

# Disruption of the Blood-Brain Barrier During Neuroinflammatory and Neuroinfectious Diseases



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**Abstract** As the organ of highest metabolic demand, utilizing over 25% of total body glucose utilization via an enormous vasculature with one capillary every 73  $\mu\text{m}$ , the brain evolves a barrier at the capillary and postcapillary venules to prevent toxicity during serum fluctuations in metabolites and hormones, to limit brain swelling during inflammation, and to prevent pathogen invasion. Understanding of neuroprotective barriers has since evolved to incorporate the neurovascular unit (NVU), the blood-cerebrospinal fluid (CSF) barrier, and the presence of CNS lymphatics that allow leukocyte egress. Identification of the cellular and molecular participants in BBB function at the NVU has allowed detailed analyses of mechanisms that contribute to BBB dysfunction in various disease states, which include both autoimmune and infectious etiologies. This chapter will introduce some of the cellular and molecular components that promote barrier function but may be manipulated by inflammatory mediators or pathogens during neuroinflammation or neuroinfectious diseases.

**Keywords** Blood-brain barrier · Neuroinfectious diseases · Tight junctions · Innate immunity · Central nervous system

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## Abbreviations

AJ	Adherens junction
ANG-1	Angiopoietin-1
APC	Antigen-presenting cell
AQP4	Aquaporin 4
BBB	Blood-brain barrier
bFGF	Basic fibroblast growth factor
BMEC	Brain microvascular endothelial cell
Cav-1	Caveolin-1
CBF	Cerebral blood flow
CHIKV	Chikungunya virus
CNS	Central nervous system
CSF	Cerebrospinal fluid
CSPG	Chondroitin sulfate proteoglycan
CTL	Cytotoxic T cell
DP1	Prostaglandin D2 receptor 1
dsRNA	Double-stranded ribonucleic acid
EC	Endothelial cell
ECM	Experimental cerebral malaria
ECM	Extracellular matrix
ERK	Extracellular signal-regulated protein kinase
ET	Edema toxin
gd-MRI	Gadolinium MRI
GDNF	Glial cell line-derived neurotrophic factor
HiV	Hendra virus
HIV-1	Human immunodeficiency virus type 1
HSV	Herpes simplex virus
ICAM-1	Intercellular adhesion molecule 1
IFN	Interferon
IFNAR	Type I IFN receptor
IL	Interleukin
iRBC	Infected RBC
JEV	Japanese encephalitis virus
LCMV	Lymphocytic choriomeningitis virus
MAPK	Mitogen-activated protein kinase
MAV-1	Mouse adenovirus type-1
MAVS	Mitochondrial antiviral-signaling protein
MDA5	Melanoma differentiation factor 5
MerTK	Tyrosine-protein kinase Mer
Mfsd2a	Major facilitator superfamily domain-containing protein 2a
MHV	Mouse hepatitis virus
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging

MS	Multiple sclerosis
Msp	Meningococcal serine protease
NADPH	Nicotinamide adenine dinucleotide phosphate
NiV	Nipah virus
NLR	Nucleotide oligomerization domain-like receptor
NMOSD	Neuromyelitis optica spectrum disorder
NVU	Neurovascular unit
OPN	Osteopontin
PAFR	Platelet-activating factor receptor
PDGF-BB	Platelet-derived growth factor BB
PDGFR $\beta$	Platelet-derived growth factor receptor $\beta$
PECAM-1	Platelet-associated cell adhesion molecule 1
PG	Proteoglycan
PGD2	Prostaglandin D2
PI3K	Phosphatidylinositol 3 kinase
pIgR	Polymeric immunoglobulin receptor
PKB	Protein kinase B
PLC	Phospholipase C
PPMS	Primary progressive multiple sclerosis
PRR	Pattern recognition receptor
RABV	Rabies virus
Rac-1	Ras-related C3 botulinum toxin substrate
RBC	Red blood cell
RhoA	Ras homolog gene family, member A
RLR	Retinoic acid-inducible gene 1 like receptor
ROS	Reactive oxygen species
RRMS	Recovery and remission multiple sclerosis
S1P	Sphingosine-1-phosphate
SAS	Subarachnoid space
sCD40L	Soluble CD40L
SHH	Sonic hedgehog
SPMS	Secondary-progressive multiple sclerosis
ssRNA	Single-stranded ribonucleic acid
TBEV	Tick-borne encephalitic virus
TEER	Transendothelial electrical resistance
TIMP	Endogenous tissue inhibitor of MMP
TJ	Tight junction
TLR	Toll-like receptor
TMEV	Theiler's murine encephalitis virus
TNF $\alpha$	Tumor necrosis factor alpha
VCAM-1	Vascular cell adhesion molecule 1
VEEV	Venezuelan equine encephalitis virus
VEGF	Vascular endothelial growth factor
VSV	Vesicular stomatitis virus
WNV	West Nile virus

## Introduction to BBB Structure and Function

Over 100 years ago, a publication by Lina Stern, Professor and Head of the Department of Physiological Chemistry at the University of Geneva, coined the term “blood-brain barrier (BBB)” to describe the finding that systemically administered dyes are excluded from the developing mammalian brain [1]. Since then, physicians and scientists have appreciated the unique diffusion barrier between the blood and the brain and its stringent regulation of central nervous system (CNS) entry of molecules, immune cells, and pathogens [2–4]. The BBB occurs at the level of postcapillary venules and capillaries and is comprised of a variety of physical specializations including inter-endothelial tight and adherens junctions (TJ and AJ), endothelial cells with polarized expression of protein receptor influx and efflux transporters, and transcytosis systems limited to albumen and histones [5]. Vasculature at the BBB is enveloped by pericytes and astrocyte end feet, which leads to the development of dual basement membranes with a complicated extracellular matrix (ECM) separating blood contents from perivascular spaces within the CNS parenchyma. The CNS ECM is comprised of hyaluronic acid and proteoglycans (PG), mainly chondroitin sulfate proteoglycans (CSPG) [6]. Heparan sulfate proteoglycans (HSPG), especially the negatively charged heparan sulfate (HS), bind and sequester pro-inflammatory molecules, including the endothelial cell-derived chemokine CXCL12 ([7] and see below), which regulates the recruitment and effector functions of leukocytes that infiltrate the CNS during neuroinflammatory diseases [8]. BBB TJ are heterodimeric proteins comprised of occludin and members of the claudin family of proteins, including claudin-3 or -5, that link to the cytoskeleton via the scaffolding and regulatory proteins ZO-1, -2, -3, and cingulin (reviewed in [9]). Similarly, AJ are comprised of E-cadherin proteins that link to actin filaments via  $\alpha$ -,  $\beta$ -, and  $\gamma$ -catenin. The length of actin fibers, which are regulated by the activation of RhoGTPases, controls the integrity of both AJ and TJ complexes [10]. Activation of Rac1 promotes stabilization of TJ and AJ, while RhoA promotes destabilization. Junctional integrity is critical for two separate capacities of the BBB, termed “gate” and “fence” function [11]. Gate function refers to the importance of inter-endothelial junctional complexes in limiting the movement of molecules and cells from the blood to the brain parenchyma. RhoA activation may therefore reduce gate function and allow BBB penetration without loss of junctional proteins. Fence function refers to the role of TJ and AJ in the maintenance of BBB polarity, preventing the rotation and diffusion of proteins and other biomolecules within the cell membrane between abluminal and luminal surfaces. Thus, BBB permeability can also be increased without complete destruction of junctional proteins through alterations in the locations of proteins involved in transport or transcytosis.

