The Blood-Brain Barrier and Its Role in Immune Privilege in the Central Nervous System

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Abstract. The blood-brain barrier (BBB) provides both anatomical and physiological protection for the central nervous system (CNS), strictly regulating the entry of many substances and blood borne cells into the nervous tissue. Increased understanding of how the unique microenvironment in the CNS influences the BBB is crucial for developing novel therapeutic approaches to CNS diseases. In this review, we discuss those characteristics of the BBB that play an important role in maintaining immune privilege in the CNS, as well as factors that regulate immune cell invasion through the BBB and thereby modulate immune responses in the nervous tissue. In general, immune cell invasion across the BBB is highly restricted and carefully regulated. A florid invasion of activated white blood cells can create a predominantly proinflammatory local environment in the CNS, leading to immune-mediated diseases of the nervous tissue. Recent developments in cellular and molecular biological methods have allowed closer analysis of BBB function, and led to an improved understanding of the active role of the BBB in immune-mediated diseases of the CNS.

Key Words: Blood-brain barrier; CNS inflammation; Immune privilege.

HISTORICAL PERSPECTIVE

In 1885 Paul Ehrlich discovered that intravenous injection of acidic dyes into experimental animals caused staining of various organs, with the notable exception of the brain (1). Somewhat later, in 1898, Roux and Borrel observed that tetanus toxin injected into the cerebral spinal fluid (CSF) caused marked cerebral symptoms but, when administered intravenously, produced no discernible cerebral effect in these animals (2). These initial experiments prompted the earliest consideration of a blood-brain barrier (BBB) that restricted the passage of substances into and out of the brain. Subsequent experiments to test this concept were conducted by Emil Goldmann in 1908 (3). He injected trypan blue dye intravenously in large quantities and observed that the dye stained all tissues except the brain. He also demonstrated that under these conditions the choroid plexus stained blue. When he injected a small amount of trypan blue directly into the CSF, the brain was found to be colored a deep blue. He concluded that there must be a barrier between the blood and the brain tissue that is impermeable to trypan blue. He placed the site of this barrier at the small, highly vascular tufts of choroid plexus epithelium within each of the 4 ventricles of the brain. During the 1920s, numerous experiments were performed to characterize the barrier in nervous tissue and, in 1929, Walter and his colleagues came to the conclusion that there must be several distinct barriers in the CNS: the blood-CSF barrier, the brain-CSF barrier, and the BBB (4). These observations led to our current view, which holds that there exists 2 principal barriers in the CNS: the BBB that is situated along 99% of the brain’s capillary endothelium, and the blood-CSF barrier that is located at the choroid plexus epithelium of the 4 ventricles.

Anatomical Localization of Barrier Systems in the Brain

The 2 principal barriers in the CNS, the BBB and the blood-CSF barrier, can be identified immunohistochemically in tissue sections of the CNS using different antibodies against barrier proteins. An example of such a marker is the GLUT-1 glucose transporter protein, which is present in virtually all brain capillary endothelium (5). Anatomical mapping of BBB characteristics demonstrates that there are variations in the level of BBB restriction in different areas of the CNS. The potential importance of such differences indicates microenvironmental regulation of brain microvessel permeability, which is exploited in neuroendocrine-feedback mechanisms in the CNS. An example of this can be seen at the capillaries of the hypothalamus tuber cinereum, where rich and close connections between the endothelial cells and neurons result in the formation of a fenestrated endothelial cell wall that permits free diffusion of releasing and inhibitory hormones into the circulation. The absence of the BBB at the area postrema has an opposite result, allowing the diffusion of materials from the blood to the brain tissue and, thus, providing an important area of the brain with a neuroendocrine feedback mechanism. These areas, also termed circumventricular organs, not only lack a BBB but are also deficient in expression of neurothelin, another BBB marker.

Brain capillaries that are approximately the diameter of a red blood cell (7–8 μm) are the formally designated sites of the BBB. This capillary bed has a large total cross-sectional area where the blood flow is quite low. Brain capillaries derive from the 2 carotid arteries, anteriorly, and the paired vertebral arteries, posteriorly.
These major arteries branch into smaller arteries as they go through the pia-arachnoid membrane and eventually penetrate the cortex and deeper structures and branch into capillaries. Ultrastructural tracer studies indicate that extravasation of macromolecules takes place primarily in segments of large, penetrating cortical blood vessels in both venules and arterioles (6). The capillaries entering the choroid plexus are different from those of the brain parenchyma, in that the choroidal vessels do not express tight junctions (to be discussed in more detail in the next section) and are porous (7). However, the choroid plexus epithelium forms tight junctions constituting the blood-CSF barrier (7).

