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REVIEW

Disruption of the blood brain barrier is vital property of neurotropic viral infection of the central nervous system

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Summary. – The blood brain barrier consisting of astrocytes, pericytes and brain microvascular endothelial cells plays a vital role in the pathogenesis of neurotropic viruses by controlling the access of circulating molecules, immune cells or viruses into the central nervous system (CNS). However, this barrier is not impenetrable and neuroviruses have evolved to disrupt and evade it. This review aims to describe the underlying entry mechanisms of several neuroviruses such as (Japanese encephalitis virus (JEV), West Nile virus (WNV), Zika virus (ZIKV), Nipah virus (NiV), Rabies virus (RABV), Herpes simplex virus (HSV) and Human immunodeficiency virus (HIV)) into the CNS through BBB disruption. The mechanisms, through which neurotropic viruses enter the BBB, are being studied and are becoming clearer, however, some aspects still remain unknown. Some of these viruses are able to invade the brain parenchyma by a 'Trojan horse' mechanism, through diapedesis of infected immune cells that either cross the BBB paracellularly or transcellularly. Important mechanisms of BBB disruption associated with paracellular entry of viruses include alterations in expression or phosphorylation of tight junction proteins, disruption of the basal lamina and disruption of the actin cytoskeleton. In the absence of such mechanisms, indirect effects of viruses on the immune system are likely causes of barrier disruption.

Keywords: adhesion molecules; blood-brain barrier; central nervous system; neuroviruses; tight junction

Contents:

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Abbreviations: AM = adhesion molecule; AIDS = Acquired immune deficiency syndrome; BBB = blood-brain barrier, BMECs = brain microvascular endothelial cells; CAMS = endothelial cell adhesion molecules; CINC-1 = cytokine-induced neutrophil chemoattractant 1; CLEC5A = C-type lectin superfamily member 5; CNS = central nervous system; CSF = cerebrospinal fluid; HSE = Herpes simplex encephalitis; HIV = Human immunodeficiency virus; ICAM-1 = intercellular cell adhesion molecule 1; IFNAR = IFN-α receptor; IRF-3 = IFN regulatory factor 3; JAM = junctional adhesion molecules; JEV = Japanese encephalitis virus; MCP = methyl-accepting chemotaxis protein; MDA5 = melanoma differentiation-associated protein 5; MMPS = multiple matrix metalloproteinases; NF-κB = nuclear factor kappa-light-chain-enhancer of activated b cells; NiV = Nipah virus; RABV = Rabies virus; RIG-I = retinoic acid-inducible gene; TJ = tight junctions; TLR = Toll-like receptor; TEER = trans epithelial electrical resistance; VCAM-1 = vascular endothelial cell adhesion molecule 1; WNV = West Nile virus; ZO = zonula occludens; ZIKV = Zika virus

1. Introduction

Most zoonotic virus infections are highly virulent and neuroinvasive in non-natural hosts. Viral infections are usually initiated at the periphery, mostly at epithelial or endothelial cell surfaces. Upon viral infection, the tissue-specific antiviral responses, such as intrinsic immune responses and paracrine signaling, are initiated from the infected cell to surrounding uninfected cells by secreted cytokines. Additionally, the infection might be cleared by the action of virus-specific antibodies and T cells of the adaptive immune response. Nevertheless, viral infections may spread to other tissues if the virus escapes from the immune system at the site of primary infection, causing increased virus replication or overreactive innate immune responses. Subsequently, pro-inflammatory and anti-inflammatory cytokines are further enhanced in the serum, leading to vigorous systemic immune responses. This reaction can cause devastating effects in the brain resulting in meningitis, encephalitis, meningoencephalitis, or even death. Once the brain is infected with zoonotic viruses, viral clearance by the immune system is a major challenge. Particularly, CNS neurons are irreplaceable and established T-cell-mediated cytolysis of these infected cells is not a favorable strategy. Brain homeostasis is sustained by the structure and function of the blood-brain barrier (BBB). BBB plays a key role in the pathogenesis of neurotropic viruses by controlling the access of circulating molecules, immune cells, or viruses into the central nervous system (CNS). Infectious virus particles, immune cells, inflammatory mediators, and eventual neuronal dysfunction is the main feature of neurotropic virus-related neuropathy in the parenchymal tissues of the CNS. Therefore, this review aims to describe the underlying entry mechanisms of several neuroviruses (JEV, WNV, ZIKV, NiV, RABV, HSV and HIV) into CNS through BBB disruption. A profound understanding of the pathogenesis of these viruses is imperative for the development of effective therapeutic strategies.

