Viral Sensing at the Blood–Brain Barrier: New Roles for Innate Immunity at the CNS Vasculature

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Neurotropic viral infections are a major source of disease worldwide and represent a growing burden to public health. While the central nervous system (CNS) is normally protected from viral infection by the blood–brain barrier (BBB), many viruses are able to cross the BBB and establish CNS infection through processes that largely remain poorly understood. A growing body of recent research has begun to shed light on the viral and host factors that modulate BBB function, contributing to both protective and pathological disease processes. Central to these studies have been the actions of host cytokines and chemokines, which have increasingly been shown to be key regulators of BBB physiology. This review summarizes recent advances in understanding how BBB function governs both viral pathogenesis and host immune responses during neurotropic viral infections.

Viral infections of the central nervous system (CNS) cause severe morbidity and mortality in >0.5 million individuals worldwide each year.[1] Encephalitis, inflammation of the CNS parenchyma, and meningitis, inflammation within the meninges, often occur concomitantly, leading to the classic presentation of fever, meningismus, and headache with various ranges of altered mental status. Indeed, viral infections can influence neuronal function and gene expression, contributing to dementia, memory loss, and other cognitive and behavioral abnormalities.[2–4] The extent of CNS injury, neuroinflammation, and overall outcome depend on the specific pathogen and the immunologic status of the patient. With the exception of rabies virus, most neurotropic viruses gain access to the CNS in a minority of cases, suggesting that barriers to the CNS entry of virus do exist and, for most individuals, prevent infection. This review will briefly introduce clinically important neurotropic viruses, and then focus on blood–brain barrier (BBB) restriction of viral entry, and host-pathogen interactions at the BBB that contribute to virologic and immunologic responses.

NEUROTROPIC VIRUSES

The mammalian CNS is susceptible to infection by a number of viruses, the majority of which are incidental and/or opportunistic in nature. Many of the most devastating causes of viral encephalitis are zoonotic infections, caused by pathogens that are relatively benign in their primary hosts, but neuroinvasive and highly pathogenic when spread to "dead-end" hosts that do not typically transmit virus back to primary hosts. Examples in humans include rhabdovirus and flavivirus infections.[5] Arthropod-borne viruses (arboviruses) carried by mosquitoes and ticks are a major source of viral encephalitis in humans, with thousands of reported neuroinvasive infections in the United States every year[6,7] (**Table 1**). Replication of neurotropic viruses within the CNS often results in viral and immune-mediated damage to nervous tissue, leading to severe neuropathology and death. However, neuropathogenesis differs widely by virus species and by host species, age, and health.

Retroviruses

Members of the viral family *Retroviridae* are single-stranded RNA (ssRNA) viruses capable of causing severe CNS infection and disease. Neurotropic retroviruses include human immunodeficiency virus-1 (HIV-1) and the related simian (SIV) and feline (FIV) immunodeficiency viruses. In humans, HIV-1 infection is most associated with the development of acquired immune deficiency syndrome (AIDS), which is often characterized by virally mediated damage to nervous tissue, resulting in HIV-associated neurocognitive impairments and dementia.[8,9] Immunodeficiency during HIV-1 infection also significantly enhances susceptibility to other neuroinvasive infections by opportunistic pathogens, compounding already-existing neuropathology.[8] The human

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Table 1 Reported cases of major neurotropic arboviral infection in the United States

Virus	Nonneuroinvasive	Neuroinvasive	Total
West Nile			
2014	823[a]	1,262[a]	2,085[a]
2013	1,202	1,267	2,469
2012	2,801	2,873	5,674
2011	226	486	712
La Crosse			
2014	—	—	71[a]
2013	8	77	85
2012	7	71	78
2011	14	116	130
Eastern equine encephalitis			
2014	—	—	8[a]
2013	0	8	8
2012	0	15	15
2011	0	3	3
Powassan			
2014	—	—	8[a]
2013	3	12	15
2012	0	7	7
2011	4	12	16

— Data not available.

[a]Provisional data, subject to revisions. Data: Centers for Disease Control and Prevention; ArboNET.