The transcellular transport of macromolecules across endothelial barriers occurs in peripheral organs via a variety of pathways including macropinocytosis and clathrin- and caveolae-mediated endocytosis. The BBB, however, exhibits low levels of macropinocytosis and lack of clathrin expression. Caveolae-mediated

transcytosis is strictly regulated at the BBB by the major facilitator superfamily domain-containing protein 2a (Mfsd2a), which is exclusively expressed on brain endothelial cells and induced by pericytes [12]. Consistently, *Mfsd2a*<sup>-/-</sup> mice exhibit increased BBB permeability, caused by enhanced caveolae-mediated transcytosis [12]. Caveolae are flask-shaped plasma membrane invagination enriched in cholesterol and sphingolipids. They contain the major structural protein caveolin-1 (Cav-1), which undergoes extensive oligomerization prior to interacting with cavin-1 to form caveolae. Genetic ablation of either Cav-1 or cavin-1 results in a complete loss of caveolae in related tissues, suggesting their essential role in caveolae formation [13, 14]. Previous studies identified a close association between caveolae and stress fibers, a feature absent in clathrin-coated vesicles [15]. These interactions are critical for both stabilizing and entry of caveolae at the plasma membrane and are also regulated by the small RhoGTPases, including Ras homolog gene family, member A (RhoA) and Ras-related C3 botulinum toxin substrate (Rac)-1 [15]. Caveolae internalization is further regulated by kinases and phosphatases. In general, BBB endothelial cells exhibit low level of formation of caveolae due to the effects of Mfsd2a. However, levels of this protein are decreased during intracranial hemorrhage, suggesting that serum inflammatory mediators might increase BBB permeability via their effects on caveolae-mediated transcytosis.

The polarized expression of proteins at the CNS vascular barriers is also important for normal immune surveillance of the CNS. There is a growing body of evidence that lymphocytes, including effector memory CD4 and CD8 T cells, normally reside within the cerebrospinal fluid (CSF) compartment [16–22]. The CSF compartment includes both the subarachnoid space (SAS) and the ventricular system, the latter of which contains the choroid plexus, a plexus of microvessels with modified ependymal cells that form a barrier between its fenestrated capillaries and the CSF compartment (reviewed in [23]), which connects with lymphatics that provide mechanisms for leukocyte egress out of the CNS [24, 25]. The choroid plexus is the main producer of CSF, which circulates via a combination of directed bulk flow, and both pulsatile and continuous bidirectional movement at the BBB and at the borders between CSF and CNS interstitial spaces (reviewed in [26]). The SAS occurs between meningeal arachnoid and pia maters and contains fenestrated capillaries where immune cells may exit the blood and migrate along abluminal surfaces into perivascular spaces within the brain parenchyma at sites with BBB specializations. The localization of lymphocytes along CNS vasculature is accomplished via polarized expression of chemokines, including CXCL12 [27], which promotes interactions between T and perivascular antigen-presenting cells (APCs) in the setting of neuroinfectious diseases. Infiltrating T cells express CXCR4, a G protein-coupled signaling receptor of CXCL12 that is downregulated after T cell receptor activation, which allows T cell egress out of perivascular compartments [28, 29]. The abluminal localization of CXCL12 stands in stark contrast to its expression pattern at high endothelial venules within lymph nodes, where luminal CXCL12 promotes the homeostatic circulation of lymphocytes between the blood and lymphoid compartments [30], whereas BBB CXCL12 instead limits T cell entry into the CNS parenchyma [27, 28]. The level of CNS expression of CXCL12 vascular barriers is

accomplished at both transcriptional and protein expression levels, the latter of which occurs via the CXCL12 scavenging receptor CXCR7 [31]. As the CXCR7 promoter contains eight NF- $\kappa$ B binding sites, multiple cytokines may alter the level of its expression at the BBB during neuroinflammation, including interleukin-1, -8, -17, and interferon- $\gamma$ . Alterations in the patterns of localizing cues at the BBB could promote excessive leukocyte entry, which may lead to further alterations in the BBB functions.

## Cellular Constituents of the NVU Regulate BBB Formation and Function

The NVU is comprised of brain microvascular endothelial cells (BMECs), abluminal pericytes, and astrocyte terminal processes, known as end feet, the latter of which receive neuronal signals that modulate BBB influx and efflux transporters in response to parenchymal demands or damage [5]. Pericapillary pericytes extend their processes along pre- and postcapillary vessels, receiving signals from BMECs, astrocytes, and neurons that induce them to form, maintain, and regulate BBB function [32]. Studies in pericyte-deficient and transgenic mice with aberrant signaling between endothelial-derived platelet-derived growth factor BB (PDGF-BB) and platelet-derived growth factor receptor  $\beta$  (PDGFR $\beta$ ) in pericytes have thus identified critical roles for these cells from embryonic development to adulthood [33]. *Pdgfb* and *Pdgfr $\beta$*  homozygous knockout mice completely lack pericytes, which causes embryonic lethality via cerebral blood vessel rupture and microhemorrhages. While *Pdgfr $\beta$* <sup>+/-</sup> mice and mice with modified PDGF-BB bioavailability are viable, they exhibit reductions in pericyte coverage along vasculature, leading to poor maintenance of BBB function and increased permeability [33]. These mice also exhibit dysregulated cerebral blood flow (CBF) leading to eventual loss of neurons in the cortex and hippocampus. These data indicate the importance of maintaining adequate pericyte numbers for proper BBB function.

Both pericytes and astrocytes are important in the preservation of BMEC TJs through the regulation of junctional proteins occludin, claudin, and ZO-1. Astrocyte end feet also contact the abluminal surfaces of BMECs and enwrap neuronal synapses, enabling simultaneous modulation neuronal activity and blood flow in response to elevations in intracellular Ca<sup>2+</sup> levels [34, 35]. Astrocyte end feet are also highly polarized and express specialized molecules such as Kir4.1 K<sup>+</sup> channels and aquaporin 4 (AQP4), which each regulate BBB ionic concentrations, and protein transporters such as glucose transporter-1 and P-glycoprotein, the latter of which promotes the efflux of toxic substances away from brain parenchyma [36, 37]. Astrocytes may exchange signals through gap junctions forming a functional syncytium that coordinates BBB responses and communicates with neurons [38, 39]. Astrocytes critically develop and maintain BBB characteristics through the release of vascular endothelial growth factor

(VEGF), glial cell line-derived neurotrophic factor (GDNF), basic fibroblast growth factor (bFGF), and angiopoietin (ANG)-1 [39, 40], which form TJ, promote enzymatic systems, and polarize expression of transporters [41].

The full integration of NVU responses that regulate and maintain BBB function relies on multiple signaling pathways and proteins that regulate TJ integrity, including calcium, protein kinase A, protein kinase C, G proteins, calmodulin, cAMP, and phospholipase C [42, 43]. Heterotrimeric G proteins and protein kinase C signaling pathways, in particular, act via altering intra- and/or extracellular levels of calcium, which promotes TJ integrity [44]. Phosphorylation additionally regulates transmembrane and accessory proteins of TJs. Both serine and threonine phosphorylation of occludin, which regulates its subcellular localization, are highly correlated with the reassembly of TJs following alterations in BBB integrity [45]. The PAR3-PAR6-APKC pathway and the evolutionarily conserved signaling complex related to the *Drosophila* Stardust-Disc lost-Crumbs complex (equivalent to the mammalian Pals1-PATJ-Crumbs complex) [43] have also been implicated in regulation or modulation of TJ assembly. As PAR-complex APKC and PAR3 may be downregulated upon activation of NF- $\kappa$ B and, in turn, act to inhibit NF- $\kappa$ B-mediated signaling, these pathways may provide additional mechanisms for the alteration of BBB function during neuroinflammation.