2. Interaction between viruses and CNS immune system

The innate immune system is a network of pattern recognition receptors (PRRs) (Toll-like receptors (TLR), nucleotide oligomerization domain (NOD)-like receptors (NLRs), retinoic acid inducible gene (RIG)-I-like receptors (RLRs) and DNA sensors) that can identify conserved pathogen-accompanied molecular patterns (PAMPS) on microbes. PRR triggers signaling cascades that encourage nuclear translocation of latent transcription factors (e.g. IFN regulatory factor 3 (IRF-3) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and induce transcriptional activation of genes that direct and mediate cell immunity against viruses, including secretion of antiviral cytokines (IFN-α and IFN-β). Specific innate immune signaling and effector pathways have been found to confine or contribute to the pathogenesis of different viruses in the CNS. For example, TLR signaling has defensive or pathogenic effects in the CNS. It was demonstrated that TLR3 and TLR7 signaling restricted WNV infection in neurons (Daffis *et al.*, 2008; Town *et al.*, 2009), whereas other studies showed that TLR3 and TLR7 enhanced BBB permeability and viral neuroinvasion after WNV infection (Wang *et al.*, 2004; Welte *et al.*, 2009). In RABV, it was revealed that mice lacking TLR3 had a lower viral load and hence enhanced survival rate following infection, suggesting that TLR3 contributes to pathogenesis. Conversely, TLR3 signaling pathway was shown to have protective effects against encephalitic herpesviruses in humans with primary immunodeficiencies (Nair and Diamond, 2015). Furthermore, it was demonstrated that ZIKV can activate TLR3 in neural progenitor cells (NPCs), thereby resulting in activation of pro-apoptotic pathway or dysregulation of cell integrity (Dang *et al.*, 2016). The second type of recognition receptor in the CNS innate immune system upon viral infection are the RIG-I-like receptors (RLRs), which are able to generate antiviral responses during several neurotropic RNA viruses engagement. RLR signaling preferentially mediates type I IFN production in response to RABV, as mice deficient in the downstream adaptor molecule mitochondrial antiviral signaling protein (MAVS) had improvement in limb paralysis upon infection (Faul *et al.*, 2010). In addition, RLR signaling also restricted WNV replication in the spinal cord and brain, mainly in neurons (Fredericksen *et al.*, 2008). The third type of recognition receptors in CNS innate immune system are DNA sensors. They also have antiviral effects during neurotropic viral infections. For instance, cGAMP synthase (cGAS), a cytoplasmic viral DNA sensor, signals via an adaptor molecule that can stimulate IFN genes (STING). STING recruits threonine-protein kinase 1 (TBK1), leading to the activation of IRF-3, induction of type I IFN and proinflammatory cytokines. A study showed that STING-deficient mice suffered higher HSV-1 infection in the brain and greater mortality (Ishikawa *et al.*, 2009). Similarly, mice lacking cGAS were more susceptible to HSV-1 and failed to produce type I IFN (Li *et al.*, 2013).

Type I and type III interferons are cytokines that are critical to control early steps of viral infections. The type III IFN family comprises of three subtypes, IFN-λ1, IFN-λ2 and IFN-λ3. The signaling pathway of type III IFNs happens

through a receptor distinct from that of type I IFNs but the same signal transduction pathway is induced. Nevertheless, the types of cells that respond to type I and type III IFNs are different. Type I IFN receptor can be found on most cell types, but the type III IFN receptor is preferentially expressed by epithelial cells. In the CNS, the expression of type III IFNs is lower than that of type I IFNs in response to viral infections. Different types of cells of the CNS were reported to respond to IFN produced upon viral infection including astrocytes, oligodendrocytes and neurons. However, the specific reaction of CNS cells to IFN-λ is very low. An overall weak expression of the IL28R-α subunit of the IFN-λ receptor has been shown in the CNS as compared to other tissues. *In vivo* expression of IFN-λ3 was used to identify the cells that can respond to circulating IFN produced by muscle cells in the periphery. In this experiment, the Mx1 protein, used as a marker of the IFN response, was detected only in the epithelial cells of choroid plexus and in few meningeal cells. These data are consistent with the epithelial specificity of the IFN-λ response. It was also recently observed that IFN-λ can inhibit HSV-1 infection in primary human astrocytes (Sorgeloos *et al.*, 2013). However, type I IFNs are the main element of the innate immune response, which perform antiviral activity within both infected and neighboring cells (Sorgeloos *et al.*, 2013). Upon viral entry, the IFN response is triggered, and released/synthesized viral components, such as double-stranded RNA intermediates, that trigger transcription factors such as IRF-3, IRF-7, NF-κB and activating transcription factor 2 (ATF2/c-Jun). Subsequently, IFN-α/β is transcribed (Fitzgerald *et al.*, 2003). The release of IFN-α/β results in binding to the IFN-α receptor (IFNAR) on the surface of infected and nearby cells, leading to the activation of Janus kinase (JAK) / signal transducer and activator of transcription (STAT) pathway. One study showed that IFN-α/β protects animal hosts against viral infections in mice deficient in the IFN pathway (Durbin *et al.*, 1996). Another study suggests that IFN-α/β controls WNV infection by restricting tropism and viral burden by preventing the death of infected neurons (Suthar *et al.*, 2013). It was also shown that induced endogenous IFN could control RABV infection (Marcovistz *et al.*, 1987) and suppress HIV-1 replication in *in vivo* and *in vitro* studies (Poli *et al.*, 1989). However, several viruses encode proteins neutralizing the innate immune system by targeting different parts of the IFN production and signaling pathways, leading to the evasion of the IFN-induced antiviral state of the host cell (Fontana *et al.*, 2008; Goodbourn and Randall, 2009). On the other hand, if the innate immune system fails to confine the virus, the adaptive immune system will be activated as it is slow, systemic, and pathogen-specific, leading to stimulation of the immunological memory. The adaptive immune response includes cell-mediated immunity and humoral immunity and involves the action of CD4+ T helper cells, CD8+ cytotoxic T cells (CTLs) and B cells. The humoral immune system produces antibodies against different pathogens. Activated of virus-specific CD4+ helper T cells, both Th1 and Th2 type, recognize virus-derived MHC class II-associated peptides on antigen-presenting cells, followed by expression of co-stimulatory molecules. Activation of CTLs leads to their migration to the infection site, where they detect virus-infected cells and eliminate them via lytic activity or apoptosis induction and hence inhibit the virus progeny production. Predominantly, nervous system tissues depend on the intrinsic and innate immune responses and avoid the extensive inflammation and cytotoxic effects of the adaptive immune response due to their mostly irreplaceable nature (Koyuncu *et al.*, 2013).

