

Review

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Advances in viral encephalitis: Viral transmission, host immunity, and experimental animal models

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ABSTRACT

Viral infections have led to many public health crises and pandemics in the last few centuries. Neurotropic virus infection-induced viral encephalitis (VE), especially the symptomatic inflammation of the meninges and brain parenchyma, has attracted growing attention due to its high mortality and disability rates. Understanding the infectious routes of neurotropic viruses and the mechanism underlying the host immune response is critical to reduce viral spread and improve antiviral therapy outcomes. In this review, we summarize the common categories of neurotropic viruses, viral transmission routes in the body, host immune responses, and experimental animal models used for VE study to gain a deeper understanding of recent progress in the pathogenic and immunological mechanisms under neurotropic viral infection. This review should provide valuable resources and perspectives on how to cope with pandemic infections.

Keywords: Neurotropic viruses; Viral encephalitis; Meningeal immunity; Experimental animal models

INTRODUCTION

Viral encephalitis (VE) is a major global disease, with an incidence rate of 1.4 cases per 100 000 inhabitants (Silva, 2013). Following infection with a variety of neurotropic viruses, VE can cause acute intracranial inflammatory injury of the meninges and brain parenchyma (Ludlow et al., 2016). Clinical VE pathogens are primarily neurotropic RNA viruses, such as Japanese encephalitis virus (JEV), Zika virus (ZIKV), West Nile virus (WNV), Dengue virus (DV), and severe acute

respiratory syndrome coronavirus 2 (SARS-CoV-2), and DNA viruses, such as herpes simplex virus 1 and 2 (HSV-1 and HSV-2), varicella zoster virus (VZV), and cytomegalovirus (CMV) (Kennedy, 2005; Silva, 2013; Spudich & Nath, 2022). The typical incubation period for primary infection is approximately six days, during which the prodromal phase may present with symptoms such as mild fever, sore throat, cough, nausea, vomiting, myalgia, and fatigue (Bale, 2015). Diagnostic strategies include clinical and laboratory testing as well as neuroimaging. Acute viral invasion of the central nervous system (CNS) can increase mortality and disability if treatment is delayed. A window exists during which time epidemiological analysis and diagnosis can confirm the infection route, genome, antigen, and specific immunoglobulin M (IgM) and immunoglobulin G (IgG) of the invading virus (Venkatesan et al., 2013). However, specific therapeutic approaches for effectively curing VE after viral infection remain limited, emphasizing the need for more intensive basic investigations on viral invasion routes, VE pathogenesis, and host immunity post-infection to accelerate the development of novel diagnostic and therapeutic strategies.

The brain is a relatively well-protected organ and contains several cellular barriers, including the blood-brain barrier (BBB), which acts as a defense mechanism to prevent entry of dangerous factors and drugs from the peripheral circulation into the CNS (de Lima et al., 2020). Natural human infection from fresh or postmortem samples of VE is extremely rare, posing a challenge in the study of VE pathogenesis and immune defense in humans. However, the transmission and life cycle of neurotropic viruses have been extensively investigated in animal models, such as artiodactyls, domestic birds, and mosquitoes. With the advance of VE animal

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models, especially non-human primate (NHP) and rodent models, our understanding of virus infection routes, pathogenesis, and immunity has greatly improved. In addition to classic invasion via blood circulation and peripheral nerves, we recently showed that meningeal lymphatic vessel (MLV) endothelial cells can be infected by JEV and vesicular stomatitis virus (VSV) and transport viral particles to cervical lymph nodes (CLNs) (Li et al., 2022). The unique origin and definite role of meningeal immune cells in health and disease have become interesting fields of study (Brioschi et al., 2021; Cugurra et al., 2021; Niu et al., 2022), remaining elusive until recently (de Lima et al., 2020). In the present review, we provide a comprehensive summary of prevalent neurotropic viruses, potential routes of neurotropic viral invasion, and host immune defense. Furthermore, we discuss reported animal models with neurotropic viral loading. This review should provide useful information for further investigation into the diagnosis and treatment of VE in the future.

CATEGORIES OF NEUROTROPIC VIRUSES

Viruses that invade the nervous system are collectively known as neurotropic viruses. Neurotropic viruses include RNA and DNA viruses from various families, including Bunyaviridae, Flaviviridae, Bornaviridae, Herpesviridae, Orthomyxoviridae, Paramyxoviridae, Picornaviridae, Retroviridae, Polyomaviridae, Rhabdoviridae, and Togaviridae (see Table 1 for details). Following CNS infection, inflammation can arise in distinct anatomical regions, such as the meninges (meningitis), brain (encephalitis), and spinal cord (myelitis), or simultaneously in multiple regions (meningoencephalitis, encephalomyelitis). Neurotropic viral infection of the CNS can cause acute inflammatory lesions, as well as chronic inflammatory or non-inflammatory lesions. Various neurological disorders, such as Guillain-Barre syndrome, multiple sclerosis, narcolepsy, and lethargic encephalitis, are considered to be delayed onset virus-induced diseases

Table 1 Categories of neurotropic viruses

Viral family	Virus	Genome	Reference
Herpesviridae	Herpes simplex virus-1, HSV-1	dsDNA	Bradshaw & Venkatesan, 2016; Whitley, 2015
	Varicella zoster virus, VZV	dsDNA	Nagel et al., 2020
	Cytomegalovirus, CMV	dsDNA	Cheeran et al., 2009
	Human herpes virus 6, HHV-6	dsDNA	Agut et al., 2015; Kimberlin & Whitley, 1998
	Epstein-Barr virus, EBV	dsDNA	Houen et al., 2020; Tselis, 2014
	Pseudorabies virus, PRV	dsDNA	Liu et al., 2021
Flaviviridae	Japanese encephalitis virus, JEV	+ ssRNA	Redant et al., 2020
	West Nile virus, WNV	+ ssRNA	Klein, 2021; Sips et al., 2012
	Zika virus, ZIKV	+ ssRNA	Christian et al., 2019; Klein, 2021; White et al., 2016
	Tickborne encephalitis virus, TBEV	+ ssRNA	Cvjetković et al., 2016; Kubinski et al., 2020
	Dengue virus, DV	+ ssRNA	Trivedi & Chakravarty, 2022; Verma et al., 2011
	St Louis encephalitis virus, SLEV	+ ssRNA	Marques et al., 2017
Picornaviridae	Poliovirus, PV	+ ssRNA	Brownell et al., 2015; Verboon-Macielek et al., 2008
	Enterovirus 71, EV71	+ ssRNA	Solomon et al., 2010
	Human parechovirus, HPeV	+ ssRNA	Verboon-Macielek et al., 2008
	Coxsackievirus A16, CV-A16	+ ssRNA	Hooi et al., 2020
Rhabdoviridae	Rabies virus, RABV	– ssRNA	Hemachudha et al., 2013
	Vesicular stomatitis virus, VSV	– ssRNA	Beier et al., 2011; Sabin & Olitsky, 1937
Retroviridae	Human immunodeficiency virus, HIV	+ ssRNA	González-Scarano & Martín-García, 2005
	Human T cell lymphotropic virus, HTLV	+ ssRNA	Cabre et al., 2000
Polyomaviridae	John Cunningham virus, JCV	dsDNA	Ferenczy et al., 2012
Orthomyxoviridae	Influenza A virus	Segmented – RNA	Takahashi et al., 1995; van Riel et al., 2015
Paramyxoviridae	Measles virus	– ssRNA	Fisher et al., 2015
	Mumps virus, MuV	– ssRNA	Rubin et al., 1998
Togaviridae	Chikungunya virus, CHIKV	+ ssRNA	Das et al., 2015; Klein, 2021
	Equine encephalitis virus, EEV	+ ssRNA	Ludlow et al., 2016
	Venezuelan equine encephalitis virus, VEEV	+ ssRNA	Ludlow et al., 2016
	Western equine encephalitis virus, WEEV	+ ssRNA	Ludlow et al., 2016
	Eastern equine encephalitis virus, EEEV	+ ssRNA	Ludlow et al., 2016
Bornaviridae	Bornavirus	– ssRNA	Jordan & Ian Lipkin, 2001
Bunyaviridae	La Crosse virus, LCV	Segmented – RNA	McJunkin et al., 2001
	Rift Valley fever virus, RVFV	Segmented – RNA	Albe et al., 2019
	Toscana virus, TOSV	Segmented – RNA	Gori Savellini et al., 2019
Coronaviridae	Severe acute respiratory syndrome coronavirus 2, SARS-CoV-2	+ ssRNA	Erickson et al., 2021; Meinhardt et al., 2021

dsDNA: Double strand DNA. ssRNA: Single strand RNA. +: Positive-sense. –: Negative-sense.

