Immune Cell Trafficking across the Different CNS Barriers

Subjects: Immunology

Contributor: Carla Rodriguez-Mogeda , Sabela Rodríguez-Lorenzo , Jiji Attia , Jack van Horssen , Maarten E. Witte , Helga E. de Vries

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) known for the manifestation of demyelinated lesions throughout the CNS, leading to neurodegeneration. To date, not all pathological mechanisms that drive disease progression are known, but the clinical benefits of anti-CD20 therapies have put B cells in the spotlight of MS research.

multiple sclerosis B cells blood–brain barrier

1. Multiple Sclerosis

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) with a heterogeneous clinical presentation. MS most often starts in young adults; young women are particularly more prone to develop the disease [1]. MS is characterized by the manifestation of demyelinated lesions (or plaques) throughout the CNS, which can be visualized with magnetic resonance imaging (MRI) [1][2]. The main pathological hallmarks of MS are widespread immune cell infiltration, loss of myelin, glial activation, neuro-axonal degeneration, and blood–brain barrier (BBB) dysfunction. Generally, MS is categorized into three clinical subtypes: relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), and primary progressive MS (PPMS) [3]. About 85–95% of MS cases start as RRMS [4], where episodes of neurological dysfunction are followed by periods of remission. During a relapse, typical clinical symptoms are optic neuritis, sensory disturbances, motor impairment, and cognitive defects. After 10–20 years, approximately 80% of RRMS cases develop SPMS. In SPMS, neurological dysfunction can worsen without periods of remission. Moreover, 5–15% of MS cases develop PPMS where neurological deficits progress gradually without remission from the onset of the disease [5].

MS is thought to be initiated by autoreactive $CD8^+$ and $CD4^+$ T cells and B cells that infiltrate the brain and spinal cord by crossing distinct CNS barriers [5]. These leukocytes generate abnormal responses against CNS autoantigens, such as myelin proteins [6]. This inflammation and the damage to the myelin layer enwrapping axons it causes leads to demyelination and neuronal dysfunction and loss [7]. Histologically, lesions can be differentiated by their localization in white matter (WM) or grey matter (GM) and by their inflammatory status. Generally, early WM lesions are more inflammatory than GM lesions and have highly inflamed endothelial cells, which facilitate the migration of peripheral immune cells over the BBB into the CNS [8]. These active WM lesions, which are predominant in RRMS, are characterized by massive infiltration and accumulation of blood-derived immune cells. In contrast, the histological hallmarks that dominate progressive MS are extensive cortical pathology (brain atrophy,

widespread demyelination, synapsis loss, etc.) with less inflammatory lesions and the less apparent breakdown of the BBB [9], and chronic active WM lesions that slowly expand [10][11].

There are various experimental animal models to study MS, of which, experimental autoimmune encephalitis (EAE) is the most commonly used. In EAE, the animals are immunized with CNS antigens, such as myelin proteins (e.g., myelin basic protein (MBP) or myelin oligodendrocyte glycoprotein (MOG)), thereby inducing an autoimmune response [12]. Most of the knowledge about the breakdown of the CNS barriers and the subsequent infiltration of peripheral immune cells in MS comes from EAE experiments or in vitro experiments using different cell lines that mimic the different CNS barriers.

The unknown cause, the complexity of MS, and the consequent lack of research models that accurately mimic the full scale of the disease have made it difficult to find a cure or a preventive treatment. Current disease-modifying treatments (DMT) are mostly immunosuppressive and/or immunomodulatory, which target inflammation and reduce the frequency and severity of the new inflammatory lesions. Therefore, these drugs are especially effective during RRMS. Despite the development of various novel DMTs in the last decades, limited therapeutic options are nowadays available to halt disease progression in the chronic phase of the disease. Recently, a monoclonal antibody against B cells (ocrelizumab) showed clinical benefits in a subset of PPMS patients, highlighting the role of B cells in the pathogenesis of MS [13].

