The Blood–Spinal Cord Barrier: Morphology and Clinical Implications

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The blood–spinal cord barrier (BSCB) is the functional equivalent of the blood–brain barrier (BBB) in the sense of providing a specialized microenvironment for the cellular constituents of the spinal cord. Even if intuitively the BSCB could be considered as the morphological extension of the BBB into the spinal cord, evidence suggests that this is not so. The BSCB shares the same principal building blocks with the BBB; nevertheless, it seems that morphological and functional differences may exist between them. Dysfunction of the BSCB plays a fundamental role in the etiology or progression of several pathological conditions of the spinal cord, such as spinal cord injury, amyotrophic lateral sclerosis, and radiation-induced myelopathy. This review summarizes current knowledge of the morphology of the BSCB, the methodology of studying the BSCB, and the potential role of BSCB dysfunction in selected disorders of the spinal cord, and finally summarizes therapeutic approaches to the BSCB.

Building Blocks of the BSCB

Similarly to the BBB, the barrier function of spinal cord capillaries is based on the specialized system of nonfenestrated endothelial cells and their accessory structures, including the basement membrane, pericytes, and astrocytic end feet processes. The orchestrated interaction of these building blocks makes up the regulatory and protective functions of the BSCB (Fig 1).

Nonfenestrated Capillary Endothelium

Endothelial cells of brain capillaries, unlike those of the peripheral circulation, are characterized by the absence of cell membrane fenestrations, have a high number of cytosolic mitochondria, and lack pinocytic vacuoles.4 These cellular characteristics reflect restricted free transcellular flow of molecules and high metabolic activity linked to selective active transport mechanisms. The paracellular diffusion pathway is severely restricted by tight junctions
between individual endothelial cells. Several plasma membrane proteins forming tight junctions have been identified, including claudin, occludin, and adherens junction molecule. The cytoplasmic components of tight junctions comprise zonula occludens protein and cingulin. For a more detailed description of tight junction proteins, we refer the reader to specialized reviews.

**Capillary Pericytes**

Pericytes are small vessel wall-associated cells that are separated from the endothelial cells by the basal lamina. Brain capillary pericytes and endothelial cells communicate with each other in several ways, including gap junctions and soluble factors. Pericytes have a significant regulatory role in endothelial cell proliferation, migration, and differentiation. In harmony with astrocytes, they contribute to the unique nonfenestrated phenotype of brain capillary endothelium.

**Basal Lamina**

The basal lamina surrounds capillary endothelial cells and engulfs pericytes. Whereas the cellular components of the BBB have been well studied, the basal lamina of CNS capillaries has received relatively little attention. Pericytes probably play an important role in the synthesis of some basal lamina constituents (ie, proteoglycans, laminins), but their overall contribution has not been clarified. It seems that the basal lamina is generated and maintained by all the cellular components of the blood–brain interface: endothelial cells, pericytes, and astrocytes. It has been shown that the basal lamina contributes to the cytoskeletal morphology of brain capillary endothelium, which in turn affects tight junction proteins and the integrity of BBB.

**Astrocyte Foot Processes**

Astrocytes surround the outer surface of brain capillaries and are critical to the development and maintenance of barrier mechanisms of the brain endothelium. Rather than contributing to the physical properties of the BBB, astrocyte control over the BBB phenotype is exerted mostly via secretory mechanisms that in turn determine the properties of other cellular constituents of the BBB. Besides the aforementioned inductive role of astrocytes, astrocytic end feet processes express a high concentration of the water channel aquaporin 4 and potassium channel Kir4.1 that are involved in ion and volume regulation.

**Differences between BBB and BSCB**

There are several structural and functional differences between the BBB and BSCB as known to date (Table 1). Even if further studies are needed to elucidate the details of BSCB microenvironment, the available results already show that specific differences exist in the ultrastructure of BBB and BSCB endothelial cells. These differences might be responsible for substantial physiological differences.

![Diagram](image)

**TABLE 1: Morphological and Physiological Differences between the BBB and BSCB**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Difference from BBB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen deposits</td>
<td>Present only on BSCB microvessels</td>
</tr>
<tr>
<td>Permeability</td>
<td>Increased to tracers: $[^{3}H]$-D-mannitol, $[^{14}C]$-carboxyl-inulin; increased to cytokines: interferon-α, interferon-γ, tumor necrosis factor-α</td>
</tr>
<tr>
<td>Transporter molecules</td>
<td>Decreased: P-glycoprotein</td>
</tr>
<tr>
<td>Tight junction protein expression</td>
<td>Decreased: ZO-1, occludin</td>
</tr>
<tr>
<td>Adherence junction protein expression</td>
<td>Decreased: VE-cadherin, β-catenin</td>
</tr>
</tbody>
</table>

See text for references.