CNS is also susceptible to retroviral infection by human T-cell lymphotropic virus-1 (HTLV-1), which can result in progressive neurodegeneration and spinal cord myelopathy.[8,10]

Flaviviruses

Another prominent family of neurotropic ssRNA viruses are the *Flaviviridae*, which include West Nile virus (WNV), Japanese encephalitis virus (JEV), dengue virus (DenV), tick-borne encephalitis virus (TBEV), and others. Flavivirus infections are a major cause of viral encephalitis worldwide. WNV, in particular, is an emerging human pathogen which, over the past decade, has become the leading cause of epidemic viral encephalitis in the United States[11] (**Table 1**). While most flavivirus infections are mild or asymptomatic, a subset of infected individuals develops severe neuroinvasive disease and encephalitis, often resulting in permanent neurological injury or death.[11,12] While recent progress has been made with JEV and TBEV, there are still few effective vaccines or treatments for other flaviviruses, including WNV and DenV, greatly hindering attempts to address the growing public health burden caused by these viruses.[13]

Other viruses

Newly emerging viruses, including the paramyxoviruses Hendra virus (HeV) and Nipah virus (NiV), are also causes of encephalitis in human hosts, but remain poorly understood.[14,15] Other more common infections such as herpes simplex virus-1 (HSV-1) establish dormant infection in peripheral nerves, but can cause sporadic, severe encephalitis in some individuals.[5,16] Other neurotropic viruses, including rabies virus (RabV) and mouse adenovirus-1 (MAV-1), can cause significant neuropathology, but access the CNS via alternative means compared to retroviruses and flaviviruses.[5,17,18] The broad variation among neurotropic viruses highlights the considerable challenge of understanding when and how these viruses infect the CNS. Recent research, however, has shed much light on how viruses and the host factors they elicit impact both viral neuroinvasion and subsequent pathogenesis.

THE BLOOD–BRAIN BARRIER

A common feature of CNS viral infections is breakdown of the blood–brain barrier (BBB). The BBB is a multicellular interface of glial and vascular cells that tightly restricts the movement of solutes and cells in the circulation into the CNS parenchyma. Microvasculature within the CNS features nonfenestrated brain microvascular endothelial cells (BMECs) joined by tight junctions (TJs) and adherens junctions (AJs). These junctions serve to limit paracellular permeability as well as maintain the polarized expression of key BBB transporter proteins, ion pumps, and other molecules that establish the metabolic gradients that are also a key component of BBB function and CNS homeostasis.[19] BMECs at the BBB are supported by close association with pericytes and the endfeet projections of astrocytes, both of which are important sources of soluble factors that enhance junction integrity and barrier function.[20,21] While BMECs are the major structural component of the BBB, pericytes and astrocytes exert considerable regulatory control on BMECs, and thus, all three cell types are targets for modulation of BBB function by viruses and host immune factors.

Under normal conditions, viruses and other pathogens are completely excluded from the CNS by the BBB. However, diminished BBB integrity during infancy, advanced age, or illness renders some individuals susceptible to neuroinvasive infections. Preterm infants and neonates, in particular, do not yet have a completely developed BBB,[22] making them particularly susceptible to neurotropic viruses.[23] However, even in otherwise healthy adults, many viruses are capable of crossing the BBB and establishing CNS infection. Thus, discovering the viral and host factors that impact BBB permeability has been a key goal of recent research aiming to understand how neuroinvasive viral infections occur, with the ultimate goal of finding targets for effective treatment and prevention strategies. At the center of this research have been investigations of host cytokine and chemokine responses, which are known to be powerful regulators of BBB permeability and junction integrity,[24] but, in some cases, have only recently been well studied in the context of CNS viral infections. Viral factors and host cytokines and chemokines can impact the BBB directly and indirectly via the induction of inflammatory responses and immune cell migration. These impacts on BBB function have been shown to result in both protective and pathological processes.

CYTOKINE REGULATION OF BBB FUNCTION DURING VIRAL INFECTIONS

A complex network of pathogen and host factors governs BBB permeability during viral infections. Recent studies have furthered the work of clarifying how networks of cytokine and chemokine signals work together to modulate BBB function. Inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and interferon (IFN)-γ, are among the best-characterized regulators of BBB permeability during CNS viral infections. Most significantly, these molecules have been widely shown to enhance BBB permeability, both via direct signaling mechanisms as well as by reciprocal induction of other inflammatory mediators such as CXCL10. In contrast, recent work has also begun to identify how other host cytokines, such as the type I IFNs, can preserve BBB integrity and act as a counter-regulator of BBB permeability against inflammatory stimuli. Together, these cytokine and chemokine signals regulate BBB function dynamically across time and CNS regions, impacting access of viruses to the CNS, as well as subsequent leukocyte infiltration into the CNS parenchyma. Thus, regulation of BBB function by host immune factors plays a pivotal role in disease pathogenesis and recovery during CNS infection by many different viruses.