3. The main entry receptor of neurotropic viruses into CNS

Immune receptors usually exist on cell membranes and they bind to factors like cytokines, resulting in a response of the immune system. Several immune receptors have been identified to limit or contribute to the pathogenesis after viral infection. For example, after JEV infection, macrophages are the main cells in the brain, where on these cells, lactin receptors interact with virus playing an important role in JEV-induced lethality. Previous research reported that JEV interacts with CLEC5A and induces DAP12 phosphorylation in macrophages (Chen *et al.*, 2012). This CLEC5A activation is accompanied by proinflammatory cytokines secretion such as TNF-α and IL-1α. In WNV recognition, TLR3 is considered as a main receptor that can induce IFN responses to protect the CNS from WNV infection, where previous research confirmed that mice deficient for TLR3 and TLR7 showed viral replication enhancement in the CNS after WNV infection (Szretter *et al.*, 2010; Lazear *et al.*, 2011). In ZIKV infections, AXL is an attachment molecule receptor for this virus (Hamel *et al.*, 2015), where it is stimulated in neural stem cells, microglia, radial glial cells and astrocytes (Nowakowski *et al.*, 2016). Recent ex vivo studies demonstrated that AXL receptor was decreased in human astrocytes infected with ZIKV (Retallack *et al.*, 2016; Xu *et al.*, 2016). Additionally, ZIKV-infected primary human skin fibroblast can induce TLR3, RIG-1 and melanoma differentiation-associated protein 5 (MDA5) activation (Hamel *et al.*, 2015). This activation is accompanied by an increase in type 1 IFN production. TLR3 was also activated in cerebral organoids and human neurospheres after neural stem cells were infected with African strain of ZIKV (MR-766) as detected by RT-qPCR (Dang *et al.*, 2016). Induction of transcription of TLR3, RIG-1 and MDA5 by Zika virus infection is observed as well as that of several IFN-stimulated genes such as OAS2, ISG15, and MX1. Increased gene expression of IFN-α and IFN-β and IFN-stimulated genes, cytokines, and other im-

mune modulators such as CCL5, CXCL10, AIM2, and IL-1β was also observed (Nayak *et al.*, 2016). Ephrin B2 (EB2), the main entry receptor of NiV (Bonaparte *et al.*, 2005; Negrete *et al.*, 2005), is a transmembrane protein that is greatly preserved among all mammalian species. EB2 is a ligand of EphB4 receptor and is involved in angiogenesis and neurogenesis (Poliakov *et al.*, 2004). Although EB2 is basically activated in arteries, different organs can also express EB2. In addition to EB2, EB3 is considered as an alternative receptor for NiV, since EB3 is expressed in the CNS (Negrete *et al.*, 2006). *In vivo* research showed that EB3 is expressed also on endothelial cells (Argyris *et al.*, 2007). This revealed that EB3 is expressed in brain parenchyma and might therefore be used in some cell types as an alternative receptor in the absence of EB2. EB2 and EB3 are a large family of tyrosine kinase receptors expressed by neurons and brain endothelium (Lee, 2007; Maisner *et al.*, 2009). Interestingly, neural cell adhesion molecule (NCAM) (Thoulouze *et al.*, 1998), p75 nerve growth factor receptor (p75NTR) (Tuffereau *et al.*, 1998) and nicotinic acetylcholine receptor (nAchR) (Lentz *et al.*, 1982) are cellular membrane components that may act as receptors for RABV glycoprotein G, which is responsible for the neurotropism of RABV (Schnell *et al.*, 2010). Once RABV binds to these receptors through viral glycoprotein G, a neutralizing antibody response is induced (Wiktor *et al.*, 1973). After internalization, glycoprotein G mediates fusion of the viral envelope with the endosomal membrane (Gaudin *et al.*, 1992). However, these studies need more research to fully understand the mechanisms used by this virus to enter the cell, multiply, replicate and cause disease. TLR2 and TLR9 act synergistically in response to HSV infection in the brain parenchyma (Sørensen *et al.*, 2008). It was shown that HSV burdens the brain at a much higher rate in TLR2 and 9 double knockouts as compared with the brains of single knockouts (Sørensen *et al.*, 2008). Furthermore, TNF-α and CXCL9 expression after HSV infection were dependent on TLR2 and TLR9. Therefore, in terms of an effective immune response to HSV, TLR2 and TLR9 are required mainly in the brain (Sørensen *et al.*, 2008) and provide resistance against HSV infection in the brain. TLR7, 8 and 9 can also confer protective immunity against herpes simplex encephalitis (HSE) in humans (Casrouge *et al.*, 2006). Hence, either suppression or activation of these receptors might be helpful to reduce the pathogenesis of the virus and can provide new insights into the treatment of the severe inflammatory consequences of infection.