Blood returning to the heart from the capillary beds flows initially into the postcapillary venules then sequentially through collecting venules and small, medium, and large veins. It was demonstrated that, in rat brain, capillary endothelium exhibits a complex network of continuous multistranded tight junctions. Continuous capillary-type tight junctions extend, although in a simpler beltlike fashion, into the endothelium of postcapillary venules, however, the endothelium of collecting veins possess widely discontinuous single- or double-stranded tight junctions associated with gap junctions. Arteries have endothelial tight junctions containing focal discontinuities associated with gap junctions (6). When in vitro BBB models are established, brain microvessels containing capillaries are trapped on small pore size filters and the brain microvascular endothelial cells (BMECs) are isolated and cultured.

**MOLECULAR ORGANIZATION OF BRAIN ENDOTHELIAL JUNCTIONS**

The specialized brain microvessel endothelial cells not only exhibit different metabolic characteristics compared to endothelial cells of other organs, but also possess well-developed intercellular tight junctions or so-called zona occludens (8).

Tight junctions provide a continuous seal around the apical regions of lateral membranes of the brain endothelial cells. The presence of tight junctions determines the tightness, and thus the permeability, of the brain endothelium, resulting in a transendothelial electrical resistance (TEER) across brain endothelium that measures between 1,000 and 1,500 Ω/cm² (9). In vitro models often monitor both intercellular tightness and paracellular diffusion of marker molecules as indices of the integrity of the endothelial monolayer. The integrity of the BBB in vitro and in vivo is drastically compromised under inflammatory conditions (10, 11).

Cellular junctions are composed of a network of intracellular and transmembrane proteins that are specific to each type of junction (Fig. 1). Two major transmembrane components of the tight junctions have now been identified: occludin (12) and the claudins (13). Occludin spans the membrane 4 times with both the amino and the carboxy termini located intracellularly and it is one of the primary sealing proteins in the tight junction (14). The other transmembrane proteins of the tight junction belong to the claudin multigene family, which is comprised of at least 20 members. Structurally, claudins contain 2 extracellular loops and 4 transmembrane domains (review, 14). Claudins are believed to be the major transmembrane proteins of tight junctions, as occludin knockout mice are still capable of forming these inter-endothelial connections (15), while claudin knockout mice are nonviable (16). With specific regard to brain endothelium, both claudin-1 and -5 were found to be expressed (17).

The carboxy-terminal cytoplasmic tail of occludin and claudins can interact with a number of cytoplasmic zonula occludens proteins (ZO), like ZO-1, ZO-2, and ZO-3 (18, 19). These proteins belong to the membrane-associated guanylate kinase protein family that can interact with other plaque domain molecules such as cingulin (20) and 7H6 antigen (21). ZO proteins can also interact with signaling molecules and cytoskeletal proteins such as cortactin and actin. Intracellular molecules like rab3, symplekin, AF-6, and 19B1 are reported to be associated with epithelial tight junctions, but their role in BMECs is unknown (review, 17).
Junctional adhesion molecules (JAMS) are transmembrane molecules that colocalize with tight junctions. JAM-1 is a member of the immunoglobulin superfamily (IgSF) (possessing 2 variable-region [V]-type Ig domains), and co-distributes with tight junction components at the apical region of the junction. The carboxy-terminal cytoplasmic tail of JAM-1 has been shown to bind guanylate kinase and/or the acidic domain of occludin, the PSD95/dlg/ZO-1 (PDZ) domain of ZO-1, and cingulin (22). Three different members of the JAM family have been identified and they are heterogeneously expressed throughout epithelial and endothelial cells (23). Recently, it has been reported that brain endothelium expresses the endothelial cell specific adhesion molecule ESAM (or 1G8 antigen), which is considered a structural equivalent of JAM. ESAM also expresses a PDZ domain, although this does not associate with ZO-1 or with ASIP/PAR3, in contrast to JAM-1 (24).

A number of other proteins are found closely associated, or colocalized, with cellular junctions. Platelet endothelial cell adhesion molecule (PECAM-1; CD31) is concentrated at the apical domain of the intercellular junction, but is not structurally associated with tight junctions (25). PECAM is also a member of the IgSF and is a single chain transmembrane glycoprotein consisting of 6 extracellular Ig-like domains and a cytoplasmic tail. PECAM-1 is involved in cell-cell adhesion through either homophilic interactions with other PECAM molecules or heterophilic interactions (26) with other proteins, such as integrin αβ3 (27). Recently, altered vascular permeability was detected in PECAM-1 deficient mice, suggesting a role for this protein in vascular composition (28). PECAM-1 has also been demonstrated to play a role in several other functions, such as the transendothelial migration of monocytes across CNS endothelia (29). Besides PECAM, the heavily-glycosylated molecule CD99 was also found to be located at the intercellular borders of human umbilical vein endothelial cells, which suggests that CD99 may be present in endothelial junctions (30). Whether this molecule is also present in endothelial cell junctions along the brain microvasculature remains to be established.