(Ludlow et al., 2016). Perineural virus infection-induced nerve inflammation, nerve damage, and neurological complications pose considerable threats to human and animal health and can cause substantial economic losses. A deeper understanding of the mechanism underlying the invasion of neurotropic viruses in the nervous system will provide a valuable theoretical foundation for proper treatment.

Flaviviruses (FVs) are enveloped, positive-sense, single-stranded RNA viruses (ssRNA) carried by mosquitoes and ticks and are regarded as neurotropic viruses due to their significant neuroinvasive characteristics. A small percentage of infected individuals may exhibit neurological symptoms, such as acute encephalitis, meningitis, and acute flaccid paralysis, while long-term effects include Parkinsonism, dystonia, and cognitive changes. Classical neurotropic FVs include the JEV, ZIKV, WNV, DV, tick-borne encephalitis virus (TBEV), and St Louis encephalitis virus (SLEV).

Horses, pigs, birds (corvid species), and dogs are natural reservoirs of FVs. For example, the JEV replicates and remains in porcine tonsils for up to 25 days, enabling persistence in seasons when mosquitoes are inactive (García-Nicolás et al., 2018). WNV is maintained in nature in a mosquito-bird-mosquito transmission cycle primarily involving *Culex* mosquitoes (Turell et al., 2001). *Aedes* mosquitoes, namely *Aedes aegypti*, *Aedes albopictus*, *Aedes scutellaris*, and *Aedes polynesiensis*, are recognized vectors for the transmission of DV infection. TBEV is transmitted from the saliva of infected ticks within minutes of a tick bite (Lindquist & Vapalahti, 2008). Humans are regarded as “dead-end hosts” because they are infected accidentally by FV-carrying mosquitoes.

The primary sites of FV infection include subcortical nuclei (substantia nigra and thalamus), anterior horn neurons, and neocortex, with different neurological signs occurring in some individuals. About 80% of human WNV infections are asymptomatic (Mostashari et al., 2001), while those with symptoms are characterized by the acute onset of fever, headache, fatigue, malaise, muscle pain, weakness, difficulty concentrating, and neck pain or stiffness (Watson et al., 2004). In neuroinvasive WNV disease, infection of spinal motor neurons (anterior horn cells) causes acute asymmetric flaccid paralysis, similar to that seen with poliomyelitis (Li et al., 2003). Many patients with WNV-induced encephalitis exhibit movement disorders, including severe tremors and parkinsonism (Sejvar et al., 2003). While most JEV infections present with either mild symptoms (fever and headache) or remain asymptomatic, those that develop encephalitis can suffer significant morbidity and mortality. Patients with meningoencephalitis may progress to a permanent neurological deficit or ultimately death (Salimi et al., 2016). JEV neuroinvasion in patients can cause reduced levels of consciousness associated with seizures, movement disorders, and flaccid paralysis, as well as perivascular and CNS inflammation (Johnson et al., 1985). ZIKV infection during pregnancy leads to an increased risk of fetal growth restriction and fetal CNS malformations, resulting in long-term structural and neurological defects (da Silva & Gao, 2016). Similarly, tick-borne encephalitis can cause acute meningoencephalitis with or without myelitis.

HSV-1, HSV-2, and VZV are members of the herpes family of DNA viruses and are characterized by double-stranded DNA genomes located within a capsid consisting of 162 capsomers. Among the herpes family viruses that infect the

nervous system, HSV is one of the most common pathogens of infectious human encephalitis.

Herpesviruses have developed very specific mechanisms to evade host defenses and establish latency by shutting down lytic replication. Following primary HSV-1 infection, which is typically asymptomatic, the virus becomes latent in trigeminal and other cranial nerve ganglia, after which it spreads via axons of the trigeminal nerve into the frontal and temporal lobes. In immunocompetent adults, more than 90% of herpes simplex virus encephalitis (HSE) cases are due to HSV-1. HSE symptoms include headache, fever, and neck stiffness, with associated convulsions and dysfunction of the frontotemporal lobes (Bradshaw & Venkatesan, 2016). Approximately 80% of neonatal encephalitis cases are caused by HSV-2. Neonates present with systemic findings (alterations in body temperature, lethargy, respiratory distress, anorexia, vomiting, cyanosis) and neurological signs (irritability, bulging fontanelles, seizures, and coma) (Overall, 1994). VZV causes varicella (chickenpox) and herpes zoster. Varicella usually results in mild to moderate illness in immunocompetent patients but may cause serious complications in infants and elderly individuals, such as CNS involvement, pneumonia, secondary bacterial infections, and death (Heininger & Seward, 2006). Typical herpes zoster presents with vesicular eruptions distributed unilaterally within a dermatome, sometimes preceded by paresthesia, itching, and pain, a condition termed preherpetic neuralgia (Gilden et al., 1991).

Rabies (RABV) and rabies-related viruses belong to the *Lyssavirus* genus of the *Rhabdoviridae* family. The small, negative-stranded RNA genome (12 kb) of RABV encodes five proteins. RABV is a prototypical neurotropic virus transmitted in the saliva of infected animals (predominantly dogs but also other species such as bats, foxes, raccoon dogs, raccoons, mongooses, and skunks) via bites and scratches, which infects host neurons almost exclusively. After successful completion of the virus cycle, host death occurs due to the exhaustion of infected neurons, accompanied by structural damage and severe neuronal dysfunction (Hemachudha et al., 2013).

Recent studies have reported that SARS-CoV-2 infection is associated with encephalopathy, encephalitis, especially meningoencephalitis, and other complications (Pilotto et al., 2021). More than one-third of patients show mild or moderate disturbance in consciousness (Pilotto et al., 2021) and autopsy reports have revealed the presence of SARS-CoV-2 in the brain tissue of COVID-19 patients (Maury et al., 2021). At present, however, the mechanism underlying SARS-CoV-2 neurotropism remains unclear.

Prions, which are unusual proteinaceous infectious agents, can cause neuropathies (Aguzzi et al., 2007), with some strains targeting the CNS as the primary target organ. Among these prions, the scrapie prion protein (PrP^{Sc}), a misfolded host-derived membrane glycolipoprotein cellular prion protein (PrP^C), can cause various fatal neurodegenerative diseases, including transmissible spongiform encephalopathies (TSEs) such as scrapie in sheep, chronic wasting disease (CWD) in deer, bovine spongiform encephalopathy (BSE) in cattle (known as “mad cow disease”), and Creutzfeldt-Jakob disease (CJD) in humans. Aberrant prion protein conformations accumulate in the CNS, causing spongiform changes in the brain and eventually death. Generally, prion transmission between distinct species (e.g., transmission of human prions

into hamsters) is restricted by the species barrier. The infectious conformer of this protein (PrP^{Sc}) is predicted to recruit and convert the normal conformer (PrP^C) into the PrP^{Sc} form by interacting with specific regions of the protein, thus completing the 'replication' process during infection (Tuite & Serio, 2010).

TRANSMISSION ROUTE OF VIRUS IN THE BODY

Most viral infections started at barrier sites, such as epithelial or endothelial cells on the peripheral surface, causing a tissue-specific antiviral response. If the virus is not effectively eliminated at the site of primary infection, it will spread to other tissues and organs. Once the virus reaches its target tissue, it rapidly replicates within the cells, leading to an overactivation of the innate immune response, causing a local or systemic "inflammatory storm" (Koyuncu et al., 2013).

The CNS possesses unique anatomical features, including the BBB and an absence of conventional lymphatic vessels within the parenchyma. The BBB is a special structure of blood vessels composed of endothelial cells, astrocytes, and

pericytes (Ballabh et al., 2004). Tightly connected vascular endothelial cells in the BBB precisely regulate the exchange of ions, macromolecules, and cells between blood vessels and the brain to ensure the normal operation of nerve function and prevent toxic substances and pathogens from entering the brain (Daneman & Prat, 2015). Despite the highly complex defense system that protects the CNS, certain viruses can evade the protective barriers through different strategies. There are three main routes for invading CNS defenses (Figure 1).