In summary, the BBB has evolved numerous cellular, subcellular, and molecular mechanisms to stringently regulate the CNS access of solutes, molecules, cells, and pathogens. BBB function, however, may become dysfunctional or derailed via intrinsic and/or extrinsic effects in the setting of neuroinflammatory diseases, including those caused by autoimmune, infectious, or neurodegenerative processes.

## **Mechanisms of BBB Disruption During Pathological Conditions**

During CNS disease, the NVU may undergo cytoarchitectural modulations that promote BBB permeability without significant alterations in structural integrity. TJs and their associated proteins are dynamically regulated and able to undergo alterations in transcription, translation, and posttranslational modifications, subcellular localization, and protein-protein interactions in normal and diseased states. Thus, acute and subtle changes in BBB permeability with accompanying mild elevations in CSF protein levels may occur without severe CNS symptoms. Prolonged alterations in NVU structure and function, however, can lead to complete TJ disruption leading to brain edema and neural cell damage and irreversible in brain injury. Here, we will discuss the role of primary or secondary BBB dysfunction in the etiology, progression, and repair of neuroinflammatory diseases.

## ***BBB Disruption During CNS Autoimmunity***

Failure of BBB function is a critical event during the development and progression of autoimmune diseases of the CNS, including neuromyelitis optica spectrum disorders (NMOSD) and multiple sclerosis (MS). NMOSD are rare, relapsing immune-mediated CNS disorders characterized by inflammation and demyelination of the optic nerves and spinal cord with evidence of BBB dysfunction in up to 70% of cases and two-thirds of patients exhibiting elevations in serum anti-AQP4 autoimmune IgG antibodies (classified as NMO patients) (reviewed in [46]). While patient serum levels of anti-AQP4 IgG are not a predictive biomarker for overall disease course [47], they are positively correlated with the extent of spinal lesions; BBB permeability, as assessed via albumin index; levels of CSF myelin basic protein concentration; and serum C3 [48]. Anti-AQP4 IgG contribute to pathogenesis via effects at astrocyte end feet within the NVU, which express AQP4, and bind to the abluminal surfaces of microvessels in NMO patients, in conjunction with lesions containing complement proteins, infiltrating neutrophils and eosinophils, and loss of AQP4 [49]. Human data are consistent with a significant role for anti-AQP4 IgG in the pathogenesis of NMO, which is further supported by the clinical efficacy of plasma exchange and B cell depletion [50]. In animal studies, targeted deletion of AQP4 or administration of anti-AQP4 IgG-positive sera plus complement from NMO patients leads to loss of BBB integrity and impaired water homeostasis within astrocyte end feet [51–53]. Similarly, using an *in vitro* human BBB model administration of human anti-AQP4 IgG and complement increased the migration of granulocytes across BMECs and led to astrocyte injury and decreased transendothelial electrical resistance (TEER) [54]. While the mechanism of anti-AQP4 IgG entry at the BBB, including access to astrocyte end feet, is unclear, endothelium-specific antibodies, VEGF, and matrix metalloproteinase (MMP)-9 are all elevated in NMO [52, 55]. Activation of BMECs via endothelium-specific antibodies may lead to concomitant upregulation of intercellular adhesion molecule (ICAM)-1 [52], promoting capture of leukocytes, and secretion of TNF and VEGF. The release of MMP-9 from infiltrating neutrophils could play a role in the degradation of the BBB ECM [55] by allowing anti-AQP4 IgG access to astrocyte AQP4. Further development of animal models of NMO could help identify therapeutic targets to prevent these effects.

The role of BBB dysfunction in the induction and progression of MS is a subject of controversy [56], mostly due to the lack of models that faithfully reproduce the diseases observed in patients. MS is a heterogeneous group of demyelinating syndromes in which patients may present with a relapsing-remitting form, characterized by periods of disease exacerbation followed by recovery and remission (RRMS). RRMS may be followed by the onset of continued progression of disease (i.e., secondary-progressive (SP)MS) or a primary progressive form in which patients continue to develop neurologic deficits without remission (PPMS) [57]. The characteristic CNS lesion observed in MS patients is a focal area of



inflammatory-mediated demyelination surrounding postcapillary venules within white matter [58]. In severe cases of MS, patients may also exhibit demyelinating lesions within cortical gray matter, often adjacent to meninges. Defects in BBB function are observed in all lesions, with gadolinium extravasation observed using magnetic resonance imaging (MRI). However, while frank TJ disruption is not observed in MS lesions, as assessed in early studies using electron microscopy [59], the exact mechanisms of BBB impairment are unclear, as is the timing of these events as primary or secondary to the effects of immune cells.

Evidence for primary causes of BBB dysfunction include altered BMEC expression of molecules involved in the stabilization of TJs, including sphingosine 1-phosphate receptor 2 (S1P2) and claudin proteins [60, 61]. S1P2 is one of the five subtypes of G protein-coupled receptors (S1P1-5) that are targeted by S1P, a signaling, blood-borne sphingolipid that regulates angiogenesis, vascular stability, and permeability and may also be important in the pathogenesis of neurodegenerative diseases (reviewed in [62]). S1P also regulates the trafficking of T and B cells within lymphoid tissues and directly suppresses TLR-mediated immune responses from T cells [63]. At the BBB, S1P1 and S1P3 activation promote Rac1-mediated tightening of inter-endothelial junctions, while S1P2 leads to their disassembly via RhoA [64]. In murine models of RRMS, disassembly of BBB TJs and AJs is associated with loss of polarized expression of CXCL12 with increased capture and CNS entry of CXCR4-expressing T cells [61]. Patients with MS exhibit loss of BBB polarity within white matter lesions [65], and women with RRMS exhibit significantly higher levels of S1P2 at the NVU within hindbrain regions compared with male MS patients [61].

Loss of polarized expression of CXCL12 may also be the result of BMEC expression of the CXCL12 scavenger receptor CXCR7. Studies in animal models of MS suggest that interleukin(IL)-17-secreting CD4 and  $\gamma\delta$  T cells may drive CNS autoimmunity, especially with regard to access to CNS parenchyma from perivascular spaces [66].  $\gamma\delta$  T cells, which do not require antigen processing and major histocompatibility complex (MHC) presentation of peptide epitopes and instead may recognize lipid antigens, are also sources of IL-1 within the inflamed CNS [67, 68]. CXCR7 reporter mice exhibit expression of the receptor along postcapillary venules, which is increased during induction of CNS autoimmune disease, leading to loss of abluminal expression of CXCL12 and increased CNS access of myelin-specific T cells [31]. In vivo targeting of CXCR7 in animal studies using small molecule inhibitors maintains polarized expression of CXCL12 and limits the egress of immune cells out of perivascular spaces during induction of EAE. In vitro studies examining the regulation of CXCR7 expression on BMECs demonstrated that IL-17 and IL-1 increase the expression and activity of the receptor, respectively, consistent with in vivo studies demonstrating roles for these cytokines in driving neuropathology and the clinical effectiveness of therapies that target IL-17 or IL-1 in patients with autoimmune diseases [69–71]. Novel therapeutics targeting CXCR4 and/or CXCR7 are under development [72] and may prove beneficial for the treatment of MS.

