Neurotherapeutics (2016) 13:514–534
DOI 10.1007/s13311-016-0443-5

REVIEW

Encephalitic Arboviruses: Emergence, Clinical Presentation, and Neuropathogenesis

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Published online: 24 May 2016

Abstract Arboviruses are arthropod-borne viruses that exhibit worldwide distribution, contributing to systemic and neurologic infections in a variety of geographical locations. Arboviruses are transmitted to vertebral hosts during blood feedings by mosquitoes, ticks, biting flies, mites, and nits. While the majority of arboviral infections do not lead to neuroinvasive forms of disease, they are among the most severe infectious risks to the health of the human central nervous system. The neurologic diseases caused by arboviruses include meningitis, encephalitis, myelitis, encephalomyelitis, neuritis, and myositis in which virus- and immune-mediated injury may lead to severe, persisting neurologic deficits or death. Here we will review the major families of emerging arboviruses that cause neurologic infections, their neuropathogenesis and host neuroimmunologic responses, and current strategies for treatment and prevention of neurologic infections they cause.

Keywords Arbovirus · Viral encephalitis · Innate immunity · Blood-brain barrier

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Introduction to Encephalitic Arboviruses

Arthropod-borne viruses (arboviruses) are transmitted to hosts during blood feeding of mosquitos, ticks, biting flies, mites, and nits. While the majority of arbovirus infections do not cause neuroinvasive disease, there are several arbovirus families with members that cause neuroinvasive disease in vertebrate hosts worldwide. The encephalitic members of the Togaviridae family alphaviruses (new-world group) consist of the Eastern, Western, and Venezuelan equine encephalitic viruses (EEEV, WEEV, and VEEV, respectively). The VEEV complex comprises 14 subtypes and varieties and includes 7 different virus species, while EEEV includes North and South American variants, with 4 major lineages [1]. Chickungunya virus (CHIKV) is another member of the Togaviridae family (old-world group) and Semiliki Forest virus complex that can cause central nervous system (CNS) disease [2]. Japanese encephalitis virus (JEV), West Nile virus (WNV), Saint Louis encephalitis virus (SLEV), and Murray Valley encephalitis virus (MVEV) comprise the Japanese encephalitis (JE) serogroup of neuropathogenic flaviviruses. Additional emerging mosquito-borne flaviviruses that can infect the CNS are dengue and Zika viruses (DENV and ZIKV, respectively). Arbovirus members of the Bunyaviridae family that cause neuroinvasive disease include viruses from 2 different genera, Orthobunyavirus and Phlebovirus. The Orthobunyaviruses California encephalitis virus (CEV) and La Crosse virus (LACV) are members of the California serogroup viruses, which include 15 related arboviruses that cause neuroinvasive and non-neuroinvasive diseases [3]. Toscana virus (TOSV) and Rift Valley fever virus (RVFV) belong to the genus *Phlebovirus*, which is predominately transmitted by sandfly and tick. Colorado tick fever virus (CTFV) is a neuroinvasive member of the Reoviridae that is transmitted by the Rocky Mountain wood tick.

Structural Aspects of Encephalitic Arboviruses

Neuroinvasive arboviruses represent all 3 groups of RNA viruses (group III, IV, and V). Alphaviruses are enveloped viruses consisting of a positive, single-strand RNA genome (group IV) with 2 open reading frames that are translated into 2 polyproteins comprising structural and nonstructural proteins, respectively. Flaviviruses are similarly structured enveloped, positive single-strand RNA viruses. However, flaviviruses are translated from a single open reading frame into a polyprotein that is cleaved into mature structural and nonstructural polypeptides by viral and host proteases. While bunyaviruses also have spherical enveloped virions similar to alphaviruses and flaviviruses, Bunyaviruses are comprised of tripartite negative, single-strand RNA genomes (group V). The large, medium, and small segments are negative sense for orthobunyaviruses (CEV and LACV). However, the small segment is ambisense in the phleboviruses (TOSV and RVFV). As a group III virus, CTFV is the structurally most divergent neuroinvasive arbovirus. CTFV virions are nonenveloped and contain a double-strand RNA genome, which is divided into 12 segments. As such, each viral family utilizes distinct strategies for infection and replication. However, the common RNA composition of these viruses allows for higher rates of mutation, which results in diverse viral populations that both impedes development of effective vaccines and contributes to instances of viral emergence.

Epidemiology of Encephalitic Arboviruses

Encephalitic alphaviruses naturally cycle between mosquitoes and birds (EEEV and WEEV), mosquitoes and rodents (VEEV enzootic cycle), or mosquitoes and horses (VEEV epizootic cycle) [4]. VEEV, WEEV, and EEEV are all widely distributed in North, Central, and South America. Human infection can progress rapidly to encephalitis, with fatality rates of ~1 % in cases of VEEV and WEEV, and 50 % to 75 % in cases of EEEV cases [5, 6]. Of the 3, VEEV is considered the most important zoonotic pathogen, with several reported human outbreaks in South and Central Americas, primarily by enzootic VEEV strains [7]. VEEV outbreaks of enzootic strains in Central America have also been followed by spread to North America with occasional fatal human cases [8]. Within North America, the human cases per year are usually 1 to 2 for WEEV and 5 to 8 for EEEV; however, in 2005 there were 21 reported cases of EEEV [9]. Focal outbreaks of VEEV have occurred in North America, with epidemics of equine and human cases numbering in the hundreds, primarily in Mexico [10]. Although the overall number of human cases reported for these viruses is small, the possibility for disease emergence is high owing to expansion and spread of mosquito vectors. Additionally, there is the potential for even moderately experienced scientists to grow VEEV, WEEV, and/or EEEV to high titer and generate aerosol forms, which would cause severe disease if dispersed in a dense urban or military setting. Despite the epidemic potential of VEEV and the high morbidity and/or case fatality rates of EEEV and WEEV, there are no approved vaccines or therapeutics for humans.

CHIKV is a re-emerging alphavirus that can cause severe and fatal disease with CNS involvement of both adults and neonates [11–14]. Prior to its re-emergence in the Indian Ocean in 2004 and ensuing worldwide spread, CHIKV infections rarely involved the CNS. However, in recent major outbreaks of CHIKV in La Reunion Island and the Caribbean, the incidence of encephalitis is reportedly 187 per 100,000 infants and 37 per 100,000 persons for adults > 64 years of age [15].

JE serogroup flaviviruses cycle between birds and mosquitoes, and cause neurologic infections in humans, whose incidences depend on age and immune status. WNV, which was first identified in the West Nile subregion of Uganda, is now endemic in temperate and tropical regions throughout the world, causing yearly outbreaks of encephalitis, with a mortality rate of 5 % to 10 % [16]. MVEV causes similar outbreaks in Australia, New Guinea, and New Zealand, while SLEV rarely causes encephalitis in the USA (<10 cases per year) [16, 17]. JEV is the most medically important member of the serogroup, causing 30,000 to 50,000 cases of encephalitis and 10,000 deaths each year in Asia [18]. While these viruses are generally transmitted via ornithophilic mosquitoes, WNV has also been transmitted via blood products, and both WNV and JEV can be transplacentally transmitted during pregnancy [19, 20]. JEV and MVEV predominantly cause encephalitis in children, whereas encephalitis due to WNV or SLEV is more likely to occur in adults. DENV, which is endemic to > 100 countries, has been reported to cause encephalitis in up to 41 % of cases [21].

ZIKV is a related, mosquito-transmitted flavivirus, first isolated from a febrile rhesus macaque in Uganda in 1947, which has emerged from obscurity to cause outbreaks in Micronesia, French Polynesia, and South and Central America [22, 23]. In adults, ZIKV infection results in a self-limiting febrile illness associated with rash and conjunctivitis, but severe neurologic disease can occur, including Guillain-Barré syndrome (GBS) and meningoencephalitis [24, 25]. The current ZIKV outbreak in South America has been associated with a 20-fold increase in the rate of babies born with microcephaly [22, 26], and spontaneous abortion or intrauterine growth restriction due to placental insufficiency [27]. Since 2007, 55 countries in America, Asia, Africa, and Oceania have detected local transmission of the virus, affecting > 1.5 million people [28]. In the USA and its territories, > 1025 cases have been reported [29]. Cases of sexual transmission of ZIKV and detection of persistent infectious ZIKV in semen have also been reported [30–33].