4. Blood-brain barrier and its components

The blood-brain barrier (BBB) acts as physical and physiological barrier that acts as a selective diffusion barrier, making it a promising neurovascular filtering system that protects the brain from any toxic molecules and infectious agents. It is comprised of the cerebral microvascular endothelium together with pericytes and astrocytes (Abbott *et al.*, 2010). Neurons and microglia are other cellular elements that play an important role in the BBB function (Wilhelm *et al.*, 2011). The BBB is composed of specialized brain microvascular endothelial cells (BMECs) and regulates the flow of molecules and factors into and out of the brain. The passive diffusion of molecules into the brain is limited by complex intercellular tight junctions (TJ); their presence results in extremely high trans-endothelial electrical resistance (TEER). Additionally, returning small lipophilic molecules capable of diffusing from BMECs back to the bloodstream are carried out using efflux transporters such as p-glycoprotein, which contribute to the barrier properties. As a result, BMECs are provided with a necessary network of specific transport systems to transport essential metabolites and nutrients across the BBB. Substantial barrier properties of BBB prevent neuropharmaceutical development by avoiding uptake of the majority of small-molecule pharmaceuticals and essentially all biologics. Conversely, BBB dysfunction and breakdown is associated with a variety of neurological diseases such as multiple sclerosis, Alzheimer's disease, stroke and brain tumors. These issues together have led researchers to develop a variety of BBB models to enable detailed mechanistic studies and drug screens *in vitro*. BMECs are the most vital cell type of the BBB involved in permeability that cover the inner surface of the capillaries, where they are connected by TJs, which form belt-like structures at the apical region of these cells (Wilhelm *et al.*, 2011). While the pericytes cover approximately 22–32% of the endothelium immersed in the basal membrane, their main role is to regulate endothelial cell proliferation, angiogenesis, and inflammatory processes (Dore, 2008). In their absence, abnormal vasculogenesis, endothelial hyperplasia and increased permeability in the brain were observed (Armulik *et al.*, 2010). The third element of BBB is the astrocytes endfeet (Kacem *et al.*, 1998), which are considered as the main sources of regulatory factors such as transforming growth factor (TGF-β), glial cell-derived neurotrophic factor (GDNF), basic fibroblast growth factor (bFGF) and IL-6 (Pekny *et al.*, 1998).

Several viruses have been known to cause disruption of the BBB or endothelial junctions. It is believed that numerous viruses enter the brain parenchyma via diapedesis of infected immune cells such as the "Trojan horse" mechanism (Ivey *et al.*, 2009; Verma *et al.*, 2009; Fletcher *et al.*, 2011). Other viruses can enhance the BBB permeability through TJ complex disruption, where they stimulate the production of inflammatory chemokines or cytokines such as IFN-α, IL-8, TNF-α, and IL-6, hence indirectly contributing to BBB breakdown (Lopez *et al.*, 2012); or they infect endothelial cells and release pro-inflammatory mediators enabling the access of the virus into the CNS (McGavern and Kang, 2011).