Although BBB disruption is clearly evident on gadolinium (gd)-MRI of MS patients, the notion that this is due to direct alterations in TJ protein expression has been controversial. Early reports examining the levels of expression of claudin-5, a major component of CNS TJs, did not reveal differences in CNS specimens from patients with and without MS [73]. More recently, claudin-11, which co-localizes with claudin-5 in CNS capillaries, was found to be significantly decreased in CNS tissue of MS patients and of mice with EAE [60]. Multiple studies, however, show leakage of serum proteins including fibrinogen, albumen, and IgG, into CNS parenchyma within MS lesions [74, 75], which is consistent with the overall loss of BBB function. Whether this extravasation is the result of loss of gate and/or fence function, the latter of which might include alterations in BMEC intracellular endocytic pathways, remains to be determined.

### ***BBB Disruption During Neuroinfectious Diseases***

The meningeal barriers, which cover the surface of the brain and spinal cord and are comprised of the dura, arachnoid, and pia maters, effectively limit the ability of a majority of bacterial, fungal, and viral pathogens to gain access to the CNS parenchyma. Thus, only neurotropic viruses, molds, and certain parasites are able to cross the BBB and infect CNS parenchyma. Certain bacteria that gain access to the subarachnoid space within the meninges may also enter perivascular spaces of postcapillary venule, leading to BBB disruption and parenchymal infection. However, this extent of infection occurs late in the course of bacterial meningitis and is generally associated with severe and fatal outcomes. Here, we will delineate mechanisms of BBB disruption during neuroinfectious diseases, focusing on pathogens that infect immunocompetent hosts (Table 1).

### **Induction of BBB Disruption and Parenchymal Invasion by Bacteria**

A variety of Gram-positive and Gram-negative bacteria display a predilection for CNS invasion, predominantly spread hematogenously within the subarachnoid space into the CSF. Most bacterial infections lead to robust inflammatory responses leading to extensive neutrophilic infiltrates throughout the meninges and, if untreated, result in vasogenic edema, disruption of the BBB, coma, and death [76]. Although bacterial infections within the CNS generally cause meningitis and are limited to the CSF compartment, host inflammatory responses and, in some cases, bacterial products may lead to BBB disruption with bacterial invasion of the CNS parenchyma. With few exceptions, most bacteria are unable to invade neural cells, leading instead to their encapsulation by glial elements and abscess formation [77]. Here, we will discuss the specific mechanisms by which bacteria interact with subarachnoid vasculature and the molecular events that may lead to parenchymal invasion.

**Table 1** Mechanisms of BBB disruption by various pathogens, including bacteria, viruses, and parasites, depicted in pink, blue, and gray, respectively

Pathogens	Mechanisms of BBB disruption	References
<i>Group B Streptococcus (GBS)</i>	GBS activates ERK1/2/MAPK signaling pathway in BMECs, leading to the induction of host transcriptional repressor Snail1, which in turn suppresses the expression of TJ proteins	[238]
<i>Listeria monocytogenes</i>	Bacterial proteins InlA and InlB interact with host cellular receptors E-cadherin and MET, respectively, on choroid plexus epithelium and brain endothelium, respectively	[86]
<i>Bacillus anthracis</i>	Reduce the expression of ZO-1 induced by bacterial edema toxin (ET) Bacterial toxins also reduce the expression of VE-cadherin by inhibiting Rab11/Sec15-dependent endocytic recycling pathway	[239, 240]
<i>Haemophilus influenzae</i>	Porin, OmpP2 causes endothelial damage via binding to the common carboxy-terminal domain of LR, and pili interact with platelet-activating factor receptor (PAFR), both expressed by BMECs	[95, 96]
<i>Neisseria meningitidis</i>	Degradation of TJ proteins and ECM via the induction of MMP8 Delocalization of TJ proteins in BMECs induced by bacterial pili	[241–243]
WNV ( <i>Flavivirus</i> )	Degradation of TJ and AJ proteins mediated by virus-induced elevation of MMP-1,-3, and-9	[104, 244]
JEV ( <i>Flavivirus</i> )	Disruption of TJ complexes by virus-induced inflammatory cytokines (e.g., IP-10 and TNF $\alpha$ ) in the CNS	[109]
TBEV ( <i>Flavivirus</i> )	Virus-induced cytokine/chemokine overproduction in the brain	[172]
HIV-1 ( <i>Retrovirus</i> )	Inducing lesion in brain ECs and activation of MMPs by gp120 Release of s-CD40L by Tat-induced platelet activation	[135, 136, 245]
HeV and NiV ( <i>Henipavirus</i> )	Syncytium formation in brain ECs. Induction of inflammatory cytokines in the brain and peripheral tissues	[129–131]
VEEV ( <i>Alphavirus</i> )	Increased expression of MMP9 Monocytes infiltration and release of inflammatory cytokines	[102, 105]
RABV ( <i>Lyssavirus</i> )	Downregulation of TJs mediated by IFN- $\gamma$ from infiltrating CD4 T cells	[182]
MHV3 ( <i>Coronavirus</i> )	Reduced expression of TJ and AJ proteins due to impaired production of IFN- $\beta$ by infected BMECs	[111]
LCMV ( <i>Arenavirus</i> )	CTL-mediated recruitment of neutrophils and monocytes into the CNS leads to vascular damage	[181]
Influenza A virus ( <i>Orthomyxovirus</i> )	Disruption of tight junction protein ZO-1, likely by virus-induced inflammatory cytokines	[246, 247]
TMEV ( <i>Cardiovirus</i> )	Degradation of TJs by perforin secreted from CD8 T cells	[184]
HSV-1 ( <i>Simplexvirus</i> )	Virus-induced upregulation of MMP9	[248]

(continued)

**Table 1** (continued)

Pathogens	Mechanisms of BBB disruption	References
<i>P. falciparum</i> (HCM)	Parasite-induced structural change in the membrane of iRBC makes them adherent to vascular endothelium, resulting in apoptosis and disruption of TJ proteins in BMECs	[193]
<i>P. berghei</i> ANKA (ECM)	Platelets deposition and leukocyte arrest on brain vascular endothelium Degradation of TJs and extracellular matrix by CD8 T cells	[204–206]
<i>Toxoplasma gondii</i>	Upregulation of CAMs and inflammatory cytokines in the CNS. Sustain and intense interaction/adherence of leukocytes with brain endothelium Production of nitric oxide and MMPs in the CNS	[35–38]
<i>Trypanosoma brucei</i>	Enhanced production of pro-inflammatory cytokines by activated microglial and astrocytes. T cell-mediated activation of MMPs	[219]
<i>Acanthamoeba</i>	Degradation of TJ proteins. Induction of cell cycle arrest and apoptosis in BMECs through activation of PI3K	[228]

### *Streptococcus pneumoniae* (aka Pneumococcus)

*S. pneumoniae* are Gram-positive, facultative anaerobic bacteria that reside in the respiratory tract. There are over 90 serotypes of *S. pneumoniae* that differ in virulence and susceptibility to antimicrobials. Pneumococcal infections generally originate in the nasal cavities but, in young children and the elderly, may become invasive, with hematogenous spread to multiple organs including the CNS. Within the subarachnoid space, *S. pneumoniae* may adhere to fenestrated endothelium via a number of interactions between bacterial and host proteins. Thus, the major adhesion protein of *S. pneumoniae* pilus-1, RrgA, binds both polymeric immunoglobulin receptor (pIgR) and platelet-associated cell adhesion molecule (PECAM)-1 on endothelial cells, while the bacterial choline-binding protein (PspC) binds only pIgR [78]. These interactions trigger Toll-like receptor-mediated expression of inflammatory mediators by meningeal endothelial cells including interleukins (IL)-1, -6, -10, tumor necrosis factors (TNF), and cytokine-induced neutrophil chemoattractant (CINC)-1 [79, 80]. The recruitment of neutrophils and lymphocytes heralds the onset of meningitis symptoms, including fever, photophobia, and meningismus [81]. Untreated, inflammatory infiltrates may gain access to the CNS parenchyma via migration along venules from the meningeal compartment. Neutrophils and macrophages secrete barrier destabilizing cytokines, IL-1, and TNF, which activate RhoA within BBB endothelial cells, which disrupts TJs [15]. In severe infections, *S. pneumoniae* may also gain access to the brain parenchyma, as animal studies demonstrate that pneumococcal pneumolysin may damage endothelial cell membranes or TJs [82].