Inflammatory cytokines

Although inflammatory cytokines have been known for decades to influence vascular permeability, recent research has begun to show how these molecules are induced and signal at the BBB during CNS viral infections. TNF-α, IFN-γ, and IL-1β, in particular, have previously been shown to increase endothelial barrier permeability, both *in vivo* and in culture models.[25–28] The most well studied of these is TNF-α, which has been implicated in increased vascular permeability, poor tissue perfusion, and endothelial cell death during viral infections.[29,30] Specifically at BBB endothelium, TNF-α signaling has been associated with enhanced BBB permeability in the context of many neurotropic viral infections, including WNV,[31,32] JEV,[33,34] NiV,[14] HeV,[14] HIV-1,[35] FIV,[36] and others. TNF-α-mediated BBB breakdown occurs via several distinct mechanisms. Recent studies have demonstrated that abrogation of TNF-α signaling during viral infections reduces the expression of other TNF-α-induced inflammatory cytokines and chemokines, with an associated rescue of BBB permeability[31,34] and/or peripheral vascular leakage.[29] In addition, TNF-α signaling acts directly on endothelial cells to enhance permeability via interaction with cytoskeletal regulatory pathways. For example, our laboratory recently demonstrated that TNF-α activates the RhoA/Rho Kinase pathway in BMECs following WNV infection or viral pattern recognition receptor (PRR) agonism, increasing endothelial permeability in *in vitro* BBB culture models. Likewise, TNF-α and RhoA have been implicated in BBB dysfunction during HIV-1 infection.[35,37]

IFN-γ is also known to disrupt BBB integrity during neurotropic viral infections. A recent study by Chai *et al.*[17] featured a pathway analysis that identified IFN-γ at the center of the cytokine network responsible for BBB disruption during laboratory-attenuated RabV infection. Subsequent experiments showed that treatment with an IFN-γ neutralizing antibody significantly rescued BBB permeability in RabV-infected animals.[17] Notably, the actions of IFN-γ in this study were associated with high expression of CXCL10, which has also been shown to enhance BBB permeability during RabV infection.[38] This finding is in line with a recent report demonstrating a positive feedback loop between IFN-γ and CXCL10 expression via astrocytes that contributes to chronic neuroinflammation and neuropathology during CNS infection with HTLV-1.[10] Another recent report suggests that astrocytes are also a prominent source of CXCL10 in the context of Theiler's murine encephalitis virus (TMEV) infection.[39] Together, these studies support a model by which migration of IFN-γ-expressing lymphocytes across the BBB activates CXCL10 expression in the CNS, which in turn dysregulates BBB integrity, allowing the influx of greater numbers of IFN-γ-expressing CXCR3+ lymphocytes into the CNS parenchyma. While CXCL10-mediated BBB breakdown and lymphocyte recruitment has been shown to promote viral clearance and survival during RabV[17,38] and WNV[40,41] infection, this process must be carefully regulated in order to prevent bystander injury and neuropathology caused by cytokines, glial activation, and cytotoxic T lymphocyte responses within the CNS.[31,42–44]

While IL-1 has been shown to increase BBB permeability *in vitro*[25,27] and during CNS injury *in vivo*,[45] the role of this cytokine at the BBB during CNS viral infections has been less well characterized. For example, despite its activity *in vitro*, we have recently shown that *Il1r1*−/− mice do not exhibit enhanced BBB permeability following subcutaneous WNV infection[43] and that WT BMECs do not produce detectable levels of IL-1β following WNV infection *in vitro*.[32] Instead, we found that IL-1 played a more indirect role at the BBB during WNV infection via its regulation of BBB chemokine expression. Most notably, we found that IL-1 signaling was critical for CXCL12-mediated localization of infiltrating lymphocytes into perivascular spaces, which was necessary for proper dendritic cell-mediated T-cell reactivation. Thus, IL-1 signaling at the BBB during WNV infection serves as an important regulator of CNS immune privilege, as loss of IL-1R1 signaling resulted in enhanced inflammatory chemokine expression in the CNS, along with greatly enhanced lymphocyte infiltration, CNS immunopathology, and loss of virologic control.[42,43] However, it is certainly possible that IL-1 signaling plays a more direct role in regulating BBB permeability in the context of other viral infections, as IL-1 family cytokines are upregulated following infection with a number of viruses that feature BBB disruption, including RabV,[17] JEV,[46] and HIV-1.[47]