Virus-infected leukocytes enter the CNS via the BBB

Certain viruses can enter the CNS via a "Trojan horse" mechanism, in which infected leukocytes carry pathogens from the blood across the BBB. Infection of monocytes and/or macrophages is a major mechanism used by lentiviruses, including simian immunodeficiency virus (SIV) and human immunodeficiency virus (HIV), to migrate across vascular barriers of the CNS (Alexaki & Wigdahl, 2008; Clay et al., 2007). Infected monocytes pass through the BBB during normal turnover of perivascular macrophages or due to the

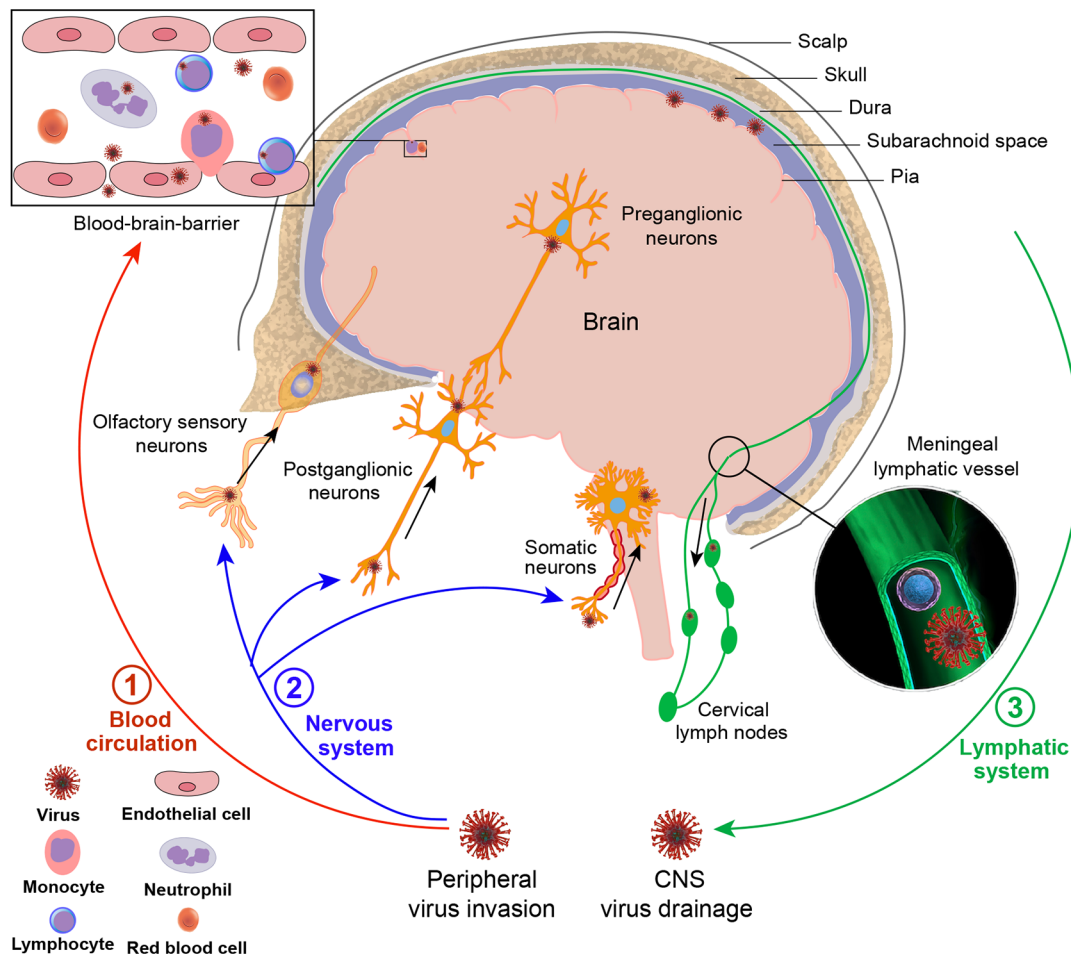


Figure 1 Viral transmission routes in the body

Although the CNS is protected by a highly complex barrier system, certain viruses still manage to enter the CNS and cause disease. One pathway is via blood circulation, whereby viruses infect leukocytes in the blood, which then pass through the BBB into the CNS during normal turnover of perivascular leukocytes or tight junction disruption of vessel endothelial cells. Viruses can also directly enter the CNS via the BBB by infecting vascular endothelial cells. In addition, viruses can migrate through peripheral nerve infection to enter the CNS, e.g., via peripheral motor neurons at axonal terminals, peripheral sensory neurons, and olfactory nerves. In addition to viral invasion routes, viruses can also drain from the CNS to the CLNs via the MLVs.

production of proinflammatory mediators that compromise the barrier, such as CC-chemokine ligand 2 (CCL2) (Ancuta et al., 2006; Roberts et al., 2010). Adhesion molecules also play a crucial role in cellular migration, with vascular cell-adhesion molecule-1 (VCAM-1) mediating mononuclear cell migration into the brain during HIV and SIV infection (Sasseville et al., 1994). Furthermore, JCV is believed to remain latent in the lymphoid organs, neuronal tissue, and kidney, but may reactivate under severe immunosuppression and infiltrate the brain via the "Trojan horse" mechanism, resulting in progressive multifocal leukoencephalopathy, a demyelinating disease of the CNS with a high mortality rate (Chapagain & Nerurkar, 2010). In addition, following WNV infection, the systemic levels of tumor necrosis factor α (TNF- α) and interleukin 6 (IL-6) increase macrophage inhibitory factor (MIF) and adhesion molecule (ICAM) expression at the BBB and promote tight junction disruption, thereby facilitating the adhesion and entry of WNV-infected neutrophils into the CNS (Arjona et al., 2007; Bai et al., 2010; Dai et al., 2008; Roe et al., 2012).

Virus enters the CNS by infecting microvascular endothelial cells

Other viruses can directly enter the CNS via the BBB by infecting vascular endothelial cells. Epithelial barrier integrity relies on tight junction complexes composed of transmembrane proteins located on the plasma membranes of adjacent endothelial and epithelial cells. Disruption of these tight junction complexes is a well-documented route for the invasion of certain viruses, such as the influenza virus, Ebola virus (EBOV), hepatitis C virus (HCV), and HIV (Meertens et al., 2008). The HIV-Tat protein increases the permeability of brain endothelial cells by down-regulating occludin mRNA levels in microvascular brain cells to increase HIV neuroinvasion (Xu et al., 2012a). Arboviruses can enter the skin through insect bites, then transmigrate to secondary lymphoid tissues and eventually enter the bloodstream, causing systemic infections and inflammation-induced disruption of the BBB, thus allowing the virus to invade the CNS (Ransohoff et al., 2003; Wu et al., 2000). WNV capsid degradation of the claudin protein disrupts epithelial barrier tight junctions and function (Medigeschi et al., 2009; Xu et al., 2012b). Replication of ZIKV, JEV, and HCV in brain microvascular endothelial cells (BMVECs) does not cause cytopathy but can increase vascular endothelial monolayer permeability (Al-Obaidi et al., 2017; Mustafá et al., 2019). Epstein-Barr virus (EBV) can infect human BBB cells, leading to increased production of proinflammatory mediators that result in immune cell adherence, which is implicated in the onset of MS (Casiraghi et al., 2011). Some viruses (e.g., WNV, HCV, HTLV-1, JCV, EBV, and human cytomegalovirus (HCMV)) can enter the CNS through the transcytosis of cerebrovascular endothelial cells and infection of nerve cells (Liou & Hsu, 1998; Papa et al., 2017). Poliovirus (PV) enters human brain microvascular endothelial cells (HBMECs) through dynamin-dependent caveolar endocytosis, facilitated by the association between the PV receptor (PVR) and SH2 domain-containing protein tyrosine phosphatase 2 (SHP-2) following virus attachment to the PVR, causing paralytic poliomyelitis by replicating within motor neurons of the brain and spinal cord (Ohka et al., 2012). Some regions of the CNS, such as the choroid plexus and periventricular organs, are not fully protected by the BBB and can be targeted as viral entry

points (van den Pol et al., 1999).