In addition to the recognized tight junction-associated proteins, other proteins could potentially affect the function or integrity of the junctional complex. For example, also found concentrated at tight junctions are several cytoplasmic signaling molecules (review, 31), the activation of which may influence BBB permeability. Two types of heterotrimeric G-proteins, Gα0 and Gαi2, localized around tight junctions were suggested to act as negative regulators for tight junction function (32). Protein kinase C has also been implicated in the regulation of tight junctions and is concentrated around them. Tight junction proteins are coupled to the actin cytoskeleton and are under the control of a number of intracellular signaling molecules.

The small GTPases RhoA, Rac, and Cdc42 are essential mediators of actin reorganization (33). Recently, it was shown that occludin is a target for this GTPase activity, and that RhoA and its downstream kinase, p160ROCK, are components of a signaling pathway leading to changes in tight junction permeability (34). Thus, the organization of tight junction-associated proteins may be directly or indirectly targeted by signaling molecules, thereby providing a means for regulating the permeability of the BBB.

THE BBB IN VITRO

Background

Nearly 3 decades have passed since the earliest descriptions of tissue culture-based paradigms of the BBB. During this time, such in vitro systems have undergone many reiterations and have been derived from a wide variety of species, but are still largely comprised of enriched populations of endothelial cells derived from fragmented segments of the brain microvasculature. As the number of reports detailing the isolation and culture of BMECs is far too extensive to be discussed here, the reader is referred to other excellent and up-to-date reviews that describe specifications regarding technique (35, 36). Briefly, in most of these protocols, brain tissue is either homogenized or pressed through filter membranes of relatively large porosity (e.g. 1,000 μm diameter) to initially fracture and disperse the parenchymal microvessels, which are then typically separated from contaminating cell types by density-dependent centrifugation and/or retention on filter meshes of graded pore size (typically ranging from 20 to 70 μm in diameter).

Resulting microvessels are then treated with collagenase to liberate BMECs. Depending on the extent of enzymatic digestion, small microvascular fragments that are partially denuded of basement membrane, or completely dissociated BMECs, are generated. The microvascular fragments or BMECs are then routinely cultured on collagen substrates, mainly either collagen I, collagen IV, or gelatin. As brain microvessels are associated with varied types of perivascular cells in situ, such as smooth muscle cells, pericytes, microglial cells, and astrocytes (37), it is not uncommon for primary cultures derived from these structures to contain components other than endothelial cells. Hence, additional procedures have been adopted to further purify BMECs, including fluorescence-activated cell sorting, cell-selective lysis, differential substrate adherence, cell-selective nutrient utilization, and paramagnetic bead separation (36, 38).

These models have allowed unfettered access to BMECs, enabling their physiologic properties and patterns of gene expression to be explored in great detail. While growth of these cells in standard culture dishes has permitted a plethora of data to be obtained, perhaps most
significant in this area of research has been the advent of commercially available filter inserts or permeable membrane supports (pioneered by Costar, Inc. under the brand name Transwell), which have enabled configuration of dual-compartment systems. In the simplest of these designs, endothelial cells are plated directly onto a filter insert that has been coated with a collagen substrate. The filter insert, in turn, is suspended within a microwell of a cluster plate. When grown in this configuration, BMECs display a physiological polarity, with the apical culture surface reflecting the luminal microvascular surface in vivo and the basolateral culture surface representing the abluminal face of the microvasculature. The dual-compartment system also allows for perivascular or parenchymal cells to be grown in apposition to the BMECs, mirroring somewhat the in vivo arrangement of the different cell types found at or near the BBB. For example, astrocytes, which project their foot processes onto brain microvessels and are thought to impart at least some of the unique properties of the BBB (39), have been cultured on the underside of the filter insert as well as on the floor of the microwell containing the suspended filter (36, 39). Recently, this system has been modified so that the BMECs are cultured atop a hydrated collagen gel containing astrocytes (40) yielding a 3-dimensional (3D) interactive co-culture that bears even more anatomical similarity to the in vivo situation. The dual-compartment arrangement, in general, is ideally suited for analyzing transendothelial transport of soluble and cellular elements, with both upper and lower compartments being accessible. It also represents the luminal (upper) and abluminal (lower) aqueous environments. A more intricate extension of co-culturing BMECs and other cell types in a 3D format has been described by Janigro and colleagues (41), who cultured BMECs on the intraluminal surface and astrocytes on the extraluminal surface of hollow fibers. In this system the endothelial surface can be subject to fluid-based shear flow to the same extent encountered within microvessels in vivo, and thus offers the opportunity to evaluate BBB properties in a near physiological microenvironment. Most recently, the same group has advanced this technology to incorporate serotonergic neurons (42), furthering the study of neurovascular interactions at the BBB.