Type I interferons

In contrast to inflammatory cytokines, a growing body of evidence has demonstrated that type I IFN signaling at the BBB acts to promote barrier integrity. The BBB-stabilizing effects of type I IFNs have been well demonstrated using *in vitro* BBB models[32,48] and in the autoimmunity literature, where type I IFN treatment has been shown to preserve BBB integrity and limit infiltration of autoreactive leukocytes into the CNS during multiple sclerosis and its mouse model experimental autoimmune encephalomyelitis.[49] However, despite the canonical function of type I IFNs as
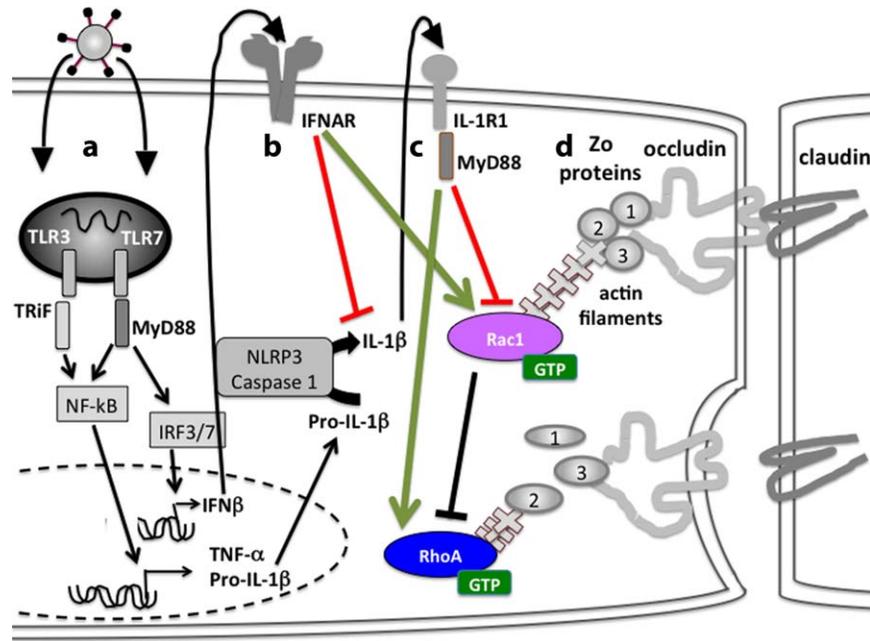
Figure 1 Innate immune responses to viruses regulate BBB permeability. (**a**) Following binding of nonself RNA ligands within the endosomal compartment, Toll-like receptor 3 (TLR3), TLR7 signal through their adaptor proteins, TRIF (TIR domain-containing adaptor inducing IFNβ), and MYD88 (myeloid differentiation 88) to promote nuclear factor kappa B (NF-κB)-, IRF3-, and IRF7-dependent gene expression, which leads to expression of Th1 cytokines, TNF-α, and pro-IL-1β, and IFN-β, respectively. (**b**) Type I IFN signaling through IFNAR inhibits production of IL-1β and activates Rac1 GTPase. (**c**) Inflammasome activation in response to viral infections results in cleavage of pro-interleukin-1β (pro-IL-1β) into the mature IL-1β form. IL-1β is secreted from the cell and binds to IL-1 receptor (IL-1R), which signals through MyD88 and activates RhoA GTPase. (**d**) Activation of Rac1 GTPase promotes BBB permeability via cytoskeletal rearrangements that assemble tight junction complexes that include claudins, occludens, and zonular occluden family members. Rac1 also inhibits RhoA, which promotes cytoskeletal rearrangements that disassemble tight junctions. Gray color indicates pathways previously identified while colorization highlights newly identified effects of IFNAR and IL-1R1.

antiviral molecules, their role at the BBB during neurotropic viral infections has only recently been addressed. Recent studies indicate that type I IFN signaling also serves to promote BBB integrity during CNS viral infections via multifaceted mechanisms, highlighting a novel protective function for these cytokines.

