Blood–brain barrier dysfunction in multiple sclerosis: causes, consequences, and potential effects of therapies

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Established by brain endothelial cells, the blood–brain barrier (BBB) regulates the trafficking of molecules, restricts immune cell entry into the CNS, and has an active role in neurovascular coupling (the regulation of cerebral blood flow to support neuronal activity). In the early stages of multiple sclerosis, around the time of symptom onset, inflammatory BBB damage is accompanied by pathogenic immune cell infiltration into the CNS. In the later stages of multiple sclerosis, dysregulation of neurovascular coupling is associated with grey matter atrophy. Genetic and environmental factors associated with multiple sclerosis, including dietary habits, the gut microbiome, and vitamin D concentrations, might contribute directly and indirectly to brain endothelial cell dysfunction. Damage to brain endothelial cells leads to an influx of deleterious molecules into the CNS, accelerating leakage across the BBB. Potential future therapeutic approaches might help to prevent BBB damage (eg, monoclonal antibodies targeting cell adhesion molecules and fibrinogen) and help to repair BBB dysfunction (eg, mesenchymal stromal cells) in people with multiple sclerosis.

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Introduction

Multiple sclerosis is the most common chronic inflammatory disease affecting the CNS. Relapsing-remitting multiple sclerosis is characterised by bouts of neurological deterioration (ie, relapses) followed by a variable degree of recovery (ie, remission). Around 10% of people newly diagnosed with multiple sclerosis have primary progressive disease, which is characterised by the relentless accumulation of neurological disability from onset. Throughout the disease course, 30–60% of people with relapsing-remitting multiple sclerosis are diagnosed with secondary progressive multiple sclerosis and show gradual clinical worsening without relapses. Although the cause of multiple sclerosis remains elusive, interactions of predisposing genetic polymorphisms with hormonal and environmental triggers ultimately lead to the formation of demyelinating plaques, which are linked to CNS infiltration by proinflammatory immune cells across a dysfunctional blood–brain barrier (BBB). Although the exact temporal sequence of pathological events is ambiguous, advanced MRI and pathological studies of apparently normal white matter show BBB breakdown before lesion formation.[1] Consequently, restricting the capacity of proinflammatory immune cells to cross the BBB and reach the CNS is potentially one of the most effective therapeutic strategies for multiple sclerosis.[2]

The BBB is established by specialised brain endothelial cells and protects the CNS parenchyma from potentially harmful circulating molecules or pathogens. Although regional differences exist, similar barriers are observed in the spinal cord. Blood–CNS barriers, including the BBB, blood–spinal cord barrier, blood–CSF barrier, and blood–leptomeningeal barrier, mean that immune cell entry into the CNS under physiological conditions is restricted to rare events of immunosurveillance.[3] Differences between brain and peripheral endothelial cells, as well as the complex immunosurveillance in the CNS, have been previously reviewed.[4] Together with CNS-resident cells, brain endothelial cells regulate cerebral blood flow to deliver nutrients and oxygen for metabolically active neurons in a process called neurovascular coupling.[5] The BBB as part of the neurovascular unit therefore controls cerebral homoeostasis and maintains an ideal milieu for neuronal activity.

In this Review, we provide an overview of BBB activation during neuroinflammation, the effect of genetic and environmental risk factors for multiple sclerosis on BBB integrity and functionality, and the consequences of BBB disturbance that might contribute to (and even accelerate) the disease pathobiology. We also discuss potential CSF and serum biomarkers of the state of the BBB, as well as potential future therapeutic options to overcome BBB dysfunction in multiple sclerosis.

Activation of the BBB during neuroinflammation

Malfunction of the BBB in acute multiple sclerosis relapses and experimental autoimmune encephalitis (EAE) rodent models is linked to proinflammatory activation of brain endothelial cells. This activation is characterised by the release of proinflammatory chemokines and upregulation of cell adhesion molecules (CAMs), and leads to hypertrophy of cerebral blood vessels and reduced surface expression of tight junction molecules, resulting in compromised BBB integrity that enables leukocytes to enter the CNS.[6] In postmortem CNS samples from people with multiple sclerosis, most leukocyte trafficking is observed within the perivascular space around postcapillary venules. Furthermore, the transcriptional profile of brain endothelial cells in mice with EAE indicates an upregulation of genes involved in extracellular matrix remodelling and leukocyte trafficking compared with mice without EAE, and downregulation of characteristic BBB-enriched genes essential for cerebral homoeostasis and angiogenesis.[7]

Brain endothelial cells come into contact with pathological cues in both the CNS and periphery, and therefore act as bidirectional sentinels (figure 1). In the CNS, brain endothelial cells express the homoeostatic chemokines C-C motif ligand 19 (CCL19), CCL21, and