Bunyaviruses exhibit a diversity of enzootic cycles. Mosquito-borne bunyaviruses include CEV and LACV, which are endemic to the western and mid-west/eastern USA, respectively, and cycle between mosquito and small mammals. CEV and LACV cause an average of 75 cases of meningitis, encephalitis, or meningoencephalitis per year with the majority of disease due to LACV [34]. RVFV has caused periodic outbreaks in Kenya, Somalia, Tanzania, Saudi Arabia, and Yemen [35]. Outbreaks in endemic areas have occurred with up to 20,000 cases and > 500 deaths [36]. RVFV is spread via mosquitoes or through extensive contact with blood, milk, and body tissues from infected livestock [37]. TOSV is transmitted by sandflies and causes 100 to 200 cases of meningoencephalitis each summer in Europe and North Africa [38]. Although the natural reservoirs for TOSV remain unidentified, canines have been identified as potential candidates [39].

CTFV primarily cycles between multiple species of rodents and Rocky Mountain wood ticks [40, 41]. CTFV causes symptomatic illnesses in all cases and occurring exclusively in the western parts of the USA and Canada [42]. A total of 83 cases occurred between the years 2002 and 2012 [43].

Clinical Features of CNS Infections with Arboviruses: Human Disease

Infections with arboviruses vary in clinical presentation from completely asymptomatic to florid encephalitis with seizures, coma, and death (Table 1). In general, symptomatic patients will initially develop a flu-like illness with headache, fever, pharyngitis, and myalgia. Depending on the virus, this may progress to nausea, vomiting, and meningismus, or undergo initial resolution followed by recrudescence of headache and fever that rapidly progresses to neurologic symptoms. Although multiple neurotropic arboviruses are endemic in the USA, including CEV, LACV, WNV, SLEV, VEEV, WEEV, DENV, CHIKV, ZIKV, and CTFV, symptomatic infections of the CNS are rare. Meningitis and encephalitis are the most common manifestations of neuroinvasive diseases with any of these viruses. In this section, we will provide a detailed review the clinical features of CNS infections with arboviruses that occur worldwide.

Togaviridae

Natural or laboratory-acquired infections have been documented in humans with all epizootic and many enzootic VEEV strains [44]. After a 2 to 4 day incubation period, all patients develop an incapacitating illness with high fever, headache, pharyngitis, malaise, and myalgia. Laboratory evaluation reveals lymphopenia and elevation in hepatic enzymes. This acute phase typically lasts 24 to 48 h and is followed by a 2 to 3-week period of lethargy and anorexia. In a small percentage of cases (0.05–4 %), patients develop encephalitis, which occurs a few days after the acute febrile illness [45]. The overall mortality of encephalitis varies with age; the mortality rate is approximately 20 % in older children and young adults but may reach 35 % in persons aged 0 to 5 years [46]. The neuropathologic and neuroimmunologic effects of VEEV in human encephalitis cases have not been well described owing to lack of autopsy specimens. Gross pathologic analysis of 21 lethal encephalitis cases revealed cerebral edema and meningeal infiltrates comprised of neutrophils, lymphocytes, and monocytes that extend to Virchow Robbins spaces and, in some cases, CNS parenchyma [47].

Most human infections with EEEV are asymptomatic; however, neuroinvasive forms of infection results in a higher mortality rate and more severe neurologic sequelae. Following an incubation period of 5 to 15 days, infection may progress to systemic then encephalitic disease [5]. Systemic disease presents with headache, fever, malaise, myalgia, nausea, and vomiting. Encephalitis additionally results in photophobia, confusion, somnolence, focal neurologic deficits, paresis, paralysis, respiratory impairment, seizures, and coma, which may persist if the patient survives the acute illness. However, in many cases (36–75 %) death occurs 2 to 10 days after onset of symptoms [9]. The mortality rate is highest (50–75 %) in patients > 60 years of age with cognitive impairment occurring in 30 to 70 % of survivors [5]. Neuropathologic findings include neuronal injury with caspase 3 activation, vasculitis and thrombosis, demyelination, necrosis, and meningeal, perivascular, and parenchymal infiltrates comprised of neutrophils, lymphocytes, and monocytes/macrophages [48]. Brain lesions in fatal cases occur predominantly within the basal ganglia, thalamus, and brainstem.

WEEV infection results in more mild disease than EEEV. Most vector-borne WEEV infections are asymptomatic or present as a nonspecific febrile illness. The incubation period is 5 to 10 days, with a short prodromal phase lasting approximately 1 to 4 days. However, the signs and symptoms of encephalitic WEEV are similar to those described for VEEV and EEEV. These signs include somnolence, seizures, coma, and motor neuron dysfunction [6]. The mortality rate in human WEEV encephalitis ranges from 3 % to 15 %, depending on age and, possibly, viral factors [49]. Unlike vector-borne infections, where virus is deposited subcutaneously, the process of neuroinvasion by aerosolized alphaviruses is more direct, via the olfactory tract, and causes increased severity and incidence of encephalitis [50]. For example, a laboratory accident resulting in aerosolization and exposure to WEEV resulted in encephalitis with a mortality of 40 % [51]. Similar to VEEV and EEEV, neurologic sequelae of WEEV encephalitis may persist for months to years or be permanent, and postmortem brain specimens of fatal cases exhibit perivascular cuffs of lymphocytes and

Table 1 Arbovirus families: vectors, geographical distribution, and the illnesses they cause

Family	Virus	Vector	Geographical distribution	Systemic illnesses	Neurologic diseases
Togaviridae	EEEV	Mosquito (*Culiseta*, *Aedes*)	Eastern and Gulf coasts of USA, Caribbean islands, Central America, north-east South America	Flu-like illness with nausea and vomiting	Meningoencephalitis, coma
	WEEV	Mosquito (*Culiseta*, *Culex*)	Mid-west and western USA, Canada	Febrile illness	Encephalitis
	VEEV	Mosquito (*Culex*, *Aedes*)	South and Central Americas, south-east and south-west USA	Flu-like illness	Encephalitis
	CHIKV	Mosquito (*Aedes*)	Africa, India, Southeast Asia, Caribbean islands, south-east USA	Fever, rash, arthralgias, myalgias	Rare encephalitis, GBS
Flaviviridae	SLEV	Mosquito (*Culex*)	North, Central, and South America	Flu-like illness with nausea	Meningitis, encephalitis, coma
	JEV	Mosquito (*Culex*)	Japan, North-East, South-East, and Central Asia, Indian subcontinent	–	Meningoencephalitis with seizures
	WNV	Mosquito (*Culex*)	Africa, Mediterranean region, Central Asia, India, Europe, North, Central, and South Americas	Flu-like illness	Meningitis, flaccid paralysis, encephalitis
	ZIKV	Mosquito (*Aedes*), sexual transmission	Africa, India, South-East Asia, Caribbean islands, Central, North, and South Americas	Flu-like illness with arthralgias, conjunctivitis	Meningoencephalitis, ADEM, GBS, IUGR, microcephaly
	DENV	Mosquito (*Aedes*)	Asia, tropical and subtropical regions of the world	Fever, rash, headache, myalgias, hemorrhagic fever	Encephalopathy, rare encephalitis
	MVEV	Mosquito (*Culex*, *Aedes*)	Australia, New Zealand, New Guinea	Flu-like illness with nausea	Encephalitis
Bunyaviridae	CEV	Mosquito (*Aedes*)	Western USA	Flu-like illness with nausea and vomiting	Meningoencephalitis with seizures
	LACV	Mosquito (*Aedes*)	Mid-west and eastern USA	–	Meningoencephalitis with seizures
	TOSV	Sandfly (*Phlebotomus*)	Europe, North Africa	–	Meningitis
	RVFV	Mosquito (*Culex*, *Aedes*)	East and South Africa, Saudi Arabia	Fever, hepatitis, hemorrhagic fever	Encephalitis
Reoviridae	CTFV	Ticks (Dermacentor)	Rocky Mountains of the USA	Flu-like illness with nausea, vomiting, hepatitis, rash, hemorrhagic fever	Meningitis, encephalitis

EEEV = eastern equine encephalitis virus; WEEV = western equine encephalitis virus; VEEV = Venezuelan equine encephalitis virus; CHIKV = Chikungunya virus; GBS = Guillain-Barré syndrome; SLEV = Saint Louis encephalitis virus; JEV = Japanese encephalitis virus; WNV = West Nile virus; ZIKV = Zika virus; ADEM = acute demyelinating encephalomyelitis; IUGR = intrauterine growth retardation; DENV = Dengue virus; MVEV = Murray Valley encephalitis virus; CEV = California encephalitis virus; LACV = La Crosse virus; TOSV = Toscana virus; RVFV = Rift Valley fever virus; CTFV = Colorado tick fever virus

neutrophils, multifocal necrosis, and gliosis throughout the basal ganglia, thalamus, and brainstem [48].