BBB = blood–brain barrier; BSCB = blood–spinal cord barrier; VE = vascular endothelial.
between the 2 barrier systems, including a potential propensity for certain pathological conditions.

**GLYCOGEN DEPOSITS.** The microvessels of the spinal cord contain glycogen deposits, which are not normally seen in the cerebral vasculature. The functional significance of these deposits is not fully understood. One possibility is that they may serve as an endogenous reservoir for energy storage and metabolism.

**PERMEABILITY.** There are reasons to believe that the permeability of the BBB and BSCB differ. Several lines of evidence suggest that the BSCB is more permeable than the BBB. In a rabbit experimental model studying the permeability of blood–nervous system barriers, the transfer constant values for samples of the spinal cord showed significantly increased uptake of [3H]-D-mannitol and [14C]-carboxyl-inulin compared to samples of brain. Another series of experiments showed that in normal conditions the spinal cord is more permeable to cytokines than the brain. Pan and colleagues demonstrated the regional differences in the permeability of the spinal cord to 3 cytokines. Radiolabeled interferons including interferon (IFN)α, IFNγ, and tumor necrosis factor (TNFα) were injected intravenously into the jugular vein as a bolus in mice and their tissue/serum ratios measured. For each cytokine, the cervical spinal cord had the highest permeability, following the lumbosacral and thoracic regions, whereas the brain was the least permeable. IFNα and IFNγ have similar molecular weights and structure; however, IFNα was more permeable than IFNγ, suggesting there may be spatial differences in binding sites and hydrophilicity throughout the spinal cord epithelium. Passage of IFNγ into the brain and cervical spinal cord reached saturation at a low dose, whereas the thoracic and lumbosacral spinal cord did not become saturated. TNFα produced saturation in the distal spinal cord and in the brain. These results suggest that IFNγ and TNFα enter from the periphery with regional differences. The authors explain that this greater permeability at the distal spinal cord could contribute to disorders such as experimental autoimmune encephalomyelitis (EAE), which has been shown to be more prominent and to occur earlier in this region. It is also possible that these cytokines could exert neurotrophic effects for spinal cord regeneration. This may suggest an association between regional differences of permeability and neuroanatomical function in the spinal cord.

**TIGHT JUNCTION PROTEINS, ADHHERENS JUNCTION PROTEINS, TRANSPORTER MOLECULES.** The increased permeability of the BSCB might well be explained by differences in cell junction protein expression between BBB and BSCB endothelial cells. In an in vitro study of cultured microvascular endothelial cells from murine spinal cord, Ge and Pachter demonstrated decreased expression of tight junction-associated proteins, ZO-1 and occludin, when compared to cultured brain microvasculature endothelial cells. This decreased expression was specific, because other tight junction-associated proteins, claudin-1 and claudin-5, remained unchanged. Given the dependence of tight junction assembly on adherence junction proteins, the authors also investigated the expression of vascular endothelial (VE)-cadherin and β-catenin. Both proteins showed significant reduction in spinal cord microvessels and cultured spinal cord endothelial cells. The overall gross morphology of cultured microvessel populations, as well as the uptake of modified low-density lipoprotein and the expression von Willebrand factor and PECAM-1, did not differ between brain and spinal cord-derived cultures. These results show that specific differences in the ultrastructure of BBB and BSCB endothelial cells exist, which in turn may have an impact on physiology and propensity to diseases.

**Methods for the Study of BSCB**

**Electron Microscopy**

Under pathological conditions, electron microscopy can be used to evaluate mitochondria degeneration in endothelial cells, swollen astrocyte end feet processes, degenerating and ruptured capillary vessels, and disorganized tight junction proteins as BSCB damage progresses. Electron microscopy has also been used to study transcytotic vesicle trafficking across endothelial cells.

**Fluorescent Tracers**

Intravenous injection of low molecular weight tracers followed by microscopic fluorescence imaging has been employed to provide a temporal and spatial evaluation of BBB and BSCB permeability after traumatic injury. Evans blue has a very high affinity for serum albumin. Thus, Evans blue binds tightly to serum albumin and does not detach, making it unlikely that the dye moves across the BBB or BSCB as a free molecule resulting in false-positive staining. Following injury, a compromised BSCB allows the leakage of Evans blue–albumin complex into the parenchyma of the spinal cord. Evans blue is commonly visualized using a light microscope, although
it can also fluoresce with an excitation peak at 470 and 540nm and an emission peak at 690nm.\(^{18,21}\)

The protein luciferase can provide a similar quantitative evaluation of the temporal and spatial profile for BSCB disruption.\(^{19}\) Luciferase undergoes an enzymatic light-emitting reaction with its substrate luciferin via oxidation. Intravenously injected luciferase can penetrate through the compromised BSCB, leaking to the extracellular space in the spinal cord and catalyzing luciferin oxidation in tissue. Using fluorescent microscopy, the generated bioluminescent signal can be detected at an excitation of 330nm, with an emission peak at 530nm. Luminosity can also be measured in injured spinal cord homogenates using a colorimetric luciferase assay and luminometer.\(^{19}\)

**Autoradiography**

Intravenously administered radiolabeled tracers can be used to correlate radioactivity in spinal cord tissue and serum. Tracers such as \(^{[14C]}\)-alpha-aminobutyric acid (AIB) have been used to assess BSCB permeability following injury.\(^{22}\) Radioactivity in collected specimens can be quantified using scintillation. Spatial distribution of tracers can be imaged and obtained as an autoradiogram.

