The Blood-Brain Barrier
Implications for Vasculitis

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KEYWORDS

• Blood-brain barrier • Primary • Secondary • Vasculitis • Autoimmune
• Nervous system

KEY POINTS

- There has been extraordinary research in the blood-brain barrier over the past decade.
- The blood-brain barrier is fully functional in development and vital in cerebrovascular angiogenesis.
- The cellular components and other molecular constituents of the blood-brain barrier, contained in a neurovascular unit, protect the central nervous system from injury and systemic diseases.
- Blood-brain barrier disruption is now recognized as an important factor in a variety of primary neurologic diseases.
- This article reviews the history, neurodevelopment, ultrastructure, function, and clinicopathologic correlation and relevance to central nervous system vasculitis.

INTRODUCTION

The past decade has witnessed an expansion of knowledge in the properties possessed by the blood-brain barrier (BBB) in health and disease summarized in several excellent recent reviews.[1–5] In essence, the neurovascular unit of the BBB is composed of capillary vascular and neural cells, extracellular matrix components, and a variety of immune cells that mediate local immunity contained in the neurovascular unit (NVU). The schematized and electron microscopic (EM) appearance of cerebral capillaries in the BBB, shown in **Figs. 1** and **2**, demonstrates layers of pericytes adherent to the abluminal or parenchymal surface of endothelial cells,

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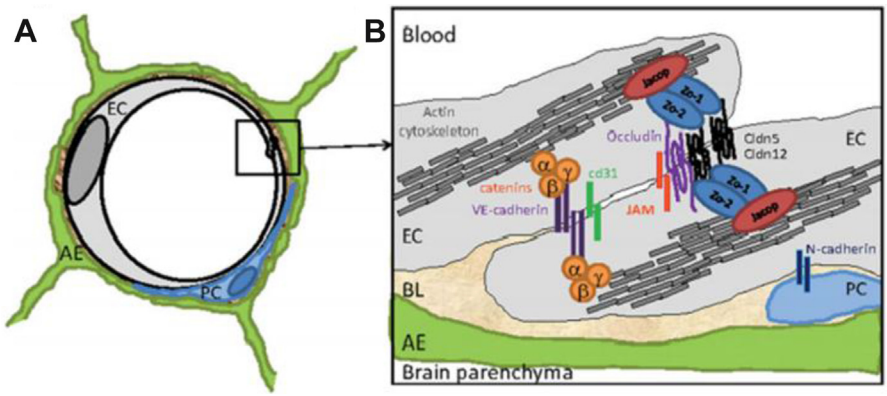

Fig. 1. (*A*) A capillary in the human BBB over an endothelial TJ. (*B*) The insert shows the molecular composition of tight and adherens junctions. See text for details. (*From* Daneman R. The blood-brain barrier in health and disease. Ann Neurol 2012;72:649; with permission.)

together surrounded by a layer of basal lamina composed of extracellular matrix protein molecules. The end-feet of neighboring astrocyte processes ensheathes the blood vessels. Monolayers of adjacent endothelial cells that form tight junction (TJ) strands, shown in **Fig. 1**, connect adjacent endothelial cells by adhesions of transmembrane (occludin, claudin, and junctional associated molecules [JAM]) across the intercellular space, whereas cytoplasmic scaffolding and regulatory proteins, such as zona occludens type 1 and 2 (ZO-1, ZO-2), provide linkage to the actin cytoskeleton and initiate several signaling mechanisms via protein-protein interactions. Endothelia BBB cells are also linked by adherens junctions composed of vascular