CHIKV infection predominantly results in an acute illness with high fever and severe arthralgias lasting weeks to months but sometimes for years [52, 53]. However, severe and fatal disease with CNS involvement in both adults and neonates has been reported and include encephalitis and postinfectious syndromes, including acute demyelinating encephalomyelitis and GBS [11–15]. Neonatal infections occur via vertical transmission during pregnancy or at birth [54].

Flaviviridae

Most individuals infected with flaviviruses are asymptomatic; however, up to 25 % of infections may present with symptomatic disease that is mild or neuroinvasive [55]. JEV and MVEV cause mild, febrile illnesses in a majority of symptomatic patients, with only 1 in 250 patients developing meningoencephalitis characterized by the rapid onset of high fever, headache, neck stiffness, disorientation, coma, seizures, spastic paralysis, movement disorders, and, in 30 % of cases, death. Of those who survive, 20 % to 30 % exhibit persistent motor and/or cognitive deficits with recurrent seizures [56, 57]. These illnesses occur in a bimodal distribution, affecting the very young or nonimmune visitors to endemic regions, and the elderly, whose immunity has waned. The neuropathology of JEV and MVEV involves neuronal damage and inflammatory infiltrates, and viral antigen may be detected within neurons of the thalamus, hippocampus, substantia nigra and medulla oblongata [58, 59].

In contrast, SLEV and WNV cause symptomatic infections in adults, especially those that are chronically ill, immunosuppressed, or elderly. In the USA, WNV transmission has also occurred through infected organ transplants and blood products [60, 61], necessitating massive screening practices. Half of symptomatic infections are limited to a febrile illness with pharyngitis, myalgia or arthralgia, and rash [62]. Neurologic diseases occur in the remainder, and depend on the site of infection within the CNS. Thus, patients can present with meningitis, encephalitis, or myelitis with flaccid paralysis [63]. Patients with encephalitis may present with seizures, movement disorders, or diaphragmatic paralysis necessitating permanent dependence on mechanical ventilation [64, 65]. Neuropathologic findings include perivascular and leptomeningeal inflammation, microglial nodules, and neuronophagia, predominantly involving the temporal lobes and brainstem. These findings may also occur in the spinal cord [62, 66, 67].

Primary infection with DENV causes a self-limited, acute febrile illness with headache, malaise, retro-orbital pain, myalgia, arthralgia, and a generalized, maculopapular rash [68]. Dengue hemorrhagic fever (DHF) results from secondary infection and is characterized by a recurrent high fever with vascular leak causing widespread bleeding and multisystem disorder. Presentations of CNS disorder were therefore initially attributed to DHF; however, recent studies indicate that patients with DHF infection may present with acute encephalitis, GBS, and polyradiculopathy, and that DENV is directly neurotropic [68–71].

Prior to the current outbreak, ZIKV infections in humans were reportedly mild, with 70 % to 80 % of infections being asymptomatic [72]. Clinical symptoms, when present, are similar to a flu-like illness with fever, maculopapular rash, nonpurulent conjunctivitis, fatigue, and arthralgia, and last approximately 1 week [73]. However, since 2007, an increase in cases of microcephaly, retinal lesions, and GBS associated with ZIKV infection has been reported, especially in Latin America [27, 74–76]. ZIKV-associated GBS is a pure motor axonal variant. Infection in pregnant women causes catastrophic fetal abnormalities, including microcephaly, spontaneous abortion, and intrauterine growth restriction due to placental insufficiency [27]. Newborns with microcephaly typically have significant neurologic defects and seizures, with variable effects on developmental milestones and cognitive abilities [77, 78].

Bunyaviridae

Encephalitides due to CEV and LACV occurs after initial incubation periods of 3 to 7 and 5 to 15 days, respectively. Both initially present as a summertime febrile illness with headache, nausea, vomiting, abdominal pain, and lethargy [3]. Encephalitis is characterized by fever, focal neurologic abnormalities with altered mental status that can progress to seizures and coma. However, the mortality rate is < 1 %. Severe illness is more common in young children and 20 % may continue to have seizures after recovery. The low incidence and mortality rate has led to a lack of autopsy specimens for neuropathologic analyses.

Most RVFV infections are asymptomatic or cause a mild febrile illness with headache, myalgias, and mild hepatitis. In < 2 % of symptomatic cases, the illness progresses to hemorrhagic fever, meningoencephalitis, and/or necrotizing retinitis leading to blindness [37]. While the overall mortality rate for severe RFVF infections is < 1 %, it may approach 50 % in hemorrhagic cases. Neuropathologic findings include diffuse perivascular infiltrates of lymphocytes and macrophages, multifocal meningitis, and focal areas of neuronal necrosis and aggregates of macrophages, lymphocytes, and neutrophils throughout all regions of the brain [79].

Similar to other members of the Bunyaviridae, TOSV infection is generally asymptomatic or causes a flu-like, febrile illness with headache and myalgia, mostly in younger people [38]. This newly emerging pathogen causes outbreaks of meningitis and meningoencephalitis mostly in older individuals. There have also been reports of severe cases complicated by

hydrocephalus, deafness, and ischemic events [38]. Neuropathologic studies have been performed in a single lethal case of meningoencephalitis and showed extensive perivascular and parenchymal infiltration of CD8 T cells with microglial activation in a variety of CNS regions, including the neocortex, basal ganglia, hypothalamus, thalamus, limbic areas and brain stem, and infiltration of immune cells within the meninges [80].

Reoviridae

After CTFV infection, the virus may persist in the bloodstream for up to 4 months, which has previously led to transfusion-acquired infections [81]. Persons infected with CTFV usually exhibit signs and symptoms within 3 to 6 days of a tick bite [82]. The illness is two staged, with an initial episode of fever, chills, headaches, photophobia, myalgia, malaise, abdominal pain, hepatosplenomegaly, nausea and vomiting, and a rash. The second stage is heralded by a high fever with return of symptoms and an increase in their severity with progression to meningitis, meningoencephalitis, or hemorrhagic fever. Children may exhibit the most severe symptoms and require extended hospitalization; death is extremely rare [82].

Recovery from Arbovirus Encephalitis

Survivors of arbovirus encephalitis may continue to exhibit significant neurocognitive deficits that persist for years after clearance of virus. For example, studies evaluating the rates of persistent impairment in patients previously diagnosed with WNV encephalitis via serum or cerebrospinal fluid (CSF) IgM using memory (Hopkins Verbal Learning Test) and visuospatial (Rey Complex Figure Copy and Recall) tests report that 40 % to 70 % exhibit cognitive symptoms for up to 5 years after the episode of acute infection [83–93]. Thus, for WNV encephalitis, at a > 90 % rate of survival with ~50 % incidence of cognitive disturbance, there are currently approximately 10,000 people living with this sequelae of neuroinvasive WNV infections, with additional cases occurring at rates of 1000 to 3000 per year. Additional encephalitic arboviruses that lead to neuroinvasive disease with neurocognitive sequelae in American patients include SLEV, LACV, EEEV, and Powassan virus, with total case numbers in the hundreds [94–97]. Finally, worldwide, encephalitic arboviruses, including JEV and RVFV, cause neurologic illness at the rate of 50 to 100,000 cases/year with neurocognitive sequelae in survivors [98–100]. There are currently no diagnostic or treatment modalities for cognitive sequelae in patients that recover from arbovirus encephalitides.