**Dynamic Contract-Enhanced Magnetic Resonance Imaging**

Dynamic contract-enhanced magnetic resonance imaging (DCE-MRI) offers a powerful, noninvasive, and qualitative technique for evaluating BSCB permeability.\(^{23-28}\) DCE-MRI involves the acquisition of T1-weighted magnetic resonance images following intravenous administration of paramagnetic contrast agents such as gadopentetate dimeglumin (Gd).\(^{28}\) Because the BSCB is impermeable to large molecules such as Gd, which has a molecular weight of 938Da, it can only leak from systemic circulation and into the spinal cord parenchyma when the BSCB is compromised. The regional distribution of the escaped contract agent (Gd) can be visualized in the T1-weighted images and evaluated according to signal intensities. Furthermore, an appropriate pharmacokinetic model can be used to qualitatively analyze the temporal changes and severity of BSCB permeability.\(^{25}\) In spinal cord injury (SCI), 2 enhancements can be visualized, diffuse enhancements that result from the vasculature disruption caused by the mechanical insult at the epicenter and focal enhancements that result from BSCB leakage at the penumbra.\(^{29}\)

**Morphological Studies of the BBB and BCSB In Vitro and In Vivo**

The BBB as a functional collective of neurons, astrocytes, pericytes, and microglia is also called the neurovascular unit. Studies using microscopic analysis of the BBB and BCSB contributed to our current understanding of the morphology of its components under physiological and pathological conditions.\(^{30}\) The aim of these studies is to provide evidence of structural changes to BBB and BSCB components to correlate with functional changes observed in vivo. In 1 of the first in vitro models of BBB, the combined use of morphological and electrophysiological studies revealed the potential of this model for drug delivery across the BBB.\(^{31}\) Numerous other studies further contributed to elucidate histological changes to the BBB and BSBC in in vitro models of BBB.\(^{30,32-40}\) More recent publications have summarized the gain in knowledge and provide the most current insight.\(^{41-50}\) Using immunohistochemical and immunofluorescence technique and fluorescent live imaging, Zehender et al, demonstrated the role of tight junction proteins, such ad ZO-1 and CL-5, in physiological properties of BBB.\(^{51}\) The interaction between astrocytes and the endothelial cells of the neurovascular unit is crucial for the maintenance of the barrier function. Communications between these cells and their histological correlates were investigated by a combination of imaging techniques, such as freeze fracturing, electron microscopy, and immunohistochemical staining in mouse brain samples.\(^{52}\) Based on their own studies and results published in the literature, the authors give a very thorough overview of the current knowledge in molecular elements, such as aquaporins, ZO-1, claudin 1 and 5, intercellular adhesion molecule-1 (ICAM-1), VE-cadherin, and \(\beta\)-catenin, and transport mechanisms of the neurovascular unit. These components are discussed throughout this review.

**The BSCB in Disease**

Increasing evidence show that the BSCB might play a pivotal role in the development or progression of several diseases of the CNS. Damage to the BSCB occurs parallel to other pathophysiological phenomena that ultimately lead to the clinical picture and course of the disease. The most extensively studied in this respect is BSCB disruption after traumatic spinal cord injury. Other diseases that have been associated with BSCB dysfunction are post-traumatic syringomyelia, amyotrophic lateral sclerosis (ALS), radiation injury to the spinal cord, multiple sclerosis (MS), neuromyelitis optica (NMO), spinal cord ischemia, and neuropathic pain.
SCI

Damage to the vasculature and breakdown of the BSCB is a universal consequence of SCI clinically as well as in animal models. Following the mechanical disruption of capillaries at the moment of the impact, blood-borne molecules and cells readily cross into the injured parenchyma. Pathophysiological cascades develop that further contribute to spinal cord damage as well as to dysfunction of the BSCB.22 These complex secondary pathomechanisms are responsible for additional extension of damage into the originally uncompromised segments of the spinal cord.22,61