Virus enters the CNS through peripheral nerve infection

Viruses can also enter the CNS via migration through peripheral nerve infection. RABV can infect myocytes through saliva and subsequently enter peripheral motor neurons at axonal terminals, where it eventually infects the CNS through strictly unidirectional (retrograde) transneuronal transfer (Ugolini, 2011). Similarly, PV can infect mucosal epithelial cells after ingestion and invade the CNS via peripheral motor nerves (Racaniello, 2006). Retrograde axonal transport in neuronal cells may represent a major transmission route of enterovirus 71 (EV71) in mice, spreading from skeletal muscle to motoneuron junctions, peripheral motor nerves, then motor nuclei in the CNS (Tan et al., 2014). In addition, HSV-1 can infect keratinocytes and migrate to peripheral sensory neurons and may invade the CNS via the trigeminal nerve or olfactory sensory neurons after primary oropharyngeal infection (Mori et al., 2005). FVs can spread to the CNS via axonal transport from the periphery during viremia, as found in other neurotropic viruses such as RABV, PV, and HSV. Furthermore, WNV exhibits bidirectional spread in neurons, with axonal transport promoting viral entry into the CNS, followed by acute limb paralysis (Samuel et al., 2007). Moreover, VSV, Nipah virus (Munster et al., 2012), influenza virus (van Riel et al., 2015), RABV (Constantine, 1962), bovine herpesvirus 5 (Lee et al., 1999), and equine herpesvirus 9 (Narita et al., 2001) are proposed to enter the CNS via the olfactory nerve. SARS-CoV-2 may also invade the CNS through the olfactory bulb, spreading into functional areas such as the hippocampus, thalamus, and medulla oblonga to induce brain inflammation (Meinhardt et al., 2021).

Virus is drained from the CNS to CLNs via meningeal lymphatic vessels (MLVs)

While previous studies on viral dissemination have primarily focused on the BBB and peripheral nerves, recent research has revealed the potential involvement of MLVs in viral spread during neurotropic virus infection. Specifically, Li et al. (2022) demonstrated that JEV migrates from the CNS to CLNs, with inoculation of suckling mice with deep CLNs (dCLNs) and superficial CLNs (sCLNs) tissue homogenates from intracerebrally JEV-infected mice resulting in a morbidity rate exceeding 40%. These results indicate that viruses draining from the CNS to the CLNs maintain their infectivity and may trigger an immune response in the CLNs. Li et al. (2022) also intracerebrally injected a recombinant VSV expressing green fluorescent protein (VSV-GFP) into the mouse brains and observed colocalization of VSV-GFP and lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1), podoplanin (PDPN), and prospero homeobox 1 (PROX1) in the meninges, suggesting that the virus can drain from the CNS to the CLNs via the MLVs (Figure 1).

IMMUNE RESPONSES TO VE

Clinical observations and experimental models have provided strong evidence that both innate and adaptive immune responses play important roles in the pathophysiology of VE (Figure 2) (Chen et al., 2019; Suthar et al., 2013; Yshii et al., 2015). Following neurotropic viral entry into the CNS, antiviral immune responses are immediately induced by innate immune cells, such as microglia, astrocytes, dendritic cells (DCs), and infiltrated macrophages, as well as other immune

cells. Given the toxic effects of proinflammatory cytokines/chemokines, and a breached BBB, peripherally circulating leukocytes, such as monocytes, neutrophils, and lymphocytes, can infiltrate the CNS. Viral antigens, presented by antigen-presenting cells (APCs), activate CNS-infiltrating CD8⁺ T cells, which differentiate into effector cytotoxic T lymphocytes (CTLs). CTLs directly eliminate infected cells by producing cytotoxic molecules, such as perforin and granzyme B (Wong & Pamer, 2003). CTLs also clear infected cells by releasing apoptotic ligands, including Fas ligand (FasL) and TNF- α -related apoptosis-inducing ligand (TRAIL) (Shrestha & Diamond, 2007; Shrestha et al., 2012). Ferroptosis is also involved in neurotropic viral clearance and brain injury (Yan et al., 2023; Zhang et al., 2022a). Furthermore, CD8⁺ T cell-derived interferon γ (IFN- γ) plays an essential role in restraining intracranial viral infection and clearing viruses from infected neurons (Garber et al., 2019; Griffin & Metcalf, 2011). Additionally, a small group of CD8⁺ T cells can transform into brain-resident memory T (bTRM) cells (Steinbach et al., 2016). After viral reinfection, bTRM cells rapidly produce cytotoxic molecules to prevent virus infection (Mockus et al.,

2019). Finally, successful antiviral immunity is acquired with virus-specific IgM and IgG/IgA antibodies secreted from B cell-transformed plasma cells to decrease viral spread and neutralize circulating viral particles (Lam et al., 2020).

The role of meningeal immunity has gained increasing attention in recent years, particularly regarding its contribution to VE (de Lima et al., 2020). MLVs can transport viruses into the CLNs to regulate peripheral immunity (Li et al., 2022), while meningeal macrophages directly protect against lymphocytic CMV (LCMV) neuroinfection (Figure 2) (Rebejac et al., 2022). Furthermore, evidence suggests that innate and adaptive immune responses exhibit both beneficial and detrimental roles in antiviral effects (Reagin & Funk, 2022). Regulating immunological balance between viral clearance and neuronal damage is important to increase survival and decrease the sequela in infected patients.

Innate immunity in VE

The innate immune system establishes the first line of defense against neurotropic viruses in the CNS. Microglia, astrocytes, monocyte-derived macrophages, neutrophils, natural killer