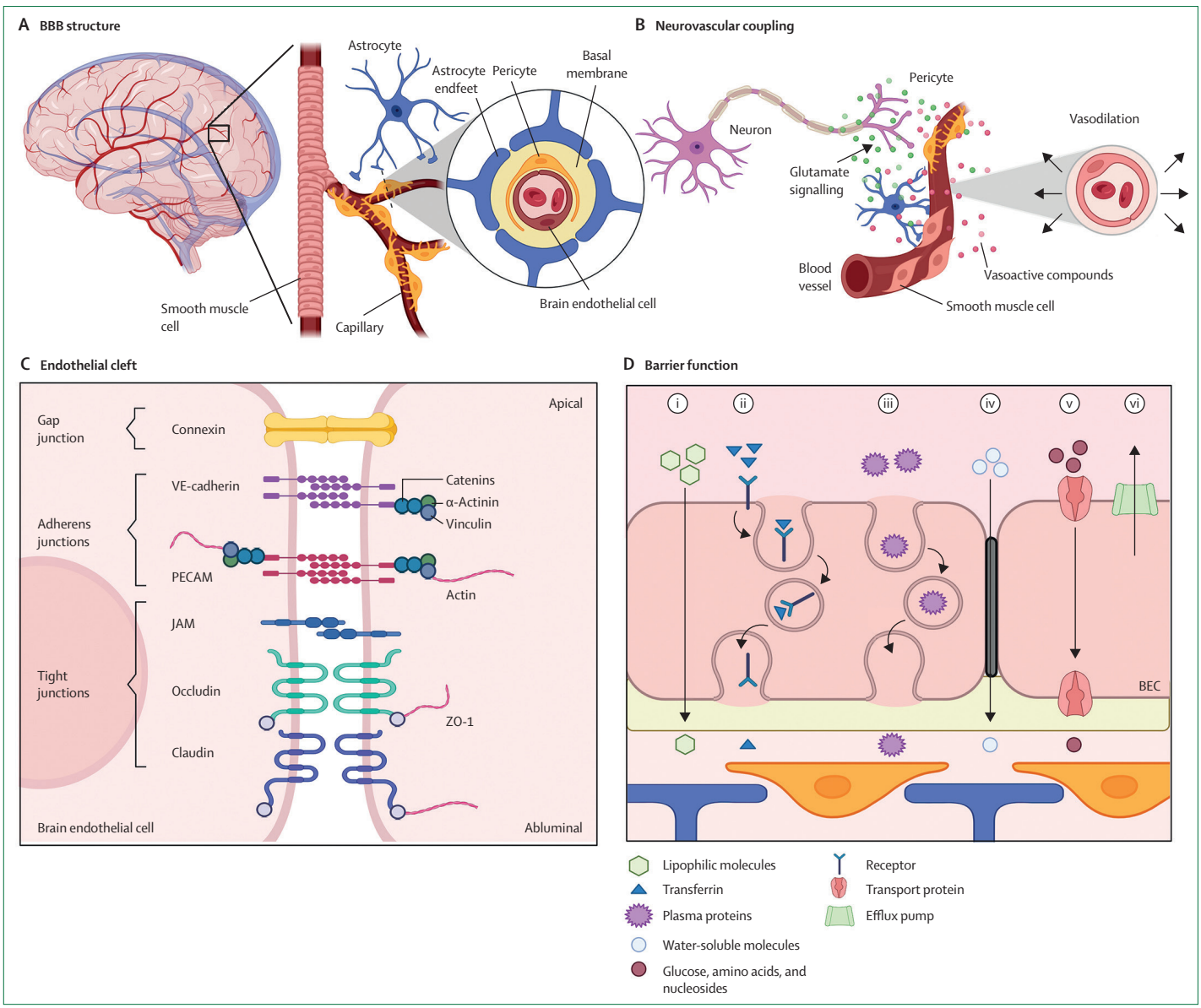

Figure 1: **Structure and function of the BBB and neurovascular unit**
(A) Arteries (in red) enter the brain and branch into smaller vessels: arterioles (red) and capillaries, and further into postcapillary venules (not shown) and veins (pale blue). Smooth muscle cells surround arterioles, whereas pericytes enwrap capillaries. The cross-section shows the BBB as part of the neurovascular unit, with brain endothelial cells embedded in the basal membrane and further support from pericytes and astroglial endfeet surrounding the capillary or postcapillary venule (not shown). (B) Active neurons stimulate release of glutamate and vasoactive compounds from astrocytes to stimulate smooth muscle cells around arteries and arterioles (and probably pericytes around smaller vessels), resulting in relaxation of the blood vessel (ie, vasodilation) and increased cerebral blood flow to supply active neurons with oxygen and nutrients. (C) From apical (ie, towards the blood) to abluminal (ie, towards the CNS), the endothelial cleft between adjacent brain endothelial cells harbours gap junctions (ie, connexin), adherens junctions (ie, VE-cadherin and PECAM), and tight junctions (ie, JAM, occludins, and claudins). Adherens junctions are anchored to the cytoskeleton through catenins, vinculin, and α-actinins, whereas tight junctions are supported by zonula occludens. (D) Brain endothelial cellscontrol trafficking across the BBB into the CNS tissue: small lipophilic molecules freely diffuse across the membrane (i); receptor-mediated trafficking of transferrin (which regulates iron transport; ii); plasma proteins such as albumin are taken up by endocytosis (iii); water-soluble molecules traffic across junction complex molecules at the endothelial cleft (iv); transport proteins enable uptake of molecules such as glucose, amino acids, and nucleosides (v); and efflux pumps such as P-glycoprotein support efflux of possible toxic compounds (vi). Figure created with BioRender.com. BBB=blood–brain barrier. VE-cadherin=vascular endothelial cadherin. PECAM=platelet endothelial cell adhesion molecule. JAM=junctional adhesion molecule.

C-X-C motif ligand 12 (CXCL12; stromal cell-derived factor 1); however, chemokine expression by endothelial cells is altered in EAE, and changes in expression of *CCL19* and *CXCL12* genes were found in brain lesions of people who had multiple sclerosis.[8] During inflammation, activation of the atypical chemokine receptor 1 mediates movement of chemokines across the brain endothelium and their release to the abluminal side, which creates high concentrations of chemokines (eg, CCL2 and CCL5, which are mainly produced by astrocytes) at the BBB and attracts peripheral immune cells.[9] Leukocytes such as $CD4^+$ T-helper cells secrete a plethora of cytokines and

can further disrupt BBB integrity or promote its barrier function. Interleukin 17 (IL-17) secretion by Th17 cells can interfere with tight junction molecule expression at the BBB and is associated with infiltration of interferon γ (IFNγ)-expressing pathogenic Th17 cells. By contrast, a subpopulation of Th17 leukocytes secrete the anti-inflammatory chemokine IL-26, increasing claudin-5 expression at the BBB and promoting the recruitment of T-regulatory lymphocytes and amelioration of EAE.[10]