Mechanisms of Peripheral Infection, Pathogenesis, and Host Defense

While the pathogenesis of arboviruses in humans has not been extensively explored, the use of animal models has elucidated conserved mechanisms and strategies in both arbovirus infection and host defense. During the course of a mosquito or tick bite up to 10^6 plaque-forming units of virus can be transferred into the host [101]. In experimental models, animals receive viral inoculum subcutaneously to mimic arthropod-borne infection. During the early phase of infection, arboviruses undergo an initial period of replication in the skin. Flaviviruses, such as WNV, replicate in both keratinocytes and skin dendritic cells (DCs), including Langerhans cells [102–104]. Infected DCs migrate to draining lymph nodes, leading to an additional round of infection and subsequent entry into the circulation via efferent lymphatic system and the thoracic duct. Similar early replication and trafficking to draining lymph nodes has been observed in VEEV [105]. However, EEEV, a related alphavirus, does not replicate readily in lymphoid tissues but instead replicates in fibroblasts before targeting osteoblasts [106, 107].

Once in the blood, infection spreads to visceral organs, including the spleen and kidney. The specific cellular targets in these tissues are not well identified, but likely targets are subsets of DCs, macrophages, and neutrophils [108–110]. The level of viremia has been shown to correlate with the viral dissemination to the CNS [111]. Therefore, the early immune responses may be critical to limiting the neuropathogenesis of neurotropic virus.

Infection of leukocytes within secondary lymphoid tissues leads to activation of the innate immune response, including antiviral cytokine expression, expansion of leukocytes, antigen processing, and presentation to T cells. Pathogen-recognition receptors (PRRs) in infected cells recognize pathogen-associated molecular patterns associated with RNA viruses at multiple stages during the replication cycle. Endosomal nucleic acids sensors Toll-like receptor (TLR)3 and TLR7 are capable of sensing viral double-stranded RNA and single-stranded RNA, respectively, during cell entry, while the cytoplasmic double-stranded RNA sensors, retinoic-acid inducible gene I (RIG-I) and melanoma differential-associated gene 5 (MDA5) can identify viral RNA products during replication. RIG-I and MDA5 have been demonstrated to recognize WNV and VEEV *in vitro*. Although their role in shaping VEEV infection *in vivo* has not been explored [112, 113], RIG-I and MDA5 are essential for control of WNV replication in the periphery. Mice lacking mitochondrial antiviral-signaling protein (MAVS), the central adaptor to both RIG-I and MDA5 signaling, exhibited increased viremia and viral load in peripheral tissues [114]. However, MDA5 itself has been demonstrated to play a limited role in controlling WNV replication in the periphery

[115]. Ablation of TLR3 results in mildly increased WNV burden in peripheral tissues [116, 117]. However, these studies have described opposing phenotypes regarding survival during WNV infection of TLR3$^{-/-}$ mice. TLR7$^{-/-}$ mice are more vulnerable to WNV infection and exhibit increased viremia, independently of regulation of cytokine expression [118]. For the bunyavirus, LACV, both endosomal cytosolic PRRs are necessary for survival and type 1 interferon (IFN) production [119].

The type I IFN response is a critical component of the regulation of viral infection. Ablation of the IFN-α/β receptor results in enhanced lethality in many arboviruses, including WNV [120, 121], VEEV [122–124], and LACV [125, 126]. Additionally, exogenous IFN-α promotes survival by inhibiting peripheral replication of WNV and VEEV [127, 128]. Activation of PRRs results in activation of IFN regulatory factors (IRFs) 3 and 7, expression of type 1 IFNs, IFN-α and, IFN-β, which induce the expression of IFN-stimulated genes (ISGs). Several antiviral ISGs, including protein kinase RNA-activated (PKR), 2'5'-oligoadenylate synthase (OAS), members of the poly (ADP-ribose) polymerase family and IFN-induced transmembrane protein family members have global effects on the cell in order to restrict viral replication. PKR inhibits both host and viral translation by regulating eIF-2α. Viremia is increased in PKR$^{-/-}$ mice, an effect that is independent of PKR-dependent augmentation of IFN expression [110, 129]. OAS generates 2'5'-oligoadenylates to activate RNase L to degrade viral and cellular RNAs. RNaseL$^{-/-}$ mice exhibit higher WNV viral loads in peripheral tissues. Members of the poly (ADP-ribose) polymerase family block translation and replication of VEEV [130, 131]. IFN-induced transmembrane protein family members play a key role in limiting bunyavirus, including RVFV and LACV, replication by inhibiting viral membrane fusion in the endosome [132].

The innate immune response is critical for the limitation of viral propagation, especially during early infection. However, viral clearance in the periphery is dependent on the adaptive immune response. Both T and B lymphocytes are critical, as severe combined immunodeficiency, RAG1, or B cell-deficient, μMT mice all exhibit enhanced lethality to both WNV and VEEV [133, 134]. Activation of B cells by infected DCs within lymph nodes induces neutralizing antibody responses, which is critical for viral clearance in the periphery [135]. Specifically, it is IgM that is essential for clearance of WNV or VEEV from the blood [136]. IgG is more critical for limiting dissemination and clearance from infected tissues.

Type II IFN (IFN-γ), which is produced by γδT cells, CD8 T cells and natural killer cells, also exerts an antiviral role in the periphery. Mice lacking IFN-γ or IFN-γ receptor exhibit increased viremia and viral burdens in lymphoid tissues leading to early CNS invasion and decreased survival [137]. Similarly, γδT cells, in part through action as IFN-γ producers, are necessary to limit WNV viremia [138]. However, in an intranasal inoculation model of WNV-induced seizures, IFN-γ was immunopathogenic, as IFN-γ$^{-/-}$ mice displayed resistance to induction of limbic seizures.

Entry of Arboviruses into the CNS

Shortly after viremia, arboviruses enter the CNS via several mechanisms, depending on host and viral factors. Despite years of intensive research, the exact mechanisms by which arboviruses enter the CNS remain unclear. However, several pathways have been described as outlined below (Fig. 1).

Arboviral infections typically occur in highly enervated dermis, which may lead to viral entry into the CNS through peripheral neurons. Both *in vitro* and *in vivo* studies have shown that WNV can spread along neurons via retrograde axonal microtube-mediated transport [139, 140]. Indeed, intrasciatic inoculation of hamsters with WNV resulted in infection of spinal cord and acute flaccid paralysis. Similarly, pretreatment of mice with nocodazole, a microtubule destabilizing agent, delayed WNV infection of the brain, further supporting the involvement of retrograde axonal transport in CNS invasion [141].

CNS invasion via olfactory bulb has been demonstrated for several encephalitic arboviruses, including SLEV [142], MVEV [143], VEEV [144], EEEV [145], and LACV [146]. Soon after onset of viremia, viral particles exit the fenestrated capillaries beneath the mucosa of the nasal cavity. There, they are directly exposed to olfactory sensory neurons (OSNs), which are highly susceptible to infection. OSNs reside in the olfactory neuroepithelium with dendritic terminals that project into the nasal cavity and axonal projections that extend the cribiform plate into the glomerular structures of the olfactory bulb, thereby providing an axonal pathway for neurotropic viruses to gain access to the CNS. Infection of OSNs is followed by viral spread into the olfactory bulb via the anterograde axonal pathway [142, 144]. Once inside the olfactory bulb, the virus disseminates rapidly to the brain tissues. Importantly, previous reports have shown that VEEV and EEEV infection of nonhuman primates through the aerosol pathway does not require development of viremia for neuroinvasion [147, 148].