The time course of BSCB breakdown after SCI has been extensively studied. Solid evidence exists that dysfunction of the BSCB occurs in 5 minutes after spinal cord trauma.62 The volume of extravasation of 3 markers of distinct size (labeled hydrazide, bovine serum albumin, and red blood cells) increased significantly with the intensity of injury and decreasing marker size. Extravasation volumes for all 3 markers were greater in gray matter than in white matter and were better correlated to the rate of spinal cord compression than to the depth of spinal cord compression.62 These results suggest that the speed of spinal cord parenchyma displacement is at least as important as the actual extent of tissue displacement with respect to spinal cord microvasculature pathology.62

The time course of re-establishment of BSCB function is less evident, and results vary greatly between studies. Depending on the tracer technique used to evaluate BSCB permeability, various time points have been established. In a study of horseradish peroxidase extravasation in rats, the function of BSCB was re-established by 14 days after injury.61 Popovich et al assessed vascular permeability by the tracer AIB.22 Quantitative analysis of AIB extravasation showed a biphasic pattern of BSCB dysfunction, with secondary elevations of AIB transfer in the spinal cord white matter 14 to 28 days postinjury.22

Another methodological approach is the use of DCE-MRI. DCE-MRI is a noninvasive technique that enables quantification of BSCB permeability in the same group of animals. It has been shown that although BSCB permeability gradually decreases with postinjury time, BSCB remains compromised as long as 56 days after SCI.29 The gradual restoration of BSCB function over time correlated well with improvement in the locomotor capacity of rats.29

The cellular and molecular mechanisms responsible for the long-term increase in BSCB permeability after SCI are numerous. Morphological studies have shown that post-traumatic separation of the perivascular basement membrane facilitates the expansion of secondary inflammation, providing conduit for inflammatory cells into the spinal cord parenchyma. Other morphological changes include increased vesicular transport across the capillary endothelium and widening of tight junctions between endothelial cells. At the molecular level, oxidative stress and free radicals seem to play a crucial role in BSCB disruption. Nitric oxide contributes to the BSCB breakdown through generation of free radicals and tyrosine nitration of peroxynitrite.65

Endothelin-1 is a 21-amino acid peptide with potent vasoconstriction effect. There is evidence that implicates endothelin-1 in BSCB disruption after SCI. The increased expression of the peptide has been correlated with the pattern of BSCB breakdown. Intrathecal administration of endothelin-1 in the intact spinal cord resulted in disruption of the BSCB, and pharmacological blockade of endothelin-1–mediated vasoconstriction attenuated the breakdown of the BSCB after SCI. The mechanism whereby endothelin-1 contributes to BSCB dysfunction may be related to prominent vasoconstriction and consequent ischemia and oxidative insult.

A significant role has been attributed to the inducible isoform of heme oxygenase (HO), HO-1, in modulating BSCB function and inflammation in the acutely injured spinal cord. HO catalyzes the conversion of heme to the potent antioxidant biliverdin. The inducible isoform HO-1 has been shown to have a stabilizing effect on the BSCB and limits oxidative stress and white matter damage in acutely injured murine spinal cord.

Other molecular mechanisms of putative BSCB dysfunction have been identified during spinal cord injury. Aquaporin-4 (AQP4) is a molecular water channel in the brain and spinal cord predominantly expressed in astrocytic end-feet processes. It has been shown that contusion SCI in AQP4−/− mice leads to significantly worse outcome relative to control mice in the sense of worse locomotor function, prolonged bladder dysfunction, greater tissue damage, and increased tissue water content. These results suggest that AQP4 as part of the BSCB plays a protective role after SCI by facilitating the clearance of tissue water.

Traumatic disruption of the spinal cord microvasculature triggers post-traumatic vessel regression and neovascularization. This process is based on the harmonized action of several angiogenic factors. Investigation of the temporal expression of angiogenic genes disclosed a constant downregulation of vascular endothelial growth factor (VEGF), and a transient decrease of angiopoietin-1, platelet-derived growth factor-BB, and placental growth factor. Hepatocyte growth factor was the only angiogenic factor with a constant increased expression. These data provide evidence on the decreased expression of the majority of angiogenic factors after SCI. This
phenomenon should be strongly considered in the development of new therapeutic approaches in this field.

**Post-Traumatic Syringomyelia**

Post-traumatic syringomyelia refers to the development of fluid-filled cysts in the spinal cord during the chronic stage of SCI. In 3 to 4% of patients, the disease has a progressive course, leading to further loss of neurological function and chronic neuropathic pain.\(^{74,75}\) The general consensus on the pathogenesis of post-traumatic syringomyelia is based on obstruction of cerebrospinal fluid flow in the subarachnoid space of the spinal cord. It is hypothesized that due to post-traumatic arachnoiditis and obstruction of free flow of cerebrospinal fluid there is a shift of cerebrospinal fluid along the pressure gradient into the spinal cord parenchyma.\(^{74,75}\) Recent evidence suggests that chronic dysfunction of the BSCB also plays an important role in the development of this disease.\(^{76}\)