Central in regulating neuroinflammation is the brain endothelial expression of CAMs: adipocyte CAM; intercellular CAM (ICAM)-1; vascular CAM (VCAM)-1; activated leukocyte CAM (ALCAM); melanoma CAM (MCAM); platelet and endothelial CAM (PECAM); and dual immunoglobulin domain-containing CAM (DICAM).[11–16] Upon interaction with their cognate receptors on immune cells, endothelial CAMs enable the first steps of trans-endothelial immune cell infiltration. Interactions between VCAM-1 and ICAM-1 and their ligands on leukocytes mediate the capture of immune cells from the blood and the arrest of these cells at the brain endothelium in inflammatory conditions. In a 2018 study, the extracellular matrix protein epidermal growth factor-like protein 7, secreted by brain endothelial cells, was shown to have beneficial properties in EAE by reducing MCAM expression and CNS immune cell infiltration.[17] DICAM is another promoter of encephalitogenic $CD4^+$ Th17 cell migration, being upregulated in patients with active relapsing-remitting and progressive multiple sclerosis, and a monoclonal antibody targeting DICAM has been shown to ameliorate both relapsing and progressive EAE.[11] Hence, CAMs and their interaction partners are attractive targets for novel therapies to treat multiple sclerosis.

Possible causes for BBB dysfunction in multiple sclerosis

BBB structure and function undergo changes during a person's life (panel). The course of multiple sclerosis is affected by ageing and age-related comorbidities, which also influence BBB integrity. Ageing is a major factor in the development of progressive multiple sclerosis: reduced angiogenesis, decreased cerebral blood flow, and lower vascular density in people older than 60 years might promote neurodegeneration. Genetic and environmental factors associated with multiple sclerosis can also directly and indirectly contribute to BBB disturbance (figure 2).

Genetic polymorphisms

A genome-wide association study revealed more than 200 genetic variants linked with susceptibility to multiple sclerosis.[25] The most robust susceptibility polymorphisms map to the *human leukocyte antigen* (*HLA*)-*DRB1* locus. Other variants are mostly located in immune-response-related genes not involved in the major histocompatibility complex (MHC) II.[25] Non-MHC II polymorphisms seem to be associated with proliferation and activation of cells of the adaptive immune system, which might also increase these cells' ability to cross the BBB. This hypothesis is supported by increased spinal cord inflammation and immune cell infiltration in people with multiple sclerosis and *HLA-DRB1*15*$^+$. Single-cell RNA sequencing in acute EAE indicates that brain venous endothelial cells upregulate genes linked to inflammation and antigen presentation in neuroinflammatory conditions.[12,26]

Single-nucleotide polymorphisms (SNPs) of genes encoding VCAM1 (rs11581062; odds ratio [OR] 1·13, 95% CI 1·09–1·18) and ALCAM (rs6437585CT; OR 2·34, 95% CI 1·22–4·51), CAMs expressed by brain endothelial cells under inflammatory conditions that mediate immune cell adhesion and transmigration, are risk factors for the development of multiple sclerosis.[15,27,28] Furthermore, SNPs for *CD6* (rs17824933; OR 1·18, 95% CI 1·07–1·30), the cognate receptor of ALCAM, and for *MMP9* (rs3918242; OR 3·20, 95% CI 1·87–5·46), which encodes an extracellular matrix degrading enzyme, are associated with onset of multiple sclerosis, implying direct and indirect effects of SNPs in genes linked to BBB disturbance on disease development.

Diet, vitamin D, and the gut microbiome

In mice, a complex, fibre-enriched diet promotes the production of anti-inflammatory and EAE-protective short-chain fatty acids (SCFAs) by the gut microbiome.[29] SCFAs support BBB integrity during embryonic development and in adult mice, suggesting the importance of a healthy gut microbiome for BBB function. Conversely, obesity, and a diet containing high amounts of red meat and foods high in fat and sugar, which are risk factors for multiple sclerosis, are associated with a less diverse microbiome.[30] Obesity promotes microglial and astrocytic activation and BBB dysfunction owing to alterations in the structure, transport, and expression of CAMs and oxidative stress at the BBB.[30] Reduced amounts of SCFAs in faeces, increased permeability of the intestinal barrier, and reduced SCFA-producing bacteria in the gut microbiome were reported in patients with multiple sclerosis compared with paired household healthy controls or sex, age, and race-matched healthy controls.[31,32]

High latitudes and reduced amounts of serum 25-hydroxyvitamin D are linked to an increased risk of multiple sclerosis onset and disease activity.[33] Furthermore, increased mean serum concentrations of 25-hydroxy-vitamin D predict better cognitive function and reduced serum concentrations of neurofilament light chain (a biomarker of neuroaxonal injury) in individuals with clinically isolated syndrome upon 11 years follow-up in the BENEFIT-11 study.[34] In a placebo-controlled phase 2 trial (n=229), vitamin D_3 with IFNβ versus placebo with IFNβ reduced the number of new gadolinium-enhancing lesions in patients with relapsing-remitting multiple sclerosis, indicating a role for vitamin D in protecting BBB function, which could be mediated by various direct and indirect biological mechanisms (appendix p 7).[35]