Features of In Vitro BBB Models

Seminal features of the BBB are variably displayed by the myriad of in vitro models, and include tight junctions (with corresponding reduced paracellular permeability and high TEER value), specific transporter systems and enzymatic activities, and low numbers of vesicles. Tight junctions, which form the anatomical substrate of the BBB, are features generally acknowledged to be largely imparted by astrocyte-derived factors (for a recent review on astrocyte influence on the BBB see [39]). Extended culturing of BMECs in the absence of such factors leads to monolayer cultures exhibiting heightened paracellular flux and overall attenuated barrier properties, a situation that can partially be prevented or reversed by co-culture of BMECs with astrocytes or astrocyte-conditioned media. While identification of these astrocyte factors remains uncertain, there is evidence that angiotensin metabolites (43) and basic fibroblast growth factor (44) might be among them.

In addition to the anatomic restrictions imposed by tight junctions, the presence of both specific transport systems (many of which are carrier- or receptor-mediated and polarized in their membrane distribution) and enzymatic activities on BMECs provide for biochemical and metabolic qualities of the BBB, respectively. Both gamma glutamyltranspeptidase (γ-GT), which catalyzes the transfer of the γ-glutamyl residue of the tripeptide glutathione to amino acids and has been postulated to function as an amino acid transport system (45), and alkaline phosphatase have enriched expression within endothelial cells of the brain microvasculature in situ (46). They have also been cited as salient markers of a maintained BBB phenotype in cultured BMECs (47). Some other transport systems present in the BBB and detected in cultured BMECs include those for amino acids, peptides, hexoses, monocarboxylic acids, organic cations, nucleosides, vitamins, and various xenobiotics (review, 48). Many of these transporters facilitate the transcellular passage of specific solutes that are both necessary for CNS homeostasis and are unable to be synthesized de novo within this compartment. Other transporters (e.g. P-glycoprotein and the multidrug resistance-associated proteins) serve as efflux proteins that preclude the entry of potentially toxic metabolites from the blood and/or effect the export of CNS-derived substances for targeted action in the periphery. Enzymatic activities that purportedly function in BBB capacity include monamine oxidase and catechol-O-methyl-transferase, which may act to lessen the degree of transport of amine neurotransmitters and/or their precursor amino acids, as well as potentially toxic xenobiotics, into the CNS.

Immune Responses of In Vitro BBB Models

Like their in vivo counterparts, cultured BMECs can be induced to express various mediators of inflammation. For example, expression of the adhesion molecules intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin have been shown to be modulated by different treatments associated with leukocyte extravasation in vivo. A consistent finding by numerous independent laboratories is the upregulated expression of these 3 adhesion molecules on cultured BMECs treated with lipopolysaccharide (LPS) and proinflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and interferon
gamma (IFN-\(\gamma\)) (29, 49–52). Cytokine-stimulated ICAM-1 expression on cultured BMECs has further been shown to display polarized membrane expression (53).

The fact that cultured BMECs display sensitivity to immune signals has prompted their use in investigating mechanisms regulating CNS inflammation. The dual-chamber, filter format in vitro model has been most vigorously exploited in investigations of leukocyte migration across the BBB—particularly when assaying the effects of chemoattractants. A variety of means of quantifying the extent of leukocyte migration across BMECs in this configuration have been utilized, such as counting radio-labeled cells that have entered the lower compartment (54, 55), and using conventional bright-field microscopy to enumerate transmigrated cells that have completely traversed the filter and adhered to a coverslip (56). Most recently, confocal microscopy combined with 3D image reconstruction has been used to both qualitatively and quantitatively evaluate leukocyte transendothelial migration across a 3D BBB model grown on a hydrated collagen matrix (40).

Modulation of Permeability

By far, the most extensive use of in vitro BBB models has been to assay permeability and drug transport. Though the vastness of this topic precludes it from being discussed here in detail (see reference 60), suffice it to say that efforts have largely focused on whether particular stimuli cause disruption of tight junction integrity. For example, the proinflammatory cytokines IFN-\(\gamma\), TNF-\(\alpha\), IL-1(\(\alpha\) and \(\beta\)), and IL-6 have all been observed to cause either increased flux of hydrophilic macromolecules or diminished TEER (57, 58) across cultured BMECs, possibly through modulation of cyclooxygenase and plasma membrane–associated tyrosine phosphatase activities. In contrast, the antiinflammatory cytokine IFN-\(\beta\) was reported to counteract proinflammatory mediator-induced translocation of the tight junction–associated molecules ZO-1 and-2 in these cells (59), thus preserving the integrity of the BBB in this model.