In an experimental model of arachnoiditis and progressive syrinx formation, a loss of structural and functional integrity of BSCB has been identified.\(^{76}\) The authors found that the most dramatic disruption of the BSCB was in the tissue adjacent to the syrinx. It is possible that dysfunction of the BSCB after trauma leads to cyst formation in the spinal cord parenchyma. The cyst steadily enlarges via extravasation of intravascular fluid due to prolonged disruption of the BSCB in the vicinity of the cyst.\(^{76}\)

**ALS**

ALS is a chronic, progressive, and ultimately fatal neurodegenerative disease of motor neurons in the brain and spinal cord.\(^{77}\) Mutations in superoxide dismutase-1 (SOD1) have been identified in inherited forms of ALS; nevertheless, about 90% of cases are sporadic.\(^{78}\) Besides SOD1, several other genes and loci have been found that predispose to ALS. These include ALS2, senataxin, vesicle-associated membrane protein-associated protein B, angiogenin, TAR DNA-binding protein, dynactin 1, and microtubule-associated protein tau.\(^{78}\) These molecular pathways include those that affect RNA processing, axonal transport, and mitochondrial function.\(^{78}\)

Besides the aforementioned genetic mechanisms, increased permeability of the BSCB has also been implicated in the pathogenesis of ALS.\(^{79}\) Leonardi found increased levels of albumin and immunoglobulin G (IgG) in the cerebrospinal fluid of patients with ALS.\(^{80}\) Subsequently, IgG, complement protein C3 deposits, and active macrophages were discovered in the motor cortex and spinal cord, providing evidence of concomitant immune activation in these patients.\(^{81,82}\)

The implication of BSCB in the pathogenesis of ALS came a step further with the demonstration of Evans blue leakage around spinal cord vessels in the mice model of ALS (SOD1\(^{G93A}\) mice), indicating dysfunction of the endothelium and basement membrane.\(^{15,83}\) Zhong et al used mouse SOD1 mutant models to determine serum protein leakage, Prussian blue staining, MCP-1 immunostaining, ZO-1 immunostaining, and total capillary length in these animals.\(^{84}\) The result showed disruption of the BSCB in the mice SOD1\(^{G93A}\) model of ALS with reduced levels of tight junction proteins ZO-1, occludin, and claudin-5 between endothelial cells. Moreover, significant 10 to 15% reduction in the total length of capillaries in the lumbar spinal cord of SOD1 mutants was detected, with electron microscopic identification of edema and collapsed capillary beds. Importantly, all these changes preceded motor neuron loss, implicating BSCB breakdown as the primary cause for the subsequent neuronal damage.\(^{84}\) These findings were paralleled by the study of Nicaise et al using 3 different BBB leakage markers: Evans blue, IgG, and hemosiderin, to study BBB/BSCB leakage in the SOD1-linked ALS rat model at presymptomatic and symptomatic stages.\(^{85}\) The study showed IgG and hemosiderin leakage at presymptomatic stage with Evans blue extravasation only in the symptomatic animals. The impairment of microvascular permeability was associated with decreased expression of mRNA for tight junction proteins, and the basement membrane protein agrin has been demonstrated in SOD1 rats.\(^{85}\) These findings from animal models of ALS are in concordance with recent revelations of decreased mRNA expression of tight junction proteins in lumbar spinal cords of patients with ALS.\(^{86}\)

Interestingly, at the electron microscopic level the aforementioned molecular changes were not reflected in disintegration of tight junctions between endothelial cells; rather, increased endothelial cell membrane permeability has been found. The spinal cord capillary endothelium showed mitochondrial damage, swelling of the endoplasmic reticulum, cytoplasmic vacuolization, and cell death in the SOD1\(^{G93A}\) mice.\(^{83}\) Similar morphological changes have been detected in perivascular astrocytes and other constituents of the neuropil.\(^{83}\)

It is important to note that despite the aforementioned strong evidence indicating a possible hemodynamic mechanism for ALS,\(^{87}\) some recent studies bring controversy in this respect. For example, investigation of patients with ALS at 7T MRI could not reveal any cerebral microbleeds or hemosiderin deposits in the cerebrum of these patients.\(^{88}\) Another human study found a reduced number of circulating endothelial cells in peripheral blood of ALS patients, indicating a different
mechanism of endothelial cell damage and repair, rather than only detachment of dysfunctional endothelial cells.89

**Radiation Injury**

Vascular dysfunction following radiation to the CNS for therapy of tumors plays a primary role in the development of postradiation edema and necrosis. In the experimental rat model of spinal cord radiation injury, Li et al demonstrated early (24 hour) apoptotic endothelial cell death associated with increased BSCB permeability as shown by increased albumin immunostaining around microvessels.90 The decrease in endothelial cells persisted for 7 days after radiation, with recovery apparent by day 14. These results are consistent with an association of endothelial cell death and acute BSCB disruption after irradiation.90