The first indication that B cells contribute to MS was the detection of oligoclonal bands (OCB) in the cerebrospinal fluid (CSF) and, consequently, early studies on the role of B cells in MS focused solely on antibodies [14]. More recently, studies with anti-CD20 therapies have shown that B cell depletion significantly decreases disease activity without any changes in the OCB pattern or levels of immunoglobulins [2]. Thus, there is growing evidence that the role of B cells in MS extends well beyond the secretion of antibodies. Lately, B cell research in MS has shifted more towards their role in T cell and glial cell activation, cytokine secretion, and antigen presentation [15]. In the brains of MS patients, B cells have been found to accumulate, particularly in the perivascular and subarachnoid spaces of SPMS patients [16][17], which correlates well with local demyelination and neurodegeneration [18]. Hence, migration of B cells over the CNS barriers appears to be a crucial step in MS pathology. Studying the route of B cell entry into the brain and how the inflamed CNS milieu can sustain B cell survival is a crucial next step to better understanding the progression of this disease.

2. Immune Cell Trafficking across the Different CNS Barriers

Limited numbers of peripheral immune cells can migrate into CNS compartments during homeostasis to act as sentinels in the surveillance of the CNS, whereas under neuroinflammatory conditions, a multitude of cells crosses the CNS barriers. Extravasation of cells from the blood into the tissue is a multi-step process. In general, the immune cell is apprehended from the bloodstream by selectins (e.g., E-selectin, P-selectin) located on endothelial cells, which interact with leukocyte glycoproteins, such as P-selectin glycoprotein ligand-1 (PSGL-1). This weak and transient interaction results in the tethering and rolling of the immune cell along the vessel wall [19]. Subsequent firm adhesion of the leukocyte to the inflamed endothelial cells halts the immune cell, which is

mediated by cellular adhesion molecules (CAMs), such as immunoglobulin family members, cadherins, or integrins. For example, leukocytes express integrins, such as lymphocyte function-associated 1 (LFA-1) or very late activation antigen-4 (also known as α4β1 or VLA-4) that respectively bind to endothelial vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) [19]. Other examples of endothelial CAMs are the melanoma cell adhesion molecule (MCAM) and activated leukocyte cell adhesion molecule (ALCAM) [20]. Chemokines are also essential regulators of the transendothelial migration of immune cells since they enhance the affinity of leukocyte integrins to bind strongly to endothelial CAMs [21][22]. Following this firm adhesion, leukocytes can cross the endothelium via paracellular or transcellular migration [23].

Three types of CNS barriers have been described through which immune cells can infiltrate the brain. A large bundle of research focused on the BBB, while fewer studies paid attention to the blood–meningeal barrier (BMB) and the blood–CSF barrier (BCSFB) in the choroid plexus (**Figure 1**). Together, these barriers protect the brain from peripheral damage and control the movement of molecules and cells from the periphery into the CNS. Although the three barriers share some features, their localization and anatomy can help understand how they differentially shape the CNS compartments.