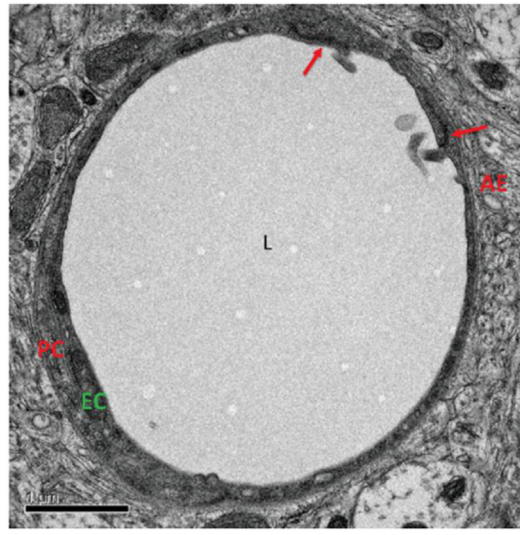

Fig. 2. EM of a capillary in the adult murine BBB. Endothelial cells are held together by TJs (*red arrow*). L, lumen. (*From* Daneman R. The blood-brain barrier in health and disease. Ann Neurol 2012;72:648–72; with permission.)

endothelial (VE)-cadherin, which mediates cell-cell adhesion interactions, linking adherens junctions to the actin cytoskeleton via catenins.[2,3] Perivascular macrophages that reside between astrocyte end-feet and the vessel wall, mast cells associated with specific regions of the central nervous system (CNS), resident microglia that act as antigen-presenting cells, circulating leukocytes that can penetrate the intact BBB via interactions with endothelial cell adhesion molecules (CAM) to mediate bidirectional cross-talk between immune cells and endothelium for normal surveillance, constitute the extended neurovascular unit.[2]

Breakdown or disruption of the BBB that accompanies a variety of inflammatory and autoimmune, neoplastic, infectious, and neurodegenerative CNS disorders, notably stroke, multiple sclerosis, brain trauma, human immune virus, infection, and Alzheimer disease. These disorders are associated with the abnormal entry of plasma components, immune molecules, and cellular elements that lead to further neural dysfunction and varying degrees of irreversible neural degeneration. Although there is little known about the role of BBB breakdown in primary and secondary CNS vasculitis, future progress could lead to improved understanding of primary and secondary forms of CNS vasculitis[6] with the prospect of even improving the outcome of 2 potentially devastating disorders, childhood and adult primary angiitis of the CNS.[7–9] According to Weiss and colleagues,[10] further understanding of the BBB could envision the use of new therapeutic strategies that bypass it, taking advantage of the selective expression of membrane-bound proteins expressed by brain endothelia cells or circulating leukocytes to target new drugs as well as improve the effectiveness of conventional systemic immunosuppression. This article reviews the history, neurodevelopment, ultrastructure, function, and clinicopathologic correlation and relevance to CNS vasculitis.

HISTORICAL BACKGROUND

In 1885, Ehrlich[11] provided the first suggestion of the presence of a barrier when a parenteral injection of vital dye into the bloodstream of mice penetrated practically every systemic organ except the brain, turning them dark purplish-blue, leaving the brain and spinal cord pale white-yellow. Ehrlich himself thought that this difference was due to a low binding affinity. The existence of a barrier at the level of the cerebral vessels was postulated by Bield and Kraus[12] and later by Goldman[13] and Lewandowsky[14] at the turn of the twentieth century, who jointly interpreted their experience in favor of a true BBB, later termed *Blut-Hirn-Schranke*. In 1967, Reese and Karnovsky[15] demonstrated a structural barrier to an intravenous injection of horseradish peroxidase (HRP) demonstrating exogenous peroxidase to the lumina of blood vessels and in some micropinocytotic vesicles within endothelial cells, but none beyond the vascular endothelium. The relatively scarce number of vesicles was a morphologic feature of a functioning BBB. Their findings localized at a fine structural level a barrier composed of the plasma membrane and the cell body of endothelial cells and TJ between adjacent cells of the cerebral cortex. In 1969, EM studies by Brightman and Reese[16] in the mouse conclusively demonstrated endothelial and epithelia TJ that occluded the interspaces between blood and parenchyma or cerebral ventricles, constituting the ultrastructural basis for the blood-brain and blood-cerebrospinal fluid barriers. Feder[17] noted active exclusion of the small electron-dense tracer microperoxidase by intact TJ after parenteral injection supplementing the findings of Reese and Karnovsky.[15] Nagy and colleagues[18] examined fracture faces of cerebral endothelium in normal and hyperosmolar mannitol-treated rat brains to elucidate the organization of TJ in various segments of the cerebral vascular bed and the structural basis of BBB opening in hyperosmotic conditions. Their findings provided no direct evidence