The molecular mechanisms underlying these vascular changes implicate increased expression of VEGF and ICAM-1 in endothelial cells and astrocytes in the experimentally irradiated spinal cord.91,92 Upregulation of VEGF has been postulated to play a role in mediating vascular permeability following irradiation of the nervous system.93 It has been shown that there was a dose-dependent temporal and spatial association of VEGF up-regulation and radiation-induced BSCB dysfunction, with hypoxia being a possible causal stimulus of VEGF expression.91

The increase in ICAM-1 expression was pronounced at late time periods, when BSCB disruption and tissue injury are most prominent.92 ICAM-1 has a central role in leukocyte binding and infiltration in inflammatory processes. Processes involving ICAM-1 contributing to BSCB disruption include ICAM-1–mediated leukocyte binding, cytoskeletal rearrangement, signaling to tight junctions, and effects of cytokines released by ICAM-1–expressing astrocytes.92,94,95 ICAM-1 expression may be involved in the interaction of activated astrocytes with other stimulating factors that cause the release of cytokines or other molecules contributing to the deleterious microenvironment after spinal cord irradiation. Astrocytes are also the source of VEGF, which is associated with BSCB disruption in radiation injury.91,93

**MS**

MS is a demyelinating disease characterized by attacks of myelin destruction throughout the CNS, perivascular edema, and inflammatory infiltrates.96 EAE is an animal model of MS. In this model, neuroantigen-specific autoimmune T cells contact an intact BBB and extravasate into the neural parenchyma. These cells are retained in the CNS due to presentation of appropriate antigens and undergo further activation.97

It has been shown that this mononuclear cell infiltration is associated with compromised BBB and BSCB function, with demonstration of infrared dye penetration into the spinal cord and brain during the acute phase of EAE.98,99 The disruption of the BSCB is greatest at the onset of the disease, followed by inflammation and demyelination, indicating that increased BSCB permeability precedes the destructive inflammatory process.26 Applying the technique of intravital fluorescence videomicroscopy, it has been demonstrated that T cells interact with the spinal cord white matter microvasculature via a 4-integrin–mediated capture and subsequently G-protein–dependent strengthening of T cells adhesion to microvascular cell adhesion molecule-1.100

**NMO**

NMO is an idiopathic inflammatory demyelinating disease of the CNS affecting mainly the optic nerves and the spinal cord.101 Although NMO has long been considered a subtype of MS, new evidences suggest that NMO and MS are distinct disease entities.96 Disease-specific IgG antibodies (NMO-IgG) against AQP4 play a crucial role in the pathogenesis of NMO. It is hypothesized that circulating NMO-IgG access the nervous parenchyma through endothelial transcytosis or at areas of relative barrier permeability. The astrocytic foot processes bound to the basal lamina of the endothelium make the extracellular domain of AQP4 channel accessible to NMO-IgG.103 Astrocytic damage, dysfunction of the BSCB, and consequently massive neuroinflammation would result from complement activation, polymorphonuclear cell activation, and antibody-dependent cellular toxicity.96,102

**Spinal Cord Ischemia**

It is well documented that ischemia increases vascular permeability; however, data on the effect of ischemia on the BSCB are rare.103 In a rabbit model of spinal cord ischemia, Jacobs demonstrated a biphasic pattern of BSCB dysfunction.104 Severe vascular permeability disruption was noticed as early as 30 minutes after reperfusion, with recovery after 8 hours. This recovery was followed by a secondary progressive increase in BSCB disruption. In this reproducible model, the secondary deterioration of motor function was in good correlation with the progressive increase in BSCB permeability.104,105 These experimental data show that cellular and molecular pathomechanisms responsible for the disruption of the BSCB during ischemia may be similar to those leading to disruption of the BBB.
Neuropathic Pain
Discharge activity of primary afferent fibers, such as injury to sensory nerves, may have long-lasting consequences in the CNS, leading to chronic pain. One of the consequences of peripheral nerve injury is the entry of monocytes and T cells from the circulation into the spinal dorsal horn parenchyma. It has been shown that injury to the peripheral nerve and electrical stimulation of C fibers both cause increase in the permeability of the BSCB. This increase in BSCB permeability could be prevented by applying lidocaine to the nerve prior to injury or stimulation and also could be mimicked by the application of capsaicin. These findings indicate that discharge activity in the TRPV1-expressing C fibers triggers a delayed increase in BSCB permeability following peripheral nerve injury.