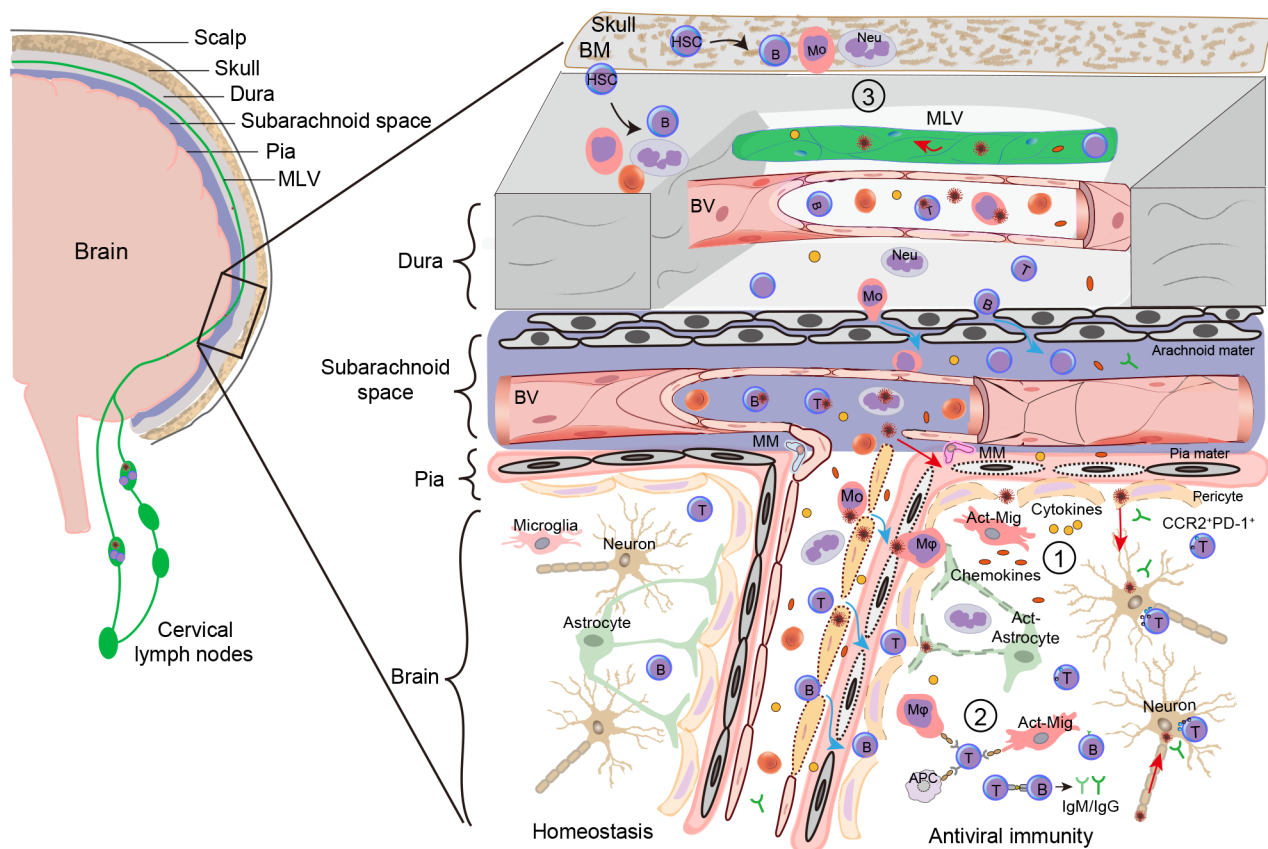


Figure 2 Meningeal and parenchymal immunity during VE

VE refers to acute intracranial inflammatory lesions and involves the meninges and brain parenchyma. Various viruses invade the CNS through blood/lymphoid circulation and peripheral nerve migration. Damaged neurons release signals (such as ATP and cytokines) to recruit microglia, which initiate innate immunity ①. Cytokines and chemokines are released from activated microglia (Act-Mig) and astrocytes (see text for details). Thereafter, peripheral neutrophils (Neu), monocytes (Mo), and APCs (including DCs) infiltrate the brain parenchyma via increased BBB permeability. IFN is produced to enlarge the antiviral effects and APCs present antigens to T cells and later to B cells, constituting adaptive immunity ②. CD8⁺ T cells produce granzyme and perforin to clear infected cells, including neurons, vascular endothelial cells, and pericytes. Plasmacytes differentiated from mature B cells secrete specific IgM and IgG antibodies to neutralize viral particles and restrict their spread. Meningeal immunity ③ has also recently been reported to play an important role in VE. Both myeloid and B cells differentiate from HSCs, which originate from skull bone marrow (BM) or meninges, and meningeal macrophages (MMs) extravasate from the pia mater or cross the arachnoid mater into the brain parenchyma. Additionally, viruses can infect and transmit from MLVs into CLNs to enhance peripheral immunity. Red arrow: Viral invasion/transmission; Blue arrow: Immune cell infiltration.

(NK) cells, and DCs play critical roles in the innate response against viral invasion. Type I IFN is important for host survival, with mice lacking IFN- α/β receptors showing significantly increased susceptibility to neurotropic viruses (Byrnes et al., 2000; Fiette et al., 1995; Müller et al., 1994). IFN- β has an important neuroprotective effect in the CNS and can induce the production of neurotrophic factors (Boutros et al., 1997). Rapid IFN- β response after infection reduces viral transmission and inhibits viral replication before the initiation of specific adaptive immune responses (Boutros et al., 1997). Virally infected neurons can also produce a relatively low level of type I IFN for CNS defense (Delhay et al., 2006).

Innate immune responses are mediated by pattern recognition receptors (PRRs), including retinoic acid-inducible gene-I-like receptors (RLRs), nucleotide oligomerization domain-like receptors (NLRs), C-type lectin receptors (CLRs), Toll-like receptors (TLRs), absent in melanoma-2 (AIM2)-like receptors (ALRs), and cytoplasmic DNA sensor cyclic GMP-AMP synthase (cGAS) (Miller et al., 2021). Following recognition of pathogen-associated molecular pattern molecules (PAMPs), such as viral RNA or DNA, mRNA metabolism, and viral protein expression, PRRs can modify their conformational structures to initiate downstream production of type I IFN and proinflammatory cytokines by infected cells (Wilkins & Gale, 2010). Virus-infected cells can also produce virus-derived small RNAs (vsiRNAs), including small interfering RNAs (siRNAs), microRNAs (miRNAs), and Piwi-interacting RNAs (piRNAs) (Ding, 2010; Parameswaran et al., 2010; Pfeffer et al., 2004). Multiple herpesviruses use viral miRNAs to regulate innate receptor recognition and the signaling pathways of IFN production and function (Chen et al., 2022). Moreover, designed peptides targeting viral suppressors of RNAi (VSR) can effectively silence cognate EV-71 RNA *in vivo* and *in vitro*. This evidence implicates the involvement of vsiRNAs in the modulation of antiviral immunity and potential therapeutic strategies (Fang et al., 2021). Thus, both PRRs and vsiRNAs play important roles in host immune defense against viral infection (Ding, 2010).

Damaged neurons can also produce chemokine CX3CL1, which binds to its receptor CX3CR1 expressed in microglia and macrophages. Activation of these cells plays an important role in immune protection of the body during the early stage of infection (Jung et al., 2000; Maciejewski-Lenoir et al., 1999). In addition to sensing adenosine triphosphate (ATP) signals through the purinergic receptor P2Y12, residential microglia are also recruited and activated around infected neurons to enhance IFN production, proinflammatory cytokine release, and phagocytic activity (Fekete et al., 2018). Microglia and astrocytes respond quickly, producing antiviral and proinflammatory mediators. During JEV infection, proinflammatory molecules, such as RANTES, TNF- α , IL-1- α , IL-6, IL-12, IL-18, IL-1 β , CCL2, C-X-C motif chemokine ligand 9 (CXCL9), CXCL10, and CXCL11, and proinflammatory enzymes, such as cyclooxygenase-2 and inducible nitric oxide synthase (iNOS), are up-regulated (Cheeran et al., 2005). *In vivo*, astrocytes can produce CXCL10, CXCL11, and CCL5 to transfer virus-specific CD4⁺ and CD8⁺ T cells (Glass et al., 2005; Klein et al., 2005; Lane et al., 2006). DCs also play important roles in T cell activation and adaptive immune response initiation during JEV infection (Li et al., 2011; Sooryanarain et al., 2012). DCs can be rapidly activated following neurotropic virus infection to release proinflammatory cytokines and chemokines, such as type I IFN, TNF, IL-1 β ,

CCL2, CCL3, and CCL5 (Martina et al., 2008; Shrestha et al., 2008; Silva et al., 2007). Following BBB breach and chemokine release, infiltrated monocytes are differentiated into macrophages, which are then recruited and activated in the CNS. Removal of polymorphonuclear leukocytes by mouse monoclonal antibodies Gr-1 treatment has highlighted their crucial functions in viral clearance (Bai et al., 2010). Using single-cell RNA-sequencing (scRNA-seq), a recent study identified a novel subset of cells, named microglia-like cells, during herpes simplex encephalitis, which show high expression of Retnlg, Cxcr2, and Il1f9 and contribute to increased CNS inflammation (Uyar et al., 2022). Another study based on WNV-inclusive scRNA-seq reported that only a few L929 cells respond and exhibit robust transcription of IFN- β (O'Neal et al., 2019). The use of scRNA-seq to investigate heterogeneity of innate immunity will greatly expand our understanding of innate immunity in VE. Collectively, innate immunity is involved in the recognition of neurotropic viruses, presentation of viral antigens, and initiation of antiviral responses.

Adaptive immunity in VE

Under physiological conditions, the BBB prevents immune cells in blood circulation from entering the brain parenchyma, and APCs, such as DCs, are absent in the brain parenchyma (McMenamin, 1999). It is widely accepted that during CNS infection, pathogenic antigens are transported by cerebrospinal fluid (CSF) to draining lymph nodes (dLNs), where antigen presentation immediately occurs (Cserr & Knopf, 1992; Harling-Berg et al., 1999).