for the structural basis of BBB opening in hyperosmolar mannitol-treated rat brains, noting extended TJ regions in capillaries and postcapillary venues. Shivers and coworkers[19] studied isolated rat brain capillaries using freeze-fracture images of interendothelial ZO revealing complex arrays of intramembrane ridges and grooves characteristic of TJ. The ZO of these capillary endothelial cells were considered very tight.

NEURODEVELOPMENT

In contrast to neuronal development, the vascular system undergoes blood vessel formation through the 2 distinct processes: vasculogenesis and angiogenesis. The former commences with endothelial differentiation from angioblasts to vascular plexuses, whereas the latter is associated with sprouting of new vessels from existing ones. Both vasculogenesis and angiogenesis are influenced by vascular endothelial growth factor (VEGF), a major attractive molecule for extending blood vessels, especially endothelial tip cells,[20] as well as by other molecules, blood flow, and contact with surrounding tissues. Found at the growing end of extending vessels, the shaft of extending endothelial tip cells is composed of an endothelial cell chain made of stalk cells similar to the axon growth cone and its associated axonal shaft. In vivo imaging using green fluorescent protein (GFP) depicts the advancing endothelial tip cell navigating the environment and sprouting from existing vasculature.[21]

Several experimental observations have suggested the importance of the neurodevelopment of the BBB.[5]

First, there are shared molecular and cellular mechanisms in both neurogenesis and angiogenesis.[22,23] Various axon guidance pathways from members of the 4 major families of axon guidance ligand-receptor pairs, including Slit/Robo, semaphoring/plexin/neuropilin, Netrin/Unc5/DCC, and Ephrin/Eph, that mediate complex cellular navigational programs within axons as both chemoattractants and repellents, also direct angiogenic tip cells toward their final destinations. Neuropilin-1 is necessary for endothelial tip cell guidance in the developing CNS.[24] Moreover, axonal terminal arborization parallels vessel sprouting. Similar to hypoxic tissue that secretes VEGF via hypoxia-inducible factor, a transcription factor that promotes cell survival through the downstream activation of numerous genes including VEGF,[25] axonal terminals devoid of synaptic input secrete nerve growth factor, the expression of which is downregulated when innervation occurs.

Second, there is coregulation of these 2 systems in developing embryonic and adult brains.[22,26,27] Stubbs and colleagues[26] found that blood vessels provided a supporting niche in regions of adult neurogenesis. The investigators[26] used Tbr2-GFP transgenic mice that served as a correlate for the expression of the intermediate progenitor cell (IPC) T-box transcription factor Tbr2, to examine the proximity of dividing cells in the subventricular zone (SVZ) and ventricular zone of the shaking rat Kawasaki and reeler mutant mouse in relation to blood vessels throughout neurogenesis. Their findings, which included the extension of neuritis toward and along labeled blood vessels, supported the notion of vascular-neuronal interactions in development. Javaherian and Kriegstein,[27] who likewise studied IPC in the SVZ of embryonic Swiss Webster mouse cortices, used confocal microscopy to image the vast network of capillaries in the SV and SVZ. The investigators[27] noted that Tbr2 cells divided near vascular branch points, suggesting endothelial tip cells contributed to the neurogenic niche for IPC, with ectopic overexpression of VEGF-A in a pattern that followed that of blood vessel development. These findings indicated that the developing cortical vasculature

provided a microenvironment within the SVZ in which IPC accumulated and divided during neurogenesis.