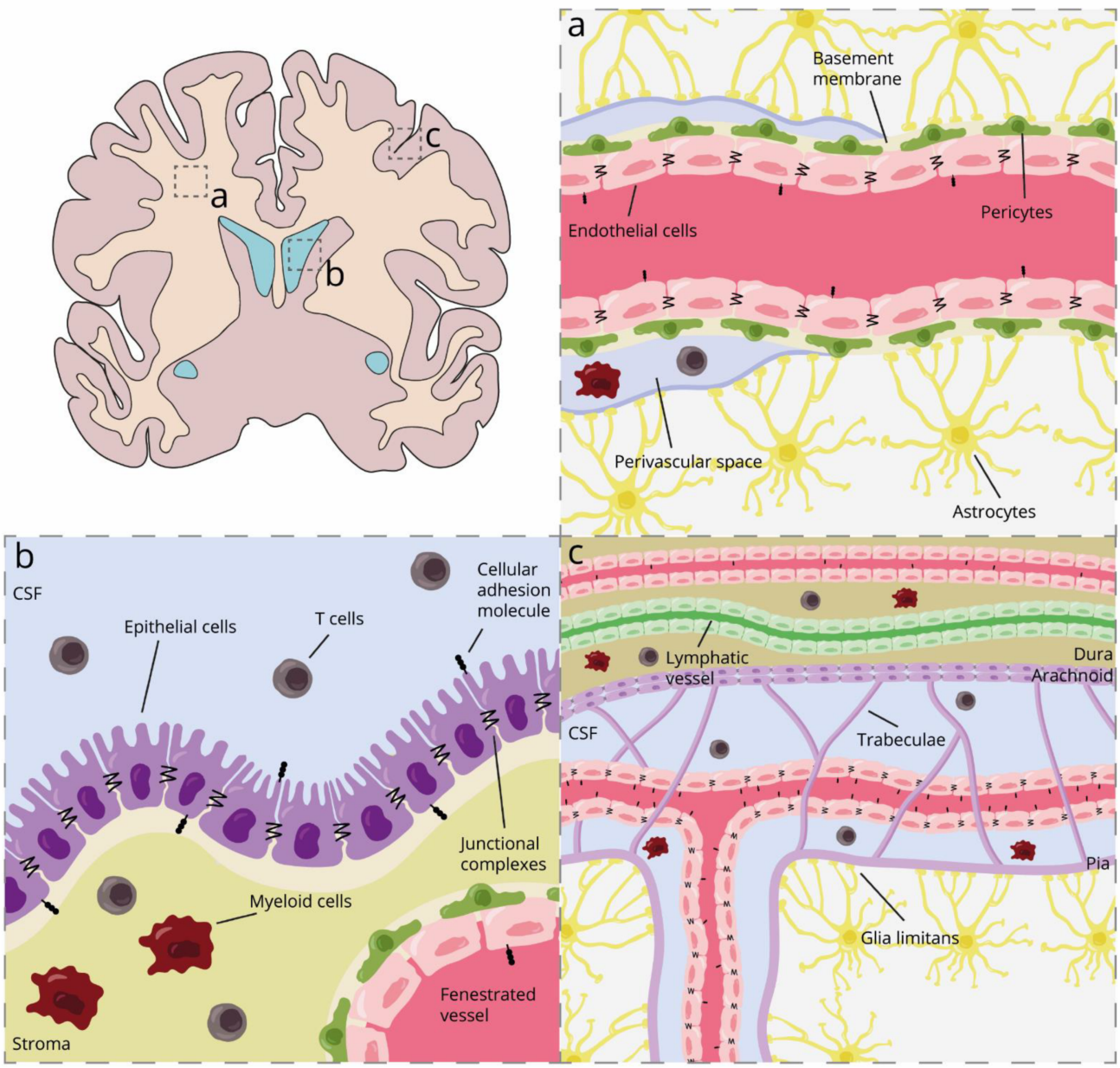

Figure 1. Schematic representation of the different CNS barriers and their localization in health. (**a**) Blood–brain barrier; (**b**) blood–CSF barrier in the choroid plexus; and (**c**) blood–meningeal barrier. CSF, cerebrospinal fluid.

2.1. Blood–Brain Barrier in Health and MS

The microvasculature of the brain parenchyma has several unique properties, creating a tightly regulated barrier known as the BBB. The BBB consists of unique continuous non-fenestrated cerebral endothelial cells (BECs) tightly connected by tight (TJ) and adherens junction (AJ) complexes. The barrier function of the BECs is further supported by the interaction with astrocyte endfeet, pericytes, neighboring microglia, and a continuous basement membrane. Altogether, this structure is called the neurovascular unit (**Figure 1**a) [24][25].

