destruction and vasculitis both perivascular and parenchymous at the forebrain, basal ganglia, cortex, midbrain, and brain stem. Neither cerebellum nor spinal cord are greatly involved in infection (Bastian, et al., 1975; Calisher, 1994; Zacks & Paessler, 2010) Histopathological changes are consistent with vasculitis, hemorrhage, and encephalitis and represent the primary manifestations of encephalitis (Deresiewicz, et al., 1997). Virus appears in oligodendroglia though primary tropism is neuronal (Gardner, et al., 2008; Kim, et al., 1985). Virus can also be isolated from other tissues post mortem.

4.4 Disease manifestations in experimental models

EEE causes disease in a number of animals including mice, guinea pigs, hamsters, NHP and some bird species such as chickens (Adams, et al., 2008; Cole & McKinney, 1969; King, 1940; Liu, et al., 1970; Ogata & Byrne, 1961; Olitsky & Cox, 1936; Steele & Twenhafel, 2010;

Wyckoff & Tesar, 1939). Experimental infections of mice, guinea pigs and rhesus monkeys provide the primary understanding of EEE pathogenesis (Hurst, 1934; King, 1938; 1939; Nathanson, et al., 1969; Olitsky & Cox, 1936; Pursell, et al., 1972; Sorrentino, et al., 1968; Wyckoff & Tesar, 1939).

4.4.1 Mouse model

While mice are an accepted model for many alphaviruses infections, they fail to reproduce the vascular component of disease typical for human infections making, in this case, the hamster model more representative to study acute vasculitis and encephalitis for EEE (Zacks & Paessler, 2010). However, multiple studies have been conducting in mice with valuable information on the pathogenesis of EEE derived from these experimental infections. Mice are susceptible to infection with EEE virus by cutaneous, JN, and IC inoculation as well as aerosol administration. Mice infected with the virus reproduce a typical biphasic disease course exemplified by viral replication the peripheral tissues followed by viremia, CNS invasion, and encephalitis. While infected mice exhibit seizures, they do not exhibit paralysis as seen in humans (Gardner, et al., 2008; Steele & Twenhafel, 2010). Interestingly no differences in mortality between 15 or 21 day and 1 year old mice are observed following IN infection with EEE, unlike the age dependent resistance seen in WEE infections. Infection by a more rigorous IC route ablates these differences in mortality(Olitsky, et al., 1936).

EEE causes lethal encephalitis in suckling and adult Swiss albino mice following IC infection and causes a pantropic infection with encephalitis following SC inoculations (Liu, et al., 1970). EEE infected mice develop minimal clinical signs of febrile illness early after peripheral inoculation, but progress directly and rapidly to neurological disease though severity varies by age of animal, route of infection, and strain of virus used . Following footpad inoculation, neuroinvasion results in rapid, efficient replication in the CNS inducing widespread infection, direct cytopathogenicity of neurons, and severe encephalitis. In young mice, abnormal bone formation characterized by loss of osteoblasts, reduced osteoid production and cartilage hypertrophy is a defining feature (Steele & Twenhafel, 2010; Vogel, et al., 2005). By four days PI, the virus presents in neurons and glial cells throughout the brain with the caudate nucleus, thalamus, and pons being the most strongly infected areas. Major histological changes in the brains of mice are associated with rarefaction of neuropil in the gray matter as well as some white matter tracks and mild inflammation characterized by neutrophil and eosinophil like infiltrates. In the periphery, EEE targets fibroblasts, skeletal muscle, and cardiac myocytes, osteoblasts, ovarian stromals cells, keratinocytes, sebaceous gland epithelium, odontoblasts, and ameloblast in the teeth, retinal ganglion in the eyes and a few olfactory neuroepithelial cells (Steele & Twenhafel, 2010; Vogel, et al., 2005). Lymphocyte apoptosis throughout the thymus and secondary lymphoid tissues is widespread, but is likely not associated with active viral replication due to failure to replicate in these cells *in vitro* situations unlike WEE (Gardner, et al., 2008; Steele & Twenhafel, 2010).

Rather than entry through olfactory routes as has been suggested for WEE and VEE, EEE appears to enter the brain directly from the bloodstream based on very rapid onset of disease (24 h PI), widely dispersed infection in the CNS, and absence of pathology in the olfactory neuroepithelium (Vogel, et al., 2005). Aerosol infection of animals indicated that mice infected with EEE show neuronal tropism and brain lesions similar to peripherally infected mice. In these animals, infection is presented at 24 hours as with peripheral

infection, but only presented in the olfactory bulbs and evidence of infection of the olfactory neuroepithelium suggesting neuroinvasion via direct infection of the neuroepithelium in contrast to peripherally infected animals (Roy, et al., 2009; Steele & Twenhafel, 2010). Thus, route of infection likely plays a significant role in mechanism of CNS entry.

4.4.2 Hamster and guinea pig models

Like mice, EEE also causes lethal encephalitis in hamsters and guinea pigs (Liu, et al., 1970; Morgan, 1941; Morgan, et al., 1942; Murphy & Whitfield, 1970). Hamsters develop biphasic illness like humans when infected with virulent EEE strains, and have the advantage of reproducing the vascular component of disease not present in mouse models (Dremov, et al., 1978; Paessler, et al., 2004).