Neutrophils and DCs are primarily recruited to the CNS after viral infection (Templeton et al., 2008; Zuo et al., 2006). Neutrophils interact with endothelial cells through adhesion molecules that promote the disintegration of tight junction complexes, leading to the breakdown of the BBB. Neutrophils can also secrete matrix metalloproteinase-9 (MMP-9) to degrade the extracellular matrix and basement membrane of the BBB, further promoting BBB permeability (Kjeldsen et al., 1994). After BBB impairment, DCs appear within a few days of CNS virus infection and migrate from the CNS to CLNs via chemokine CCL3, thereby sensitizing virus-specific T cells (Trifilo & Lane, 2004). Mouse CD11c^{hi} DCs can induce the differentiation of CD4⁺ T cells into inflammatory T-helper 17 (Th17) cells, increase the number of anti-inflammatory regulatory T (Treg) cells in lymphoid tissue and CNS, and play a protective role in the CNS during fatal neuritis (Chiou et al., 2005; Kim et al., 2015). CD4⁺ and CD8⁺ T cells are recruited into the infected CNS, to some extent, by chemokines CXCL9 and CXCL10 (Stiles et al., 2006; Walsh et al., 2007). In addition, CCR5 contributes to T cell recruitment in the CNS (Chen et al., 2001; Glass & Lane, 2003). CD4⁺ T cells secrete IFN- γ to support the function of CD8⁺ T cells (Weinger et al., 2013), which are the main antiviral effector cells during CNS infection. IFN- γ is also critical for the elimination of viruses in glial cells (Bergmann et al., 1999, 2003, 2006). CD8⁺ T cells produce IFN- γ , granulosa B, and perforin (Ramakrishna et al., 2004), which participate in the elimination of virus-infected astrocytes (Lin et al., 1997). IFN- γ helps oligodendrocytes control viral replication (González et al., 2006; Parra et al., 1999). Recent mass cytometry of infiltrating immune cells revealed a new subset of PD-1⁺CCR2⁺CD8⁺ T cells that may play important roles in viral defense (Zhang et al., 2019a). In MHV and Sindbis virus (SINV) encephalitis models, T cells

promote B cell proliferation and differentiation via secretion of cytokines IL-10 and IL-21 (Linterman et al., 2010; Phares et al., 2011; Puntambekar et al., 2011). B cells clear virions from the CNS through powerful non-complement-dependent, non-cytolytic mechanisms. During RABV infection, antibodies against RABV glycoproteins inhibit viral RNA transcription and prevent viral spread between cells (Dietzschold et al., 1992). Antibodies can also sensitize NK cells and macrophages, inducing antibody-dependent cell-mediated cytotoxicity of virus-infected cells (Dietzschold et al., 1992). In acute infection, virus-specific antibody-secreting cells (ASCs) play an important role in achieving non-cytolytic viral clearance. In addition, because viral RNA is difficult to completely eradicate from target tissues, the long-term presence of ASCs in the CNS can prevent viral reactivation (Metcalfe & Griffin, 2011). Human memory T cells contribute to defense against JEV infection (Turtle et al., 2016). Previous studies have indicated that IFN- γ responses of asymptomatic individuals infected with JEV are primarily mediated by CD8 $^{+}$ T cells, whereas IFN- γ responses of JEV-recovered individuals are primarily mediated by CD4 $^{+}$ T cells, suggesting that distinct clinical outcomes in JEV infection may be associated with CD4 $^{+}$ and CD8 $^{+}$ T cell responses. (Aleyas et al., 2009, 2012; Falasco et al., 1990).

Understanding the relationship between different immune cell infiltration and disease prognosis can help guide the prediction and treatment of clinical VE.

Meningeal immunity in VE

The CNS also relies on meningeal immune defense, which consists of the meningeal lymphatic system, glymphatic system, immune cells, and cytokines (de Lima et al., 2020; Louveau, 2018; Rua & McGavern, 2018). The crucial roles of meningeal immunity have been confirmed in many studies of different CNS diseases, such as stroke, Alzheimer's disease (AD), VE, and cancer (Chen et al., 2020; Da Mesquita et al., 2018; Hu et al., 2020; Li et al., 2022; Song et al., 2020).

Recent research revealed that hematopoietic stem cells (HSCs) reside in the meninges under steady-state conditions. These meningeal HSCs are an important origin of leukocytes that supplement immune cells in the CNS (Niu et al., 2022). Reports also indicate that meningeal B cells derived locally from the calvaria at the CNS border are educated and negatively selected by CNS-specific antigens and may play an essential role in maintaining immune privilege within the CNS (Brioschi et al., 2021; Wang et al., 2021). In addition, recent study found that a pool of meningeal monocytes and neutrophils is supplied from the adjacent skull and vertebral bone marrow, but not from circulated blood. Under spinal injury and neuroinflammation, the meningeal myeloid cells can infiltrate the CNS and may serve a critical function in affecting the infection of these diseases (Cugurra et al., 2021). Furthermore, in our previous work, we found that neurotropic viruses, including JEV and HSV-1, can infect and replicate in lymphatic endothelial cells (LECs). *In vivo*, JEV can spread into dCLNs through the MLVs to activate the peripheral immune response for CNS viral clearance. Moreover, pretreatment of vascular endothelial growth factor C (VEGF-C), a well-known cytokine for MLV expansion, can improve the effects of antiviral infection (Li et al., 2022). MHC-II $^{+}$ meningeal macrophages are also reported to play a critical role in protecting against LCMV neuroinfection via regulation of the IFN-I signaling pathway (Rebejac et al., 2022). These

studies indicate that MLVs and meninge-resident immune cells may exhibit unique functions in immune defense of the CNS, including protection against neurotropic virus infections. The recent confirmation of functional lymphatic vessels in the brain meninges raises the possibility of an alternative drainage route of macromolecules and immune cells in the cerebrospinal fluid (CSF) into the CLNs. Following the initial discovery of MLVs, whole-mount immunolabeling and imaging revealed that most sinus T cells and MHCII $^{+}$ cells, as well as some CD11c $^{+}$ and B220 $^{+}$ cells, are found within the MLVs (Louveau et al., 2015). Several studies have reported that naive CD4 $^{+}$ T cells and Tomato-labeled CD19 $^{+}$ splenocytes primarily accumulate in the dCLNs and sCLNs after intracisternal magna injection into the CSF of naive mice (Brioschi et al., 2021; Louveau et al., 2018). Thus, MLVs may serve as a migratory route for B and T cells exiting the CNS compartment. MLVs are also involved in the regulation of immune cells under pathological conditions. Enhanced drainage of MLVs promotes the transport of tumor-related antigens and DCs from intracranial tumor tissue to dCLNs, thereby promoting the enhancement of CD8 $^{+}$ T cell initiation in dCLNs and the rapid clearance of tumors (Da Mesquita et al., 2018). Ablation of dorsal MLVs can reduce CNS-derived autoantigen drainage, thus alleviating the inflammatory response of brain-reactive T cells, delaying experimental autoimmune encephalomyelitis (EAE) onset, and diminishing pathology (Hsu et al., 2019; Louveau et al., 2018). However, the molecular mechanism underlying the cross-talk among the MLVs, skull, vertebral bone marrow-derived or meningeal immune cells, and cytokines is still unclear. Thus, further investigations focusing on the skull, meningeal ecosystem, and local immunity among these regions are required.

EXPERIMENTAL ANIMAL MODELS OF NEUROTROPIC VIRAL INFECTION

Different experimental animal models are required to investigate the viral life cycle, viral invasion routes in hosts, antiviral immunity, neuropathogenesis, clinical outcomes, and therapeutic strategies. Here, we focus on experimental animal species, including NHPs, artiodactyls, domestic birds, and mosquitoes, and individual routes in experimental animal models of neurotropic viral infection.

NHPs

NHP-based research has played a crucial role in understanding the neuropathogenesis of neurotropic viral infection, especially fetal infection from ZIKV (Haese et al., 2021). ZIKV infection in adult macaques is generally limited to pathologies of rash, fever, and conjunctivitis (Hirsch et al., 2017). Following subcutaneous infection, viremia can be observed as early as one day post-infection (dpi) and is usually cleared by 10 dpi (Dudley et al., 2016). During this period, ZIKV RNA can also be detected in saliva, lacrimal fluid, CSF, urine, semen, and vaginal swabs (Li et al., 2016c). These evidences suggest that the virus will develop rapid and widespread infection in the body. Fetal death in pregnancy and microcephaly in newborn babies are the most serious outcomes of ZIKV infection in humans. In NHP models, a four-fold higher rate of fetal loss occurs in ZIKV-infected rhesus macaques compared to ZIKV-unexposed animals (Dudley et al., 2018). The neurological pathologies and histopathologies found in the brains of macaque newborns are similar to those found in human neonates, including loss of

neuroprogenitor cells and reduced brain size (Adams Waldorf et al., 2018; Seferovic et al., 2018). In several NHP models, certain CNS abnormalities have not yet been manifested clinically (Mavigner et al., 2018). NHP models have also been used to study the pathologies of TBEV, WNV, and DV infection. However, the low quantity of offspring and high experimental costs restrict large-scale basic and translational research. Surprisingly, the Chinese tree shrew shows benefits of safety, efficacy, and predictability for studying the neural mechanisms underlying brain diseases, including VE (Yao, 2017). With the successful application of gene-editing technology in tree shrew models (Li et al., 2017) and the release of the tree shrew genome database (Fan et al., 2014), a more powerful animal model for investigating VE should be developed in the coming years.