The junctional complexes TJ and AJ are connected to the cytoskeleton and can alter the morphology of the endothelium. These dynamic structures change depending on the local microenvironment and can also activate

intracellular signaling pathways. Well-known TJ proteins of the brain endothelium are occludin, claudins, and junctional adhesion molecules (JAMs), while vascular endothelial cadherin (VE-cadherin) and platelet endothelial cell adhesion molecule 1 (PECAM-1 or CD31) are part of the AJs [24][25][26][27][28]. In addition, these complexes regulate the polarization of BECs by differentiating between apical and basal domains. Moreover, BECs express specific polarized transporters that allow the active transport of essential molecules and waste products [24][25][26][27][28]. Pericytes are contractile cells that regulate cerebral blood flow by interacting physically with BECs. In the neurovascular unit, pericytes are situated in between the BECs and astrocyte endfeet (**Figure 1**a) [29]. The astrocyte endfeet interact with the brain endothelium creating the glia limitans, which regulate the blood (and ion) flow and volume that passes through the capillaries [30][31]. Another component of this unit is the basement membrane. Astrocytes and BEC can secrete fibrous proteins or proteoglycans and generate this extracellular matrix, which maintains the structure of the neurovascular unit. In addition, it modulates BBB function and permeability since matrix proteins can influence the expression of TJs [32]. Finally, microglia are the CNS-resident immune cells involved in the homeostasis and protection of the CNS against pathogens or damage. Microglial processes can take over the coverage of pericytes or astrocytes on BECS and physically interact with the endothelium [33][34].

BBB dysfunction is one of the early key hallmarks of MS pathogenesis. Peripheral immune cells and CNS-resident cells (e.g., microglia or astrocytes) induce inflammation in BECs by secreting pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) or interferon-γ (INF-γ) [35]. This neuroinflammation associates with several molecular changes. (1) Inflamed BECs increase the presentation and secretion of chemokines and (2) enhance the expression of CAMs, which promote leukocyte transendothelial migration, also called diapedesis [20]. (3) This inflammatory state further modifies the structure and location of TJ and AJ, leading to a mesenchymal state and thereby increasing BBB permeability [36]. Consequently, leukocytes, such as B and T cells, cross the inflamed endothelium more easily. Immune cells can then stay in the perivascular spaces to create the perivascular immune aggregates characteristics of MS, but also later cross the glia limitans to infiltrate the brain parenchyma. Furthermore, in MS, these perivascular spaces are enlarged [37][38]. Once within the CNS, activated lymphocytes propagate a cascade of neuroinflammatory reactions leading to disease onset and progression and associated clinical symptoms [39][40].

2.2. Blood–CSF Barrier in Health and MS

The BCSFB is located in the choroid plexus in each of the brain ventricles. The choroid plexus is responsible for producing the CSF, supplying nutrients to the brain, clearing toxic molecules, and, thereby, maintaining brain homeostasis. It is a highly vascularized tissue and, consequently, a door for peripheral immune cell migration into the CSF. The architecture of the choroid plexus consists of a vascularized stroma surrounded by a layer of cuboidal epithelial cells (**Figure 1**b). The choroid plexus epithelium presents apical villi to increase the flux of solutes and water from the blood to the CSF. Moreover, apical motile cilia contribute to the CSF flow throughout the ventricular system [41]. The CSF contains compounds that help the CNS to develop and function normally, including water, ions, glucose, growth factors, amino acids, lipids, and hormones, among others [42]. Most of these components are imported from the blood or produced by the choroid plexus epithelium [41].

The choroid plexus stroma is abundantly populated by immune cells, mostly antigen-presenting cells [43][44], which guarantee immunosurveillance for the maintenance of a healthy brain. In contrast to most BECs, the choroid plexus endothelial cells are fenestrated and have higher constitutive expressions of ICAM-1 and P- and E-selectin [45][46] and lower amounts of TJ (**Figure 1**b) [47]. Hence, the choroid plexus capillaries allow easy immune trafficking to the choroid plexus stroma. Instead, the blood–CSF barrier is mainly formed by the choroid plexus epithelial cells. As a highly polarized barrier, the basal side of the epithelium interacts with the stroma, while the CSF-facing apical side is tightly connected with TJ, which hinders the migration of immune cells.