*Listeria monocytogenes*

*Listeria monocytogenes* is a facultative intracellular bacterium that is tenfold more effective at invading the CNS other than neuroinvasive Gram-positive bacteria [83]. *L. monocytogenes* spreads hematogenously from the gastrointestinal tract after the consumption of contaminated food, gaining access to the CNS parenchyma through a variety of routes including invasion of meningeal endothelium, transportation across the BBB within infected monocyte, or retrograde migration along cranial nerve axons [84, 85]. Bacterial proteins, including internalins (InIA and InIB), interact with host cellular receptors E-cadherin and mesenchymal-epithelial transition (MET), respectively, and are expressed by choroid plexus epithelium and brain endothelium, respectively [86]. Listeriolysin O (LLO), a pore forming toxin, activates NF- $\kappa$ B within brain endothelial cells in vitro, leading to increased expression of P- and E-selectin, ICAM-1 and VCAM-1, as well as IL-6, -8, and CCL2, which may promote the adhesion and recruitment of neutrophils and monocytes [87]. Invasion and infection of brain endothelial cells with the ensuing activation of glial cells and recruitment of leukocytes in patients with severe CNS *L. monocytogenes* infections may lead to abscess formation or cerebritis [88].

*Bacillus anthracis*

*Bacillus anthracis*, a spore-forming Gram-positive bacterium, causes the disease anthrax, which has three clinical forms: cutaneous, inhalational, and gastrointestinal [89]. Untreated, anthrax disseminates hematogenously to the CNS, causing fatal hemorrhagic meningitis. Anthrax toxins, such as InhA and BsIA, induce destruction of brain endothelial cell TJs, leading to increased BBB permeability and hemorrhage [90, 91]. BsIA has also been demonstrated to act as an adherence factor for all endothelial cells and to be required for CNS infection [92]. Finally, the anthrax toxin pXO1 downregulates innate immune responses, allowing dissemination of the pathogen throughout the CNS [93].

*Haemophilus influenzae*

*Haemophilus influenzae* is a Gram-negative bacterium that was a leading cause of childhood meningitis until its near eradication through the introduction of a the highly effective conjugate HiB vaccine [94]. In vitro studies have implicated *H. influenzae* porin, OmpP2, in endothelial damage via binding to the common carboxy-terminal domain of LR, and *H. influenzae* pili have also been shown to interact with platelet-activating factor receptor (PAFR), which are both expressed by BMECs [95, 96]. In vivo studies have shown that targeting leukocyte CD11/CD18 integrins in conjunction with systemic treatment with corticosteroids reduces life-threatening CNS inflammation and prevents TJ disruption [97], the latter of which is now standard of care in the treatment of patients with *H. influenzae* meningitis [98].

### *Neisseria meningitidis*

*Neisseria meningitidis*, a Gram-negative bacterium that may colonize the oropharynx and genital tract, causes fulminant meningococemia and meningococcal meningitis, which often occur together [99]. *N. meningitidis* adheres to host endothelial cells via pili surface proteins Opa and Opc followed by bacterial adhesin PilQ interaction with the common carboxy-terminal domain of LR [96]. Additional determinants of host cell binding include complex protein ACP and the autotransporter meningococcal serine protease (Msp) A [100, 101].

In summary, while bacterial invasion of the CNS is primarily limited to the meningeal compartment, numerous species exhibit pili surface proteins that are able to interact with BMECs via binding to pIgR and/or LR, which lead to endothelial cell activation, with upregulation of PAFr, CD31, and/or intercellular adhesion molecules [95]. PAFr activation leads to dilation of vessels, aggregation of platelets, and increased BBB permeability, which are all terminal events during bacterial meningitis.

### **Effects of Viruses on BBB Structure and Function**

Many neurotropic viruses with barrier disrupting properties (e.g., Japanese encephalitis virus (JEV), West Nile virus (WNV), Venezuelan equine encephalitis virus (VEEV)) enter the CNS in the absence of BBB opening, suggesting that barrier disruption results from the local virus replication in the CNS [102–106]. Viruses can compromise the integrity of BBB by either infecting or inducing cellular damage to the NVU or by eliciting innate and adaptive immune responses leading to neuroinflammation. Thus, a combination of host and virus-related factors contributes to BBB opening during neurotropic viral infection.

#### **Virus Factors that Impact BBB**

Infection of mice with mouse adenovirus type-1 (MAV-1) induces BBB disruption in the absence of inflammation, suggesting that the barrier loss is primarily caused by viral infection rather than inflammatory responses [107]. MAV-1 infects brain vascular endothelium *in vivo* [108] and dampens expression of TJ proteins *in vitro* [107]. Indeed, reduced expression of TJ and AJ proteins is a characteristic feature of BBB disruption by neurotropic viruses such as JEV, WNV, and human immunodeficiency virus type 1 (HIV-1) *in vivo* [104, 109, 110]. Viruses accomplish this either by downregulating transcription levels of TJ mRNA or promoting protein degradation [104, 111].

Disruption of TJ complexes is often associated with enhanced generation of reactive oxygen species (ROS). Viral infection in target cells can induce mitochondrial damage or NADPH oxidase activation, resulting in robust ROS generation [112, 113]. While low levels of ROS are required for normal cell function, unchecked

level of these reactive intermediates can exert detrimental effects. Indeed, ROS can target virtually all biological molecules, including lipid, protein, and nucleic acid, resulting in the release of various cytokines and proteases that damage vasculature. Cellular component of NVU can be a source and target of ROS. While brain endothelial cells are highly susceptible to oxidative stress, astrocytes are less prone to such damages. However, exposure to viral proteins (e.g., HIV-1 Nef) augments astrocyte sensitivity to redox insults [114]. Activation of metalloproteinases (MMPs) is one of the mechanisms by which ROS dysregulate TJ complexes [115–117]. Elevated levels of MMPs have been reported in brain tissue of mice infected with neurotropic viruses such as WNV, JEV, and VEEV [104, 105, 118] and in cerebrospinal fluid (CSF) of human patients infected with WNV [118]. Infected microglia and astrocytes robustly elevate the expression of MMP-2 and -9 *in vitro* and *in vivo* [119, 120]. MMPs are known to disrupt the BBB integrity by cleaving TJ proteins, AJ proteins, and the extracellular matrix (ECM) [115]. Activity of these MMPs is controlled by regulating gene expression, activation, and inhibition mediated by endogenous tissue inhibitors of MMPs (TIMPs) [121]. Nonetheless, viral infection (e.g., HIV-1) can perturb the fine balance between MMPs and TIMPs, resulting in enhanced MMP levels and BBB leakage [122]. Consistently, pharmacological blockage or genetic ablation of MMPs is reported to protect BBB integrity upon viral infection in murine models [118, 123].