Aside from direct application of proinflammatory cytokines, hypoxic conditions and the generation of reactive oxygen species have also been shown to cause perturbation of tight junction complexes and heightened para-cellular permeability across cultured BMECs (60). On the other hand, nitric oxide affords protection against hypoxia/reoxygenation-mediated injury of the BBB in vitro, possibly by scavenging reactive oxygen species (61).

Proteins of human immunodeficiency virus-1 (HIV-1) have also been shown to cause disruption of BBB integrity in vitro, thereby highlighting the means by which this pathogen might invade the CNS and ultimately cause HIV-1–associated dementia. Specifically, HIV-1 envelope protein gp120 has been shown to both alter the morphology and elevate the permeability of BMEC monolayer cultures, the latter feature being antagonized by spantide (a substance P antagonist) as well as anti-substance P antibody (62). The HIV Tat protein, known to be secreted extracellularly, has been reported to stimulate IL-8 synthesis by cultured BMECs (63), a response that could signal loss of barrier integrity.

Caveats of In Vitro BBB Models

Notwithstanding the considerable advancements made, one would be remiss not to view such systems with a healthy degree of skepticism—particularly regarding their use in investigating neuroimmune processes. In this regard, it is important to refer back to the primary descriptions of the cellular site of the BBB and to keep in mind the physiological context in which these were made. Specifically, the BBB has been described as residing at the level of brain capillaries (64, 65), which demonstrate significantly reduced solute transport due to the presence of high-resistance tight junctions and specialized transport systems. However, whether such barrier properties are similarly manifest in postcapillary venules, where leukocyte extravasation is primarily thought to occur, remains a matter of at least some conjecture. That significant morphological and biochemical distinctions have been described, even between endothelial cells from successive segments of the vascular tree (66), legitimates such concern and underscores the cynicism regarding use of other than brain microvascular–derived endothelial cells to faithfully model the BBB in vitro (67, 68). It is thus important to remember that BMEC cultures derived from “microvascular fragments,” comprised of a collection of capillaries, arterioles, and venules, are likely to generate, at best, mosaics of different endothelial types. Also, cultures obtained predominantly from larger microvessels might not be able to display the barrier properties attributed to capillaries, while cultures generated specifically from capillaries might not properly support leukocyte adhesion and/or transendothelial migration.

Aside from these matters regarding inherent endothelial differences, there is the often neglected—but nonetheless significant—issue relating hemodynamics and blood flow to solute and cell transport across BBB models. Conventional transport assays performed with Tranwell or Transwell-type, dual-chamber filter formats are considered to be “static,” as they are not subject to flow or other than gravitational forces. However, awareness that flow–induced shear stress across the surface of monolayers of peripheral vascular endothelial cells modifies both leukocyte diapedesis (69) and solute permeability (70) has naturally raised speculation that flow conditions might be similarly important in BMEC function. Indeed, Stanness et al (41), in describing their tridimensional BBB model cultivated on pronectin-coated hollow fibers, argued that “endothelial cells grown under flow develop greater differentiation than after conventional culture.”
In vitro models are clearly imperfect in reflecting the entire complexity of the BBB. Despite this handicap, however, their use has allowed specific BBB properties to be evaluated that otherwise would not have been amenable to experimental scrutiny. It is expected that with continual refinement, these model systems will come ever closer to recapitulating the in vivo scenario.

**BBB AND IMMUNE PRIVILEGE IN THE CNS**

**Is There Antigen Presentation at the BBB?**

Antigen presentation is a crucial event for activating naïve T cells (for more details, see review [71]). Throughout this process, naïve T cells recognize their specific antigen presented in the context of MHC class I or class II molecules on antigen presenting cells (APCs). This is considered to be the first signal for naïve T cell activation. In order to complete the pathway of naïve T cell activation, secondary signals provided in the form of costimulatory molecules expressed on APCs are also necessary. This activation results in changes in gene expression that drive T cell proliferation, differentiation, and effector function in T cells. However, this activation requires a stable zone of contact formation between the T cell and the APC. This structure is known as the immunological synapse, where there is a distinct segregation of antigen receptors, adhesion, and signaling molecules (72). It has been demonstrated that several hours of T cell-APC contact were required to induce the proliferation of naïve T cells in response to antigen pulsed APC (73). This long-term naïve T cell and APC interaction is facilitated in specialized tissues known as the secondary lymphoid tissues. The CNS has been considered an immunologically privileged tissue, where naïve T cell activation could not take place due to the absence of APCs and/or insufficient expression of co-stimulatory molecules. However, in light of the potential importance of this problem, many laboratories have revisited the question of antigen presentation at the BBB. If BMECs are capable of presenting antigen in situ to naïve T cells, this could potentially be important in the initiation of autoimmune diseases of the CNS. The conclusions of these studies are quite divergent. Expression of MHC class II molecules on BMECs in vitro, which is a prerequisite for antigen presentation by APCs to T helper cells, has been demonstrated by several laboratories (74). Co-stimulatory molecule expression by in vitro cultures of BMECs has also been demonstrated (75). The process and presentation of myelin basic protein (MBP) antigen to T cells by in vitro cultures of murine BMECs was also reported (76). Others have shown that both murine and guinea pig BMECs can act as APCs for presentation of MBP to CD4+ T cells, but not for presentation of purified digested or whole ovalbumin (77). Bourdoulous et al (78) have demonstrated that IFN-γ-activated BMECs, expressing MHC class II molecules, are able to stimulate proliferation of a syngeneic CD4+ T cell line in the presence of MBP. In contrast, Pryce et al (79) have demonstrated that BMECs are poor stimulators of T cell proliferation, despite these 2 cell types being able to engage in antigen-specific interactions in vitro.