See Online for appendix

Panel: **Age-related characteristics of the blood–brain barrier (BBB)**

- The BBB develops during embryogenesis and seems functional before birth;[18] however, studies in mice indicate that cerebral vascularisation and BBB maturation proceed postnatally.
- Children exhibit a higher relative brain mass, lower grade of myelination, more fragile vasculature, and higher cerebral blood flow than adults.[19] Tight junction molecules are present in early development, but increased BBB permeability in the infant brain due to heightened activity of transporters and reduced function of efflux pumps might contribute to the increased vulnerability of the infant brain to circulating toxic molecules.
- The cerebrovascular tree exhibits reduced angiogenesis, decreased cerebral blood flow, and reduced vascular density with age.
- Permeability of the BBB and blood–CSF barrier can increase as people get older, as shown by an abundance of the peripherally produced protein albumin in the CSF versus the serum (Q_{ALB}).[20] Q_{ALB} values increase continually with age, and men show significantly higher values than women. This divergence occurs around puberty and indicates possible sex differences in BBB permeability.[20]
- Hippocampal MRI of individuals without cognitive decline corroborated the observation that BBB integrity (determined capillary permeability measured by the volume transfer constant [K^{trans}]) is impaired with ageing. BBB integrity was further diminished in people with cognitive impairment—including people with multiple sclerosis—compared with age-matched individuals without cognitive impairment, despite similar hippocampal volumes.[19]
- Disorganisation of endothelial tight junction molecules and reduced function of the BBB-located efflux transporter P-glycoprotein (which is needed to protect the brain from an accumulation of toxic substances) probably contribute to the age-associated increase in BBB permeability.[19]
- Single-cell RNA sequencing of brain endothelial cells derived from aged mice indicate a decline of the epigenetic modifier Sirtuin-1.[21]
- Several age-related factors might negatively affect the BBB integrity of people aged 60 years or older:[22]
  - Loss of pericytes due to increased oxidative stress
  - Impaired astrocytic function favouring oxidative metabolism
  - Disturbed glutamate regulation
  - Upregulation of genes linked to complement and antigen presentation
- The high abundance of mitochondria in brain endothelial cells might render them more vulnerable than their peripheral counterparts to propagation of mitochondrial DNA point mutations linked to biological ageing in humans.[23]
- Microvesicles derived from functional brain endothelial cells can be a cargo for mitochondrial transfer to support ATP and energy production in oxygen-deprived and glucose-deprived neurons and brain endothelial cells.[24] Although speculative, age-associated progressive mitochondrial dysfunction could interfere with this support mechanism, contributing to reduced brain endothelial function upon ageing.

Epstein-Barr virus (EBV)

A longitudinal study revealed that the risk of multiple sclerosis was 32-fold higher after infection with EBV.[36] Moreover, serum neurofilament light chain concentrations increased after EBV seroconversion in people who later developed multiple sclerosis, suggesting that EBV is an important causal factor for onset of the disease. In addition to its effect on B-cell biology, EBV could contribute to the pathobiology of multiple sclerosis through molecular mimicry of CNS proteins such as anoctamin-2 and GlialCAM. Among 8746 patients with multiple sclerosis, 14·6% (*vs* 7·8% in the control cohort; n=7228) were seropositive for anoctamin-2, an ion channel expressed by neuroglia,[37] and 20–25% of tested plasma samples from 36 patients showed increased reactivity (compared with healthy plasma donors) towards GlialCAM, which is expressed at the glia limitans and on perivascular glial cells.[38] As neuroglial and perivascular cells support BBB integrity, such autoreactive responses could directly (through immune-mediated neuroglial injury) and indirectly (through T-cell priming) affect BBB function in people with multiple sclerosis. EBV viral particles can be transported across the BBB as cargo of infected leukocytes, or can directly infect (and replicate within) human brain endothelial cells.[39] In-vitro human brain endothelial cells infected with EBV show upregulation of ICAM-1 and CCL5, and adherence of leukocytes to infected brain endothelial cells is increased, possibly further driving leukocyte recruitment and CNS infiltration.

Other environmental factors

Concussion and mild to moderate traumatic brain injuries temporarily increase BBB permeability, as shown by elevated serum concentrations of S100B and glial fibrillary acidic protein (GFAP) and the presence of microbleeds. Although pathophysiological mechanisms remain unclear, traumatic brain injury during teenage years is associated with significantly increased risk of developing multiple sclerosis.[40]

Smoking is a risk factor for the onset and progression of multiple sclerosis.[41] In mice with EAE, the lungs seem to be pivotal for disease development; peripheral encephalitogenic lymphocytes adopt an invasive chemokine profile (eg, CXCL11 and CCL5) in the lungs before infiltration into the CNS. Transcriptional profiling of $CD31^{+}$-enriched brain endothelial cells by use of whole-transcriptome sequencing and Gene Ontology gene enrichment analysis showed that brain endothelial cells in subacute EAE—when BBB permeability is highest—upregulate genes characteristic for lung endothelial cells.[7] This shift in expression profile might lead to the recruitment of immune cells that have changed their chemokine expression in the lung. Furthermore, lung inflammation in smokers might promote interactions between leukocytes and brain endothelial cells and, therefore, CNS inflammation. Smoke-derived nicotine changes cerebral vascular reactivity, is linked to altered microglial polarisation, and can increase BBB permeability by altering the expression of cerebral tight junction molecules.[42,43]

Night shift work in young adults is associated with an increased risk of developing multiple sclerosis and circadian rhythm sleep disorders (ie, disturbances to sleep–wake rhythms) are more common in people with multiple sclerosis than in healthy controls.[44] Sleep regulates molecule trafficking along and across the BBB; in both nocturnal rats and diurnal *Drosophila*,