Additionally, ROS trigger the small GTPase RhoA, PI3 kinase, and protein kinase B (PKB/Akt) signaling pathways. This results in the reorganization of the actin cytoskeleton, altered localization of TJ proteins, and consequently increased BBB permeability [124, 125]. Furthermore, ROS can cause barrier dysfunction by activating inflammasome via signaling pathways involving mitogen-activated protein kinases (MAPK) and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) [126].

Viruses can also infect brain endothelial cells and induce syncytium resulting in vascular damage and hemorrhage [127, 128]. For instance, Nipah and Hendra viruses (NiV and HeV, respectively) invade the CNS by infecting brain endothelial cells. Virus infection induces syncytium in brain endothelium resulting in extensive vascular damage associated with influx of inflammatory cells [129–131]. Additionally, neurotropic viruses induce apoptosis in brain endothelial cells causing BBB dysfunction *in vitro* [132]. Secretory viral proteins also trigger barrier permeability. For instance, HIV-1 Tat protein is actively released from the infected cells and crosses the cellular membrane [133]. Intravenous injection of mice with HIV-1 Tat reduces the expression of TJ proteins in brain vasculature, partly by upregulating cyclooxygenase-2 expression [134]. Additionally, HIV-1 Tat enhances serum levels of soluble CD40L (sCD40L) by activating platelets [135, 136], a phenomenon also observed in HIV-infected patients [137, 138]. sCD40L alters barrier permeability by increasing the expression of cell adhesion molecules on brain endothelial cells in a JNK-dependent manner [139]. This culminates in enhanced leukocyte adhesion to brain endothelium leading to BBB dysfunction [135].

In summary, evidence indicates that neurotropic viruses can directly induce BBB permeability by disrupting TJs and AJs between brain endothelial cells. This is

mainly achieved by inducing ROS generation in the CNS, which in turn activates several tyrosine kinases, MMPs, and small GTPase RhoA. The cumulative effect of these activities leads to the loss of BBB function.

### Innate Immune Responses to Viruses that Impact BBB Function

Microbes possess pathogen-associated molecular patterns (PAMPs) that are recognized by pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), retinoic acid-inducible gene 1 (RIG-I)-like receptors (RLRs), nucleotide oligomerization domain-like receptors (NLRs), and cytosolic DNA sensors. These PRRs are expressed by various cell types in the CNS (reviewed in [140]). Viruses contain single- or double-stranded RNA molecules (dsRNA and ssRNA, respectively), present either in the viral genome or generated during virus replication. Recognition of viral PAMPs by TLR3 (dsRNA) or TLR-7 (ssRNA) triggers signaling pathway related to NF- $\kappa$ B, resulting in the production of pro-inflammatory cytokines and type I interferons (IFNs). Similarly, RLRs, which include RIG-I and melanoma differentiation factor (MDA)-5, are activated by dsRNA and ssRNA sequences containing 5'-triphosphate [141]. RLR activation stimulates mitochondrial antiviral-signaling protein (MAVS), which in turn elicits the expression of inflammatory cytokines via induction of the NF- $\kappa$ B signaling pathway. While PRR-induced expression of type I IFNs restricts virus replication in the CNS [142, 143], enhanced production of pro-inflammatory cytokines and chemokines can lead to neuroinflammation. Studies have shown that TLR3 signaling contributes to both enhancement and protection of CNS inflammation during WNV infection in murine models [144, 145]. Similarly, TLR3 signaling has been associated with neuropathogenesis of rabies virus (RABV) in mice [146] while limiting infection of herpes simplex virus (HSV)-2 in the CNS through the activation of IFNAR signaling in astrocytes [142].

The NLR family is involved in the maturation of pro-inflammatory cytokines, produced by other PRRs (e.g., TLRs and RLRs) in response to viral infection. Viral sensing by NLRs triggers the assembly and activation of inflammasome complex, resulting in the maturation and release of interleukin-1 $\beta$  (IL-1 $\beta$ ) and IL-18 from infected cells (reviewed in [140]). Seemingly, IL-1 $\beta$  acts in synergy with type I IFNs to suppress virus replication in cortical neurons, thus providing protection against lethal WNV infection in mice [147]. In contrast, enhanced production of IL-1 $\beta$  is linked to neuropathology associated with JEV infection in murine models [148]. IL-1 $\beta$  also abrogates the protective effect of astrocytes on BBB integrity by suppressing astrocytic expression of sonic hedgehog (SHH), a protein that upregulates the expression of TJs in BMECs [149]. Additionally, IL-1 $\beta$  and IL-18 activate microglia and astrocytes to generate more inflammatory molecules potentiating inflammation. Activation of microglia is often reported during encephalitic viral infection [104, 150–153], which is regulated by IFNAR signaling in astrocytes and neurons [154]. While microglia play a crucial role in viral clearance in the CNS [154–156], uncontrolled gliosis can disrupt BBB integrity through induction of pro-inflammatory



cytokines and matrix metalloproteases [153]. Similarly, astrocytes mount a strong innate immune response upon recognition of viral PAMPs via RLR and TLR signaling pathways. In fact, astrocytes are the main producers of type I IFNs during infection with several distinct neurotropic viruses, including La Crosse virus, rabies virus (RABV), vesicular stomatitis virus (VSV), and Theiler's murine encephalitis virus (TMEV) [157, 158]. Deletion of *IFNAR* signaling specifically in astrocytes results in severe encephalomyelitis and mortality during otherwise nonlethal mouse hepatitis virus (MHV) [159]. Nonetheless, activated astrocytes can release excess amount of IP-10 during encephalitic viral infection [109]. IP-10 enhances the expression of tumor necrosis factor alpha (TNF $\alpha$ ) in a JNK-dependent manner, leading to barrier disruption [109]. Consistently, injection of mice with neutralizing antibodies against IP-10 [109] or TNF- $\alpha$  ameliorated the decrease in TJ proteins and improved BBB integrity during JEV infection [160].

Alternatively, viral-induced inflammasome activation triggers pyroptosis, a highly inflammatory form of programmed cell death [161]. Although pyroptosis plays a crucial role in controlling virus spread [162], it can cause neuronal necrosis and gliosis [163], features associated with BBB disruption. In the CNS, inflammasome activity is regulated by mechanisms involving osteopontin (OPN) and prostaglandin D2 (PGD2), upon viral infection [164, 165]. OPN inhibits the caspase-1-dependent inflammasome activation by reducing the expression of inflammasome components in the brain [165]. However, PGD2 can exert both pro- and anti-inflammatory effects depending on the receptor involved. Engagement of D-prostanoid receptor 1 (DP1) on microglia by PGD2 upregulates PYDC3 (an inflammasome inhibitor), which protects against IL-1 $\beta$ -mediated neuroinflammation [164]. Deficiency in DP1 also correlates with reduced expression of IFN-I and -III, augmenting viral titer in the brain. Interestingly, upon viral infections, *IFNAR* signaling in BMECs reduces expression of IL-1 $\beta$  [15], likely via inhibition of inflammasome activity [166]. Additionally, type I IFNs act in synergy with MerTK (a member of TAM receptor tyrosine kinases) to activate Rac-1, which in turn improves TJ integrity [167].

Taken altogether, these studies suggest that innate immunity plays a central role in restricting viral replication in the CNS. This has the potential to be protective or detrimental, depending on the virus and magnitude of host immune response. The protective effect is mainly attributed to *IFNAR* signaling in CNS residential cells that not only limits local virus replication but also restricts additional viral entry or leukocyte infiltration by retaining BBB integrity.