Peripheral infection results in an early visceral phase, with accompanying virema, then neuroinvasion, and subsequent death caused by encephalitis within four to six days post infection. Fever, appears within 24 hours and clinical signs of neurological systems namely head pressing, stupor and coma follow. However, unlike in mice targeting of the osteoblasts has not been reports. Virus is cleared from blood and peripheral tissues but not the brain following development of neutralizing antibodies(Paessler, et al., 2004).

As with the mouse model, virus appears to be blood-borne as evidence by first appearance of antigen in the neuronal cells of the basal ganglia and brain stem by two days post-infection (Dremov, et al., 1978). This early infection of periventricular and perivascular neuronal cells in the basal ganglia and hippocampus characterize experimental infection and may be due to the hypothesized blood borne nature of the virus, though data is inconclusive (Paessler, et al., 2004). The virus penetrates the brain rapidly and neuronal phase of disease develops quickly with a disseminated virus infection throughout the brain . Though peripheral organ infection develops with early targets including the heart, liver, lungs, kidneys, lymphoid tissues, and skeletal muscles, viral load in the brain is typically much higher (Dremov, et al., 1978; Paessler, et al., 2004).

Peripherally affected organs may display pathological changes such as congestion and micro-hemorrhages as described in fatal human EEE cases (Dremov, et al., 1978; Paessler, et al., 2004). In animals surviving five days or more, inflammatory pathology in the brain is characterized by infiltrates of macrophages, lymphocytes and neutrophils (Cole & McKinney, 1969; Dremov, et al., 1978). Given the early presence of antigen in the basal nuclei, hippocampus, and brainstem, these areas are more severely affected. However, the cerebral cortex and cerebellum also demonstrate severe pathologies. Histopathological features include destruction, of neurons, inflammatory cell infiltration of the neuropil, gliosis, and microhemorrahages as well as vasculitis (Dremov, et al., 1978; Paessler, et al., 2004).

Guinea pigs infected via aerosol by EEE develop clinical signs within 24 hours (Paessler, et al., 2004; Roy, et al., 2009). Symptomatic disease includes decreased activity, dorsal tremors, and progress to coma and death. Virus infection of the neuroepithelium and appearance of the virus in the olfactory bulbs occurs by 24 h PI, and the virus spreads to the remainder of the brain by four day PI. In the brain, neurons again represent the primary viral target of infection, and brain lesions are typified by neuronal necrosis and the presence of perivascular cuffs and infiltrates in the neuropil of macrophages and neutrophils. Vasculitis is evident in some late stage cases and characterized by fragmentation of the vessel walls, intramural infiltrates of inflammatory cells, and fibrinoid necrosis. No peripheral organs examined had detectable infectious virus, and

osteoblasts were only rarely infected again in contrast to mice (King, 1938; Olitsky & Cox, 1936; Roy, et al., 2009; Sorrentino, et al., 1968).

4.4.3 Nonhuman primate model

IC inoculation of non human primates results in lethal illness, but by peripheral routs causes subclinical infection (Steele & Twenhafel, 2010; Wyckoff & Tesar, 1939). In cynomolgus macaques, IN or aerosol infections result in lethal neurologic disease (Holbrook & Gowen, 2008; Reed, et al., 2007). Aerosol infection of older cynomolgus macaques resulted in clinical disease characterized by fever, neurological signs, and death within five to nine days after challenge in 66% of infected animals. Symptomatic animals also displayed elevated white blood cell (WBC) counts and elevated liver enzymes indicative of infection and systemic dysfunction. Viremia levels varied and some animals failed to develop detectable levels. Pathology indicated severe meningoencephalomyelitis with widespread neuronal necrosis and strong signs of inflammation including perivascular cuffs, cellular debris, gliosis, satellitosis, edema, and hemorrhage. Reproduction of the vascular component of disease also occurred with vasculitis affected cerebral blood vessels. Unsurprisingly, EEE antigen was disseminated throughout the brain (Reed, et al., 2007). An early study performed in the 1960's utilized virus isolated from a fatal human case to inoculate the thalamus of rhesus macaques. All animals developed acute encephalitis and were euthanized by four day PI. Histology evidenced severe encephalitis with neuronal necrosis and loss of neurons in the forebrain. Inflammation was mild in comparison to the effects on neuronal cells populations and consisted of leptomeningitis and perivascular cuffs (Nathanson, et al., 1969; Steele & Twenhafel, 2010). Adult marmosets inoculated IN with an attenuated strain of EEE responded with asymptomatic disease following administration, and meningeoencephalitis when inoculated with virulent strain. Again animals did not develop detectable viremia, but signs of perivascular hemorrhage affecting the cerebral cortex were present (Adams, et al., 2008). Owl monkeys are also susceptible to infection (Espinosa, et al., 2009).

4.5 Host response in experimental models

As with the other alphaviruses, the mechanism of host defense against EEE is poorly understood. However, data from experimental models corroborates the importance of type I IFN, but indicates that type II IFN (IFN-γ) is not crucial for protection. Furthermore, mechanisms of attenuation related to the type I IFN response are well understood for EEE and require exploration in the other alphaviruses.