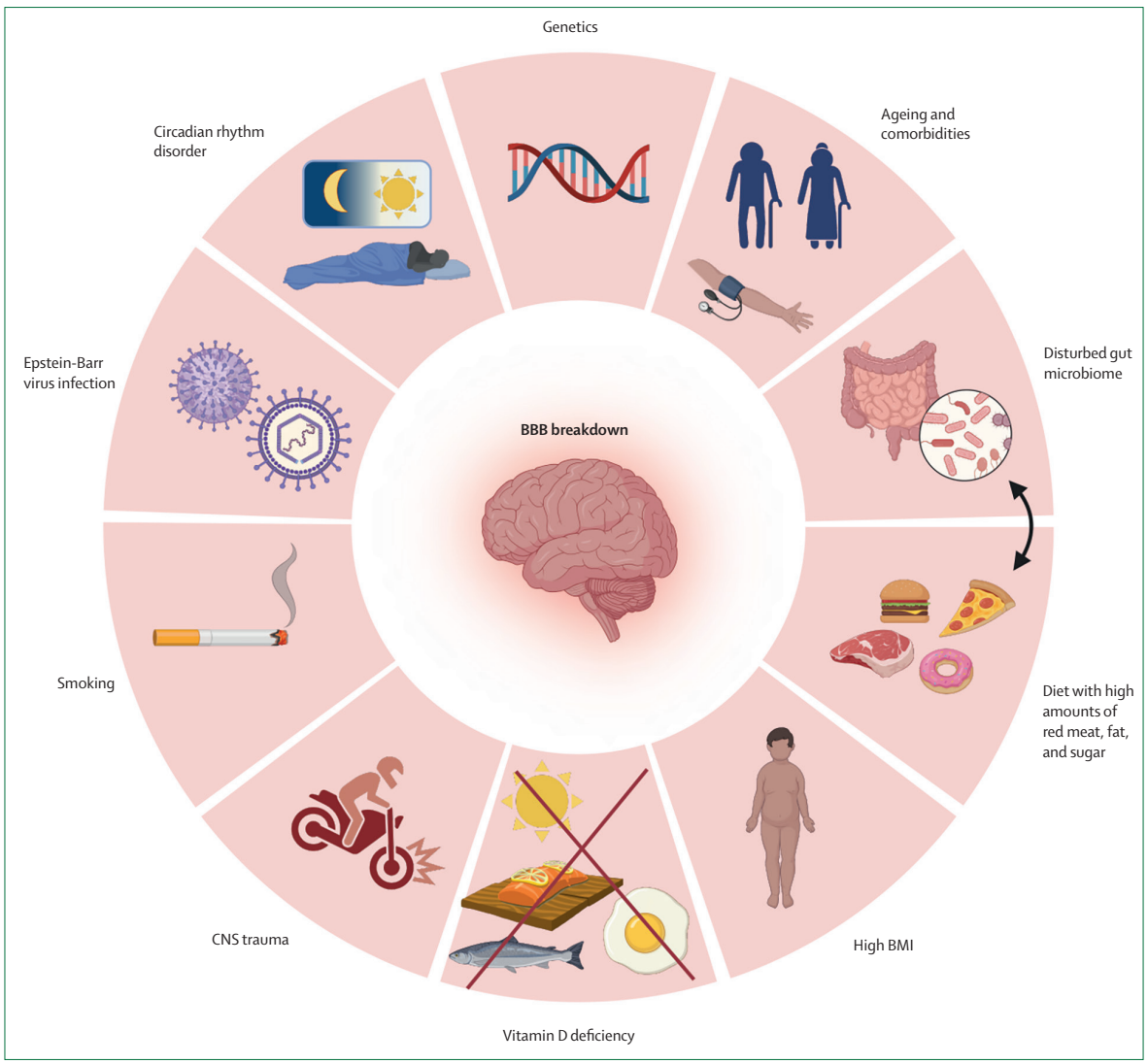

Figure 2: **Possible causes for BBB dysfunction in multiple sclerosis**
BBB integrity in people with multiple sclerosis is directly and indirectly influenced by genetic and environmental factors that are associated with increased risk of developing the disease. Single-nucleotide polymorphisms in human leukocyte antigen (HLA)-related and non-HLA-related immune response gene loci might indirectly promote immune cell trafficking across the BBB. Single-nucleotide polymorphisms in the cellular adhesion molecules VCAM and ALCAM, the ALCAM receptor CD6, and the extracellular matrix degrading enzyme MMP9, could exert a direct influence on BBB permeability and immune cell transmigration in multiple sclerosis. Ageing is the main risk factor for progression in multiple sclerosis, and is associated with reduced angiogenesis and vascular repair, disturbed mitochondrial metabolism, and cardiovascular comorbidities such as hypertension, which impair BBB integrity and increase the risk of white matter lesions. Children and adults with multiple sclerosis have altered gut microbiota; a reduced abundance of short-chain fatty acid-producing microbes could negatively affect the BBB, as these short-chain fatty acids are crucial for BBB integrity. A diet containing high amounts of red meat and high-fat and high-sugar foods shapes the gut microbiome towards dysbiosis and promotes high BMI, low-grade inflammation, and, therefore, increased BBB permeability. High BMI (>30) and obesity are associated with astrocyte and microglia activation and BBB dysfunction. High BMI in children and young adults is associated with an increased risk of multiple sclerosis. Vitamin D deficiency is also associated with an increased risk of multiple sclerosis, and downstream activation of vitamin D receptors promotes BBB integrity. Mild to moderate traumatic brain injury is directly linked to BBB disturbance and in teenagers is associated with an increased risk of multiple sclerosis. Smoking promotes lung inflammation, which could influence leukocyte priming prior to CNS infiltration in multiple sclerosis. Nicotine exhibits vasoactive properties and might change cerebrovascular reactivity, causing dysfunction of neurovascular coupling. Epstein-Barr virus infection can promote the destruction of GlialCAM on astroglial endfeet and increase expression of endothelial CAMs, possibly resulting in increased BBB permeability and immune cell infiltration. Sleep is essential to regulate the trafficking of molecules along and across the BBB, and therefore helps to maintain cerebral homoeostasis and BBB integrity. Shift work has been associated with an increased risk of multiple sclerosis. Figure created with BioRender.com. BBB=blood–brain barrier. VCAM=vascular cell adhesion molecule. ALCAM=activated leukocyte cell adhesion molecule. GlialCAM=glial cell adhesion molecule. MMP=matrix metalloproteinase.