CNS infection by arboviruses typically occurs shortly after viremia, suggesting the bloodstream as a critical route for CNS entry across the blood–brain barrier (BBB). The BBB is a tightly regulated interface that separates the CNS from the circulating blood. This barrier is composed of a basal membrane, brain microvascular endothelial cells (BMECs), with extensive tight junctions (TJs) and adherens junctions, which are ensheathed by pericytes and astrocyte end-feet [149]. Virus may enter via either direct infection of BMECs, passive diffusion through the BBB, transcellular transport through BMECs, or by paracellular migration between endothelial

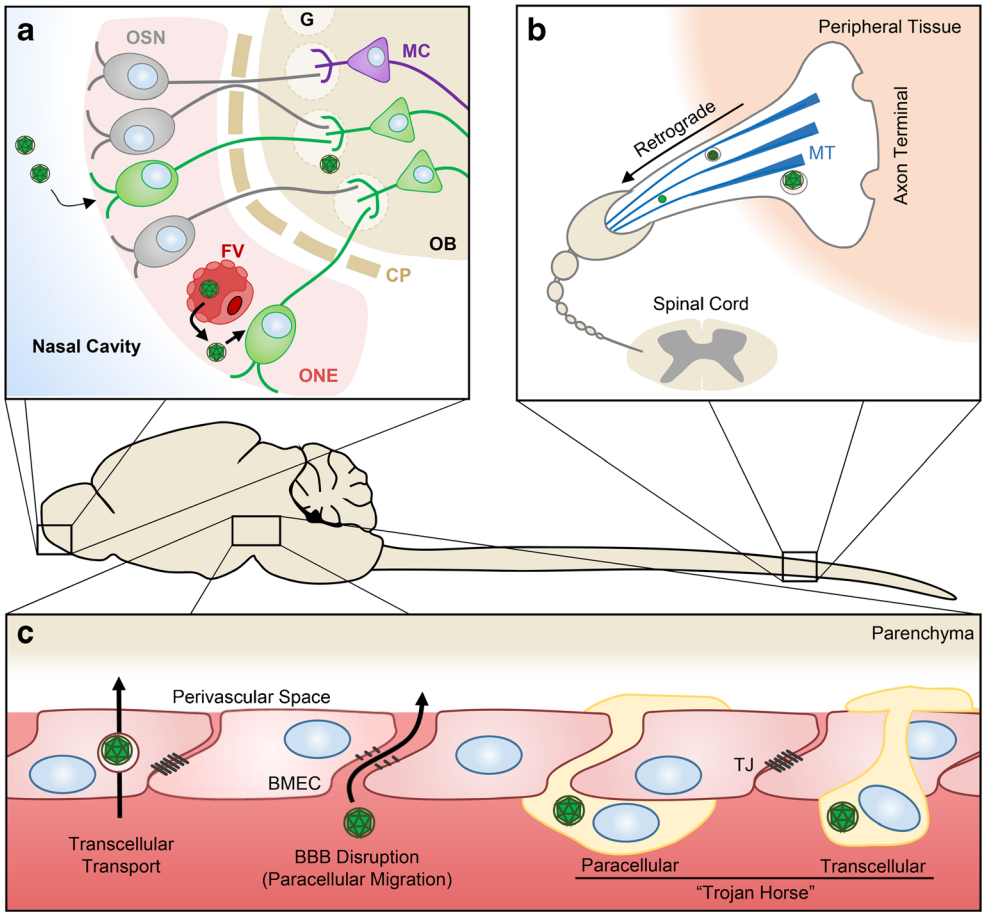

Fig. 1 Routes of arbovirus entry into the central nervous system (CNS). (A) Infection of olfactory sensory neurons (OSN) in the olfactory neuroepithelium (ONE) following intranasal inoculation or ONE infection from fenestrated vessels (FV). CNS entry occurs after viral migration through the cribiform plate (CP), subsequent infection of mitral cells (MC) at the glomeruli (G) of the olfactory bulb (OB), and dissemination along neuronal tracts. (B) Retrograde transport of virus along axon microtubules (MT) of peripheral neurons facilitates entry into the CNS at the spinal cord. (C) Virus entry through the blood–brain barrier (BBB) is dependent on transcellular transport by brain microvascular endothelial cells (BMECs) of virions or paracellular migration of virions following disruption of tight junctions (TJ). Infected leukocytes may also facilitate CNS entry via paracellular or transcellular extravasation – the "Trojan Horse" model FV = fenestrated vessel; BMEC = brain microvascular endothelial cells

TJs after BBB disruption. Although BMECs have been shown to be infected by several encephalitic arboviruses *in vitro* [150–152], there is no strong evidence of arboviral infection of BMECs *in vivo*, indicating that BMEC infection is not necessary for entry to the CNS [153, 154]. Passive diffusion of virus across the BBB has been proposed previously; yet, convincing evidence to support this hypothesis has still to be demonstrated. However, WNV is transcellularly transported from the apical to basolateral side of murine BMECs *in vitro*, independently of replication and without impairing the integrity of TJs [150, 155]. Likewise, ultrastructural studies in infected mice revealed JEV within endocytic vesicles of BMECs [156].

Paracellular entry of virus may occur following disruption of the TJs of the BBB. In a model of WNV infection using insect cell-derived WNV, BBB disruption coincided with peripheral infection and preceded CNS entry [152, 157]. However, recent studies using mice infected with JEV [151], WNV [158, 159], and tick-borne encephalitis virus (TBEV) [160] have demonstrated that CNS entry of virus can occur before BBB disruption. Paracellular entry under these conditions may contribute to a second phase of CNS infection. Indeed, initial infection of CNS by VEEV via the olfactory route impairs the BBB leading to a CNS infection through hematogenous spread [161]. This observation was further supported by other studies where intracranial inoculation of mice with VEEV replicon particles resulted in increased expression of intercellular adhesion molecule 1 (ICAM-1) on BMECs, followed by BBB disruption [162].

An alternative pathway of CNS infection is the "Trojan horse" model in which infected leukocytes cross the BBB, thereby delivering virus into the CNS. Under normal conditions, BMECs express very low levels of leukocyte adhesion molecules (i.e., platelet endothelial cell adhesion molecule 1, ICAM-1 and vascular cell adhesion protein 1). However, infection with alphaviruses and flaviviruses upregulates BMEC

expression of these adhesion molecules [151, 163, 164], promoting extravasation of leukocytes into the CNS. Consistent with this notion, increased expression of ICAM-1 has been shown to precede CNS invasion of mice infected with WNV [165]. Additionally, mice deficient in ICAM-1 displayed decreased CNS viral loads, diminished neuronal damage, and reduced BBB permeability following infection with WNV [166]. The Trojan horse model has also been suggested for JEV [167, 168]. Nonetheless, whether infected leukocytes maintain their function to migrate into the CNS remains controversial, as no ultrastructural evidence of virus infection was observed in infiltrating leukocytes after infection with MVEV, a zoonotic flavivirus [169]. Collectively, the abovementioned studies suggest that disruption of BBB can be a prerequisite for or a consequence of CNS infection, and that arboviruses seem to utilize multiple pathways for CNS entry, depending on virus, route of infection, dose, and age.

Neuropathogenesis of Arboviruses

Within the CNS, neurons are the main target cells for encephalitic flaviviruses [154, 170], alphaviruses [107, 145], and members of the Bunyaviridae and Reoviridae [146, 171, 172]. Arboviral infection of other cell types of the CNS has also been reported (Table 2), and recent *in vitro* studies with ZIKV suggest it may target neural progenitor cells [173–176]. RNA of ZIKV has also been detected in samples of brain tissue, placenta, and amniotic fluid of newborns with microcephaly and in the stillborn infants of women infected by Zika during pregnancy [28].

The envelope protein of encephalitic arboviruses is the major determinant of neurotropism and neurovirulence, owing to its role in receptor binding and virus entry [177–179]. However, sequence analysis of virulent and attenuated strains of these viruses revealed that other regions of the viral genome, including the 3′ untranslated region and nonstructural proteins, also contribute to neurovirulence and viral tropism [180].