Recent work in our laboratory demonstrated that detection of WNV by PRRs at the BBB induces local type I IFN responses that promote BBB integrity,[32] as *in vitro* BBB cultures prepared with type I IFN receptor-deficient (*Ifnar*^{-/-}) BMECs and mice lacking intact type I IFN signaling exhibited enhanced permeability following WNV infection or chemical agonism of PRRs. Type I IFNs accomplished this via two major mechanisms, the first being suppression of inflammatory cytokine production, thereby suppressing a major source of BBB disruption following infection. *In vitro*, type I IFN modestly suppressed the expression of TNF-α and completely abolished expression of IL-1β by BMECs following exposure to WNV or PRR agonism. While the mechanisms of type I IFN suppression of IL-1 signaling at the BBB have not been established, type I IFN has been shown to suppress IL-1β expression in other contexts via inhibition of inflammasome activity and/or suppression of pro-IL-1α and pro-IL-1β expression.[50,51] Similarly, a recent report by Pinto *et al.* demonstrated that loss of IFN signaling in whole animals or in subsets of myeloid cells resulted in greatly enhanced inflammatory cytokine production following WNV infection, associated with profound vascular leakage and sepsis syndrome.[29] These

findings highlight the complex interactions of type I IFN and IL-1 during CNS infections, as, while type I IFN suppressed the actions of IL-1 at the BBB in our study, a recent study has demonstrated synergistic antiviral signaling between type I IFN and IL-1 in neurons during WNV infection.[52] Thus, interactions between type I IFNs and inflammatory cytokines is likely to vary significantly across time and cell types within the CNS.

In addition to suppressing inflammatory cytokine expression in our study, type I IFNs were also shown to preserve BBB integrity via counterregulation of the BBB-disrupting effects of inflammatory stimuli. *In vitro,* induction of enhanced BBB permeability following treatment with recombinant inflammatory cytokines could be reversed by subsequent addition of recombinant IFN-β or WNV, the effect of which was shown to be type-I IFN-dependent.[32] This effect was mediated through preferential activation of cytoskeletal regulatory proteins, as type I IFN signaling resulted in greater Rac1 activation and inhibition of RhoA activation by inflammatory cytokines (**Figure 1**). These findings are corroborated by recent *in vitro* studies demonstrating the protective effects of type I IFNs on peripheral vascular permeability following DenV-2 infection.[30,53] In this report, TNF-α-induced vascular permeability was rescued following recombinant IFN-β treatment or DenV-2 infection, the effect of which was also type I IFN-dependent. DenV-2 and type I IFN-mediated enhancement of endothelial barrier integrity was shown to occur via CD73, an enzyme richly expressed in endothelial cells that

functions to increase intracellular cyclic adenosine monophosphate (cAMP) levels, which has been shown to promote endothelial barrier function.[54] Based on these findings, it is possible that viral induction of type I IFNs at the BBB promotes barrier function via multiple cell-intrinsic signaling mechanisms.

NEUROTROPIC VIRUSES AND BBB JUNCTIONS
A key determinant of BBB function is the proper assembly of TJs and AJs between BMECs. Neurotropic viral infections have been shown to alter junction protein expression and function in several ways, including direct mechanisms via viral proteins and downstream immune-mediated regulation of junction integrity. To date, the effects of retroviruses on BBB junctions have been the most extensively characterized. Several retroviruses, including HIV-1, HTLV-1, SIV, and FIV have been shown to diminish expression of BBB TJ proteins *in vitro* and *in vivo*.[8,55] Several key retroviral proteins, in particular, are known to induce endothelial cell activation and BBB breakdown. The HIV-1 protein Tat, a key retroviral transcriptional regulator, has been shown in numerous studies to activate BBB endothelium, decrease TJ protein expression, and degrade junctions via MMP-9 and RhoA-mediated cleavage of the TJ protein occludin.[55,56] Similarly, the HIV-1 virion envelope protein gp120 has been shown to enhance BBB permeability by decreasing expression of several TJ proteins, including claudin-5[57] and Zo-1,[58] and by inducing proteosome-mediated degradation of Zo-1 and Zo-2.[59] In addition to viral proteins, recent work suggests that elevated CCL2 levels during HIV-1 infection also likely contribute to BBB breakdown and junction disruption via multiple mechanisms,[55] including disruption of AJs in brain endothelium via phosphorylation and sequestration of β-catenin.[60] Other studies have linked CCL2 to endothelial barrier disruption in the context of MAV-1[18] and DenV[61] infections as well.