The presence of a continuous line of TJs at the cell-cell border is one of the most important elements of the BBB phenotype in cerebral endothelial cells (CECs). The molecular components of the TJs can be divided into transmembrane and cytoplasmic plaque proteins. Transmembrane proteins include junctional adhesion molecules (JAMs) (Martìn *et al.*, 1998), occludin (Furuse *et al.*, 1993) and members of the claudin family (Furuse *et al.*, 1998). These network of proteins and molecules control the passage of the compounds into and out of the brain (Lehner *et al.*, 2011; Mariano *et al.*, 2011). Occludin plays an important role in the formation of the TJ complex (Luissint *et al.*, 2012). Claudins form the TJ backbone and preserve the integrity of the BBB. Brain endothelial cells express claudin-5 (Morita *et al.*, 1999) and to a smaller extent claudin-3, -10, -12 (Ohtsuki *et al.*, 2008) in normal conditions. The second type of proteins (cytoplasmic plaque) act as an intermediary between transmembrane proteins and actin cytoskeleton, such as PDZ-containing proteins, non-PDZ proteins (e.g. cingulin) (Citi *et al.*, 1988; Citi *et al.*, 1989), zonula occludens (ZO)-1 (Stevenson *et al.*, 1986), ZO-2 (Gumbiner *et al.*, 1991) and junction-associated coiled-coil protein/paracingulin (JACOP) (Ohnishi *et al.*, 2004). In addition, cell adhesion molecules (CAMs) are cell surface molecules that enable intercellular binding and communication (Kobayashi *et al.*, 2007). During viral infections, CAMs are responsible for recruiting leukocytes into the vascular endothelium before extravasating into the injured tissues. Vascular endothelial cell adhesion molecule 1 (VCAM-1), intercellular cell adhesion molecule 1 (ICAM-1), and platelet endothelial cell adhesion molecule 1 (PECAM-1) are examples of CAMs. Under healthy circumstances, the endothelial cells of the BBB express very low levels of CAMs.

5. Neuroviruses and BBB disruption

As mentioned earlier, zoonotic infections are the most devastating causes of viral encephalitis caused by pathogens (Koyuncu *et al.*, 2013). It has been documented that a large number of infected subjects will develop encephalitis and other neurological consequences after viral infection (Tyler, 2009). The virus may replicate in neurons and glia after crossing the BBB to infect CNS (Hauwel *et al.*, 2005; Savarin and Bergmann, 2008). Eventually, any CNS virus infection shows a balance between the beneficial effects of the brains' protective innate immune response and detrimental effects of bystander injury due to the consequences of ineffective clearance of the virus by infiltrating systemic NK and T cells. If the balance favors the latter, determined brain inflammation will be the result with either an acute or chronic encephalitis (Schnell *et al.*, 2010).

5.1 Disruption of tight junctions via ICAM-1 and CINC-1

Acute encephalopathy is one of the phenomena of flaviviruses infection such as JEV, WNV and ZIKV, where the envelope (E) protein glycosylation has the main role as virulence determinant (Shirato *et al.*, 2004). Pathologically, the neuroinflammation observed in Japanese encephalitis (JE) infections in the CNS is due to BBB disruption stimulated by JEV (Diagana *et al.*, 2007). Several reports have revealed that the disruption of the BBB and signs of endothelial damage are concurrent with the presence of viral particles in BBB endothelial cells in a JE mouse model (German *et al.*, 2006; Mishra *et al.*, 2009). Recent findings showed that JEV-infected endothelial cells can express extra ICAM-1 and cytokine-induced neutrophil chemoattractant 1 (CINC-1) participating in some steps of leukocyte trafficking into the CNS (Lai *et al.*, 2012). This mechanism attracts immune cells into the brain and finally leads to BBB disruption (Zhang *et al.*, 1995). Furthermore, cells expressing JEV significantly compromised permeability barrier by altering the expression of claudin-1, suggesting JEV may play a role in the disruption of TJ functions (Agrawal *et al.*, 2013). A recent study revealed that the BBB integrity was compromised in brain mouse model infected with JEV by reduced TJ genes (claudin-1, claudin-5, and ZO-1 occludin,) expression and enhanced AMs expression such as ICAM1 and JAM (Chen *et al.*, 2012). In addition, JEV also induces microglial activation in the brain (Ghoshal *et al.*, 2007), leading to expression of numerous immune-related proteins such as chemokines methyl-accepting chemotaxis protein (MCP-1), macrophage inflammatory protein 1 (MIP-1α, MIP-1β), RANTES), cytokines (IL-1, IL-6, IL-18, TNF-α), vascular endothelial growth factor (VEGF), lymphotoxin, and multiple matrix metalloproteinases (MMPs) (Banati *et al.*, 1993; Shima *et al.*, 1995; Ubogu *et al.*, 2006). It has been confirmed that high levels of IL-6 and MCP-1 after virus infection led to increased vascular permeability (Lander *et al.*, 2014). Moreover, deformation of TJs and disruption of the BBB due to high levels of IL-6 have been detected in a mouse model of JE (Gupta and Rao, 2011; Yang *et al.*, 2011).