Third, a structural and functional BBB complete with TJ appears as soon as cerebral vessels penetrate the CNS parenchyma.[28,29] Johansson and colleagues[28] explained that the widely held view that the BBB was immature during development stemmed from teleologic interpretations and experimental observations of high cerebrospinal fluid protein levels in fetal cerebrospinal fluid and the apparent passive passage of biomarkers during development. Instead, the blood–cerebrospinal fluid barrier, like the BBB, is functionally and morphologically mature from very early in development. The investigators maintain that inconsistent terminology used in the literature, such as leaky, immature, and developing, used to describe the barrier gives a connotation of TJ that is more permeable than their adult counterparts without evidence to support this concept. Mølgård and Saunders[30] noted well-formed complex TJ across cerebral endothelial cells in human embryos and fetuses by freeze fracture and thin-section EM by 8 weeks of age, commensurate with the differentiation of brain capillaries. Efflux transporters are likewise expressed in cerebral endothelial and choroid plexus epithelial cells early in the fetal and postnatal rats.[31] Ballabh and colleagues[32] studied the expression and quantification of endothelial TJ molecules, including claudin-5, occluding, and JAM, by immunohistochemistry and Western blot analysis in blood vessels of germinal matrix, cortex, and white matter of fetuses and premature infants gestational age 16 to 40 weeks. The investigators[32] noted no significant decrease in the expression of the endothelial TJ molecules claudin-5, occludin, and JAM-1 as a function of gestational age in germinal matrix compared with cortex and white matter, suggesting that they were unlikely to be responsible for germinal matrix fragility and vulnerability to hemorrhage in premature infants. These findings are consistent with the concept that TJ molecules develop and perhaps mature early during human gestation. Ballabh and colleagues[33] observed that a paucity of TJ or pericytes coupled with incomplete coverage of blood vessels by astrocyte end-feet, could instead account for the observed fragility of blood vessels in the germinal matrix of premature infants. Braun and colleagues[34] found that pericytes coverage and density were less in the germinal matrix vasculature than in the cortex or white matter in human fetuses, premature infants, and premature rabbit pups. Although VEGF suppression significantly enhanced pericyte coverage in germinal matrix, it remained less than in other brain regions.

NEUROBIOLOGY AND CELLULAR INTERACTIONS

Endothelial Cell Interactions

The existence of the endothelial cell was first surmised by William Harvey, first observed by Marcello Malpighi in blood capillaries using compound microscopy in the nineteenth century, and later by EM in the mid-twentieth century, revealing the presence of plasmalemmal vesicles or caveolae. The ability to culture EC later permitted even more detailed investigation of their activation and function in vivo. Derived from mesoderm via the differentiation of hemangioblasts and angioblasts, there are a few protein/messenger RNA marker candidates, including platelet/endothelial cell adhesion molecule (PECAM)-1 in monocytes and VE-cadherin in fetal stem cells. Endothelial cells of the BBB not only provide a physical barrier between the systemic circulation and the brain but also assure the selective inward passage of ions, nutrients, and neuropeptides via specialized transport mechanisms. Sodium, potassium, chloride, hydrogen, bicarbonate, and calcium ions are transported across the BBB via transporters located mainly along the luminal surface of endothelial