Aside from retroviruses, interest in the impact of flaviviruses on BBB junctions has also received considerable attention in the literature, but with much less clear results. As others have noted, the impact of WNV infection on BBB junction protein expression has been highly variable in published reports, with conflicting findings among studies using different *in vitro* methodologies, *in vivo* models, and those looking at mRNA vs. protein readouts.[62] One explanation for these discrepancies is that junction protein regulation downstream of WNV infection may occur more indirectly through the action of inducible inflammatory mediators, as opposed to directly via viral proteins. Thus, variations such as viral inoculum and experimental time course may result in differential expression of cytokines, chemokines, MMPs, etc., resulting in different expression profiles of TJ proteins.[32,63]

For example, in our recent study[32] we observed that inflammatory cytokine signaling following WNV infection resulted in loss of TJ integrity as indicated by loss of Claudin-5 and Zo-1 colocalization in BMECs along intercellular borders. However, viral induction of type I IFNs suppressed the effects of inflammatory cytokines on TJ organization. Moreover, triple blockade of type I IFN, TNF, and IL-1 receptor signaling resulted in essentially no alteration to TJ organization following BMEC exposure to

WNV *in vitro*, indicating that these three innate cytokines represent a cell-intrinsic regulatory axis by which BBB TJ integrity is regulated following detection of WNV at the BBB. Expression of inflammatory mediators by other BBB cell types is also likely to contribute to BBB disruption during flavivirus encephalitis, as was demonstrated by a recent report showing that JEV-infected pericytes secrete IL-6, leading to proteosomal degradation of Zo-1 in BMECs in BBB coculture systems.[64] Indeed, inflammatory cytokines and chemokine expression via multiple cellular sources has been linked to suppression of BBB junction protein expression and degradation of existing junctions during many viral infections, including studies using retroviruses,[8,55,60] RabV-1,[17] MAV-1,[18] and others.

PROTECTIVE VERSUS PATHOLOGICAL OUTCOMES OF BBB DISRUPTION DURING VIRAL INFECTIONS
While enhancement of BBB permeability has traditionally been thought of as a hallmark of neuropathology, recent research has expanded our appreciation of the nuanced and dynamic nature of BBB function in the context of viral infections. While BBB disruption is a putative mechanism by which many neurotropic viruses gain access to the CNS parenchyma, BBB disruption is also necessary in some contexts to facilitate CNS immune trafficking and subsequent viral clearance and recovery. However, even this protective function of BBB breakdown can contribute to pathogenesis, as infected leukocytes may participate in viral seeding of the CNS (the so-called "Trojan horse" method of neuroinvasion) and the antiviral activity of infiltrating cells can cause significant tissue injury within the CNS, a site with limited capacity for repair. Thus, the physiological outcomes of BBB disruption during neurotropic viral infections are multifaceted and are influenced by myriad viral and host factors.

BBB breakdown and CNS viral entry
While the methods by which most neurotropic viruses access the CNS remain poorly understood, the trans- or paracellular trafficking of free virions across the disrupted BBB is thought to be a major route of viral neuroinvasion. A growing body of studies using *in vitro* BBB models have shown the ability of free viruses, including WNV and HIV-1, to traffic across BBB endothelium and have shown that enhanced permeability and/or junction disruption is linked to higher rates of viral migration in culture systems.[32,35,65,66] In the case of WNV infection, BBB permeability has been shown to increase days before initial detection of virus within the CNS in mouse models, likely as a result of circulating inflammatory mediators, and this early increase in BBB permeability during high viremia has been linked to initial WNV neuroinvasion, as abrogation of inflammatory signals that preserve BBB integrity also delay or prevent neuroinvasion.[31,32,67,68] Despite this understanding, the precise route of entry for free flaviviruses across the BBB is still poorly understood. While the transcellular trafficking of WNV and JEV through BBB endothelium has been suggested in culture systems,[66,69] there is little evidence that either virus enters or infects BMECs *in vivo*,[62] although such events may be so infrequent and/or stochastic that detection with traditional histological methods is difficult. In our

recent study, we showed that trafficking of WNV across *in vitro* BBB cultures could occur independently of BMEC infection, and that trafficking of free virus was correlated closely with paracellular permeability.[32] Thus, it is possible that free flaviviruses cross the BBB via both trans- and paracellular routes.