5.2 Uncontrolled entry of immune cells via Trojan horse mechanism

West Nile virus (WNV) is a mosquito-borne enveloped flavivirus that causes encephalitis and meningitis in a small percentage of infected humans after the initial replication in keratinocytes and Langerhans cells in the skin (Lim *et al.*, 2011). WNV can access the CNS either by infecting sensory nerve endings, olfactory neurons or through blood circulation (Lim *et al.*, 2011). The hallmark of WNV neuropathogenesis is the disruption of the BBB resulting in uncontrolled entry of immune cells into the brain via "Trojan horse" mechanism (Wang *et al.*, 2008b), where the leukocyte traffic across the

BBB in a harmonized process, including tethering, rolling, adhesion and transmigration, that is directed by the interactions of CAMs with their ligands, MMPs and chemokines (Stanimirovic and Satoh, 2000; Dietrich, 2002). A previous study demonstrated that BBB disruption in WNV-infected mice was accompanied with increased MMPs and loss of TJ proteins (Wang *et al.*, 2008a; Xu *et al.*, 2012), leading to the entry of the virus into the brain. It was also revealed that WNV-infected astrocytes induced TJ proteins degradation associated with loss of TEER and barrier integrity (Verma *et al.*, 2010). Moreover, high levels of pro-inflammatory cytokines and chemokines resulted in the entry of the virus into the brain after WNV infection (Kumar *et al.*, 2010). These studies confirm that WNV has an effect on BBB function and may result in encephalitis and meningitis.

5.3 BBB disruption via direct infection

The pathological properties of ZIKV were discussed by Dick (1952). Analysis of ZIKV-infected brains of mice following intraperitoneal infection showed an increase of viral titers over the course of several days, suggesting the virus may cross the blood brain barrier. Other findings conducted by Bell *et al.* (1971) observed that ZIKV indirectly infected CNS in mice via both glial cells and neurons, creating a variety of intracytoplasmic inclusions or virus factories. There is a strong evidence showing a correlation between ZIKV and microcephaly, where ZIKV was isolated from microcephalic brains of three aborted fetuses and two newborns from mothers with a suspected Zika virus infection. In addition, mothers of microcephalic fetuses/infants demonstrated the presence of ZIKV between 6 and 13 weeks of gestation (Brasil *et al.*, 2016; Martines, 2016; Meaney-Delman, 2016; Mlakar *et al.*, 2016). Viral antigen was localized to the placenta, microglia, and neurons (Bayer *et al.*, 2016). Recent studies have demonstrated that type I IFNAR-deficient mice crossed with wild-type C57BL/6 mice and C57BL/6 mice treated with blocking anti-IFNAR antibody showed transplacental transmission of ZIKV and some signs of microcephaly (Miner *et al.*, 2016). Another study had also demonstrated that adult IFNAR-deficient mice and other mouse strains lacking one or more components of the type I IFN system are prone to numerous ZIKV strains with high viral loads in the CNS and testis (Lazear *et al.*, 2016; Rossi *et al.*, 2016). Similar to other flaviviruses, we can speculate that a possible mechanism of ZIKV entry into the brain is via BBB disruption. However, further investigations are needed to understand the exact effects of this virus on BBB disruption.

5.4 Transendothelial permeability leading to compromised BBB

A different mechanism of BBB disruption is described for Nipah virus (NiV), where this virus can enter the CNS through the hematogenous route as detected in vasculitis patients (Wong *et al.*, 2002). It has been shown that NiV infects neurons and may spread through the cribriform plate and enter into the olfactory bulb as a mode of entry into the CNS (Munster *et al.*, 2012). In humans, the virus disease is categorized by respiratory distress and encephalitis, with histopathologic changes in the lung and brain showing multinucleated giant cells in the microvasculature (Luby and Gurley, 2012). Approximately 19% of patients that survive the NiV infection still suffer from long-term neurological deficit that continues for more than four months after the initial outbreak (Sejvar *et al.*, 2007). However, some patients showed late-onset of encephalitis that occurred up to many years after the initial infection (Abdullah *et al.*, 2012). Endothelial cells are the major target cells during the systemic phase of NiV infection, which is characterized by a systemic vasculitis, discrete inflammation in most organs and parenchymal necrosis mainly in the CNS. Vasculitis of the small arterioles, arteries, capillaries and venules of the CNS in patients with NiV encephalitis was detected in autopsies (Wong *et al.*, 2002). NiV-induced endothelial damage observed in cultured peripheral blood mononuclear endothelial cells (PBMECs) is confirmed by an increase in the transendothelial permeability late in infection, leading to compromised BBB as well as penetration of leukocytes in small brain vessels during *in vivo* infection (Weingartl *et al.*, 2005). Neurological signs of the disease with BBB disruption were also shown in hamsters infected with NiV (De Wit *et al.*, 2011). Thus, the pathogenesis of NiV infection mostly appears to be due to the endothelial destruction, multinucleated syncytia, vasculitis-induced thrombosis, ischemia and microinfarction in the CNS, followed by the infection of neurons and glial cells in the brain parenchyma, allowing the virus to overcome the BBB (Chua *et al.*, 2000; Wong *et al.*, 2002).