4.5.1 Innate immune response to EEE

The discovery that cellular infection with EEE virus was suppressed in the presence of type I IFN led to a body of research on the effect of IFN on EEE infection and vice-versa (Wagner, 1961). A subsequent study examining the temporal effects of IFN and EEE infection of chick embryo and L-cells at a high multiplicity of infection showed production of high levels of IFN occurring with peak viral production and extensive cytopathogenecity (Wagner, 1963). Corroborating the protective, antiviral effects of type I IFN, studies in murine models determined that mice deficient in type I IFN receptors developed higher viremia and had a more rapid time to death. Additionally, pre-treatment of mice with type I IFN inducer (poly(I-C)) results in dose dependent protection (Aguilar, et al., 2005). Unlike VEE, EEE does not induce high levels of IFN rapidly in the serum of infected CD-1 mice. The ability of EEE

to antagonize type I IFN induction is cell-dependent, and the failure of EEE to quickly induce IFN production may be due to its inability to replicate in myeloid lineages (Burke, et al., 2009; Ryman & Klimstra, 2008).

Type I IFN's mode of action was pinpointed as antiviral and the cytokine demonstrated the ability to interfere with synthesis of EEE viral RNA (Armstrong, et al., 1971). Mechanistic studies demonstrated that artificial attenuation of EEE results in a marked increase in sensitivity to type I IFN associated with decreased virulence of the virus (Aguilar, et al., 2005; Brown & Officer, 1975; Brown, et al., 1975; Gardner, et al., 2009; Murphy, 1975; Wagner, 1963). Combined with studies in WEE, this led researchers to hypothesize that differences in disease presentation and incidence in humans between South American and North American isolates could be attributed to IFN sensitivity (Brown & Officer, 1975; Brown, et al., 1975). Indeed, comparison of replication of North American and South American strains in IFN pre-treated Vero cells showed a depressive effect on replication of the South American isolates. However, *in vivo*, no differences in IFN induction were observed (Aguilar, et al., 2005). Identification of an avirulent, IFN type I and II sensitive South American strain capable of replication more efficient than virulent strains in mice brains unlike IFN resistant North American strains, identified both structural and non-structural genes as important to generation of virulence and IFN sensitivity (Aguilar, et al., 2008a; Aguilar, et al., 2008b). More specifically, the C protein is associated with host cell gene shut-off likely resulting in cell toxicity (Aguilar, et al., 2007). Amino acids 55 to 75 were critical to the capsid's ability and associated with viral evasion of the anti-viral type I IFN mechanisms (Aguilar, et al., 2008a; Aguilar, et al., 2008b). In addition to inference with host cell systems, EEE may partially avoid the IFN response due to its failure to replicate in myeloid lineages (Ryman & Klimstra, 2008). Thus, host response and viral susceptibility to the host response appears to be a critical determinants of outcome

Mice with a deficiency in the type II IFN (IFN-γ) receptor demonstrate equivalent levels of viremia and mortality rates to wild type animals indicating that IFN-γ is not crucial to control of murine EEE infection (Aguilar, et al., 2008a; Aguilar, et al., 2008b). Induction and modulation of the innate immune response helps to modulate the adaptive immune response, and may have unexplored downstream effects following infection.

4.5.2 Adaptive immune response to EEE

4.5.2.1 Antibody response

Humans develop neutralizing antibody following infection or vaccination (Eklund, et al., 1951). Horses, chickens, and other avian hosts develop neutralizing antibody as well following infection or vaccination (Barber, et al., 1978; Bedenice, et al., 2009; Calisher, et al., 1986c; Clark, et al., 1987; Cole, et al., 1972; Edelman, et al., 1979; Elvinger, et al., 1996). High levels of neutralizing antibody result in mice vaccinated with EEE virion proteins. Humans develop diagnostically useful IgM levels allowing discrimination of different arbovirus populations for serological determination of infection (Calisher, et al., 1986a; Calisher, et al., 1986b). In humans, virus specific IgM antibodies can develop as early as 24 h following onset of disease and persist for upwards of 3 months. IgG cannot be detected till the middle of the 2nd week of disease (Calisher, et al., 1986b; Calisher, et al., 1986c; Knoroz, et al., 1986). Individuals immunized with investigational inactivated EEE (PE-6) develop high titer neutralizing antibodies against E1 and E2 glycoproteins of the parent strain. However, the neutralizing antibody titers against a related South American strain are minimal for these

epitopes (Strizki & Repik, 1995). Taken together data indicated that humoral viral immunity is highly specific for different EEE strains.

Immunization of guinea pig and hamster models results in protection and vaccination of certain avian populations has been reported to reduce numbers of infected birds (Clark, et al., 1987; Olsen, et al., 1997). Chimeric vaccines of EEE and WEE have also been proposed as have trivalent vaccines utilizing immunogens from all three encephalitic alphaviruses or recombinant genetic systems (Atasheva, et al., 2009; Barber, et al., 1978; Pedersen, 1976; Schoepp, et al., 2002). In murine models with pre-existing immunity generated via vaccination, neutralizing antibody is typically detected ten days following infection (Brown & Officer, 1975; Brown, et al., 1975). Neutralizing antibody also develops in the spinal fluid of rabbits vaccinated with either a live or formalin-inactivated vaccine, and such animals are able to resist vigorous challenge routes by IC injection of active virus. Interestingly, failure to resist CNS infection occurred in animals with detectable levels of neutralizing antibody in the serum, but lacking antibody in the cerebral spinal fluid (Morgan, 1941; Morgan, et al., 1942). Such data indicates the importance of the immune responses in the microenvironment of the CNS and indicate that once virus reaches the CNS peripheral measures of protection may be insufficient to determine prevention of disease.