Another potential mechanism of viral entry into the CNS invokes the "Trojan horse" phenomenon. In this case, virus-infected peripheral immune cells cross the BBB, either due to BBB damage or in the normal process of immune surveillance. Once there, they shed infectious virions within the CNS parenchyma and perivascular spaces, establishing CNS infection. This method of neuroinvasion is most frequently associated with retroviruses such as HIV-1, which access the CNS via trafficking of infected CD4^{+} T cells and monocytes,[8] a process enhanced by HIV-1 mediated inflammatory signals that disrupt BBB function.[47,56,57] The Trojan horse mechanism may also be yet another means by which flaviviruses access the CNS, as blockade or deletion of several immune trafficking molecules has been shown to inhibit BBB disruption and neuroinvasive disease in mouse models of WNV infection.[67,68,70]

The BBB and antiviral CNS immune trafficking

The regulation of CNS immune trafficking during viral infections is an incredibly complex process, which, although it may expose the CNS to viral pathogens in some contexts, is also often critical for effective antiviral immune responses within the CNS.

Indeed, BBB breakdown observed during many CNS infections is thought to primarily serve to facilitate antiviral leukocyte trafficking. This is particularly true for viruses known to enter the CNS via non-BBB routes, such as transneuronal trafficking by RabV and HCV,[16,17] resulting in increased BBB permeability that is largely secondary to CNS infection. In the case of RabV infection, recent studies have demonstrated that induction of BBB permeability is associated with ameliorated disease pathogenesis and enhanced antiviral immunity within the CNS.[17,38]

Even for viruses that may utilize BBB breakdown and/or immune trafficking to access the CNS, these processes are often critical for effective virologic control and clearance. In the case of WNV, even though the abrogation of some leukocyte trafficking molecules can inhibit WNV neuroinvasion,[67,68,70] multiple studies have shown that intact cellular immune trafficking within the CNS is absolutely essential for survival and recovery from WNV encephalitis.[40,41,71,72] Despite these findings, other reports have shown that immune cell trafficking during WNV and other neuroinvasive infections is a significant source of CNS injury.[73,74] Thus, host inflammatory responses must be carefully regulated, such that viruses are effectively cleared without the induction of major immune pathology. This can be accomplished in regulation of both the numbers and the activation state of antiviral leukocytes. As we have recently shown, in the context of WNV infection, loss of proper IL-1 mediated T-cell reactivation at the BBB resulted in greatly enhanced immune infiltrates during WNV encephalitis, but these cells were ineffective at viral clearance and, instead, contributed to enhanced immunopathology and glial cell activation.[42,43] In contrast, prior work in our laboratory demonstrated that blockade of CXCL12-mediated sequestration of

virus-specific T cells in perivascular spaces via pharmacological inhibition of CXCR4 resulted in enhanced penetration of T-cells into the CNS parenchyma, where they both cleared virus and decreased immunopathology.[75] These findings and others illustrate the potential benefits of carefully regulated disruption of normal BBB function during viral infections of the CNS, and provide clues as to how these processes may be targeted for therapeutic interventions.

CONCLUSION

The notion that viruses interact with and are sensed by the BBB is a novel concept that highlights evolutionary adaptations in mammals that limit CNS infection. The signaling pathways that directly impact BBB permeability and function outlined here are just the beginning of a new direction in infectious diseases research on neurotropic pathogens that focuses on mechanisms of neurotropism and host responses critical for preventing neuroinvasion. Most patients infected with the viruses discussed do not develop viral encephalitis, suggesting that the innate immune mechanisms operative at the BBB normally succeed in preventing viral entry into the CNS. These mechanisms are also likely to increase the stringency with which leukocyte entry into the CNS is regulated, providing avenues to prevent neural injury by both viruses and the ensuing inflammation triggered by CNS infections. Further studies are needed to determine whether viruses alter BBB apicobasal polarity, leukocyte capture, and transendothelial cells migration, and to identify additional signals involved in BBB stability. Understanding the impact of neurotropic viruses on BBB biology is a critical part of the neuropathogenesis of disease in those patients who develop encephalitis and may lead to new targets to treat immunopathology in both infectious and autoimmune diseases.

CONFLICT OF INTEREST
The authors declared no conflict of interest.

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