5.5 Reduction in expression of tight junction proteins and penetration of inflammatory cells

Similar to JEV mentioned earlier, Rabies virus (RABV) can also induce a deadly neurological disease and have a devastating influence globally. There are two types of RABV: i) laboratory-attenuated RABV and ii) wild-type (wt) RABV. Numerous studies have revealed that the laboratory-attenuated RABV can be cleared from the CNS, due to its ability to induce innate and adaptive immunities such as the production of chemokines, cytokines, and activation of immune cells and its ability to permeate the BBB (Chai *et al.*, 2015). A previous study demonstrated extensive inflammation, apoptosis, and expression of innate immune genes in the CNS of mice infected with laboratory-attenuated RABV (Jackson *et al.*, 2008; Zhao *et al.*, 2009), whereas wt RABV stimulates little or no inflammatory responses (Chai *et al.*,

2014). Within the peripheral nervous system and CNS, axonal transport process mediates the spread of RABV in a microtubule network-dependent process (Ceccaldi *et al.*, 1989). This process allows RABV to infect and disseminate to all brain neuronal subtypes and spreads to peripheral glands such as salivary, adrenal and lacrymal gland (Charlton, 1988; Fields *et al.*, 2007). The mechanism, by which RABV infection initiates BBB permeability enhancement in mice has been described by Chai *et al.* (2015), where it was observed that RABV infection enhances BBB permeability by reducing the TJ protein expression and inducing penetration of inflammatory cells into the CNS. The enhancement of BBB permeability and the reduction of TJ protein expression is associated with the expression of chemokines/cytokines. Enhancement of BBB permeability is vital in RABV attenuation by allowing immune effectors to access the CNS to clear RABV (Kuang *et al.*, 2009). According to Phares *et al.* (2006), increased BBB permeability and inflammation occur differently in various parts of the brain, are accompanied by clearance of the virus and a lack of the neurological sequelae in RABV infections of mice.

5.6 Degrading MMPs cause BBB disruption via capillary congestion

HSV is another neurotropic virus that can induce HSE. It is one of the ultimate devastating infectious diseases of the CNS with the mortality of up to 20% and neurological sequelae in over 50% of the survivors (Raschilas *et al.*, 2002). The olfactory and trigeminal nerves were suggested as potential pathways of this virus to the CNS (Johnson *et al.*, 1968; Schlitt *et al.*, 1986). Interestingly, former studies have indicated that brains of elderly Alzheimer's patients have shown the presence of HSV-1 within the brain regions affected by Alzheimer's disease (Jamieson *et al.*, 1991). Hudson *et al.* (1991) stated that the intranasal inoculation of HSV-1 in mice produces focal lesions localized to the temporal lobe, similar to what is observed in humans. A more recent study showed that parenchymal injury is mediated by direct lytic effects of the HSV on neurons and glial cells, resulting in inflammatory reaction and finally collateral damage (Sellner *et al.*, 2005). It was shown that early signs in the course of the disease comprise vascular alterations with disruption of the BBB, congestion of capillaries and petechiae in experimental models of HSV and human cases (Arsenio *et al.*, 1975; Farkas *et al.*, 1975). Degrading MMPs are believed to play a main role in stimulating BBB disruption in CNS infections (Lo *et al.*, 2002). In a mouse model of HSE, MMP2 and MMP9 activity are increased, where MMP9 activity is centered on meninges and parenchymal blood vessels in the brain *in situ* zymography (Sellner *et al.*, 2006). In humans, high levels of MMP-9 were found in the cerebrospinal fluid (CSF) in viral meningitis (Kolb *et al.*, 1998) and also in the serum of patients with viral meningoencephalitis (Beuche *et al.*, 2000). Additionally, high levels of MMP-2 and MMP-9 activity were observed within the CNS in the acute phase of HSV infection (manuscript under review) that persist for several months thereafter in experimental encephalitis (Martínez *et al.*, 2004). The other probable mechanism of BBB disruption during HSV infection is via enhancement of several markers of AM such as ICAM-1, VCAM-1 and selectins on human dermal microvascular endothelial cells (Kim *et al.*, 2000).

5.7 Enhancing permeability via alterations in tight junctions and increasing MMP expression

With the same BBB disruption mechanism as other viruses, human immunodeficiency virus (HIV) is one of the most studied neuroviruses in *retrovirus* family with respect to viral and host processes involved in encephalitis and BBB disruption. It causes severe neurological disorder known as HIV-associated neurocognitive disorder (HAND), leading to HIV-related encephalitis. In the early stages of HIV infection, the virus may enter CNS, infecting microglia and macrophages of the perivascular space and resulting in HIV encephalitis at the later stages of HIV infection (An *et al.*, 1999; Gartner, 2000). The most common and pathogenic strain of HIV is HIV-1. It was found that BMEC dysfunction can be directly involved in the process of HIV-1 entry into the CNS (Wu *et al.*, 2000). HIV-1 may lead to BBB disruption resulting in acquired immune deficiency syndrome (AIDS) neuropathogenesis (González-Scarano and Martín-Garcia 2005). The possible mechanism responsible for BBB disruption in HIV-1 encephalitis is alteration of TJ protein expression (Afonso *et al.*, 2008; Gralinski *et al.*, 2009; Strazza *et al.*, 2011). A previous study revealed that primary human brain-derived microvascular or umbilical vein-derived endothelial cells infected with HIV-1 enabled the virus crossing the endothelial cell monolayers by altering TJ protein expression, enhancing permeability and matrix metalloproteinases (MMP2 and MMP9) expression (Eugenin *et al.*, 2006). In addition, both transcellular and paracellular diapedesis of infected leukocytes is involved in HIV-1 transit across the BBB (Ivey *et al.*, 2009). It is also hypothesized that HIV-1 trafficking into the brain may occur through a "Trojan horse" mechanism, where HIV infects CD4+ T-lymphocytes and circulating monocytes, leading to CNS entry through breaches in the BBB, followed by the induction of inflammatory reactions that may play a critical role in HIV-1 entry into the brain (Liu *et al.*, 2000). Additionally, high levels of soluble ICAM-1 were found in CSF of the patients suffering from HIV-associated neurological diseases (Heidenreich *et al.*, 1994). It was also shown that HIV-infected monocytes/macrophages stimulate up-regulation of TNF-α, IL-6, VCAM-1, and E-selectin expression (Persidsky *et al.*, 1997), resulting in neuronal injury and thus increasing the BBB permeability (de Vries *et al.*, 1996; Abbott, 2000). Viral glycoprotein gp120 of HIV breaches the BBB through acti-