BBB permeability for xenobiotics is increased during resting time.[45,46] This increased permeability is accompanied by elevated endocytosis of brain endothelial cells and reduced function of the P-glycoprotein efflux pump, which is controlled by circadian rhythm genes. Conversely, inhibition of the

pump and of endocytosis increases sleep in flies, possibly due to an influx of circulating sleep-promoting molecules.[47] By contrast, mice deprived of sleep show increased BBB permeability, marked astrocytic activation, elevated proinflammatory signalling, and reduced tight junction molecule expression.[48]

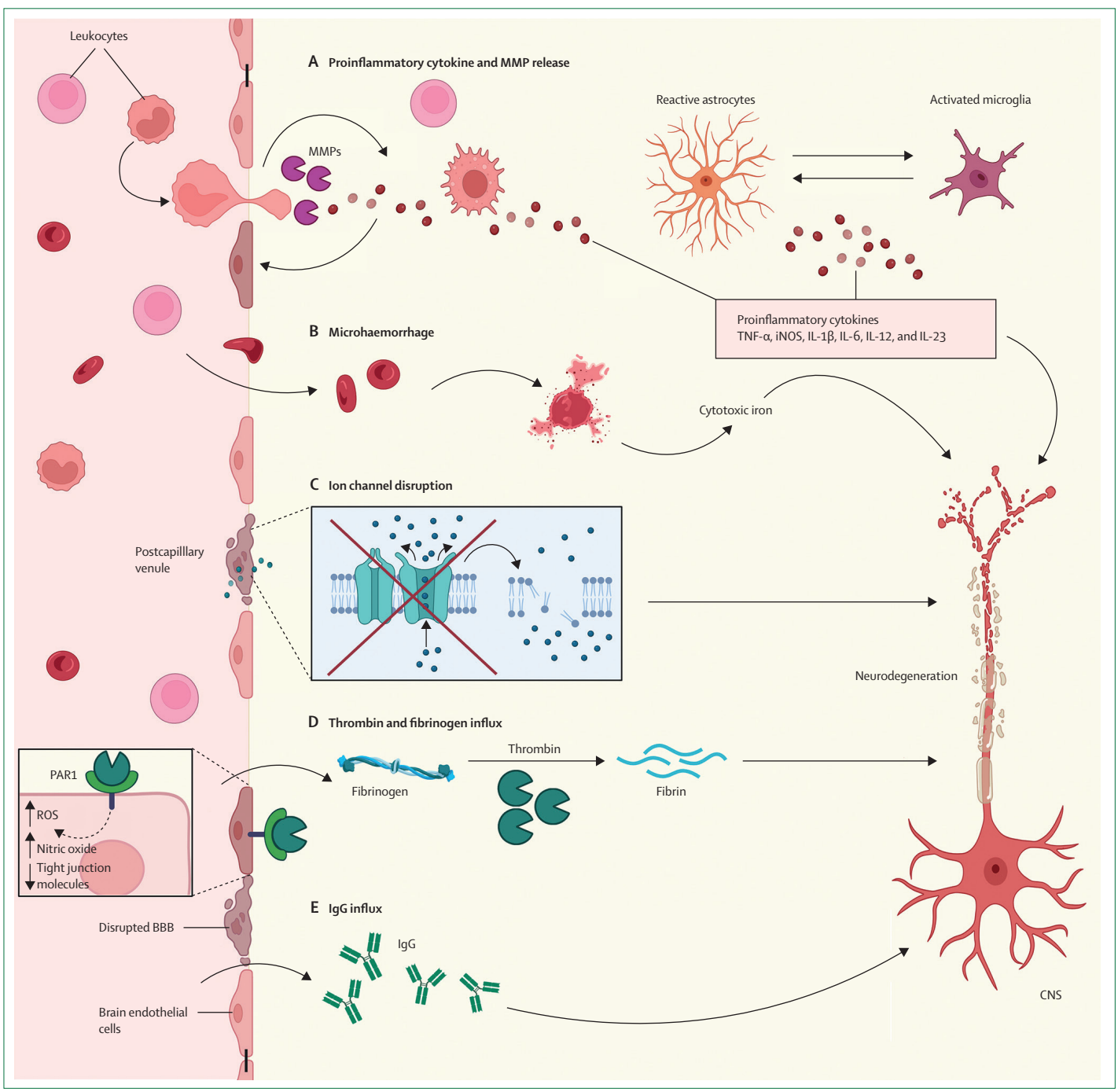

Figure 3: **Consequences of BBB disturbance in multiple sclerosis**
The BBB, the interface between blood and CNS parenchyma, is crucial for the regulation of molecule trafficking and restriction of immune cell entry under physiological conditions; during neuroinflammation, its disturbance leads to disruption of cerebral homoeostasis, neuronal dysfunction, and neurodegeneration. The BBB disruption in multiple sclerosis and experimental autoimmune encephalomyelitis, especially at the postcapillary venule, has several effects: the release of proinflammatory cytokines by both CNS-resident cells (eg, astrocytes and microglia, which influence each other) and infiltrating leukocytes (A); extravasation of erythrocytes and elevated levels of cytotoxic iron (B); disruption of ion channels at the brain endothelium, which can lead to unregulated passage of ions and neuronal damage (C); an influx of thrombin and fibrinogen, which leads to brain endothelial cell activation through an interaction between PAR1 and thrombin, downstream nitric oxide and ROS production, and downregulation of tight junction molecules (D); and an influx of immunoglobulins, including autoantibodies and activation of the complement cascade (E). Figure created with BioRender.com. BBB=blood–brain barrier. MMP=matrix metalloproteinase. PAR1=protease-activated receptor 1. TNF=tumour necrosis factor. iNOS=inducible nitric oxide synthase. IL-6=interleukin 6. ROS=reactive oxygen species.