4.5.2.2 Other cell-mediated immune responses

Early studies in T-cell mediated immunity indicate that T-cells proliferate in a virus-specific manner and are predominately of a Th1 phenotype. (Mathews, et al., 1994). As mentioned previously EEE virus replicates poorly in lymphoid tissues, but preferentially infects osteoblasts in murine models. Compared to VEE, the ability of EEE to infected dendritic cells (DC) or macrophage populations is severely limited. However, both viruses replicate efficiently in other cell populations of mesenchymal lineage. Translation of EEE is inhibited in lymphoid tissues of myeloid lineage, but not other cells. This inhibition was shown to be independent of IFN responses both *in vitro* and *in vivo*. (Gardner, et al., 2008; Gardner, et al., 2009).

EEEV does not replicate well in lymphoid tissues *in vivo* on in *vitro*. Specifically, EEE fails to replicate in either macrophages or dendritic cells (Gardner, et al., 2008). Additionally, replication is restricted in human leukocytes *in vitro* (Levitt, et al., 1979). This corroborates with evidence from mouse models demonstrating the primary site of EEE replication occurs in fibroblasts, skeletal muscle and osteoblasts. Failure to replicate in lymphoid organs may explain the later appearance of EEE symptoms than the WEE or VEE (Gardner, et al., 2008; Vogel, et al., 2005). Thus, the failure of EEE to initiate significant signs of early febrile disease may be due to this primary tropism for the oesteoblast lineage of cells rather than lymphotropic nature of the VEE or WEE virus. In fact, while antigen is noted in the draining lymph node (DLN), serum viremia is low (Vogel, et al., 2005).

5. Venezuelan equine encephalitis

5.1 History and significance

Despite lower mortality rates of VEEV compared to EEEV, of the New World alphaviruses, VEEV is the most important human and equine pathogen due to large epizootic and epidemic outbreaks associated with the virus (Weaver, et al., 2004). Isolated in 1938 from the brain of an infected equine, VEEV was not recognized as a significant cause of illness in man till the 1950's though the virus was isolated from a lethal case of encephalitis in the early 1930s (Beck &

Wyckoff, 1938; Kubes & Rios, 1939b; Saleh, et al., 2009; Sanmartin & Arbelaez, 1965; Sanmartin, et al., 1973). Since that time, the virus has been identified as the causative agent for epizootic outbreaks occurring sporadically at approximately 10 year intervals from time of discovery through the early 1970's. One of the largest outbreaks of VEE originated in South America, spread through Central America, and reached southern Texas in 1971 causing thousands of equine and human cases of disease (Franck & Johnson, 1971; Gibbs, 1976). Circumstantial evidence and isolation of vaccine strain from mosquitoes in Louisiana led to speculation that inoculation of equines with incompletely inactivated live-attenuated vaccine, or the presence of mixed virus populations in vaccine preparations led to the extensive disease spread (Brown, 1993; Pedersen, et al., 1972). However, evidence remains inconclusive. Following this outbreak, epidemics and epizootics ceased only to reappear in the early 1990's. The most recent major outbreak occurred in 1995 in Venezuela and Colombia in which 75,000 to 100,000 human cases were reported (Weaver, et al., 2004).The sporadic nature of these outbreaks is likely related to the viability of virus transmission to equine amplifying hosts. In the presence of sterilizing immunity in survivors of outbreaks or following vaccination, viral spread in amplifying hosts is greatly limited. Thus, the interepoozotic periods can be partially explained by resistance of the amplifying host as can the disappearance of epizootic or epidemic outbreaks following 1972 through effective vaccination campaigns. However, changes in mosquito control programs and alterations in rural and urban living conditions impacting the mosquito vector may also be partially responsible for the long hiatus of epidemic outbreaks though true cause is uncertain(Calisher, 1994; Forrester, et al., 2008; Weaver, et al., 2004).

While the majority of VEE cases from natural disease outbreaks are asymptomatic, approximately 10% of infected individuals develop an apparent infection. Of apparent infections less than 1% are lethal, but 14% develop neurological disease and, in pediatric populations mortality in apparent infections is higher (~4%) (Sanmartin-Barberi, et al., 1954; Sanmartin, et al., 1973; Steele & Twenhafel, 2010). In cases with CNS manifestations, mortality increases to as high as 35% in children and 10% in adults (Bowen & Calisher, 1976; Bowen, et al., 1976; Gibbs, 1976; Steele & Twenhafel, 2010). As with the other encephalitic alphaviruses, the prevalence of severe infections greatly increases at the extremes of the age spectrum with the majority of deaths seen in pediatric patients. Virus is highly infectious via aerosol and the virus has been responsible for numerous laboratory related exposures (CFSPH, 2008; Hanson, et al., 1967; Weaver, et al., 2004).