Rodents

Rodents, such as mice and rats, are the most common animals used for studies of neurotropic virus pathogenesis. Wild-type (WT) mice and rats are sensitive to certain neurotropic viruses, such as JEV and HSV-1, but exhibit less consistent development of encephalitis under ZIKV, DV, and WNV infection (Kennedy, 2005; Miura et al., 1988). Thus, several gene-editing and humanized mouse models have been developed to study VE. AG129 mice, which lack both type I and II interferon (IFN) responses, generate reproducible viremia and neurological symptoms, including tremors, following ZIKV infection, with peak viremia (10^7 plaque-forming units (PFU)/mL) at 2 dpi, high viral titers in the spleen (1 dpi) and brain (3 dpi), and robust viral replication in the testes. (Rossi et al., 2016). Recently, several groups have reported on human angiotensin-converting enzyme 2 (hACE2) transgenic mouse models for SARS-CoV-2, confirming that hACE2 is the target of SARS-CoV-2 and that the virus can rapidly spread into tissues (Bao et al., 2020; Jiang et al., 2020; Sun et al., 2020). Rodent models are also valuable tools for studying pathology and immune responses and for testing potential therapeutics and vaccines (see Table 2). Although rodents are genetically and evolutionarily distant from NHPs and humans, their dependable reproductive ability and gene-editing capability make them useful tools for studying neurotropic encephalitis.

Other animal models

Artiodactyls, domestic birds, and mosquitoes have also been used for studying the transmission cycles of neurotropic viruses. Some viruses can replicate in mosquitoes and their zoonotic life cycle can be maintained in vertebrate hosts. While pigs and domestic birds can potentially act as amplifying or reservoir hosts, humans are considered dead-end hosts (Hameed et al., 2021). Understanding these transmission cycles will help to develop preventive measures, such as vector control and vaccination in animals.

Diagnosis of neurotropic virus diseases

In 2013, the International Encephalitis Consortium released guidelines related to case definitions, diagnostic algorithms, and priorities for diagnosing encephalitis (Venkatesan et al., 2013). The diagnostic strategies present clinical, neuroimaging, and laboratory tests, including major and minor criteria, with presumed viral infectious encephalitis given priority examination (as per Table 3) (Fillatre et al., 2017; Venkatesan et al., 2013). The Consortium also proposed an etiological examination algorithm, including CSF examination,

skin and serum antibody detection, and other peripheral examinations, including skin changes, tracheoscopy biopsy, throat swab, and stool and urine culture.

Perspectives for animal model use in VE research

For decades, scientists and physicians have pursued innovative therapeutic strategies to combat viral infections, including IFN, immunoglobulin, and ribavirin treatment, due to increasing clinical demands. Standard therapeutic compounds that target receptors or enzymes involved in essential viral functions have focused on host cell factors, with drug resistance, cytotoxicity, and cellular side effects remaining significant disadvantages. As such, computational screening of small molecular drugs, nucleic acid-based antivirals, and monoclonal antibodies that target virus-conserved proteins provides an alternative strategy to target the development of viral replication (Joe et al., 2022; Laulund et al., 2020; Lundin et al., 2006). Classic drug screening is a cost-effective and time-efficient technique to identify potential drug candidates, allowing hundreds of candidates to be tested at the cellular level *in vitro*, using viral titers as readouts. For example, remdesivir and chloroquine, which are effective at inhibiting SARS-CoV-2 replication, can be rapidly screened *in vitro* (Wang et al., 2020). However, further study is required to examine pharmacokinetics and drug metabolism *in vivo*, particularly given the presence of the BBB.

Animal models are indispensable for investigating human diseases and therapeutic interventions. Although rodents are widely used, certain pathological phenotypes and immune responses cannot be fully recapitulated in small animals and *in vitro* culture systems. NHP models are ideal experimental tools for studying pathology, immunity, and therapeutic efficacy. However, limitations in terms of animal feeding, inbreeding, and long experimental periods have restricted the use of NHP models in VE research. Thus, the development of viable animal models, such as the Chinese tree shrew, may provide early diagnostic tools and contribute to the development of effective therapies.

CONCLUSIONS AND FUTURE PERSPECTIVES

Identifying and classifying neurotropic virus species can help epidemiologists and clinicians to respond quickly and accelerate basic research. In the current review, we discussed the categories of common neurotropic viruses (Table 1), which remain the primary pathogens of VE. Newly identified SARS-CoV-2 can enter the CNS and generate neuroinflammation (Meinhardt et al., 2021), thus we included SARS-CoV-2 as a new neurotropic viral candidate. Compared to DNA viruses, neurotropic RNA viruses are endowed with the ability to mutate frequently, leading to larger infectious populations. The invasion and transmission routes of neurotropic viruses in humans are becoming increasingly diverse. Beyond classical invasion routes of the BBB, peripheral nerve migration, and microvascular endothelial cells, the MLV system also exhibits the ability to infect and transport neurotropic viruses from the CNS to the periphery (Li et al., 2022). This beneficial behavior can enhance peripheral immunity against intracranial viral infection. However, the efflux function of MLVs can be damaged (Li et al., 2022). With aged individuals exhibiting recession MLV function, dysfunction of MLVs can promote amyloid- β deposition in the meninges and aggravate parenchymal amyloid- β accumulation in transgenic mouse models of AD (Da Mesquita et al., 2018). These findings

Table 2 Animal models of neurotropic viruses

Species/Strain	Viral strain	Route of infection	References
NHPs			
<i>Macaca sylvanus</i>	TBEV	s.c.	Kenyon et al., 1992; Süss et al., 2007
	ZIKV	s.c.	Adams Waldorf et al., 2016, 2018
Rhesus macaque	WNV	i.d.	Verstrepen et al., 2014
	DV	s.c./i.d.	Li et al., 2013
	HCMV	i.p.	Tarantal et al., 1998
	ZIKV	s.c.	Dudley et al., 2016; Martinot et al., 2018
	SARS-CoV-2	Intratracheal/i.n./ocular	Gao et al., 2020; Munster et al., 2020; Shan et al., 2020
<i>Callithrix jacchus</i>	WNV	i.d.	Verstrepen et al., 2014
Marmoset	ZIKV	s.c.	Dudley et al., 2018
Squirrel monkey	ZIKV	i.d.	de Alcantara et al., 2021
Olive baboon	ZIKV	s.c.	Gurung et al., 2019
Tree shrew	HSV-1/2	i.v./i.p./s.c./ocular	Darai et al., 1978; Li et al., 2016b
	Influenza	i.n.	Yang et al., 2013
	Coxsackie virus A16	Nasal spraying	Li et al., 2014
	ZIKV	s.c.	Zhang et al., 2019b
	SARS-CoV-2	Oral/i.n./ocular	Xu et al., 2020
Rodents			
C57BL/6	JEV	i.v./i.p./footpad	Grossberg & Scherer, 1966; Miura et al., 1988
	DV	i.p.	Byrne et al., 2021
	HSV-1	i.n./i.v./corneal	Xiao et al., 2001
	EV71	Intracranial	Luo et al., 2019
BABl/c	JEV	i.p.	Saxena et al., 2008
	DV	i.p./i.c.	Byrne et al., 2021; Li et al., 2013
µMT	WNV	Footpad	Diamond et al., 2003a
slgM-/-	WNV	Footpad	Diamond et al., 2003b
RAG1-/-	WNV	Footpad	Throsby et al., 2006
Ifnar1-/-	ZIKV	i.v./footpad	Lazear et al., 2016
IRF3-/-IRF5-/-IRF7-/-	ZIKV	Retro-orbital	• Lazear et al., 2016; Li et al., 2016a
AG129	ZIKV	i.v./i.p./footpad/s.c.	Aliota et al., 2016; Rossi et al., 2016; Sumathy et al., 2017; Xie et al., 2011
	DV	i.p./i.v.	Brewoo et al., 2012; Fuchs et al., 2014
NSG transplanted with CD34+ HSPC	DV	Footpad/era via mosquito biting	Cox et al., 2012
HepG2-grafted SCID	DV	i.p.	An et al., 1999
hSCARB2-transgenic	CV-A16	i.n.	Chen et al., 2021
HFH4-hACE2	SARS-CoV-2	i.n.	Jiang et al., 2020
hACE2 transgene	SARS-CoV-2	i.n./i.g.	Bao et al., 2020; Sun et al., 2020
K18-hACE2	SARS-CoV-2	i.n.	Winkler et al., 2020
C3H/He	RABV	i.m.	Mifune et al., 1980
ICR	RABV	Footpad	Smith, 1981
Kunming	RABV	i.m.	Zhang et al., 2016
	ZIKV	s.c.	Yu et al., 2017
Rat	HSV-1/2	Oral mucosa/i.c.	Bergström et al., 1991; Hirsch et al., 1984
	RABV	i.m.	Ren et al., 2021
Golden hamster	RABV	i.m.	Zhang et al., 2016
	SARS-CoV-2	i.n.	Chan et al., 2020
Artiodactyls			
Pig/swine	JEV	Oronasal/s.c.	Ricklin et al., 2016
Beagle	RABV	i.m.	Fekadu et al., 1982
Horse	WNV	s.c.	Meyer et al., 1931
Cattle	JEV	s.c.	Kimura et al., 2010
Domestic birds			
Chicken	JEV	s.c.	Fan et al., 2019
Duckling	JEV	s.c.	Xiao et al., 2018
Great egret	JEV	s.c.	Nemeth et al., 2012