### Adaptive Immune Responses to Viruses that Impact BBB Function

Leukocyte migration across the BBB requires expression of ICAM-1 and VCAM-1 on brain endothelial cells (ECs). As mentioned above, under normal conditions, these molecules are expressed minimally on brain ECs to restrict immune cell interaction and extravasation into the CNS. However, elevated expression of ICAM-1 and VCAM-1 has been frequently observed in infection with several neurotropic

viruses [168–172]. Altered expression of these adhesion molecules mainly results from the activation of NF- $\kappa$ B by ROS or ERK signaling pathway. For instance, HIV-1 Tat protein induces NADPH oxidase in astrocytes, which results in the upregulation of CAMs expression via NF- $\kappa$ B signaling [173, 174]. Similarly, JEV infection augments ICAM-1 expression on brain endothelial cells through activation of ERK signaling pathway [171]. Interestingly, expression of CAMs on brain endothelium is downregulated by IFNAR signaling in astrocytes, which promotes BBB integrity during encephalitic viral infection [170]. Additionally, IFNAR signaling in astrocytes influences the composition of inflammatory cells recruited to the CNS upon viral infection [159].

Although immune cell infiltration is crucial for viral clearance in the CNS [147, 175, 176], it can cause BBB disruption and neuronal damage by potentiating neuroinflammation [104]. Studies have shown that immune cell infiltration precedes BBB disruption in mice infected with VEEV and tick-borne encephalitic virus (TBEV) [102, 172]. This is associated with increased expression of RANTES, CCL2, IP-10, ICAM-1, TNF- $\alpha$ , IL-6, and IL-1 $\beta$  in brain tissues [172]. Similarly, enhanced levels of CCL2 and RANTES have been reported in serum samples of TBE-infected human patients [177]. Activated monocytes produce CCL2 in response to viral infections, which promotes barrier permeability via alteration in the actin cytoskeleton and localization of TJ proteins [178, 179]. Additionally, infiltrating neutrophils and monocytes produce high levels of MMP8, which promotes myelomonocytic cell extravasation and vascular leakage upon infection with lymphocytic choriomeningitis virus (LCMV) [180]. Consistently, depletion of both monocytes and neutrophils in LCMV-infected mice promotes BBB integrity and prolonged survival [181]. Notably, individual depletion of either cell type does not protect against vascular permeability.

As with monocytes, infiltrating lymphocytes can also induce BBB disruption by secreting inflammatory cytokines. Infected neurons produce CXCL10, which is a chemoattractant for CD4 and CD8 T cells [176]. Upon infection with rabies virus, CD4 T cells infiltrate into the CNS and differentiate into Th1 and Th17 that produce IFN- $\gamma$  and IL-17, respectively [182, 183]. While IFN- $\gamma$  reduces the expression of TJ proteins (i.e., occludin, claudin-5, and ZO-1), elevated levels of IL-17 disrupt TJ complexes in infected mice [182]. Consistently, blockage of IFN- $\gamma$  ameliorated BBB integrity *in vivo*, presumably by restoring expression of TJ proteins in brain endothelial cells [182]. Administration of IFN- $\gamma$ -neutralizing antibody also alleviated BBB disruption in JEV-infected mice [103]. CD8 T cells are also involved in vascular leakage during viral infection. They promote BBB disruption during infection with TMEV (Theiler's murine encephalomyelitis virus), by releasing perforin that disrupts TJ proteins [184]. Additionally, it has been suggested that interaction of CD8 T cells with neurons upregulates VEGF, which in turn promotes barrier dysfunction by dysregulating TJ complexes [184, 185]. Another mechanism by which cytotoxic T cells (CTLs) can cause vascular leakage involves degradation of the basal membrane via secretion of granzyme B. This allows CTLs to extravagate across brain vasculatures [186]. Upon extravasation, CD8 T cells release several

chemoattractants (e.g., CCL2, CCL3, and CCL4), which recruit monocytes and neutrophils to the CNS, thus indirectly leading to the loss of BBB function during viral infection [181].

Notably, physical interaction of infiltrating leukocytes with ICAM-1 on brain endothelium per se can promote vascular permeability by triggering generation of ROS in a NADPH oxidase and Rac-1-dependent manner [187–189]. Enhanced generation of ROS activates downstream tyrosine kinases (e.g., c-Src and PYK2), resulting in phosphorylation of VE-cadherin. This phosphorylation dissociates interaction of VE-cadherin with the actin cytoskeleton resulting in the disruption of adherent junctions. Likewise, TNF $\alpha$  and VEGF that are generated during viral infection [109, 120] trigger Rac-1-mediated ROS generation. ROS in turn promotes phosphorylation and internalization of VE-cadherin, leading to barrier dysfunction [188, 190, 191].

Collectively, the above studies suggest that upon viral infection, CNS residential cells release inflammatory cytokines/chemokines, which activate brain endothelium allowing immune cell infiltration. Infiltrating leukocytes provide microglia with costimulatory signals to eliminate infected cells. Additionally, cytotoxic T cells can directly kill infected cells contributing to viral clearance. Nonetheless, an excessive immune cell infiltration imposes severe structural damages to the cells of NVU, leading to barrier dysfunction.

### Effects of Parasites on BBB Structure and Function

As with other pathogens, neurotropic parasites have evolved several strategies to disrupt the BBB promoting their entry into the brain. These include infection and lysis of brain ECs (e.g., *Toxoplasma gondii*), secretion of proteases and toxins (e.g., *trypanosoma* and *acanthamoeba*), and induction of inflammatory cytokines or matrix metalloproteinases [192].

#### Cerebral Malaria and BBB Function

*Plasmodium falciparum*, the causative agent of human cerebral malaria (HCM), is associated with disruption of BBB and severe vasculopathy. Infection of red blood cells (RBCs) by *P. falciparum* induces structural changes in their membrane that make them adhesive to other cell types. This results in the formation of microaggregates that can obstruct blood flow, leading to hypoxia, hypertension, and alteration of metabolites in the CNS [192]. Additionally, infected RBC (iRBC) can directly interact with brain vascular endothelium and promote BBB dysfunction. Indeed, adhesion and sequestration of iRBCs in brain vasculature are linked to the loss of BBB function in humans [193, 194]. In vitro studies also have shown that adherence of iRBCs to brain endothelium triggers barrier permeability via induction of apoptosis and disruption of TJ proteins (e.g., ZO-1) [195–197]. In mouse models

of experimental cerebral malaria (ECM), BBB dysfunction correlates with platelet deposition and leukocyte arrest on the endothelium of postcapillary venules [198, 199]. Activated platelets augment BBB permeability by potentiating vascular damage induced by iRBC and impairing vascular repair. Similarly, leukocyte arrest, along with increased production of vasoconstrictive factors, impairs venous efflux from the CNS. This results in enhanced intracranial hypertension, vascular leakage, and hemorrhages [200, 201]. Among leukocytes, antigen-specific CD8 T cells and ICAM-1<sup>+</sup> macrophages are particularly involved in the development of ECM [198]. *Plasmodium* species upregulate the expression of ICAM-1, VCAM-1, P-, and E-selectins both in human and mice, thus promoting immune cell extravasation into the CNS [202, 203]. Antigen-specific CD8 T cells trigger BBB permeability by secreting granzyme B and perforin that target TJ proteins and induce apoptosis in brain endothelial cells [204–206]. Nonetheless, endothelial cell death is not correlated with barrier dysfunction or development of ECM in other studies [198, 199]. Instead, BBB disruption is related to increased paracellular or transcellular transport, mediated by the interaction of leukocytes with postcapillary venules [199]. Furthermore, antigen-specific CD8 T cells activate brain endothelial cells by releasing IFN- $\gamma$  [207], which upregulates the expression of adhesion and antigen-presenting molecules selectively on cerebrovascular ECs but not peripheral ECs [207, 208]. Consistently, deficiency in IFN- $\gamma$  or depletion of CD8 T cells results in complete protection against ECM by preventing barrier permeability and vascular hemorrhage [207, 209]. Likewise, co-infection with chikungunya virus (CHIKV) protects mice from ECM mortality by preventing pathogenic CD8 T cells from migration into the CNS [210].