5.2 Transmission on cycles and geographic range

VEEV circulates in an enzootic cycle between a mosquito vector and vertebrate host, namely rodents and humans. In epidemic or epizootic cycle, the virus transmits between horses, humans and mosquitos (Watts, et al., 1998; Zacks & Paessler, 2010). Typically, multiple mosquito(Weaver, et al., 2004) vectors present during epizootic outbreaks, but, *Ochlerotatus taeniorhynchus*, *Aedes* and *Psorphora*, are believed to be the principal vectors responsible for transmission in this scenario with *Culex (Melanconion*) species believed to transmit the enzootic strains (Weaver, et al., 2004). Domestic goats, sheep, and pigs can be infected, but rarely show disease. Dogs and domestic rabbits are susceptible to infection and develop symptomatic disease though none of these hosts develop high titer viremia. The primary, enzootic host is thought to be rodents such as the cotton rat, but species likely vary based on geographic location (Johnson and Martin 1974; Johnson 1974; Gibbs 1976; Johnson and Varma 1976). Horses act as amplifying hosts generating a very high titer viremia, and are capable of transmitting the virus back to mosquitoe-to-human populations (Gibbs, 1976;

Paessler & Weaver, 2009; Zacks & Paessler, 2010). Long thought to be a dead end host with levels of viremia below the limit able to reinfect mosquito populations, humans may be capable of acting as the primary vertebrate host for some strains of VEEV. Namely, VEEV ID strains circulate in an urban cycle in Peru with transmission occurring in a primary cycle between humans and mosquitoes. Restriction to geographic range is based primarily on habitat of the mosquito vector and primary vertebrate host (Watts, et al., 1998).

5.3 Disease manifestations in humans

Humans present with a spectrum of diseases ranging from inapparent infections to lethal encephalitis. However, human infections are typically mild or asymptomatic, and severe encephalitis is less commonly seen. Fever, headache, convulsions, disorientation, ataxia, and mental depression appear in a subset of symptomatic cases, but primarily in patients under 15 years of age (de la Monte, et al., 1985; Rivas, et al., 1997; Weaver, et al., 1996). In the event neurological symptoms occur and the patient survives, sequelae are common (Weaver, et al., 2004). Virus has been isolated from throat swabs, serum and brains. Most of what is known of onset of disease has been determined based on accidental infection of laboratory workers where incubation period appears to be two to five days followed by sudden appearance of flu like symptoms though reports from natural disease outbreaks vary (CFSPH, 2008; Steele & Twenhafel, 2010). Acute disease typically lasts approximately four to six days. However, the disease can be biphasic with a secondary fever developing four to eight days after onset. During this second phase, neurological symptoms develop, though again a range is present from somnolence and mild confusion to seizures, ataxia, paralysis, and coma (Tsai, 1991). Following recovery from acute disease, patients develop generalized asthenia lasting one to two weeks. CSF protein and liver enzymes are typically elevated with cell counts in the CSF ranging from 12 to 900 WBC/mm^3. An EEG tracing in one patient identified diffuse irregular slowing (Johnson, et al., 1968).

Gross findings in neurological cases include cerebral edema, but gross pathology of infections is poorly described in the literature (de la Monte, et al., 1985; Johnson, et al., 1968; Steele & Twenhafel, 2010). Fatal human cases are histopathologically characterized by edema, congestion, meningitis, and encephalitis in the brain. Vasculitis and hemorrhage are more rarely found (Johnson & Martin, 1974; Paessler & Weaver, 2009; Weaver, et al., 2004). Lymphocytes, mononuclear cells, and neutrophils infiltrate the meninges with cells extending into the Virchow Robins space and neuropil in a subset of cases. In the peripheral organs in the majority of cases, interstitial pneumonia associated with cellular infiltration, alveolar hemorrhage, congestion and edema present in the lung with diffuse hepatocellular degeneration and few infiltrates presenting in the liver (Walton & Grayson, 1988). In lymphoid tissues, lymphocyte degeneration, lymphoid depletion and follicular necrosis are accompanied by infiltrates of neutrophils as well as vasculitis (Steele & Twenhafel, 2010).

5.4 Disease manifestations in experimental models

Mouse and NHP models make the largest contribution to the understanding of VEE pathogenesis. However, hamsters and guinea pigs are also susceptible to infection, and early studies in these models initiated understanding of the host response to infection.

5.4.1 Mouse model

Mice are highly susceptible to VEEV infection (Holbrook & Gowen, 2008; Paessler & Weaver, 2009; Walton & Grayson, 1988; Zacks & Paessler, 2010). The mouse model mimics

both human and equine disease with mice developing neurotropic disease characterized by lethal encephalitis and lymphotropism following a biphasic disease course (Gardner, et al., 2008; Gleiser, et al., 1962; Steele, et al., 1998). Following peripheral routes of infection, mice display human like disease with progression from infection of the lymphoid tissue and ultimate destruction of CNS tissues. Clinical symptoms in mice include: lethargy, huddling, dehydration, weight loss, tremors, and paralysis or paresis with a minority of animals developing seizures (Davis, et al., 1994; Grieder, et al., 1995).

The lymphotropic nature of VEE results in severe myeloid depletion in rodents and lymphocyte destruction in lymph nodes and spleen (Davis, et al., 1994; Grieder, et al., 1995; Steele & Twenhafel, 2010; Zacks & Paessler, 2010). Peripheral infection is not present late in disease, but high levels of infectious virus are found in the CNS with death in immunocompetent mice occurring five to seven days after infection (Ludwig, et al., 2001; Vogel, et al., 1996).