Induced pluripotent stem cells derived from people with multiple sclerosis that differentiate into BBB endothelial cells show higher permeability, reduced efflux pump activity, and increased expression of CAMs compared with stem cells from healthy donors of a similar age, suggesting that genetic and epigenetic modifications might cause dysfunction of brain endothelial cells in people with multiple sclerosis.[49]

Consequences of a disturbed BBB function

As the BBB regulates molecule exchange between the blood and CNS tissue, its disturbance leads to disruption of cerebral homoeostasis, neuronal dysfunction, and neurodegeneration. Alteration of BBB integrity in people with multiple sclerosis and rodents with EAE promotes transcytosis, dysregulation of cerebral ion metabolism, influx of erythrocytes, and accumulation of cytotoxic iron in the brain, as well as an influx of fibrinogen, thrombin, and immunoglobulins, which might further drive pathology of the disease (figure 3).[50]

Influx and accumulation of toxic molecules

Although the activation of the plasminogen cascade is linked to matrix metalloproteinase activity and BBB breakdown in acute multiple sclerosis lesions,[6,51] the downregulation of fibrinolysis appears to be involved in progressive forms of the disease. Fibrinogen aggregates accumulate in various neurological diseases and traumatic injuries associated with BBB disruption.[52] By regulating growth factor receptor signalling and promoting an inflammatory response and scar formation, fibrinogen deposition has been associated with cognitive decline and inhibition of repair. A histochemical study of postmortem brain tissue showed that patients with progressive multiple sclerosis exhibited increased fibrin and fibrinogen deposition within areas of the motor cortex, which significantly correlated with expression of plasminogen activator inhibitor-1 and reduced neuronal density.[53] A monoclonal antibody recognising fibrin can enter the CNS of mice with EAE and selectively inhibit fibrin-induced inflammation and oxidative stress without interfering with clotting in the periphery.[54] However, the safety and efficacy of this antibody as a therapeutic intervention in humans remains untested.

Plasma-derived extracellular vesicles from patients with relapsing-remitting multiple sclerosis are enriched in fibrinogen.[55] The injection of vesicles containing fibrinogen aggravates EAE induced by myelin oligodendrocyte glycoprotein, and resembles spontaneous relapsing-remitting EAE, which might be mediated by activated $CD8^+$ T cells.[55] Therefore, fibrin and fibrinogen deposition, which possibly occurs after BBB breakdown in acute multiple sclerosis lesions, might exert long-term neurotoxic effects that contribute to pathological mechanisms in progressive forms of the disease.

The influx of blood-derived thrombin mediates further BBB breakdown by binding to endothelial protease-activated receptor 1. This breakdown elicits a cellular influx of Ca^{2+}, production of nitric oxide and reactive oxygen species, formation of stress fibres, and disruption of tight junction molecule complexes.[56] Although pathological iron deposition in the brains of people with multiple sclerosis[57,58] is mainly linked to the death of iron-enriched oligodendrocytes and destruction of their myelin sheaths,[51] microhaemorrhages in patients might also represent a source for cytotoxic iron influx. In this regard, iron accumulation was previously linked to increased production of reactive oxygen species, lipid peroxidation, reduced amounts of antioxidants, and neurodegeneration in patients with multiple sclerosis.[51]

To maintain cerebral homoeostasis and an ideal interstitial fluid for neuronal function,[5] brain endothelial cells control ion transport through modulating their expression of tight junction molecules (to inhibit paracellular transport), receptors, and ion channels.[59] Disturbance of BBB integrity in multiple sclerosis, however, might dysregulate selective ion exchange across the BBB and might be neurotoxic.

Cognitive decline and dysfunctional neurovascular coupling

Up to nearly 45% of patients with relapsing-remitting multiple sclerosis, and 75% with secondary-progressive multiple sclerosis, have cognitive slowing, as evidenced by standardised neuropsychological tests (eg, the symbol digit modalities test), that can affect their daily tasks of working memory and verbal fluency.[60] Functional MRI studies found that cerebral vascular reactivity (ie, the change of cerebral blood flow upon stimulation with vasoactive compounds) is significantly reduced in the grey matter of patients with multiple sclerosis compared with healthy controls.[61] This disturbance was significantly correlated with grey matter atrophy and lesion volume in multiple sclerosis.[61] Upon stimulation with the vasoactive mediator CO_2, cognitive slowing was significantly associated with disturbed arterial compliance, indicating dysfunctional neurovascular coupling.[62] Because grey matter atrophy can influence cerebral vascular reactivity, whether disturbed cerebral vascular reactivity is a cause or consequence, or both, of neurodegeneration linked to cognitive impairment in multiple sclerosis remains unclear.

Fluid biomarkers as indicators for BBB disturbance

MRI is the gold standard method to assess BBB disturbance and lesion distribution and is indispensable for diagnosing and monitoring multiple sclerosis. However, MRI is expensive, time-consuming, and not always accessible, and its usefulness is restricted by resolution and artifacts; circulating biomarkers that reflect BBB dysfunction would be more convenient for routine measurements. BBB disturbance is associated with the presence of white matter lesions and increased CSF:serum

albumin index (Q_{ALB}).[20] Although CSF is particularly informative in multiple sclerosis, lumbar puncture is an invasive procedure, making peripheral blood biomarkers preferable (table 1). Ultrasensitive single-molecular array bead technology enables detection of molecules linked to CNS damage in the blood (eg, neurofilament light chain, GFAP, and S100β). Concentrations of these proteins presumably increase in the blood after CNS damage—as seen in multiple sclerosis—by CSF drainage towards the peripheral compartment and through a disrupted BBB.

Neurofilament light chain concentrations in blood and CSF are increased in individuals newly diagnosed with multiple sclerosis compared with healthy controls of a similar age, and correlate with relapses, new lesion formation (indicated by MRI), disease severity, and prognosis.[63] During the first demyelinating event, elevated concentrations in the serum correlate with Q_{ALB} and IgM indices, and with the presence of CSF-located $CD80^+$ B cells and gadolinium-enhancing lesions, suggesting that increased BBB permeability might contribute to the increased amounts of serum neurofilament light chain.[64] Treatment with disease-modifying therapies reduces serum neurofilament light chain concentrations;[65–67] however, increased serum concentrations can also indicate peripheral nervous system damage[68] and do not necessarily correlate temporally with BBB breakdown.[69]