### Toxoplasmosis

Toxoplasmosis is one of the most common parasitic diseases that is caused by *Toxoplasma gondii*. In healthy individuals, infection is either mild or asymptomatic; however, it can cause life-threatening CNS complications in developing fetus or immunocompromised patient [211]. Upon infection, *T. gondii* can enter CNS via Trojan horse as well as direct infection of brain endothelial cells [192, 212]. During acute phase, the parasite induces an exacerbated inflammatory response, which then subsides during the chronic phase. Inflammatory response upregulates expression of VCAM-1 on brain endothelium to promote migration of CD4 T cells into the CNS, which is required for controlling parasite replication [213]. Nonetheless, sustained and intense leukocyte-endothelium interaction in postcapillary venules leads to the formation of plugging, which can interfere with blood flow and cause cerebral hypoperfusion [214, 215]. Additionally, *T. gondii* can infect, lyse, and induce structural and functional defects in brain endothelial cells [192, 215]. Furthermore, elevated levels of MMPs, inflammatory cytokines, and nitric oxide are reported during parasite infection, features associated with BBB disruption [216, 217].

## Trypanosomiasis

Human African trypanosomiasis (HAT), also known as “sleeping sickness,” is caused by *Trypanosoma brucei*. HAT is divided into two clinical stages: during the first stage, parasite replicates in the blood and lymphatic system. This is followed by a second stage when the parasite enters and establishes infection in the CNS, which can cause meningoencephalitis [218]. *T. brucei* initially enters the CNS through choroid plexus and circumventricular organs, likely by secreting a protease that degrades the basal lamina [219, 220]. During the early phase of CNS infection, production of IL-6 and IL-10 protects against neuroinflammation [221]. However, later in disease, activated microglia and astrocytes produce high levels of inflammatory cytokines (e.g., IL-1 $\beta$ , CXCL-8, CCL-2, and TNF- $\alpha$ ), which can lead to severe neuropathology [222]. These inflammatory cytokines upregulate the expression of cell adhesion molecules (i.e., ICAM-1, VCAM-1, and E-selectin) on brain endothelial cells, which promotes leukocyte migration into the CNS. Infiltrating lymphocytes are particularly involved in the entry of parasite across BBB. They secrete IFN- $\gamma$ , which in turn activates MMP9, a protease that degrades astrocytic basement membrane allowing paracellular entry of parasite into the CNS [219]. Consistently, enhanced expression of MMPs is reported during *Trypanosoma* infection [223, 224]. Additionally, IFN- $\gamma$  augments the expression of CXCL-10 by astrocytes, which recruits more lymphocytes into the CNS [225]. Furthermore, *T. brucei* releases cysteine proteases that trigger protease-activated receptors (PARs) on BMECs, thereby promoting BBB dysfunction through enhancement of intracellular calcium level [226]. Interestingly, production of nitric oxide by perivascular macrophages restricts the entry of both parasites and activated T cells into the CNS by preserving BBB integrity [224].

## Amoebic Encephalitis and BBB Effects

*Acanthamoeba castellanii* is a fatal infection of immunocompromised individuals and is associated with BBB dysfunction and has been shown to cause granulomatous encephalitis in immunocompromised patients. It invades the CNS through hematogenous pathway following disruption of the BBB [227]. *A. castellanii* interacts with BMECs through a mannose-binding protein that is expressed on the surface of its trophozoites. These interactions trigger degradation of TJ proteins (e.g., occludin and ZO-1) in a Rho kinase-dependent manner [228]. Additionally, parasite interaction can induce cell cycle arrest and apoptosis through activation of phosphatidylinositol 3 kinases (PI3K) and inhibition of proteins that are involved in cell cycle progression [229, 230]. Similarly, cell cycle arrest and apoptosis of brain endothelial cells have been reported for *Balamuthia mandrillaris*, another parasite that is known to cause fatal amoebic meningoencephalitis [231]. Notably, host immune response plays a major role in the disruption of BBB during infection with *A. castellanii* and *Naegleria fowleri* [227, 232]. Since these amoebae are relatively

large in size, they elicit an amplified immune response that not only compromises the BBB integrity but also causes neuronal damage [232].

Neurotropic parasites (e.g., *Trypanosoma*, *Acanthamoeba*, and *Balamuthia* species) are also known to produce and release a variety of proteases (e.g., cysteine and serine proteases and metalloproteinases) that target TJ proteins and the basal membrane of the BBB, leading to barrier dysfunction [233–236]. Proteases interact with protease-activated receptors on BMEC and stimulate calcium release from intracellular stores by activating phospholipase C (PLC) [226]. Increased calcium levels result in calmodulin activation of myosin light chain, which in turn augments intracellular contraction, leading to disruption of TJs between brain ECs [237].

## Future Perspectives

Since the initial demonstration and appreciation of the specialized nature of the CNS microvasculature, researchers have learned that it is less an impermeable barrier and more a dynamic interface that senses and responds to the periphery. These responses are generally neuroprotective, such as the IFNAR-mediated increase in TJ integrity during viral invasion or the stringent regulation of T cell access that can promote efficient clearance of pathogens, such as *T. gondii*, without excessive immunopathology. Pathogens have evolved various mechanisms to exploit cellular and molecular processes that control the CNS access, such as the reduction in expression of ZO-1 induced by the *B. anthracis* edema toxin (ET). Host responses, in turn, regulate immune cell infiltration into the CNS via antigen-specific events that allow leukocyte localization, interactions, and egress from perivascular spaces such that inflammation is efficiently directed at pathogen elimination. The interaction of immune cells with BMECs destabilizes junctional molecules via cytokine-mediated signaling events that alter the structural properties of these cells. Thus, the most severe outcomes in the context of neuroinfectious diseases that enter the CNS via the BBB arise from host inflammatory responses rather than due to direct effects of pathogens themselves. This is particularly evident in the context of autoimmune diseases of the CNS where leukocytes gain inappropriate access to the CNS and cause extensive damage without acute infection.

While we continue to improve our understanding of these processes, the challenge will be to better identify mechanisms that promote efficiency in immune-mediated pathogen clearance while enhancing the CNS' own neuroprotective mechanisms. The use of animal models of neuroinfectious diseases that focus on various aspects of these processes in conjunction with the development of methods to isolate cellular participants, such as RiboTag or single cell RNA sequencing, in conjunction with cell-specific gene deletion strategies will permit cell-type-specific evaluation of mRNA expression and protein functions during the course of in vivo pathogen invasion, infection, and clearance. The advent of techniques in which human-induced pluripotent stem cells (hiPSCs) can be differentiated into all members of the NVU which are then incorporated into three-dimensional, fluid-based

models of the human BBB also holds promise for identifying molecular players in this process and validating results in human systems. Future studies are likely to uncover novel neuroimmune pathways that may be safely targeted to prevent or treat infections of the CNS while also providing strategies for manipulating BBB function for the purposes of drug delivery or immunotherapies for noninfectious neurologic diseases.

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