Species/Strain	Viral strain	Route of infection	References
Mosquitoes			
<i>Culex tritaeniorhynchus</i>	JEV	Intrathoracic	Buescher et al., 1959
<i>Culex pipiens</i>	JEV	Intrathoracic	Hameed et al., 2019
<i>Aedes aegypti</i>	DV	Midgut	Choy et al., 2020
<i>Aedes albopictus</i>	DV	Salivary glands	Pompon et al., 2017
<i>Aedes aegypti</i>	ZIKV	Intrathoracic	Boorman & Porterfield, 1956
<i>Aedes aegypti/ unilineatus/ vittatus/ luteocephalus</i>	ZIKV	Oral	Diagne et al., 2015
<i>Aedes albopictus</i>	ZIKV	Oral	Wong et al., 2013
<i>Culex p. quinquefasciatus</i>	ZIKV	Oral	Guo et al., 2016
<i>Culex annulirostris</i>	ZIKV	Oral	Duchemin et al., 2017

i.c.: Intracutaneous; i.d.: Intradermal; i.m.: Intramuscular; i.n.: Intranasal; i.p.: Intraperitoneal; i.v.: Intravenous; s.c.: Subcutaneous.

Table 3 Diagnostic tests preferred for suspected etiology

Causative agents	Diagnostic tests
HSV-1/2	HSV-1/2 PCR: if negative and highly suspected, repeat within 3–7 days with CSF sent for HSV PCR; if test available, consider HSV CSF IgG and IgM in addition
VZV	CSF: VZV IgG
Enterovirus	CSF: EV PCR; Sensitivity may be low, if test available, consider throat swab and stool sent for EV PCR
EBV	EBV serology: VCA IgG and IgM and EBNA IgG
HHV-6	CSF: HHV-6 PCR/Photoconductive relay
Influenza	Culture/Antigen detection/Respiratory secretion PCR
JEV	CSF/serum: JEV PCR/CSF: IgM/Serology: IgM
ZIKV/DV/ CHIKV	CSF/serum: RT-PCR/CSF: IgM/Serology: IgG and IgM
Measles virus	Plasma/CSF serology/CSF: PCR
CMV	CSF: PCR/IgM
JCV	CSF: RT-PCR/IgM/Serology: IgG and IgM/Plasma antigen
Rabies/ABLV	Rabies/ABLV testing: serological analysis of serum and CSF; viral isolation or RT-PCR from saliva; tests for viral antigen or histopathology on either a brain biopsy or full-thickness biopsy of nape of neck

CSF: Cerebrospinal fluid; EBNA: Epstein-Barr nuclear antigen; RT-PCR: Reverse transcription polymerase chain reaction; VCA: Viral capsid antigen.

suggest that neurotropic virus-infected aged individuals may experience an increased risk of developing neurodegenerative diseases, such as AD or Parkinson's disease. Further investigation is necessary to understand the impact of neurotropic virus-infected MLVs in triggering or accelerating the development of neurodegenerative diseases. Studies have shown that IL-6 induced by genotoxic stress may promote lymphangiogenesis in the bones, including the cranium, which may contribute to bone and hematopoietic regeneration (Biswas et al., 2023). However, it is still unclear whether and how the intracranial inflammatory cytokines induced by viral infection in the CNS, contributes to the expansion and functional impairment of MLVs. Apart from VEGF-C, the specific factors, such as inflammatory cytokines and chemokines, that modulate the proliferation, migration, and differentiation of LECs and influence the structure and function of MLVs in the meninges during viral infection are yet to be determined.

The generation of type I IFN in response to intracranial neurotropic viruses has been demonstrated in parenchymal neurons and immune cells, including residential microglia and peripheral-infiltrating leukocytes. Upon mobilization, B lymphocytes will encounter viral antigens that stimulate their maturation and differentiation into plasmacytes, which then secrete virus-specific antibodies to neutralize viral particles (Figure 2). Interestingly, meningeal immunity was recently discovered to play an important role in antiviral immune defense in the CNS (Li et al., 2022; Rebejac et al., 2022).

Immune cells, including many myeloid cells, are harbored in the subarachnoid lymphatic-like membrane (SLYM) and may participate in CNS immunity (Møllgård et al., 2023). Mucosal-associated invariant (MAIT) cells in the meninges preserve meningeal barrier integrity and restrict neuroinflammation in the brain (Zhang et al., 2022b). These findings imply that resident immune cells in the meningeal ecosystem possess many unexplored features in immune defense against diseases, including VE. The importance of peripheral-infiltrating immune cells in the clearance of intracranial pathogens is indisputable. However, it remains unclear which cells take priority in the mobilization of resident immune cells in the meninges or periphery. In addition, the routes through which meningeal immune cells shuttle between the peripheral lymph nodes and the brain parenchyma are yet to be explored. Of course, several barriers still exist in the field. First, using LEC-specific Cre recombinase or photoinitiators to ablate MLVs can result in fetal death or low-deleting area and efficiency. Second, monitoring the transmission paths of viruses and dynamic migration of immune cells in real-time in the meninges and deep brain parenchyma is challenging. Third, observing lymphangiogenesis of the MLVs with LEC-specific lineage tracing in diseased animal models remains difficult.

As summarized in this review, common animal model-based experiments can provide first-hand evidence of the pathologies of VE, including routes of infection (Table 2). While NHP models are invaluable for pathological and

therapeutic development studies of VE, the difficulty in consecutively obtaining brain samples from individual animals has restricted cellular mechanism studies of pathogenesis. *In vitro* organoid co-culture systems are beneficial supplements, although they cannot fully simulate pathological conditions of VE. However, such systems have the advantage of cell tropism in combination with the expression of receptors necessary for viral entry and are useful models for drug screening and therapeutic testing (Antonucci & Gehrke, 2019; Depla et al., 2022). Finally, improving the delivery efficiency and accuracy of antiviral drugs remains an unsolved clinical issue. Engineering precision nanoparticle drug delivery systems (NDDS) (Mitchell et al., 2021) and virus-based nanoparticles (VNPs) (Li et al., 2019) may provide new insights to ameliorate those problems. For example, FDA-approved Plegridy is an injectable nanomedicine for relapsing forms of multiple sclerosis that offers low-dosing frequency (Mitchell et al., 2021). VNPs work well with different types of cargo, including inorganic nanoparticles and proteins (Li et al., 2019). Modulating the function of MLVs in animal models of disease, including VE, may improve disease outcomes (Da Mesquita et al., 2018; Hu et al., 2020; Li et al., 2022). Furthermore, altering drug delivery and administration sites, such as the meninges, may provide an improved therapeutic strategy.

However, how to integrate animal models and advanced live tracing systems to consecutively visualize the spread of neurotropic viruses, lymphangiogenesis, and mobilization of immune cells in the brain is still a challenge. How to improve outcomes for VE patients with neoteric therapeutics and modified drug delivery also remain urgent tasks.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

D.Y., X.J.L., and D.Z.T. wrote the original draft and constructed the figures and tables. X.L.L. and B.W. conceptualized, wrote, and edited the manuscript. All authors read and approved the final version of the manuscript.

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