Table 1. Neuroviruses and their entry mechanisms into CNS via BBB disruption

No.	Family	Virus	Virus genome	Virus entry mechanisms	References
1	*Flaviviridae*	JEV	Single-stranded RNA	Upregulation of ICAM-11 (CINC-1), and RANTES activity.	Lai *et al.*, 2012
				TJ alteration (Downregulation of claudin-1, claudin-5, and ZO-1 occludin.	Agrawal *et al.*, 2013
				Induce microglial activation in the brain leading to the expression of numerous immune-related proteins such as chemokines (MCP-1, MIP-1a, MIP-1b, cytokines (IL-1, IL-6, IL-18, TNF-α).	Ghoshal *et al.*, 2007
2	*Flaviviridae*	WNV	Positive single-stranded RNA	Trojan horse.	Wang *et al.*, 2008b
				TJ proteins degradation.	Wang *et al.*, 2008a; Xu *et al.*, 2012
				High level of pro-inflammatory cytokines and chemokines.	Kumar *et al.*, 2010
3	*Flaviviridae*	ZIKV	Positive single-stranded, sense RNA	Trojan horse and TJs deformation.	Dietrich, 2002; Wang *et al.*, 2008a; Li *et al.*, 2015
4	*Paramyxoviridae*	NiV	Negative single-stranded, sense RNA	High expression of IL-6, TNF-a, IL-1α, IL-1β, and IFN-α.	Bailey *et al.*, 2006; Mathieu *et al.*, 2011
				Increasing in the transendothelial permeability.	Weingartl *et al.*, 2005
5	*Rhabdoviridae*	RABV	Non-segmented negative strand RNA	Production of chemokines, cytokines, and activation of immune cells.	Chai *et al.*, 2015
				Reducing the TJ protein.	Chai *et al.*, 2015
6	*Herpesviridae*	HSV	Double-stranded DNA	Degrading MMPs.	Lo *et al.*, 2002
				Increasing several markers of AM such as ICAM-1, VCAM-1.	Kim *et al.*, 2000
7	*Retroviridae*	HIV	Positive single-stranded, sense RNA	Alterations of TJ protein expression.	Strazza *et al.*, 2011
				Trojan horse.	Liu *et al.*, 2000
				Up-regulation of TNF-α, IL-6, ICAM-1, VCAM-1, and E-selectin expression.	Heidenreich *et al.*, 1994; de Vries *et al.*, 1996; Abbott, 2000

vating the C-X-C chemokine receptor type 4 (CXCR4) and chemokine receptors C-C chemokine receptor type 5 (CCR5) (András *et al.*, 2005; Kanmogne *et al.*, 2005), leading to TJ protein degradation via proteasome (Nakamuta *et al.*, 2008; Wang *et al.*, 2011). Several mechanisms have been postulated to show how numerous neuroviruses enter the CNS via BBB disruption as shown in Table 1.

6. Conclusion

In conclusion, several mechanisms have been reviewed, showing how neuroviruses disrupt the BBB. Viruses such as JEV, WNV, HSV and HIV-1 disrupt the BBB by "Trojan horse" mechanism, in which the virus is carried into the brain by infected inflammatory cells via overexpression of AM, or by altering the TJ proteins to enable penetration of inflammatory cells into the CNS as shown for viruses such as JEV, WNV, RABV and HIV-1. NiV on the other hand gains entry into the CNS through infection of glial cells in the brain parenchyma, thus allowing the virus to overcome the BBB. Viruses such as HSV and WNV also tend to cause BBB breakdown via activation of MMP2 and MMP9. However, the exact mechanism of entry of ZIKV is still speculative, and it may use similar mechanisms as other flaviviruses (e.g. JEV and RBV). In this reveiw, several mechanisms have been reviewed in order to understand the pathogenesis of neuroviruses and to develop effective therapeutic schemes of each virus in further investigations.

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