The differential activation of T helper (Th) 1 and Th2 CD4+ T cell clones was also suggested as a means by which the BBB acts to maintain immune privilege of the CNS (80). In these experiments, in vitro cultures of BMECs preferred Th2 cell clones, as reflected by cell proliferation and production of IL-4 by BMEC-activated Th2 clones (80). As Th1 cells are the harmful effector cells in CNS autoimmunity, the downregulating activity of BMECs on Th1 function could potentially minimize inflammation-associated disruption of BBB integrity.

Another point to consider is that, aside from BMECs, other cell types that are in the vicinity of the BBB can also present antigens to T cells. One of these cell types might include the perivascular microglia/macrophages. The role of these cells in shaping CNS autoimmunity has been suggested in bone marrow chimeric animals by Hickey and Kimura (81). These cells are capable of phagocytosis and can constitutively express high levels of class II antigen (82). Furthermore, using irradiated bone marrow chimeras in CD45-congenic rats, highly purified populations of microglia and nonmicroglial, CNS-associated macrophages (CD45[high]CD11b/c+) have been isolated from the adult CNS. A minority of this CD45[high]CD11b/c+ macrophage population serves as effective APCs, as determined by these cells participating in experimental autoimmune encephalomyelitis (EAE) and activating CD4+, MBP-reactive T cells (83). Depletion of systemic macrophages results in elimination of the perivascular microglia/macrophage population and also leads to inhibition of the onset of EAE (84). Due both to their close proximity to BMECs and ability to induce immune responses, perivascular microglia/macrophages must be considered an important, if not the most critical, APC at the BBB.

Another mechanism by which the BBB may control immunity in the CNS is by regulating the migration of leukocytes across BMECs. Work from various laboratories has heightened awareness of the means by which T cells, monocyes, and neutrophils breach the normally restrictive BBB, invade the CNS parenchyma, and ultimately cause neurologic disease.

**T Cell Migration**

In general, T cell migration across the BBB is a highly regulated process involving adhesion molecules, chemokines, cytokines, and matrix metalloproteases. From a T
cell point of view, the brain is considered a tertiary immune tissue that is outside the line of normal T cell circulation. Under normal conditions, T cells recirculate between the blood and secondary lymphoid tissues. This recirculation is regulated by a group of adhesion molecules that are expressed on high endothelial venules of the secondary lymphoid organs. Upon T cell activation, a set of newly expressed adhesion molecules directs T cells to inflamed tissue. The generally accepted theory is that activated T cells, independent of their antigen specificity, can cross the BBB, but only those with specificity for CNS antigens will be retained in the brain parenchyma (review, 85). Leukocyte transendothelial migration across the BBB, like that which occurs in the periphery, is a multistep process involving the following sequential activities: 1) tethering of leukocytes to endothelial cells by interactions between selectin and carbohydrate adhesion molecules; 2) activation of leukocytes by chemokine stimulation of G-protein linked receptors; and 3) arrest and transmigration of leukocytes, mediated by integrin/immunoglobulin adhesion molecule interactions (Fig. 2).

T cell migration across nonactivated brain microvessel endothelial monolayers has been shown to be mediated by both LFA-1/ICAM-1 interactions and heterotrimeric G-protein signaling pathways in BMECs (86), while VLA-4/VCAM-1 interactions have additionally been implicated in migration across cytokine-stimulated BMECs (87). Following transendothelial migration, there is a noticeable shift in protein expression in T cells that is characterized by downregulation of $\alpha_4\beta_1$ integrins (VLA-4) and upregulation of matrix metalloproteinases (MMP). MMP upregulation in activated T cells enables the degradation of extracellular matrix components in order to facilitate T cell migration into the CNS parenchyma (88).