cells, including the sodium and potassium adenosine triphosphate (ATP) -dependent transport pump, the sodium-potassium-chloride cotransporter, sodium-proton, chloride-bicarbonate, and sodium calcium exchanges that assure optimal levels of brain electrolyte levels and intracellular pH. The transport of essential nutrients is assured by members of the solute-linked carriers (SLC) superfamily, located variably along the luminal and abluminal membrane, including glucose transporter-1, monocarboxylic acid-1, excitatory, organic acid, cation, amine, and choline transporters, respectively, to transport lactate and ketone bodies as alternative energy neuronal sources, and sodium-independent or -dependent removal of glutamate, aspartate, glutamine, histidine, and asparagine from the interstitial compartment of the brain. Other specific carrier-mediated transporters mediate the passage of transferrin, low-density lipoproteins, leptin, immunoglobulin G, insulin, and growth factors via receptor-mediated transcytosis via binding of the protein to specific receptors on the endothelial cell surface followed by endocytosis of the ligand-receptor complex with passage across the cytoplasm and exocytosis at the opposite side of the cell[35] and via the formation of caveolae or vesicle formation for the transport of macromolecules.[36] Transmigration of cellular elements across endothelial cells of the BBB during inflammation, including leukocytes, neoplastic cells, and pathogenic viruses, bacteria and yeasts, investigated in experimental animal models using HRP, highlighted the role of caveolae as minitransporters of the CNS.[36] Unique systems of modified caveolae that fuse together forming transendothelial cell channels and later vesiculocanalicular or vesiculotubular structures or vesiculovacuolar organelles appear to be an important gateway to the CNS in damaged endothelial cell populations.[36] Transportation of potentially toxic endogenous or xenobiotic lipid-soluble nonpolar molecules from the brain to the blood is accomplished by transporters located along the luminal membrane, such as the ATP binding cassette transporter P-glycoprotein 1 (multidrug resistance protein 1 or ATP-binding cassette subfamily B member 1), respectively, important in the distribution of CNS tumor drug treatment[37] and the active efflux of the anti–human immunodeficiency virus type 1 (HIV1) nucleoside drug abacavir at the BBB,[38–40] and breast-cancer resistance protein and multidrug resistance related protein (MRP) 1, 2, 4, and 5 efflux transporter pumps that serve as defense mechanisms and determinate bioavailability and concentration of many CNS drugs important in the treatment of CNS cancers,[41] such as the novel tyrosine kinase inhibitor dasatinib,[42] and the efflux transportation of the protease inhibitor lopinavir that contributes to its poor oral bioavailability in the treatment of HIV1.[43] The neuroinflammation and progression of damage associated with focal cerebral ischemia appear to be modulated by upregulation of other MRP protein molecules that activate Toll-like receptor signaling contributing to neuroinflammation and progression of ischemic cerebral damage.[44]

Transendothelial migration of circulating leukocytes involves a multistep process. Leukocyte adhesion molecules expressed on the surface of EC initiate binding of leukocytes as a beginning step in the in their entry in brain tissue, which later includes rolling adhesion to EC, firm adhesion, and transmigration. Although less well understood, the molecular mechanism is thought to involve endothelial CAM, including CD99, PECAM-1/CD31, vascular CAM-1 (VCAM-1) (important in firm adhesion); junctional adhesion molecule-1; and expression of leukocyte adhesion molecules E- and P-selectin (rolling adhesion); cytokine responsiveness so noted in situ and in cell culture,[45,46] and expression of the integrins alpha-4 and beta-2. Inflamed capillary endothelia support transmigration of different subsets of leukocytes. There are 2 routes for leukocytes to pass through endothelial cells: the so-called paracellular route, or through the endothelial cell itself or transcellular route. The BBB with its abundance

of TJ complexes relies primarily on the transcellular route as it does for solute and fluid transport. *Neutrophil recruitment is partially dependent on ICAM-1, and express L-selectin and lymphocyte function-associated antigen-1 but not chemokine C motif receptor 7 may explain why granulocytes roll but do not arrest for transmigration in high endothelial venules.

Pericyte Interactions

Brain endothelial cells are exposed to a myriad of pericyte interactions[47] in the regulation of brain angiogenesis, endothelial cell TJ formation, as well as the differentiation, microvascular vasodynamic capacity, structural stability, and neuroimmunologic network operations of the intact BBB.[48] Rouget[49] first ascribed capillary contractility to pericytes, but Zimmerman[50] named the cell and described its morphologic aspects. The presence of smooth muscle cells in association with pericytes and the absence of a smooth muscle layer from capillaries and postcapillary venues influenced early views ascribing contractile properties to narrow capillaries, hence regulating microvascular flow even though several subsequent experimental studies failed to substantiate it.[51,52] Smooth muscle actin was conclusively demonstrated in pericytes by immunocytochemistry using smooth muscle α-actin isoform-specific antibodies and immunogold labeling in conjunction with EM, noting that smooth muscle α-actin expression in capillaries was limited exclusively to pericytes and not present in endothelial cells.[53] Because the histochemical localization of smooth muscle α-actin is demonstrated in precapillaries and not in midcapillaries, it has been suggested that smooth muscle α-actin–containing capillaries are involved in contractility and the control of capillary blood flow in the BBB.[54]