Arboviral neuropathogenesis involves two distinct features: neuroinvasiveness and neurovirulence. Neuronal damage and loss can occur by either direct arboviral infection or indirectly by uncontrolled immune responses to the replicating virus [181, 182]. *In vitro* and *in vivo* studies have shown that neurons undergo morphologic changes that are characteristic of apoptosis, that is, nuclear and membrane condensation, after infection with several encephalitic flaviviruses, including WNV [183], JEV [154], TBEV [184], and alphaviruses [145, 185, 186]. Neuronal degeneration, necrosis, and apoptosis were also demonstrated in various CNS regions in mice infected with LACV [146].

Several mechanisms appear to be involved in the induction of apoptosis by encephalitic arboviruses. For instance, WNV and JEV induce neuronal apoptosis through caspase-3 and Bax signaling pathways [154, 187], depending on brain region or viral strain [187]. Indeed, mice lacking caspase-3 displayed

Table 2 Susceptibility of central nervous system cells to infection by encephalitic arboviruses

	Neurons		Astrocytes		Microglia		BMECs		References
	In vitro	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*	
Flaviviruses									
WNV	Yes	Yes	Yes	No	Yes	No	Yes	No	[152, 286, 287]
JEV	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes/no	[151, 288–290]
SLEV	Yes	Yes	NIA	Yes	NIA	Yes	NIA	No	[142, 291, 292]
TBEV	Yes	Yes	Yes	No	Yes	NIA	NIA	No	[198, 241, 293]
MVEV	Yes	Yes	No	No	No	No	No	No	[143, 294]
Togaviridae									
VEEV	Yes	Yes	Yes	Yes	NIA	Yes	NIA	No	[185, 204, 295, 296]
EEEV	NIA	Yes	NIA	Yes	NIA	Yes	NIA	No	[107, 145, 297]
Bunyaviridae									
LACV	Yes	Yes	NIA	Yes	NIA	Yes/no	NIA	No	[146, 218, 298]
RVFV	Yes	Yes	NIA	Yes	NIA	Yes	NIA	No	[299–301]
Reoviridae									
CTFV	NIA	Yes	NIA	NIA	NIA	NIA	NIA	NIA	[171]
POWV	Yes	Yes	NIA	Yes	NIA	Yes	NIA	No	[172, 302, 303]

BMECs = brain microvascular endothelial cells; WNV = West Nile virus; JEV = Japanese encephalitis virus; SLEV = Saint Louis encephalitis virus; NIA = no information available; TBEV = tick-borne encephalitis virus; MVEV = Murray Valley encephalitis virus; VEEV = Venezuelan equine encephalitic virus; EEEV = Eastern equine encephalitic virus; LACV = La Cross virus; RVFV = Rift Valley fever virus; CTFV = Colorado tick fever virus; POWV = Powassan encephalitic virus

reduced neuronal death compared with wild-type mice, despite having comparable viral loads in their brain [183]. Similarly, inhibition of caspase activity reduced WNV-mediated neuronal death in primary neuronal culture [183]. Both structural and nonstructural proteins of flaviviruses were involved in caspase-mediated neuronal apoptosis [188–190]. A second mechanism of neuronal apoptosis involves RIG-I-like receptor (RLR) signaling. While RLR-induced activation of MAVS exhibits antiviral activity [191], it can cause neuronal death by triggering sterile alpha and TIR motif-containing 1 (SARM1) protein [192]. In this regard, mice lacking SARM-1 appeared to be more resistant to LACV-mediated neuronal damage than wild-type mice, despite having similar viral loads in their brain [192]. Nonetheless, an earlier study demonstrated a neuroprotective role for SARM-1 against lethal WNV infection [193]. Finally, neuronal apoptosis can be induced by increased expression of apoptotic-related genes, that is, *TNFα*, *FasL*, and *TRAIL*, which have been shown following infection with some encephalitic arboviruses such as TBEV and VEEV [186, 194]. In VEEV-infected mice, neuronal apoptosis was observed in areas of the brain that contained astrogliosis and inflammation in the absence of viral antigens [185, 186]. Similarly, apoptosis of uninfected neurons was identified in a primate model of JEV infection [154], indicating an alternate, indirect mechanism of neuronal death. A better understanding of molecular elements involved in arboviral-mediated neuronal apoptosis will provide insights into mechanisms of neurodegeneration and will aid in development of antiviral therapies.

While inflammatory responses within the CNS play an important role in both control and clearance of viral infection, uncontrolled responses can be neuropathogenic. Indeed, neuropathogenesis of arboviruses has been often associated with neuroinflammation, which can cause neuronal death and disruption of the BBB. Following CNS infection by arboviruses, both infected target cells and bystander cells release an array of chemokines and proinflammatory cytokines, which trigger neuroinflammation. Neurons release several inflammatory cytokines, such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-6 in response to infection by encephalitic flaviviruses, such as WNV and JEV [195]. As well as impairing BBB integrity [152], elevated levels of IL-1β can induce cell cycle arrest and apoptosis in neural precursor cells (NPCs) [196]. TNF-α impairs glutamate uptake by astrocytes, leading to neuronal death due to excitotoxicity [197]. Consistent with this, it has been shown that increased expression of TNF-α after infection with WNV and JEV is correlated with neuronal death [154, 195], and that neutralizing antibodies specific for IL-1β and TNF-α protected neurons from WNV-induced cell death [195]. The release of inflammatory cytokines by neurons can trigger gliosis (i.e., microglial and astrocyte activation), which is one of the major hallmarks of arboviral neuropathogenesis [154, 186, 198].

Although glial cells are not generally as permissive to arboviral infection as neurons, they release many more inflammatory mediators, which is likely to perturb the balance between protective and pathogenic immune responses [199]. Activated astrocytes and microglia play an important role in inflammatory responses during natural infection with encephalitic flaviviruses, namely JEV [200], WNV [201], and TBEV [198], as well as encephalitic alphaviruses [186]. Upon activation, astrocytes and microglia release several chemokines and cytokines such as TNF-α, IL-6, C-X-C motif chemokine ligand (CXCL)10, chemokine (C-C motif) ligand 2, monocyte chemoattractant protein-1, RANTES, IFN-γ-induced protein 10, and matrix metalloproteinases [154, 195, 202–204]. Elevated levels of matrix metalloproteinases disrupts BBB by degrading TJ proteins [203, 205], allowing unrestricted entry of leukocytes into the brain, which further exacerbates neuroinflammation. Activated microglia also release nitric oxide (NO) in response to infection with flaviviruses [202, 206]. While NO clearly has antiviral activities, a high level of NO is toxic to neurons, and its antiviral effects can also be virus specific [206]. Consistently, high levels of NO was correlated with accelerated death in mice infected with VEEV [186], and its inhibition prolonged survival time in TBEV-, MVEV-, and VEEV-infected mice [204, 206, 207]. Alternatively, inflammatory responses can result in formation of reactive oxygen species, which, in turn, triggers neuronal death. Notably, attenuated strains of VEEV and TBEV induced delayed or no cytokine response in the CNS, despite displaying similar tissue tropism or replication kinetics [186, 208]. A recent study also showed that it is not virus itself but the inflammatory responses that cause BBB disruption following JEV infection of mice [151]. Thus, the extent of host immune responses is positively linked to neuropathogenesis of some encephalitic arboviruses.

Innate Immune Response to Arboviruses in the CNS

As in peripheral organs, the innate intracellular responses are the first line of defense against invading pathogens in the CNS. These rapid antiviral responses are particularly important in controlling viral replication and thus preventing irreversible loss of neurons. Type I IFN signaling is a key component of these responses, and it is triggered by recognition of arboviral pathogen-associated molecular patterns via PRRs (i.e., TLRs, NOD-like receptors, and RLRs), which are expressed by neurons and glial cells in the CNS [209]. RIG-I and MDA5 appeared to be involved in the induction of type I IFN against WNV, such that disruption of these pathways abolished activation of the antiviral response to WNV [191]. In addition, mice deficient in MAVS showed increased inflammation in CNS with no protection against WNV [114]. Similarly, lack of MAVS in primary cortical neurons was

associated with enhanced virus production after infection with WNV. These cells were also less efficient at expressing IFN-β and ISGs relative to wild-type cells [114]. Thus, RLR signaling appears to play a regulatory role in modulating innate immune responses in the CNS against flaviviruses. RIG-I-mediated induction of type I IFNs has also been shown following infection with other encephalitic arboviruses, such as LACV and JEV [210, 211].