As a model system, the human cell line of choroid plexus epithelial cells (HIBCPP) is frequently used. While the HIBCPP cell line expresses ICAM-1, researchers found no evidence for the expressions of ICAM-1 or VCAM-1 in choroid plexus epithelial cells in healthy humans, only on the endothelial cells of the choroid plexus [43][45][48]. This suggests that other adhesion molecules might be involved in the transepithelial migration to guarantee immunosurveillance in healthy humans. Of note, the HIBCPP cell line comes from a human choroid plexus papilloma and the results obtained from this research model should be carefully considered [48]. Interestingly, both ICAM-1 and VCAM-1 are constitutively expressed on choroid plexus epithelial cells in mice, but not on the fenestrated endothelial cells [49]. Furthermore, the localization of ICAM-1 on the apical side of the epithelium suggests that it can be involved in cellular migration from the ventricular CSF to the stroma, as was shown in mice [50]. This process may be related to the reactivation of T cells and CSF monitoring and is thought to occur at a much lower rate than the infiltration into the CNS via the BBB [50][51]. Hence, the choroid plexus epithelium might allow a bi-directional migration of immune cells between blood and CSF in health.

In the early stages of MS, the choroid plexus has been described as an immunological niche for T cell activation in response to peripheral inflammation [51]. In EAE, pathogenic Th17 cells infiltrate through the choroid plexus via upregulation of CCR6 [52]. At the same time, choroid plexus epithelial cells in mice and humans express CCL20, a chemotactic signal for pathogenic Th17 cells [52]. Accordingly, human Th17 cells preferentially migrate through the cell line HIBCPP compared to other $CD4^+$ T cell subsets [50]. Additionally, in EAE, there is increased expression of ICAM-1 in the epithelium of the choroid plexus [53]. Hence, it might be an important route of immune cell migration in MS. Furthermore, patients with MS had enlarged choroid plexi than the controls [54], which could be explained by the accumulation of leukocytes and/or edema [44].

In progressive phases of MS, the choroid plexus expresses low levels of the TJ claudin-3 compared to choroid plexus tissue from control donors [55], inflammation becomes chronic, and unknown triggers (such as hypoxia) may sustain the upregulation of adhesion molecules and chemokines [43][56]. Interestingly, B cells and plasma cells are virtually absent in the choroid plexus from control and MS donors, suggesting that the BCSFB is not the preferred route of entry for those cells in MS [43]. Furthermore, the choroid plexus may become an “educational gate” [44][57][58] and shift to selective recruitment of suppressive immune cells as seen with a specific accumulation of $CD56^{bright}$ NK cells in MS [57].

2.3. Blood–Meningeal Barrier in Health and MS

The meninges consist of three layers of connective tissue that wrap the brain parenchyma: pia mater, arachnoid mater, and dura mater (**Figure 1**c) [58]. The structural role of the meninges is to structurally protect the CNS by anchoring the brain to the skull and preventing side-to-side movement of the brain and spinal cord injury [59]. The meninges also serve as an additional barrier to control the movement of molecules and cells between the periphery and the CNS [60].

The dura mater is the outer membrane located adjacent to the skull. It consists of two epithelial layers of dense collagen fibers [61][62]. The outermost layer is called the periosteal layer and is tightly adhered to the skull cap [61]. Internally attached to the periosteal layer is the meningeal layer. Nerves, arteries, veins, and lymphatic vessels run between the two dural layers similar to peripheral tissue [60]. There is no strict barrier between the blood vessels and the dura mater because dural vessels are fenestrated, thereby allowing the transport of small molecules to move the blood to the dura mater [61][63][64]. Furthermore, at the dural venous sinuses, there is a unique communication interface between the CNS and the immune system. While there is a low expression of TJ claudin-5 and occludin in the dural endothelial cells, the high expression of VCAM-1 and ICAM-1 in these cells together with the presence of dural lymphatics suggests that the dura mater might be an important site for immunological surveillance [63]. This is illustrated by the presence of different immune cell subtypes in the dural sinuses, including macrophages, dendritic cells, neutrophils, innate lymphoid cells, T cells, and B cells [64][65][66]. The subdural meninges comprise the arachnoid and pia mater, and in combination, they are often called leptomeninges as they are structurally connected [67]. Directly attached to the meningeal dural mater layer is the arachnoid mater, a translucent multilayer of dense leptomeningeal cells [62]. The outer layer of the arachnoid mater is joined by TJs and desmosomes and expresses efflux pumps forming an impermeable barrier for molecules and cells similar to the BBB [62][68]. In between the arachnoid mater and pia mater lies the subarachnoid space (SAS), which is filled with CSF that can be reabsorbed to the systemic circulation or lymph nodes through arachnoid granulations and villi and the meningeal-dural sinuses [69]. Thus, the arachnoid barrier controls the passage of CSF from the SAS into the dura mater and the entry of immune cells and molecules derived from dural arteries into the SAS [62]. Within the SAS, strands of collagen covered by a layer of leptomeningeal cells, called subarachnoid trabeculae, connect the inner layer of the arachnoid mater to the pia mater [67]. The leptomeningeal cells enclosing the subarachnoid trabeculae create a continuous cellular monolayer over the pia mater. This layer of leptomeningeal cells is connected by gap junctions, including connexins 26 and 43, which serve as semipermeable membranes for solutes [62][68][70]. The pia mater, the innermost layer of the meninges, houses blood vessels that penetrate the brain parenchyma and will form part of the BBB (**Figure 1**c).