Unlike other perivascular cells, they lie within the microvessel basal lamina and contribute to its formation, and typical CNS pericytes are flattened or elongated, stellate-shaped solitary cells with multiple cytoplasmic processes encircling the capillary endothelium and contacting a large abluminal vessel area. Brain pericytes are characterized by granular deposits present in lysosomes that strongly react with acid phosphatase, a finding that led to consideration of a phagocytic role.[55] They rapidly phagocytose an intravenous injection of HRP, which can be used as a pericyte histochemical stain. The number of granular lysosomes in brain pericytes increases with disruption of the BBB. Several other markers have been used in the identification of pericytes, including smooth muscle α-actin, desmin, polydendrocytes (NG2 cells), platelet-derived growth factor receptor-β, aminopeptidase A and N, regulator of G-protein signaling 5, and the promoter trap transgene *XlacZ4*.[56]

An active role of pericytes in the BBB was inferred from the localization of γ-glutamyl transpeptidase (GGTP) in brain capillary endothelial cells and pericytes, both in vivo and in vitro.[57] This heterodimeric glycoprotein distributed on the external surface of the cell catalyzes the transfer of γ-glutamyl from glutathione to accept peptides and functionally appears to be concerned with transport of large neutral amino acids across the BBB. Detectable amounts of GGTP are found in other regions of the brain with an intact BBB but not in those that lack one, such as the median eminence. Abnormal platelet-derived growth factor (PDGF)-B and PDGF-β signaling play a critical role in the recruitment of pericytes to newly formed vessels, and when deficient, as in knockout of *pdgfb* and *pdbfrb*, lead to perinatal death due to vascular dysfunction with associate vascular leakage and hemorrhage.

Pericyte-endothelia cell signaling factors have been identified. Sphingosine-1-phosphate signaling triggers cytoskeletal, adhesive, and junctional changes, affecting cell migration, proliferation, and survival.[58] Angiopoietin-Tie2 signaling in the vascular wall is involved in reciprocal communication between endothelial cell

and pericytes, such as may be seen in *ang1*-or *tie*2-null mice deficient in Ang1, which leads to defective angiogenesis and poorly organized BM and reduced coverage and detachment of pericytes. Conversely, overexpression of Ang1 leads to expanded and stabilized leakage-resistant microvasculature.[59,60]

The importance of CNS pericytes has been underscored by their proposed role in neuroimmunologic networks associated with BBB function. First, CNS pericytes may be actively involved in the regulation of leukocyte transmigration, antigen presentation, and T-cell activation. They constitutively express low levels of VCAM-1 and ICAM-1, which have costimulatory activity in main histocompatibility cell class II dependent antigen presentation, and leukocytes cluster on pericytes in culture,[48] suggesting a role in inflammation. Smooth muscle pericytes present antigen in vivo and differentially activate Th1 and Th2 $CD4^-$ T cells. Moreover, CNS pericytes produce several immunoregulatory cytokines, including interleukin (IL) 1β and granulocyte-macrophage colony stimulatory factor.[61] Transforming growth factor (TGF)-β produced in an active form in pericytes/endothelial cocultures may function as an endogenous immunoregulator at the BBB.[62] It is therefore of interest that TFG-β1 inhibits cytokine-induced CNS endothelial cell activation in isolated rat CNS microvessels.[63] To further emphasize the importance of pericyte interactions in association with endothelial cells, there are no known genetic human diseases due to pericytes deficiency.

