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Sphingosine 1-phosphate signaling at the blood–brain barrier

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The characterization of molecular pathways that modulate blood–brain barrier (BBB) function and integrity has been fueled by a growing body of literature implicating BBB dysfunction in a wide range of neurologic diseases. Sphingosine 1-phosphate (S1P) is a pleiotropic signaling molecule that has been effectively targeted by the immunomodulatory $S1P_1$ functional antagonist fingolimod in the treatment of multiple sclerosis (MS). Investigation into the pathways modulated by S1P has revealed its important role in regulating BBB integrity via signaling through receptor isoforms on astrocytes and endothelial cells (ECs). Current evidence supports a significant role for S1P signaling as a key determinant of BBB permeability and hence as a potential pathogenic player or therapeutic target in diseases characterized by BBB dysfunction.

The BBB

The BBB (see Glossary) is a selective and specialized barrier that physically and functionally separates the central nervous system (CNS) from the circulatory system. The evolutionarily conserved and crucial role of the BBB is the maintenance of CNS homeostasis. The BBB protects extracellular ionic concentrations to enable neuronal activity and mediates nutrient influx and the extrusion of toxic metabolites and xenobiotics. It is probably an adventitious correlate of these functions that the BBB also governs the migration of immune cells into the brain [1]. The principal constituents of the BBB are ECs, pericytes, astrocytes, and the extracellular matrix (ECM) [2]. ECs at the BBB create a selective and polarized transport system characterized by low pinocytotic activity, paracellular clefts sealed by tight junctions (TJs) and adherens junctions (AJs), and a high transendothelial electrical resistance [3,4].

Integrated functions of astrocytes and pericytes are crucial for the maintenance of an effective BBB

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Glossary

Adherens junctions (AJs): responsible for contact and communication between neighboring cells. E-cadherin is the primary component of AJs and interacts with several intracellular proteins to transmit cytoskeletal signals and regulate cell spacing.
Astrocytes: a critical component of the BBB, astrocytes maintain neuronal homeostasis by providing metabolic and structural support. They support the BBB endothelium through release of trophic factors and maintain BBB integrity through endfeet contacts on ECs
Astrogliosis: a state of increased astrocyte proliferation and reactivity that is characterized by inflammation and can be both beneficial and harmful to neurons. Reactive astrocytes are found in MS lesions.
Blood–brain barrier (BBB): regulates the influx of nutrients and immune cells and efflux of waste to and from the brain parenchyma. It comprises specialized ECs, astrocytes, and pericytes. Signaling between these three cell types is important for modulating BBB permeability. Peripheral signals such as inflammatory cytokines can induce upregulation of integrins and other markers on BBB endothelium, facilitating leukocyte capture and extravasation. Regulation of BBB integrity is important for physiologic homeostasis and BBB dysfunction has been implicated in several neurodegenerative, neuroinflammatory, and other neurologic conditions.
Experimental autoimmune encephalomyelitis (EAE): an experimental animal model used to study MS that is induced by the injection of myelin components, immunizing the animal against myelin and leading to inflammatory demyelination.
Fingolimod: first-in-class oral immunomodulator used in the treatment of MS. It binds four of five S1P receptors and has a complex pharmacokinetic profile that includes functional antagonism of $S1P_1$. In addition to its therapeutic efficacy, it has proved to be a valuable experimental tool in the characterization of S1P signaling pathways at the BBB and CNS.
Multiple sclerosis (MS): a neuroinflammatory disease that leads to demyelination and eventually neurodegeneration. Diagnosis is made through the identification of white matter lesions on MRI and progression of the disease results in severe disability in most patients.
Pericytes: regulate vascular development and stability by wrapping microvessels. They communicate extensively with the BBB endothelium.
S1P receptor: there are five subtypes of S1P receptor, all of which are G protein-coupled receptors (GPCRs). The profile of receptor expression varies with cell type and each receptor activates several different downstream pathways including those responsible for regulating cell proliferation, TJ and AJ formation, and vascular tone.
Sphingosine 1-phosphate (S1P): a systemic signaling molecule and key regulator of leukocyte extravasation from lymph nodes. It plays a significant role in modulating BBB integrity via activation of several receptor isoforms found on ECs and astrocytes.
Tight junctions (TJs): comprise occludins and claudins and when intact tightly adhere neighboring cells to each other, minimizing paracellular passage of solutes, cells, and other substances. Disruption of TJs increases the permeability of the BBB.
Vascular endothelial growth factor A (VEGF-A): acts on ECs to promote EC survival and proliferation, angiogenesis, and enhanced vascular permeability.
VE-cadherin: an adhesion molecule that regulates the space between adjacent ECs. VE-cadherin is involved in transducing cell–cell signaling between neighboring cells and mediating leukocyte extravasation through the endothelium.