Various vessels run through the leptomeninges and are suspended by the subarachnoid trabeculae [62]. To enter the SAS from a meningeal vessel, a cell or molecule has to cross the blood–meningeal barrier, which is composed of a layer of non-fenestrated endothelial cells connected by TJ and the pia mater [61][71][72]. While the meningeal EC characteristics remain in great part unknown, this barrier differs slightly from the features of BBB by lacking pericytes and the astrocytic endfeet [72][73]. Additionally, meningeal ECs have a higher constitutive expression of CAMs. In contrast to BECs, meningeal ECs express high levels of ICAM-1, even in a non-inflammatory environment [46], which may make vessels more permissive for immune cell transmigration in healthy states.

Initially, the meningeal barrier was thought to only provide physical protection to the CNS. However, it was recently shown that leptomeninges may be structures of immune cell reactivation before infiltrating the brain parenchyma under inflammation [16][18]. These lymphoid-like structures are called ectopic/tertiary lymphoid follicles that consist of aggregates of antigen-presenting cells (APC), T cells, and B cells. These structures are often present in chronic-progressive MS [16]. Autoimmunity is likely to be re-initiated in the meninges through the reactivation of reactive T cells by APCs in these ectopic lymphoid follicles. Progressive MS patients with these structures are characterized by a faster progression of the disease and earlier onset of neurological disability [74][75].

Under inflammatory conditions, lymphocytes and myeloid cells can easily cross the BMB as the meningeal blood vessels are permeable [61][73][76]. Hence, these vessels are primary entry sites for immune cells into the SAS [72]. In the animal model EAE, activated T cells adhere to the leptomeninges once they have migrated across leptomeningeal vessels. Non-activated T cells can be observed in the CSF, where they can exert immune surveillance or they are removed from the CNS through drainage of the CSF [77]. Previous studies in EAE showed how T cells and dendritic cells enter the leptomeningeal space before the onset of CNS inflammation [78]. This might suggest that migration across the BMB occurs earlier than over the BBB. This process could be mediated by P-selectin, which is upregulated before other endothelial adhesion molecules in the meninges and choroid plexus [79]. Moreover, meningeal inflammation also occurred in the early stages of MS before the emergence of white matter lesions [80], and it was frequently close to gray matter lesions, BBB damage, and cortical demyelination [75][80]. Thus, the entry of immune cells to the CNS cortex is likely to be preceded by infiltration of the meninges via the meningeal blood vessels.

The importance of the route of entry across the BMB has also been demonstrated with CXCR7 inhibitor-treated EAE animals. This inhibitor reduced leukocyte trafficking from the leptomeningeal vessels into the SAS and decreased the extent of parenchymal leukocyte infiltrates [81]. Furthermore, VCAM-1 is expressed under normal conditions in the human meninges and its expression is increased in the meninges of MS patients [82]. Altogether, these results indicate that the BMB represents an essential structure regulating the entry of immune cells to the CNS and that the meningeal compartment plays an active role in neuroinflammatory diseases, such as MS.

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