Encephalitic pathologies vary based on background of mouse, route of inoculation and strain of virus (Ludwig, et al., 2001; Ryzhikov, et al., 1991; Steele, et al., 1998; Steele, et al., 2006; Stephenson, et al., 1988; Vogel, et al., 1996). Thus, based on study design, encephalitic pathologies range from mild neutrophilic infiltration to neuronal degeneration, necrotizing vascultis and Purkinje cell destruction. Lesions appear that are surrounded by necrotic cellular debris, perivascular cuffs composed of mononuclear cells, rarefaction of the neuropil and infiltration of neutrophils lymphocytes and macrophages. These lesions spread following the virus by an approximately 24 h delay from olfactory bulb to more caudal regions (Jackson, et al., 1991; Jensen & Jackson, 1966; Ludwig, et al., 2001; Ryzhikov, et al., 1991; Steele, et al., 1998; Steele, et al., 2006; Stephenson, et al., 1988; Vogel, et al., 1996). Once in the CNS viral tropism is primarily neuronal though CNS macrophages as well as astrocytes may become infected with VEEV, but do not appear to be a primary target (Jackson & Rossiter, 1997a; Schoneboom, et al., 2000b; Steele, et al., 1998; Steele, et al., 2006). As a result, neuronal damage is extensive and has been ascribed to both necrosis and apoptosis (Jackson & Rossiter, 1997b; Jensen & Jackson, 1966; Schoneboom, et al., 2000b; Schoneboom, et al., 2000d; Steele, et al., 2006). Neuropathology may also be attributed to bystander activation and death as damage to glial cells, lympohcytolysis and astrocytosis are reported in areas where virus is not detected (Davis, et al., 1994; Grieder, et al., 1995; MacDonald & Johnston, 2000b; Schoneboom, et al., 2000a; Schoneboom, et al., 2000c).

Routes of infection mimicking natural, mosquito borne infection utilize SC or footpad inoculation results in infection of DC, the primary cell type for VEE infection, at the site of inoculation. These cells then carry the virus to the DLN where VEE begins replication approximately four hours after infection (Aronson, et al., 2000; Davis, et al., 1994; Grieder, et al., 1997; Grieder, et al., 1995; Grieder & Nguyen, 1996b; MacDonald & Johnston, 2000a). The virus enters the blood stream by 12 hours and reaches high levels of serum viremia followed by infection of other tissues, particularly lymphoid. As such, VEEV presents in the spleen, gut and nasal-associated lymphoid tissues, thymus, bone marrow, and non-draining lymph nodes(Grieder & Nguyen, 1996a; Vogel, et al., 1996). Histological lesions found in peripherally infected animals are both neuronal and non-neuronal (Aronson, et al., 2000; Grieder, et al., 1997; Grieder, et al., 1995; Grieder & Nguyen, 1996a; Steele & Twenhafel, 2010).

Aerosol or IN infection results in a direct infection of the olfactory epithelium (Pratt, et al., 2003; Steele, et al., 1998; Vogel, et al., 1996). Other nasal epithelia tissues (respiratory or squamous) do not appear to become infected (Charles, et al., 1995; Davis, et al., 1995; Griffin, 2007; Pratt, et al., 2003; Steele, et al., 1998). VEEV is proposed to reach the brain via the

olfactory nerve, but dental structure involvement has also been proposed (Charles, et al., 1995; Steele, et al., 1998; Steele & Twenhafel, 2010). Direct invasion of the brain via the bloodstream does not seem to be significant in VEE infection. Neuroinvasion results in caudal spread of the virus, and ultimately, overwhelming disseminated brain infection (Julander, et al., 2007; Julander, et al., 2008b). Thus, all routes of infection in mice with virulent forms of the virus result in neuroinvasion. However, infection of the nasal cavity, by either aerosol or IN infection, results in more rapid entry to the CNS depending on strain of virus used.

Strain of mice also is an important determinant of outcome. C3H/HeN and BALB/C mice vaccinated dermally with TC-83, the live attenuated vaccine strain, survive. However, aerosol or IN infection with TC83 results in 90-100% mortality in C3H/HeN animals unlike inbred counterparts BALB/C that respond with no evidence of mortality (Julander, et al., 2007; Julander, et al., 2008c; Steele, et al., 2007; Steele, et al., 1998; Steele, et al., 2006). Similar experiences have shown that route of administration can alter neuroinvasion with nonpathogenic viruses failing to enter the CNS by peripheral routes, but exhibiting the ability to enter the CNS by IC, IN, or aerosol administration. However, neuroinvasion, regardless of route, does not always correlate with mortality and virus strains avirulent in the periphery may remain so once in the CNS (Grieder, et al., 1995; MacDonald & Johnston, 2000a; Steele, et al., 1998; Steele, et al., 2006). This creates an interesting dichotomy between neuroinvasiveness and neurovirulence of the virus and indicates that outcome depends on factors beyond neuroinvasion or viral replication to generate mortality.

5.4.2 Hamster and guinea pig models

In guinea pigs and hamsters, VEEV causes acute, fulminant disease associated with extensive necrosis of lymphoid tissues, and death typically occurs prior to development of CNS disease making these models limited for studies of human infections and encephalitis (Gorelkin & Jahrling, 1975; Jackson, et al., 1991; Jahrling & Scherer, 1973a; Jahrling & Scherer, 1973b; 1973c; Walker, et al., 1976)