Although several laboratories have addressed the importance of T cell migration across the BBB, the mechanism of this event is still unclear. Greater understanding of this process would highlight potentially novel therapeutic targets to control invasion of these cells into the CNS. While there is copious information on the importance of adhesion molecules and chemokines in the recruitment phase of CNS inflammation, the adhesion molecules and chemokines that govern the initial antigen-specific T cell entry into the CNS have yet to be defined. A major obstacle in studying the T cell-mediated autoimmune process is the technical difficulty of studying these T cells in vivo. Specifically, the frequency of antigen-specific T cells represented in the total immune repertoire is below the detection limits of standard T cell assays. However, novel technical advances, such as transgenic technology, real time PCR, and ELISPOT assay, allow for the study of these rare T cells and their requirements for localization and function in the CNS. As an example of applying these new technologies, T cell receptor (TCR) transgenic mice were employed (as a source of monoclonal T cells with known antigen specificity to pigeon cytochrome c (PCC) residues 88–104) to characterize adhesion molecules participating in the early recruitment of these cells into the brain (89, 90). By monitoring the accumulation of a small number of antigen-specific T cells in the CSF through the use of monoclonal antibodies to their specific T cell receptor (V$\alpha$11 or V$\beta$3), it was demonstrated that intraventricular delivery of PCC antigen results in a strong PECAM-1-dependent antigen-specific T cell accumulation in the CSF(89). It was also suggested that early migration of CD4+ T cells into the CNS occurs independent of the lymphocyte integrin VLA-4 and endothelial VCAM, but does require increased surface expression of endothelial P selectin (91).

T cell activation and migration across the BBB is followed by recruitment of nonspecific inflammatory cells, such as monocytes. Infiltration by monocytes significantly contributes to amplification of inflammatory reactions in the CNS and is a major effector of tissue damage.

**Monocyte Migration**

Molecular mechanisms governing monocyte transmigration through brain microvascular endothelium are not as well known as those regulating lymphocyte adhesion and migration. The $\beta_2$ integrin complement receptor-3 (CR-3 or Mac-1), which is highly expressed on monocytes, has been shown to mediate monocyte migration across peripheral endothelial monolayers (92). In EAE, anti-CR3 antibodies have been shown to significantly delay the onset and diminish the severity of clinical signs of EAE, even when injections are given at the first appearance of clinical signs (93). However, in these examples, disease palliation was not accompanied by reduced cellular infiltration, implying that other CR3-mediated cellular functions are involved in EAE, such as myelin phagocytosis and reactive oxygen species production (93, 94). Studies on peripheral endothelium have suggested that the interaction of VLA-4 with VCAM-1 is required for firm adhesion to, and subsequent migration of, monocytes through the peripheral endothelial cell barrier (92). Similarly, an important role for the VLA-4/VCAM-1 pathway has been demonstrated for monocyte migration across BMECs (29). An additional or alternative pathway, employing the integrin $\alpha_5\beta_3$, has also been implicated in the infiltration of monocytes into the CNS. This integrin binds to VCAM-1, and treatment with antibodies directed against $\alpha_5$ reduced macrophage infiltration at the lesion site of spinal cord-injured rats (95). Whether this integrin is involved in monocyte recruitment into the CNS during EAE remains to be established. Together, these studies suggest that VCAM-1 may be a major candidate molecule involved in the infiltration of monocytes to the CNS. However, other IgSF members may also participate in the transendothelial migration of...
Fig. 2. Molecular mechanism of leukocyte trans-endothelial migration across the BBB. The first step in leukocyte transmigration involves initial weak adhesion that is followed by reversible rolling on the BBB surface and is regulated by interaction between selectins and their glycosylated ligands. The second step in the process of transmigration involves the stimulation or "triggering" of the leukocytes by chemokines and their receptors. The third step in this process involves integrin adhesion molecule activation on leukocytes, leading to an increased adhesion of these cells to members of immunoglobulin superfamily expressed on endothelial cells. The final step in the transmigration process is the diapedesis through the vessel wall. Molecules currently known to be present at the junction between brain capillary endothelial cells involve CD31, JAM, and ESAM. (Please see text for further details).

Monocytes. For instance, PECAM was shown to be essential in monocyte migration across human umbilical vein endothelium, particularly in the final step of the extravasation process (96), although the direct role of this adhesion molecule in transendothelial migration across BBB has not been reported. Also, other IgSF members have a part in monocyte infiltration into the CNS. For example, it was recently reported that the widely expressed CD47, also known as integrin-associated protein, mediates the final postadhesion step of monocyte migration into the CNS (97). CD47 is a member of the IgSF and contains a single extracellular Ig-like domain, 5 transmembrane segments, and a short cytoplasmic tail. CD47 can interact with thrombospondin and also with its monocytic ligand signal regulatory protein-α (SIRPα), another member of the IgSF. SIRPα, also called SHPS-1 or macrophage fusion receptor, is exclusively expressed on myeloid cells and neurons (97). SIRPα interaction with CD47 was suggested to contribute to the recruitment of monocytes into tissue during neuroinflammatory disease (97). Moreover, this process is mediated by CD47-triggered signaling events in BMECs that in turn cause a cytoskeletal rearrangement that facilitates monocyte migration (97).