Summary of Clinical Data
An increasing amount of data show that dysfunction of BSCB is intimately implicated in the cause or progression of numerous spinal cord pathologies. Theoretically, there are 2 ways dysfunctional BSCB could be involved in these disease processes (Table 2). First, BSCB dysfunction could be the primary cause; in this scenario, the alteration of BSCB function precedes the development of the disease process, and BSCB dysfunction is at the very origin of pathophysiological cascade, leading to spinal cord affliction. This very probably occurs in radiation-induced myelopathy and ALS, but also in MS and NMO. Second, BSCB dysfunction could be part of secondary damage mechanisms; in this constellation of events, the damage to the BSCB is part of broader destructive forces, and BSCB dysfunction then contributes to downhill pathological mechanisms contributing to the progression of the disease. An example of the secondary type of BSCB dysfunction is spinal cord ischemia. It is important to emphasize, however, that the distinction between primary and secondary BSCB dysfunction is not always possible, as in traumatic spinal cord damage, where both forms of BSCB dysfunction occur. Lastly, in several spinal cord afflictions the exact role of BSCB damage is not elucidated. Into this category belong disorders such as post-traumatic syringomyelia and BSCB dysfunction associated with neuropathic pain (see Table 2).

Repairing and Bypassing the BSCB
Therapeutic targeting of the BSCB is related to 2 major goals, namely repairing BSCB function and thus

<table>
<thead>
<tr>
<th>Disease</th>
<th>Primary (causative) BSCB Dysfunction</th>
<th>Secondary (contributory) BSCB Dysfunction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinal cord ischemia</td>
<td>Mechanical disruption of spinal cord capillaries</td>
<td>Neuroinflammation: nitric oxide, endothelin-1, heme oxygenase-1, loss of aquaporin-4; neovascularization: VEGF, angiopoietin-1, PDGF, PGF</td>
</tr>
<tr>
<td>Posttraumatic syringomyelia</td>
<td>Endothelium and basal lamina damage in SOD1 mutation: reduced tight junction proteins</td>
<td>BSCB dysfunction due to arachnoiditis and obstruction of CSF flow</td>
</tr>
<tr>
<td>Amyotrophic lateral sclerosis</td>
<td>Endothelium and basal lamina damage in SOD1 mutation: reduced tight junction proteins</td>
<td>Neuroinflammation: nitric oxide, endothelin-1, heme oxygenase-1, loss of aquaporin-4; neovascularization: VEGF, angiopoietin-1, PDGF, PGF</td>
</tr>
<tr>
<td>Radiation injury</td>
<td>VEGF: increased permeability of capillaries; ICAM-1: increased leukocyte binding</td>
<td>BSCB dysfunction due to arachnoiditis and obstruction of CSF flow</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>Autoimmune T-cell activation</td>
<td>Neuroinflammation: nitric oxide, endothelin-1, heme oxygenase-1, loss of aquaporin-4; neovascularization: VEGF, angiopoietin-1, PDGF, PGF</td>
</tr>
<tr>
<td>Neuromyelitis optica</td>
<td>IgG antibody against aquaporin-4 in astrocyte feet processes</td>
<td>Ischemic damage to endothelium</td>
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<td>Spinal cord ischemia</td>
<td>Ischemic damage to endothelium</td>
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<tr>
<td>Neuropathic pain</td>
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<td>Neuroinflammation: monocyte and T-cell activation during C-fiber discharge activity</td>
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</tbody>
</table>

See text for references.

BSCB = blood–spinal cord barrier; VEGF = vascular endothelial growth factor; PDGF = platelet-derived growth factor; PGF = placental growth factor; CSF = cerebrospinal fluid; SOD1 = superoxide dismutase-1; ICAM-1 = intercellular adhesion molecule-1; IgG = immunoglobulin G.
alleviating the tissue damage, and developing methods of drug delivery across or bypassing the BSCB to improve the functional outcome. In recent years, particularly the latter approach has shown significant advances, although mainly on an experimental basis so far.

Drugs used to modify the pathophysiology of the BSCB may contribute to limiting cellular damage caused by SCI. Major efforts have been directed toward slowing down or reversing the progression of associated secondary events in SCI.110,111 Numerous studies have been conducted using a variety of neuroprotective agents to target the BSCB following injury. A significant amount of data in the field have been reported by Sharma and his co-workers.16,112–116 Although this group provided a significant contribution to current knowledge, the general value of the findings is limited. The information was obtained in 1 type of SCI in 1 animal model investigated in a relatively short time period, namely 5 hours postinjury. Using a rat model of right dorsal horn incision, these authors observed reduced BSCB permeability to Evans blue and radiodiode, and reduced swelling and/or edema following mostly topical treatment with insulinlike growth factor-1,16 rat growth hormone,112 brain-derived neurotrophic factor (BDNF),16 a bradykinin receptor antagonist,113 an antioxidant compound,115 and a dynorphin A antiserum114 at short time intervals. Moreover, the stimulation of histamine H3 receptors and blockade of H2 receptors resulted in beneficial effects in this animal model.116 Other authors using contusion or compression SCI models in rats provided data on reduction of BSCB permeability and better outcome following intraperitoneal administration of magnesium sulfate117 or tamoxifen118 and combined treatment with dexamethasone and aminoguanidine.119 Conversely, topical administration of VEGF induced a long-term increase in BSCB permeability, although enhancing neurobehavioral recovery after SCI.120