5.4.3 Nonhuman primate model

Like the majority of symptomatic human infections, infection in NHP represents an acute biphasic, nonspecific febrile disease with infection of lymphoid organs (Danes, et al., 1973; Gleiser, et al., 1962; Monath, et al., 1974a; Nalca, et al., 2003b; Pratt, et al., 1998; Reed, et al., 2007; Verlinde, 1968; Victor, et al., 1956) . In a comprehensive study with rhesus macaques infected IP, animals developed a transient viremia and biphasic fever, but otherwise displayed no clinical signs of disease with complete resolution of pathologies by five weeks PI. Lymphoid depletion occurred rapidly by two days post-infection with extensive lymphoid necrosis, later followed by lymphoid hyperplasia as the animals recovered from infection. Lesions in the brain characterized by perivascular cuffs associated with lymphocytes and gliosis were also apparent and developed around six days post-infection starting at the olfactory bulb and spread caudally throughout the brain (Danes, et al., 1973; Gleiser, et al., 1961; 1962; Monath, et al., 1974b; Pratt, et al., 1998; Reed, et al., 2007; Verlinde, 1968; Victor, et al., 1956). A range of CNS involvement presents depending on strain of virus utilized and route of inoculation with IN and IC inoculation being particularly severe. In cynomolgus macaques similar findings were reported except in the case of IN or aerosol infection where CNS damage is more severe and, in the case of IC infection, lethal (Danes, et al., 1973; Monath, et al., 1974b; Pratt, et al., 1998; Reed, et al., 2007; Steele & Twenhafel, 2010;

Verlinde, 1968; Victor, et al., 1956). Thus, IN inoculation results in disease ranging from simply clinical signs of encephalitis and subsequent recovery to fulminant lethal encephalitis (Gleiser, et al., 1962; Steele & Twenhafel, 2010).

5.5 Host response in experimental models

Of the three viruses, VEEV has the best characterized animal models, and therefore the most is understood regarding the specific host response to infection. However, significant obstacles due to the high biocontaiment level and the rapid death of the host animal following infection resulted in alternatives to typical infection models. Thus, the majority studies examining host response to VEE has been generated using models of attenuated virus or pre-existing immunity, so to date little is known about the primary host response to virulent infection.

5.5.1 Innate immune response to VEE

Like other alphaviruses, VEEV is highly susceptible to the effects of type I IFN (Jordan, 1973). Attenuation in enzootic strains limits the virus' ability to interfere with type I IFN signaling pathways and partially explains the absence of typical disease symptoms following infection with enzootic strains (Grieder & Vogel, 1999; Jahrling, 1975; Jahrling, et al., 1976; Simmons, et al., 2009; Spotts, et al., 1998; White, et al., 2001). Conversely, epizootic and epidemic strains are able to limit host production of type I IFN through interference with signaling pathways, particularly STAT1 (Simmons, et al., 2009; White, et al., 2001a; Yin, et al., 2009). However, the susceptibility of enzootic and epizootic strains appears to vary significantly based on the virus strain(Anishchenko, et al., 2004). As with EEE, the ability to interfere with host transcription has been ascribed to properties of the capsid protein (Garmashova, et al., 2007a; Garmashova, et al., 2007b). Attenuating mutations in the 5′ untranslated region of the genome are associated with susceptibility of the virus to the effects of type I IFN. Animals genetically modified with deficiencies in type I IFN signaling are highly susceptible to VEE infection and, even attenuated strains are uniformly lethal in these animals(White, et al., 2001). Additionally, artificial induction of type I IFN or prophylactic administration of type I IFN delays or prevents death in animal models (Julander, et al., 2008b). In the case of IN or aerosol infection, the rapid entry of virus to the CNS results in earlier neuroinvasion and may limit or alter the effectiveness of early innate immune mechanisms such as IFN or other as yet other unexplored mechanisms.

Vaccination of immunocompromised mice genetically deficient in the IFN-γ receptor is only partially protective unlike complete protection observed in wild-type counterparts indicating type 2 IFN signaling is not required for complete protection unlike type I IFN signaling (Paessler, et al., 2007).

5.5.2 Adaptive immune response to VEE

5.5.2.1 Antibody response

Current knowledge of the humoral response of the host is largely derived using models of pre-existing immunity (vaccination) or using attenuated strains of the virus. Early work identified high-level neutralizing serum antibody as essential following peripheral infection, and the production of neutralizing antibody is utilized as an endpoint in vaccine studies as well as marking efficacy of the IND vaccine, TC83, to vaccinate at risk laboratory personnel (Alevizatos, et al., 1967; Berge, et al., 1961; Eddy, et al., 1972; Engler, et al., 1992; Feigin, et al.,

1967; Jochim & Barber, 1974; Pittman, et al., 1996; Walton, et al., 1972). However, more recent research efforts indicate that circulating antibody may be irrelevant once virus reaches, invades, and begins replicating in the CNS and the role of antibody produced in the endogenous micro-environment of the CNS has been poorly explored (Paessler, et al., 2007; Yun, et al., 2009). Research from Sindbis models using neuroadapted strains of this particular alphavirus indicate that neutralizing antibody is capable of non-cytolytic clearance of virus from neurons (Burdeinick-Kerr, et al., 2009; Griffin, et al., 1997; Griffin, 2010). Primary tropism of VEE in the periphery is for dendritic cells; however, in the CNS it replicates in neurons where non-cytolytic clearance may be of great importance(MacDonald & Johnston, 2000a; Schoneboom, et al., 1999b).