Astrocyte Interactions

The intimate relationship of astrocytes and blood vessels was appreciated by Ramon y Cajal[64] and Golgi[65] in the late nineteenth century. Since then, ultrastructural studies have shown that astrocytic end-feet in the perivascular astroglial sheath leads to a complete covering of brain microvessels.[66] Signaling at the gliovascular interface is facilitated by astrocyte-specific proteins and channels in astrocyte end-feet, including acquaporin-4, connexin 43, purinergic receptors, and potassium channels.[67] Moreover, ultrastructural studies have demonstrated that processes of vasoactive neurons for the regulation of cerebrovascular tone, in particular, those expressing noradrenaline, synapse onto astrocytes rather than directly onto blood vessels.[68] Altogether, these findings support the observation that astrocytes, one of the more numerous cells in the CNS, are important determinants of the intact BBB and crucial as well for ionic homeostasis, neurotransmitter uptake, synapse formation, and neurodevelopment. Zhang and Barres[69] have reviewed the differences in astrocyte morphology, developmental origin, gene expression profile, physiologic properties, function, and response to injury and disease. Two essential roles of astrocytes, in neurovascular coupling and the regulation of lymphocyte trafficking across the BBB, have been extensively studied.

All signaling molecules targeted to the cerebral vasculature must first act on or pass through astrocytes in order to reach smooth muscle cells in the vessel wall. It is now recognized that neurotransmitter-mediated signaling has a key role in regulating cerebral blood flow, and that much of this control is mediated by astrocytes[70]; moreover, cerebral blood flow may be controlled by capillaries as well as by arterioles. The glial and neuronal control of cerebral blood flow has been studied in brain slices.[71] Koehler and colleagues[72] demonstrated that electrical field stimulations in brain slices led to an increase in intracellular calcium in astrocyte cell bodies, which, when transmitted to perivascular end-feet, was followed by a decrease in vascular smooth muscle calcium oscillations and arteriolar dilation. The increase in astrocyte calcium after neuronal activation was in part mediated by activation of metabotropic glutamate receptors. Calcium signaling in vitro was influenced by adenosine acting on A2B receptors and by epoxyeicosatrienoic acids (EET) shown to be synthesized in astrocytes. Moreover,

prostaglandins, EET, arachidonic acid, and potassium ions are candidate mediators of communication between astrocyte end-feet and vascular smooth muscle. Astrocytes appear to be capable of transmitting signals to pial arterioles on the brain surface to ensure adequate blood flow to feeding arterioles; therefore, these cells play an important role in the coupling of dynamic changes in cerebral blood flow in association with neuronal activity.

Koehler and colleagues[72] have provided insight into the morphologic aspects of neurovascular coupling at the capillary level of the BBB. At least one astrocyte end-foot process contacts a blood vessel, and those abutting capillaries and larger vessels express connexin-43 and purinergic P2Y receptors, which together permit Ca^{2+} increases to be transmitted 60 μm or more along the abluminal side of the vessel wall. Astrocytic cells are therefore in a unique position for sensing neuronal activity, integrating that information, and communicating with blood vessels in brain parenchyma. Although neurons do not directly innervate intraparenchymal vascular smooth muscle, subpopulations of GABAergic interneurons come into close contact with astrocyte foot processes and elicit vasodilation. Such neurons might modulate vascular function through stimulation of nitric oxide synthase activity, release of vasoactive peptides, or an astrocyte signaling mechanism.

Hudson and coworkers[73] studied trafficking of peripheral blood mononuclear cells (PBMC) across feline brain endothelial cells (FBEC) in cell culture system after the addition of combinations of different configurations of astrocytes and microglia in a model of feline immunodeficiency virus. The addition of astrocytes to FBEC significantly increased the adherence of PBMC, which was suppressed by the addition of microglia, whereas the latter alone had no effect on PBMC adherence. Whereas all PBMC showed some level of trafficking across FBEC, monocytes and B cells were significantly increased if astrocytes were present. The exposure of astrocytes notably increased the percentage of trafficking CD8 T cells from 24% to 64%, whereas microglia led to a significant reversal in the preferential trafficking of CD8 cells in the presence of astrocytes. Astrocytes are capable of secreting various cytokines and chemokines in the upregulation of adhesion molecules and T-cell ligands in intact endothelial cells, such as ICAM, VCAM, E-selectin, and PECAM. Human cocultured human endothelial cells and astrocytes increase the expression of ICAM-1 due to inflammatory activation by hypoxia in vitro.[74] Other studies have demonstrated that astrocytes are a source of IL-6, tumor necrosis factor-α, and monocyte chemoattractant protein-1, which contribute to the CNS inflammatory response.[75] Trafficking of PBMC along the endothelial cell of the BBB is a complex mechanism that involves major subsets of immune cells and relies heavily on astrocyte, microglia, and endothelial cell interactions; moreover, astrocytes appear to be an active factor in the recruitment of immune cells, whereas microglia appear to curtail this activity.