VEGF, injected intravenously, increased BBB and BSCB permeability also in SOD1 mice, which are used to model ALS. The increase in barrier permeability was limited by the size of the delivered substance.121 Treatment of mutant SOD1-expressing mice with activated protein C (APC) analogs delivered after disease onset retarded progression of ALS-like disease. APC with protease activity was found to cross the BSCB via endothelial protein C receptor and act on motor neurons and their glial neighbors, especially microglia.122

EAE is widely studied as an animal model of MS. In this model, intravenous treatment with insulinlike growth factor-1 was reported to promote clinical recovery by reducing EAE-induced BSCB changes and the associated immune-mediated inflammatory lesions.123 A reduction of disease severity accompanied by diminished BSCB leakage in EAE mice was also observed following treatment with erythropoietin.124

There are several possibilities for enhancing the transfer of therapeutic macromolecular agents, such as proteins, peptides, and genes, to the site of the injury (Fig 2). The most obvious is the local, intrathecal route of drug administration. In addition to the invasiveness of such treatment, bolus intrathecal injections are not optimal, and catheters are open to infection. Recently, a new paradigm for intrathecal sustained release of therapeutic molecules to the injured spinal cord has been developed. The new intrathecal drug delivery system has been successfully used for 28-day treatment with the neuroprotective molecule erythropoietin.125,126 A noninvasive and relatively novel method that targets drugs to the brain and spinal cord is intranasal administration. This is possible because of the unique connections that the olfactory and trigeminal nerves provide between the brain and the external environment.127 For example, intranasally delivered insulinlike growth factor-1 can bypass the BBB and BSCB to rapidly elicit biological effects at multiple sites within the brain and spinal cord.128

Drugs that do not cross the barrier (see Fig 2) can reach the spinal cord via drug delivery systems. Based on preclinical research, several drug delivery systems are available. For example, a reduction of clinical severity of EAE was observed using a novel system for BDNF delivery, namely transformed BDNF producing bone marrow stem cells.129 The most promising drug delivery systems for improving therapeutic options to treat SCI include nanoparticles.130–133 Nanoparticle carriers represent a revolutionary contribution to drug delivery. The small size of the nanoparticles enables them to penetrate the BSCB. There are modern strategies used to attach biomolecules to the surface of nanoparticles.134 Various types of nanoparticles are available, ranging from
polymeric substances to liposomes. The description of composition and properties of individual nanoparticles and related substances is beyond the scope of this review. There is no doubt that nanoparticles provide a suitable carrier to locate to the lesion site, deliver the macromolecular drug via the BSCB, and treat the SCI.

Although not yet established in the treatment of SCI in patients, the nanoparticles appear to be a promising option for the future. As the clinical approval of new drugs is rather complicated and time consuming, drugs and/or delivery strategies used in other fields of medicine may help to improve treatment in SCI patients. The clinical use of nanoparticles is increasing in several applications, for example, in ophthalmology. Of course, there are several obstacles to the incorporation of nanotechnology into the clinical treatment of SCI, such as proofs of safety and efficacy. Above-described animal studies bring mainly positive support, but some types of nanoparticles can induce negative effects. Next to nanoparticles, other approaches may be worth considering. For example, rats subjected to SCI were effectively treated by direct delivery of a potent inhibitor of epidermal growth factor receptor to the injured area. As the inhibitors of this growth factor are in clinical use in cancer treatments, they might be candidates for clinical trials aimed at improving the outcome of SCI. With further investigation into the function of the BSCB, novel therapeutic strategies can be developed in hopes of slowing down the progression and severity of SCI-induced neurodegeneration and promoting sensory–motor recovery.

Conclusions

Disorders of the spinal cord have a significant impact on the morbidity, mortality, and overall quality of life in our society. Intensive scientific effort is dedicated to elucidating the pivotal cellular and molecular mechanisms that are responsible for the occurrence and progression of various spinal cord pathologies. The BSCB represents a particularly important morphological and functional entity in this respect. Despite similarities to the BBB, it seems that the BSCB also has some unique properties that distinguish it from the BBB. Dysfunction of the BSCB has been well documented in the etiology or progression of several spinal cord pathologies, such as secondary damage after SCI and post-traumatic syringomyelia, ALS, NMO, MS, radiation-induced myelopathy, and spinal cord ischemia. Modulating the function of the BSCB may significantly improve the somber course of these diseases. BSCB-based therapy certainly represents an attractive and promising direction in the near future.

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Potential Conflicts of Interest

Nothing to report.

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