Monoclonal antibodies against specific surface glycoproteins, particularly E1 and E2, may act as useful therapeutic agents and may provide passive immunity to infected animals (Hart, et al., 2000; Hart, et al., 2001; Hart, et al., 1997; Mathews & Roehrig, 1982; Roehrig, et al., 1988; Roehrig & Mathews, 1985). Alterations in the E2 protein are capable of altering the pathogenesis of the virus and preventing neuroinvasion (Davis, et al., 1995). Since aerosol and IN infection bypasses the need to develop viremia to be neuroinvasive, the IgG peripheral neutralizing antibodies may not afford protection against nasal routes of infection. Studies of the antibody response indicate that not all species are equally protected by the same vaccines and specific IgA production at the nasal mucosal surfaces may play a critical role in prevention from aerosol infection (Hart, et al., 1997). More recent studies indicate that administration of hyperimmune sera is only effective against peripheral infection, and has little effect once virus invades the CNS though transfer is able to prolong survival probably due to depression of peripheral infection (Hart, et al., 1997). However, the role of plasma cells or locally produced antibody in the brain by memory B-cells remains to be determined for this infection (Paessler, et al., 2007; Yun, et al., 2009). Research efforts in the 1970's indicated that Fc-dependent clearance of the virus does not rely on complement (Mathews, et al., 1985). Vaccinated mice with non-functional B-cells (μMT deficient) are highly susceptible to intranasal infection, and when infected with attenuated strains develop persistent viral infection (Brooke, et al., 2010; Paessler, et al., 2007; Yun, et al., 2009).

5.5.2.2 Other cell-mediated immune responses

Recent research indicates that T-cells are crucial in recovery from VEE. Specifically, CD4+ T-cells contribute to resolution of infection. While adoptive transfer of primed CD3+ and CD4+ T-cells generated via vaccination ameliorates encephalitis in vaccinated αβ T-cell receptor deficient animals, CD8+ T-cells fail to generate protection. Vaccination of γδ T-cell receptor deficient animals is partially protective, but animals develop a persistent viral infection to 28 days. Thus, while αβ T-cell subsets appear to be required for protection, γδ T-cells do not and viral persistence in these animals may be an indirect effect of a deficit in T-cell help for B cells in these animals (Paessler, et al., 2007; Yun, et al., 2009). Earlier studies of the immune response to the attenuated, live vaccine strain TC83 identified a Th 1 mediate immune response with local activation of CD4+ and CD8+ T-cells (Bennett, et al., 2000; Jahrling & Stephenson, 1984; Phillpotts, 1999; Phillpotts, et al., 2003; Phillpotts & Wright, 1999). Later data examining transcriptional profiles in the brain and sera corroborate an overwhelming proinflammatory response and support the Th1 bias in response to infection(Davis, et al., 1994; Grieder, et al., 1997; Grieder & Vogel, 1999; Koterski, et al., 2007). These studies indicated that T-cells are critical to the host defense against infection,

survival, encephalitis, and the repair of neural damage and homeostasis in the brain(Paessler & Weaver, 2009; Paessler, et al., 2007; Yun, et al., 2009; Zacks & Paessler, 2010). The role of CD8+ T-cells explored previously by Jones et al. and arrived at much the same conclusion that CD8+ T-cells were not cytolytic or immunoprotective in VEE encephalitis (Jones, et al., 2003). However, given the pleiotropic roles of CD8+ T-cells and their significance in other viral infections the elimination of a role in VEE infection is doubtful.

Functional antibody production is not required for recovery from infection with an genetically modified, attenuated VEE virus while T-cells were critical to complete protection and survival of the animals. Persistence, as seen previously in γδ deficient mice, was present in mice deficient in functional B-cell response (μMT knock out) and infection was less controlled in animals depleted of CD3, CD4 or CD8 T-cells with CD4 cells appearing to contribute the most significantly in viral control (Brooke, et al., 2010).

Additionally data indicate that IFN-y secretion from these cell populations contributes to survival. However, their role in primary infection has not been well defined. The ability of animals to sustain high levels of virus in the CNS calls into question the importance of viral replication in host pathogenesis. Vaccinated animals maintain equivalent levels of virus to uninfected counterparts with differential outcome indicated that viral load is not the best discriminator for mortality(Paessler, et al., 2007; Yun, et al., 2009). Additionally, such evidence indicates that the efficacy of antivirals once the virus invades the CNS may be limited and therapeutic efforts may be better focused on limiting or altering the host response to generate a non-pathogenic response. However, further understanding of the host response and pathogenic and protective mechanisms of resolution of infection are integral to effective therapeutics development.

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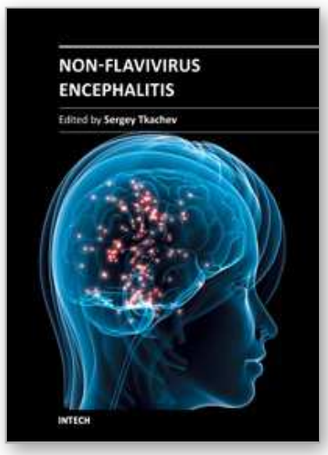

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This book covers the different aspects of non-flavivirus encephalitises of different ethiology. The first section of the book considers general problems of epidemiology such as study of zoonotic and animal vectors of encephalitis causative agents and methods and approaches for encephalitis zoonoses investigations. The members of different virus species are known to be the causative agents of encephalitis, so the second section of the book is devoted to these viral pathogens, their epidemiology, pathology, diagnostics and molecular mechanisms of encephalitis development by such viruses as HIV/SIV, herpes simplex virus type 1 and equine herpesvirus 9, measles virus, coronaviruses, alphaviruses and rabies virus. The next section of the book concerns the study of protozoan pathogens such as toxoplasma and amoebae. The last section of the book is devoted to multicellular pathogen as human Filaria Loa Loa - a filarial worm restricted to the West Africa.

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