IMPLICATIONS FOR CEREBRAL VASCULITIS

There is an extensive literature of BBB biology in health and in widely differing neurologic disorders, including stroke,[76] epilepsy,[77] multiple sclerosis,[78] Alzheimer disease,[79] motor neuron disease,[80] Parkinson disease,[81] trauma,[82] glioblastoma,[83] HIV encephalitis,[84] and neuropsychiatric systemic lupus erythematosus (NPSLE).[85] Between 40% and 70% of patients with SLE have involvement of the CNS,[86] yet unlike systemic and primary CNS vasculitides, the role of the BBB in CNS lupus has been the subject of intense study using animal models and human clinical data.

The BBB is crucial because it maintains the brain internal milieu constant, allowing optimal neuronal function. Loss of BBB integrity leads to influx of inflammatory cells,

and molecules, such as autoantibodies, causing brain injury. Earlier studies using the well-established MRL/lpr mouse model that reflects the events that occur in human CNS lupus revealed loss of BBB integrity with worsening disease[87,88] due to chronic activation of the complement cascade with the generation of the anaphylatoxins, C3a and C5a, and aggravated C5a/C5aR signaling. In cell culture, C5a causes neuronal cells to become apoptotic and binds to 2 receptors, the G-coupled, C5aR1, and the alternate receptor, C5aR2.[89] C5a/C5aR1 signaling mediates several biological processes, including chemotaxis and degranulation of mast cells, basophils, neutrophils, and eosinophils. It increases vascular permeability, increases generation of reactive oxygen species, and enhances production of cytokines from monocytes and macrophages. Whether C5a/C5aR signaling is protective or neurotoxic depends on the setting, with increases in circulating C5a leading to poor outcomes in CNS lupus.[89]

Hopkins and colleagues[90] measured levels of complement anaphylatoxin split products, C3a and C5a, in the circulation of patients with SLE who were followed serially. Means complement levels were significantly higher during periods of lupus flare compared with those during a stable period, with the highest levels seen in patients with CNS involvement. Pathologic specimens from 2 cases who died during an acute lupus flare revealed neutrophils occluding the cerebral and systemic intestinal vessels.

C5a also contributes to cellular apoptosis in lupus,[91] wherein treatment with the C5aRant, inhibiting C5a/C5aR signaling, results in significant and substantial decreases in brain pathologic condition in MRL/lpr mice, leaving upstream potentially protective complement activation events intact. There was evidence for a relationship between complement activation, inflammation, and neuronal viability in lupus brain tissue. C5aR1 is present predominantly on blood myeloid cells, but is also constitutively expressed on several brain cell types, including endothelial cells, which contribute to BBB integrity. Such studies show the possible neuroprotective role for C5aR antagonists in MRL/lpr mice and indicate potential future avenues of research for systemic and primary CNS vasculitides.

SUMMARY

There has been extraordinary research in the BBB over the past decade. Once considered a static anatomic barrier to the traffic of molecules in and out of the CNS, the BBB of children and adults is now recognized to be fully functional and vital to both cerebrovascular angiogenesis and normal homeostatic maintenance. The cellular components and other molecular constituents of the BBB, contained in the NVU, protect the CNS from injury and disease by limiting the passage of toxins, pathogens, and inflammatory effectors of the immune system. The implications of BBB disruption in cerebral vasculitis have yet to be appreciated; however, comparisons to CNS lupus may lead to a better understanding of the neural mechanisms involved in disease pathogenesis and BBB integrity, and efficacious neuroprotective treatment strategies.

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