endothelium. Both astrocytes and pericytes release growth factors and morphogens that modulate pathways important for regulating BBB maturation and maintenance [2,5,6]. Specifically, pericytes contribute to BBB stability through the release of trophic factors and modulation of vascular diameter and blood flow [7]. Interaction of astrocytes with ECs is essential for maintaining the integrity and function of the EC layer. Astrocytes are responsible for structural support by surrounding ECs with specialized endfeet and provide further support through trophic factor signaling. When isolated from astrocytes, ECs lose their barrier characteristics, while administration of astrocytes or astrocyte-conditioned media *in vitro* causes unspecialized ECs to acquire BBB properties [8–10]. Furthermore, astrocytes enhance the stability and permeability of the EC layer through release of angiopoietin [11,12]. Astrocytes regulate TJ expression and hence BBB integrity through the release of fibroblast growth factor (FGF) 2 and 5, which modulate the recycling and membrane density of vascular endothelial (VE)-cadherin, a protein involved in EC cell–cell contact [13,14]. Another factor by which astrocytes regulate and maintain BBB integrity is glial cell line-derived neurotrophic factor (GDNF), which enhances the barrier function of the BBB [15].

S1P receptors are expressed in nearly every CNS cell type including neurons, microglia, oligodendrocytes, and astrocytes [16–19]. The demonstrated role of S1P signaling in endothelial barrier integrity and cell survival has opened a new avenue of investigation into its role in modulating the permeability and maintenance of the BBB [20]. The current and growing body of literature documenting S1P activity in the CNS and at the BBB suggests a potential therapeutic application of S1P-modulating drugs through action at the BBB in diseases such as MS, stroke, and other neurologic disorders characterized by breach of the BBB. Using MS as a prototypic example of neuroinflammation and altered BBB integrity, we discuss the evidence supporting a significant role for S1P signaling in BBB regulation.

MS

MS is an inflammatory neurodegenerative CNS disorder without a spontaneous equivalent in any nonhuman species. Convergent findings from neuropathology, animal models, unbiased genetic studies, and results of treatment implicate cell-mediated neuroinflammation as the principal pathogenic element of MS. Treatments to ameliorate the inflammatory aspect of MS have been available for two decades. These treatments comprise a wide spectrum of different agents from small molecules to injected cytokines, peptide mixtures, and monoclonal antibodies. Each treatment modality addresses a distinct mechanism of action and the authentic determinants of efficacy remain obscure. MS treatments are assessed in a uniform fashion for efficacy. Clinical end points include annualized relapse rate and disability status score. Findings on cranial and spinal cord MRI also provide important secondary end points and include lesion burden as visualized on T2-weighted images and brain atrophy.

The BBB and disease

Although it is not pertinent to CNS immune privilege, the presence of a specialized BBB modulates inflammatory reactions of the CNS [21]. Alteration of BBB properties has been implicated in the initiation or perpetuation of several CNS disorders. In particular, MS is a neuroinflammatory disease in which perturbation of the BBB plays a central role in pathogenesis. Inflammatory monocytes and effector memory T cells extravasate across the activated BBB (downstream of immune re-stimulation of memory T cells within the meninges) and subsequently further permeabilize the BBB through release of cytokines [1]. Therefore, disruption of the BBB has been proposed as both a stimulus and an aggravating effect of disease processes in MS [22–24]. Leukocytes crossing the endothelium must undergo a series of events that lead to direct capture or to the sequence of rolling, arrest, crawling, and transcellular or paracellular extravasation into the perivascular space. Once there, T lymphocytes can interact with antigen-presenting cells and become licensed to invade the CNS parenchyma by penetration of the parenchymal basement membrane, which comprises mainly astrocyte endfeet [22]. This event is crucial for the pathogenesis of experimental autoimmune encephalitis (EAE), an animal model of inflammatory aspects of MS induced by immunization regimens that break tolerance to myelin protein antigens (Box 1).

While MS is the quintessential neuroinflammatory disease, recent events implicate perturbation of tightly regulated BBB permeability in the pathogenesis of several other neurologic diseases including amyotrophic lateral sclerosis (ALS), Alzheimer's disease, epilepsy, stroke, traumatic brain injury, and spinal cord injury [24–27]. As a result, novel therapeutic approaches for stroke and epilepsy, among others, target the BBB to increase the integrity and reduce the infiltration of immune cells and plasma proteins that activate microglia and astrocytes [28–30]. In neoplastic processes such as glioblastoma multiforme, BBB disruption represents both a pathologic process and an avenue for therapeutic delivery and continues to be an active area of clinical and translational research [31]. Increasing recognition of the role played by S1P signaling at the BBB suggests it may be a candidate for therapies targeted at modulating BBB integrity.

S1P biology and receptors

S1P is a signaling sphingolipid that is involved in anti-apoptotic, proliferative, and inflammatory signaling. It is produced from the metabolism of sphingosine by sphingosine kinase (SPHK) 1 and 2. S1P plasma concentrations range 200 to 1000 nM; most of the S1P is bound to low- or high-density lipoprotein, leaving only 1–2% circulating in its active form [32]. Initially considered to be a second messenger, S1P binds and activates a family of five G protein-coupled S1P receptors ($S1P_{1–5}$) previously known as endothelial differentiation gene (Edg) proteins [33,34]. Whereas $S1P_{1–3}$ are expressed on many different cell types, $S1P_4$ is predominantly confined to the immune system and $S1P_5$ is found in the CNS (particularly on oligodendrocytes) and in the spleen [16,35–37]. However, results regarding the localization of these receptors should