Junctional proteins like JAM also mediate monocyte migration across brain endothelium (98). Monocyte migration into murine brain during experimental meningitis is inhibited by using α-JAM monoclonal antibodies (mAbs), and these antibodies have similarly been shown to antagonize monocyte migration across cultured peripheral vascular endothelial cells (98). It is postulated that JAM guides monocytes through endothelial cell junctions, since it is expressed at the tight junction (98). An essential role for another junctional protein, CD99, was recently established for monocyte migration across perivascular endothelium. In particular, CD99 appears to act at the level of diapedesis through the tight junction (30). It is clear that monocyte extravasation into the brain is controlled by a number of adhesion molecule interactions and signaling events, and that many facets of monocyte migration across the BBB remain to be identified.

Monocytes recruited from the blood into the CNS differentiate into macrophages and contribute to neuroinflammatory processes by producing a wide range of mediators that stimulate inflammatory cascades. Monocyte-derived inflammatory products such as proinflammatory cytokines, reactive oxygen species, or nitric oxide can further recruit leukocytes into the CNS (99).
Conversely, a neurotrophic factor like nerve growth factor may act as an anti-inflammatory agent, since it limits the transendothelial migration of monocytes across BMECs (100).

Current treatments of neuroinflammatory diseases, like multiple sclerosis (MS), aim at dampening the inflammatory cascades in the CNS. For instance, IFN-β treatment leads to the reduction of new MS lesions as assessed by MRI (101). Reduced cellular infiltration may be the result of attenuated expression of adhesion molecules on the brain endothelium, as has been reported in EAE animals and in brain endothelial cells in vitro (29). Cannabis, now under consideration as a potential therapeutic for MS patients, reduced spasticity and clinical signs in EAE (102) and may influence the migration of monocytes across the BBB. The psychoactive form of cannabis, Δ or δ-9-tetrahydrocannabinol, has been shown to influence macrophage functions, including phagocytosis, antigen presentation, and migratory capacity across endothelium (103). Lovastatin, a potent inhibitor of 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, a key enzyme in the cholesterol biosynthesis pathway, may also be a promising new therapeutic agent. Lovastatin was shown to suppress the clinical course of EAE by inhibiting monocyte infiltration into the CNS (104). Even sunlight influences monocyte activation through a bioactive metabolite of vitamin D, 1,25-dihydroxyvitamin D3. Interestingly, treatment of EAE animals with this bioactive hormone was reported to decrease macrophage accumulation into the CNS (105).

Exploiting the ability of the dual-chamber model to allow restricted access of solutes to either the apical or basolateral surface, Andjelkovic et al (40) showed that monocyte chemoattractant protein-1 (MCP-1) was only able to stimulate monocyte transendothelial migration across cultured human BMECs when exposed to the basolateral endothelial surface. This finding is consistent with the in vivo situation wherein perivascular astrocytes are considered major sites of MCP-1 production. Describing a similar functional polarity, Giri et al (106, 107) reported that β-amyloid peptide (Aβ) interaction, specifically with the basolateral surface of cultured human BMECs derived from patients afflicted with Alzheimer disease (AD), caused enhanced monocyte transendothelial migration. This has prompted these authors to postulate that perivascular accumulation of Aβ in AD and other related cerebral vascular disorders may signal the entry of monocytes and lead to increased numbers of potentially harmful, monocyte-derived microglia.

**Neutrophil Migration**

Lastly, compared to that of T cells and monocytes, transendothelial migration of neutrophils across BBB models has not been as intensively studied. However, vascular endothelial growth factor (VEGF), which is strongly induced during hypoxia and is accompanied during this state by an infiltration of neutrophils into the brain, has recently been observed to stimulate neutrophil transendothelial migration across cultured BMECs (108). Such migration was suggested to be dependent upon VEGF-stimulated expression of the largely neutrophil-targeting chemokine IL-8.

Full disclosure of the complex of players mediating leukocyte migration into the CNS will be of great importance for the development of novel therapeutic strategies to effectively treat various neuroinflammatory conditions.

**Conclusion**

In summary, the BBB plays a very important role in maintaining the immune-privileged status of the CNS. In pathological conditions, the integrity of the BBB can be breached and the inflamed brain endothelial cells can play an important role in regulating the migration of leukocytes into the CNS. Further understanding of the unique microenvironment that influences BBB characteristics in the CNS will lead to novel therapies to manipulate CNS inflammatory diseases.

Please note: Due to space considerations and the wide breadth of the review, the list of references had to be curtailed and the authors regret not being able to cite all.

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