The role of TLR signaling in induction of protective immune responses against arboviral infections remains controversial. TLR3-deficient mice displayed increased viral load in the brain following intracranial challenge with WNV [116]. Likewise, lack of TLR3 in mice infected with JEV was associated with higher frequency of inflammatory monocytes in the CNS, enhanced number of activated microglia, and elevated levels of IL-6 and TNF-α in the CNS, along with increased BBB permeability [212]. However, ablation of TLR3 did not alter virus titers in the CNS after intracranial inoculation of mice with JEV [212]. Conversely, in another study there was no significant difference in survival between wild-type and TLR3-deficient mice after intracranial infection with WNV [117]. Additionally, TLR3 was suggested to be a risk factor for severity of TBEV infections [213]. Further studies using other encephalitic arboviruses are therefore needed to better clarify the role of TLR3 responses in the CNS against invading neurotropic viruses.

Brain-specific TLR7 knockdown ($TLR7^{KD}$) mice showed no significant differences in susceptibility to JEV compared with wild-type mice when subcutaneously infected with virus [214]. However, $TLR7^{KD}$ mice demonstrated reduced levels of IFN-α mRNA accompanied by increased viral loads and proinflammatory cytokines in their brain. In addition, they had reduced levels of two additional antiviral proteins—IFN-induced antiviral RNA-binding protein 1 and OAS-like 1 (OAS1)—in their brain tissues. Similarly, TLR7-deficient mice displayed higher viral loads in the CNS than wild-type mice after intracranial inoculation with another flavivirus, Langat virus. However, the absence of TLR7 did not significantly alter the incidence or onset of clinical symptoms [215]. TLRs are also involved in recognition of alphaviruses in the brain. In this regard, increased expression of multiple TLRs, namely TLR1, TLR2, TLR3, TLR7, and TLR9, and their downstream signaling genes (i.e. *Nfkb* and *Irf*) were observed in brain tissues of VEEV-infected mice [216].

Both neurons and glial cells release type I IFNs in response to arboviral infections [217, 218]. As well as playing a critical role in controlling viral spread, tropism, and pathogenesis, IFNs are essential for the formation of TJs [152]. Mice deficient in IFN-α/βR, PKR, or RNAase L (antiviral proteins that are induced by IFN) displayed increased viral loads in CNS and accelerated death after intracranial infection with WNV [110, 121]. Likewise, pretreatment with IFN-α, IFN-β, or IFN-γ also renders cortical neurons resistance to WNV infection [110]. The residential glial cells in the brain are activated in response to IFN-γ, and express a variety of PRRs that are involved in recognition of apoptotic-associated molecular patterns on the surface of apoptotic cells. Although IFN-γ participated in the clearance of Sindbis virus, a mosquito-borne alphavirus, from infected neurons [219], it was dispensable for clearance of WNV from CNS [137]. Collectively, these observations suggest that type I IFN signaling directly modulates viral replication in CNS tissues, and that IFN-γ is indirectly involved in clearance of apoptotic cells from the CNS.

Viral Clearance from the CNS

PRR signaling leads to the induction of adaptive immune responses, which are required for clearance of viral infections in the CNS [220–222]. Increased permeability of the BBB following arboviral infection allows entry of B cells and neutralizing Abs (nAbs) into the CNS. Once in the CNS, nAbs bind cell-free viruses, preventing new viral infections. Mice deficient for B cells and antibody production demonstrated higher viral loads in CNS and increased vulnerability to lethal WNV infection than wild-type mice [221]. Similarly, adoptive transfer of monoclonal antibodies protected mice from lethal encephalitis caused by other flaviviruses, namely JEV [223], SLEV [224], and yellow fever virus [225]. Further, high levels of JEV-specific IgM and IgG in the CSF was linked to virus clearance from the CNS and a better clinical outcome in patients [226, 227]. Development of nAbs was also associated with longer survival after intracerebral inoculation of mice with TBEV [208].

Antibody-mediated virus clearance from infected neurons have also been described for alphaviruses and RVFV [228–230]. Nonetheless, antibody response appeared to be dispensable for clearance of VEEV from CNS [231]. In addition, antibody response was not required for recovery of μMT mice from encephalomyelitis induced by attenuated strains of VEEV [232], such that these mice were able to reduce dramatically virus titers in the CNS and clear virus from CSF, in the absence of antiviral antibody.

Elimination of virus-infected cells in the CNS requires a CD8 T-cell response [233–236]. In response to WNV and JEV infections, neurons release CXCL10, which is required for recruitment of CD8 T cells into the brain via the C-X-C chemokine receptor (CXCR)3 [151, 170]. Mice lacking CXCL10 displayed high viral loads in the CNS, and increased mortality after WNV infection [170]. Interestingly, CXCL10 appeared to be important for clearance of WNV from CNS but not from peripheral lymphoid tissues [170]. Unlike CXCL10, CXCL12 is constitutively expressed on basolateral surfaces of CNS endothelial and retains CD8 T cells in perivascular space via CXCR4 and inhibits their entry into CNS. Hence, inhibition of

CXCR4 results in enhanced entry of CD8 T cells into the CNS and improves mice survival from lethal WNV infection [233]. Further, mice deficient in CD8 T cells, major histocompatibility complex I, or perforin were unable to clear WNV infection from CNS, had increased viral burden in the CNS, and displayed higher mortality rate after infection with WNV than wild-type mice [137, 236].

CD8 T-cell responses are also important for clearance of other encephalitic arboviruses from the CNS. For instance, intracranial, but not peripheral transfer, of anti-JEV effector cells protected mice from lethal intracranial challenge with JEV [237, 238]. Nonetheless, CD8 T cells contribute to both recovery and immunopathology of WNV infection [239], and the presence of regulatory T cells in the CNS of mice infected with WNV is associated with reduced immunopathology both in humans and mice [240]. In addition, the CD8 T-cell response was not required for clearance of RVFV from the CNS [229], or for protection against lethal VEEV encephalitis [231]. Furthermore, analysis of paraffin-embedded autoptic brain tissue of human TBEV cases suggest that CD8 T cells can cause significant neuronal degeneration via induction of bystander damage [241], and lack of CD8 T cells was correlated with increased survival time in mice infected with TBEV [242]. Thus, CD8 T-cell response can be beneficial, deleterious, or both, depending on the virus.

CD4 T cells can also clear virus from infected neurons via a noncytolytic pathway, which involve production of IFN-γ. Adoptive transfer of CD4 T cells into RAG knockout mice resulted in virus clearance from the CNS and conferred resistance to lethal WNV infections in the absence of any B cells or CD8 T-cell responses [243]. In addition, mice deficient in CD4 displayed prolonged WNV infection in the CNS, and eventually died 50 days postinfection. Importantly, CD4-deficient mice showed unaltered viral loads in the spleen, indicating that CD4 T cells are required for limiting WNV infection in the CNS but not in the periphery [222]. CD4 T cells are also required for protection against infection with other arboviruses, including JEV [237], yellow fever virus [244], TBEV [242], RVFV [229], and VEEV [231]. CD4 T-cell response resulted in a dramatic reduction of virus titers in the CNS and mediated full recovery of μMT mice from VEEV-induced encephalomyelitis [232].

Susceptibility Factors for Arboviral Encephalitis: Lessons from Murine Models

The clinical manifestation of arboviral infections in both mice and humans varies greatly, depending on several host and virus related factors. The clinical outcomes are also influenced by the route of viral administration. For instance, dermal inoculation of C3H/HeN and BALB/C mice with TC83, a highly attenuated strain of VEEV, is typically avirulent [50]. Nonetheless, aerosol inoculation of C3H/HeN mice with TC83 led to encephalitis and 100 % lethality [245]. Age is another determining factor that affects the clinical presentation of arboviral infection. While immunocompetent individuals of all ages may present with neurologic symptoms after infection with WNV, the risk of severe disease increases in elderly, as well as immunocompromised, hosts. Lethal infections of WNV are more prevalent in older individuals [246], despite a fairly uniform incidence rate among different age groups [247]. The age-related increased vulnerability to WNV in a mouse model was correlated to defects in CD4 and CD8 T-cell responses [248]. CD8 T cells from old mice were severely inefficient in producing IFN-γ, TNF-α, and granzyme B. In line with this, passive transfer of IFN-γ or perforin-deficient T cells into RAG knockout mice did not confer any noticeable anti-WNV protection [248]. Dysregulation of TLR3 and TLR7 responses in the elderly has also been associated with enhanced vulnerability to lethal WNV infections [249, 250].

In contrast to WNV, JEV demonstrates particular tropism for developing neurons and NPCs [251]. Hence, children are at high risk of developing neurologic problems after JEV infection. Although JEV infection does not affect viability of NPCs, it severely impairs their proliferation ability [251]. Likewise, severity of LACV infections reduces with increasing age, both in humans and mice [119, 252]. Weanling mice displayed reduced type I IFNs responses, which was associated with high viral loads in the brain and severe neurologic disease. In contrast, strong production of type I IFN in adults limited virus dissemination to the CNS and provided protection against lethal LACV. Notably, differences in myeloid DC responses between weanling and adult mice were accounted for vulnerability to LACV-induced neurologic disease [119]. Similarly, maturation of neurons *in vitro* restricted replication of several arboviruses, including Sindbis virus, VEEV, WEEV, and LACV [253, 254], and delayed virus-induced translational arrest [254], which allows a longer period for production of antiviral proteins by the host. Indeed, early restriction of virus replication in the periphery contributed to age-dependent resistance to alphaviruses [255].

Type I IFN responses also appear to be important for placental infection with ZIKV. In a recent publication, two murine models of placental and fetal disease associated with *in utero* transmission of ZIKV were established via use of genetic deletion or antibody neutralization of IFN-α/IFN-β receptor [256]. These models may facilitate the study of the teratogenicity of ZIKV and allow testing of therapies and vaccines to prevent congenital malformations.

The incidence and severity of arboviral infection is also influenced by genetic variations in the host. Results from previous cohort studies revealed a direct correlation between CCR5 Δ32 homozygosity and symptomatic disease during infections with WNV and TBEV [257, 258]. However, the results from recent follow-up studies using larger cohorts

failed to show any association between CCR5 deficiency and symptomatic infection with WNV or TBEV [259]. These discrepancies may be explained by differences in study design, cohort size, and the control populations used in these studies. Infection of mice with WNV led to an increase in the expression of CCR5 and its ligand, which was associated with enhanced migration of leukocytes into the brain and protection of mice against lethal infection [260]. Strong correlation between WNV infection and single nucleotide polymorphisms in IRF3, myxovirus resistance protein 1 and OAS1 was also observed by other studies [259, 261]. Similarly, genetic variations within the human OAS1 gene was found to modulate the outcome of infections with TBEV [262], and individuals with major histocompatibility complex B49 on CD8 T cells appeared to be more vulnerable to lethal LACV infections [263]. Elucidating the host genetic factors contributing to arboviral pathogenicity is imperative for identification of individuals that are at high risk of severe arboviral-induced disease, and providing potential therapeutic targets.

Treatment and Prevention of Arbovirus Neurologic Infections

There are currently no approved antiviral medications for the treatment of neurologic infections with arboviruses. Symptomatic relief may be provided with the use of antipyretics, fluid reconstitution in cases of severe emesis, or use of life support to maintain respiratory and circulatory systems as the infection is cleared or in the event of organ failure. In the following sections, we highlight treatments currently under investigation for encephalitis due to JEV, WNV, and LACV, and licensed and investigational vaccines used to prevent arbovirus infections.

JEV

Several investigators have highlighted the role of oxidative stress in neuronal apoptosis during JEV infection of the CNS. Consequently, several antioxidants have been studied to evaluate therapeutic efficacy during CNS infection with JEV. Minocycline, a semisynthetic derivative of tetracycline, was shown to protect mice from challenge with JEV when treatment is initiated within 24 h of infection [264]. The mechanism of action of minocycline was shown in a variety of studies to involve inhibition of oxidative stress with reduction of reactive oxygen species [265, 266]. In a recent randomized controlled clinical trial, trends towards better outcomes were observed when minocycline was administered to individuals over the age of 12 years [267]. However, larger studies are needed to determine the true clinical efficacy of minocycline for JEV encephalitis. Similarly, arctigenin, a lignin derived from the greater burdock (*Arctium lappa*), was shown to inhibit oxidative stress from microglial activation and protect mice from challenge with JEV [268]. However, this agent has not been evaluated in clinical trial. Additional antioxidants have been evaluated for effects on JEV-mediated cell injury *in vitro*. These include the peroxisome proliferator-activated receptor-α agonist fenofibrate [269], and curcumin, a naturally occurring phenolic compound extracted from the rhizome of *Curcuma longa* L. [270]. However, these have not yet been tested in animal models.

There are two JEV vaccines that are licensed in the USA. An inactivated mouse brain-derived JE vaccine (JE-VAX; The Research Foundation for Microbial Diseases of Osaka University, Osaka, Japan), was originally licensed in 1992 to prevent JE in persons aged ≥ 1 year that are traveling to JEV-endemic countries [271]. However, as production of this vaccine has ceased, supplies are limited. In March 2009, an inactivated Vero cell culture-derived vaccine (IXIARO; Valneva Austria GmbH, Wien, Austria) was licensed for use in persons aged ≥ 17 years [272, 273]. Mouse brain-derived JE vaccine is now the only JE vaccine available for use in children aged 1 − 16 years and is being reserved for this age group. Other JEV vaccines, including live-attenuated SA14-14-2 JEV vaccine, are manufactured and used in Asia but are not licensed for use in the USA [274].

WNV

The critical role of type I IFN in virologic control during WNV infection has led to the use of IFN-α in human WNV infections, with varying results [152, 275]. In 1 report, 2 middle-aged patients with encephalitis exhibited marked improvement in CNS function after 2 doses, and had limited neurologic sequelae with almost complete recovery [276]. However, despite treatement with IFN-α, an elderly patient with WNV encephalitis and flaccid paralysis died [277]. Clinical trials are needed to better evaluate the efficacy of IFN-α in patients of varying ages with WNV encephalitis.

While there are 4 US Department of Agriculture-approved, licensed vaccines to prevent illness due to WNV infection for horses, there are no licensed vaccines for prevention of disease in humans. However, there is currently an ongoing National Institutes of Health-sponsored clinical trial using WNV inactivated with HydroVax-001, a novel, hydrogen peroxide-based process that inactivates virus while preserving immunoreactive surface structures [278].

LACV

Early studies examining antiviral agents demonstrated that ribavirin inhibits the transcription of the LACV genome in host cells [279]. Several case reports of its use in the setting of severe LACV encephalitis suggested it may improve outcome [280, 281]. In phase I, IIA, and IIB clinical trials,

however, higher doses were needed to achieve appropriate CSF levels of ribavirin, which led to adverse events that necessitated trial discontinuation [282]. There are currently no licensed or investigational vaccines for LACV.

VEEV, EEEV, and WEEV

Treatment of encephalitis due to VEEV, EEEV, or WEEV is mostly supportive as there are no antiviral treatments for these viruses. A live-attenuated VEEV vaccine (TC-83) is licensed for protection of horses in endemic areas. The TC-83 strain was generated via passage of strain Tr-D 83 times in heart cells of a guinea pig; C-84 is a derivative of TC-83 [283]. It is highly reactogenic, causing illness in up to 20 % of recipients, but poorly immunogenic. Military personnel require boosters with investigational formalin-inactivated VEEV [284]. EEEV and WEEV formalin-inactivated vaccines are available, but are poorly immunogenic with unknown ability to protect against variant viruses [283, 285]. There are no prophylactic or therapeutic drugs to prevent or treat, respectively, VEEV, EEEV, or WEEV encephalitis.

Acknowledgments Funding for was provided by the National Institutes of Health (R01 NS052632, U19 AI083019, and P01 NS059560) and by Defense Threat Reduction Agency (HDTRA 1-15-1-0032).

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

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