Compared with healthy controls, serum GFAP concentrations were reported to be significantly higher in patients with actively progressive and relapsing-remitting multiple sclerosis around the time of a relapse, but not during remission.[70] GFAP production is increased in response to CNS injury, but also to BBB breakdown after mild traumatic brain injury and intracerebral haemorrhage.[71] Therefore, increased serum GFAP concentrations might indicate CNS injury, BBB disturbance, or both. By contrast, serum S100β is linked to BBB leakage independent of neuronal damage. S100β concentrations in blood and CSF are increased in relapsing-remitting multiple sclerosis at diagnosis and correlate with disease severity and progression.[72] Furthermore, myelin basic protein (MBP) is a CNS-specific myelin component and its presence in CSF and serum is a direct indicator for myelin damage. Elevated MBP was detected in people with active relapsing-remitting multiple sclerosis compared with people with stable or progressive disease. As MBP is not detectable in the peripheral compartment, it might be suitable to assess BBB damage associated with different neurodegenerative disease states, including spinal cord demyelination.[73]

Acute exacerbation in multiple sclerosis is linked to neuroinflammation and associated with the secretion of chemokines promoting leukocyte recruitment to the inflamed CNS site. Increased amounts of the chemokines CXCL8 (linked to innate immune response), CXCL10 (which mediates T-cell recruitment and Th1 response), and CXCL13 (which mediates B-cell recruitment and IgG production) can be detected in the CSF of patients with paediatric-onset multiple sclerosis compared with age-matched, non-neuroinflammatory, non-neurodegenerative controls, as well as in adults with multiple sclerosis.[74] By contrast, CCL2 concentrations in CSF are downregulated in adults compared with non-inflammatory neurological diseases, and tend to be lower in younger patients compared with children with non-inflammatory and non-neurodegenerative disease. In both paediatric-onset and adult multiple sclerosis, CCL2 concentrations show a significant negative correlation with Q_{ALB} and, therefore, BBB permeability. Potentially explaining this phenomenon, research indicates that CCL2 is consumed by $CCR2^+$ leukocytes in patients with multiple sclerosis and EAE upon CNS infiltration.[74]

Brain endothelial cell-derived CAMs, including endoglin (CD51), E-selectin, VCAM-1, MCAM, and PECAM-1 (CD31), can be shed from the endothelial plasma membrane and circulate as endothelial microparticles in plasma and serum. Patients with multiple sclerosis who have gadolinium-enhancing lesions exhibit an increased abundance of $CD31^+$ endothelial microparticles in plasma,[16] which might indicate brain endothelial cell activation and BBB disruption. Wang and colleagues further corroborated the function of MCAM on vascular brain endothelial cells, and showed that its soluble form is enriched in the CSF of patients with active disease compared with patients with non-inflammatory neurological diseases or inactive relapsing-remitting multiple sclerosis, and can be a sensitive marker for BBB damage and neuroinflammation.[75] Soluble MCAM could stem from both brain endothelial cells and inflammatory T cells. In a secondary analysis of the RAGTIME study, which included 60 participants with progressive multiple sclerosis,[76] soluble L-selectin (which is expressed on leukocytes and mediates rolling along the brain endothelium), E-selectin (expressed on brain endothelial cells and promotes leukocyte arrest), and PECAM-1 (which is expressed on brain endothelial cells and promotes diapedesis of leukocytes) were reduced after rehabilitative exercise and correlated with mobility outcome. However, whether people with progressive multiple sclerosis have higher amounts of soluble CAMs than healthy volunteers, and whether exercise contributes to preserve BBB integrity, remains to be explored.[76] Furthermore, P-selectin is rapidly upregulated on brain endothelial cells during injury, can be a marker of vascular inflammation, and can predict disease activity in EAE by use of in vivo molecular MRI. Further studies are needed to establish whether P-selectin could also aid in disease management, for example in monitoring brain endothelial activation, in people with multiple sclerosis.[77]

Soluble CD40 ligand (sCD40L) is recognised by CD40 expressed on brain endothelial cells and might also be both a biomarker and mediator of BBB disruption. sCD40L is increased in the serum of patients with multiple sclerosis compared with people with other inflammatory

	Tissue or cell type	Function	Relevance
Direct indicators of BBB disturbance			
Albumin (CSF-to-serum Q_{ALB} ratio)	Most abundant protein in human blood	Carrier protein for a wide range of endogenous molecules including hormones, fatty acids, and metabolites	Direct marker for BBB permeability; correlates with white matter and gadolinium-enhancing lesions; increases continuously with age
Indirect indicators of BBB disturbance			
Myelin basic protein	Highly enriched in CNS-derived myelin	Formation and stabilisation of myelin sheaths	Increased serum and CSF concentrations directly indicate myelin damage; might be suitable to assess BBB damage associated with different neurodegenerative disease states, including spinal cord demyelination; increased concentrations in active relapsing-remitting multiple sclerosis compared with stable and progressive multiple sclerosis
Fibrin and fibrinogen and its degradation product D-dimer	Blood-borne glycoprotein	Blood clotting; fibrinogen and derived fibrin regulate cell adhesion and spreading, display vasoconstrictor and chemotactic activities, and act as mitogens for several cell types	Plasma-derived extracellular vesicles from patients with relapsing-remitting multiple sclerosis are enriched in fibrinogen, and their injection accelerates EAE disease course; increased serum fibrinogen and fibrinogen-to-albumin ratios in patients with relapsing-remitting multiple sclerosis who present with a relapse independent of their disease duration and EDSS; CSF D-dimer values are significantly higher in patients with relapsing-remitting multiple sclerosis (as shown through contrast enhancement MRI) than in patients without BBB impairment
Glial fibrillary acidic protein	Mature astrocytes (CNS) and non-myelinating Schwann cells (PNS)	Intermediate filament protein	Serum concentrations are increased in progressive multiple sclerosis and active relapsing-remitting multiple sclerosis compared with healthy controls, but not in stable multiple sclerosis; concentrations increase in serum in response to CNS injury (eg, BBB breakdown after mild and severe TBI and intracerebral haemorrhage)
MMP9-to-TIMP1 ratio	Neutrophils, macrophages, fibroblasts, and many other cell types	Breakdown of extracellular matrix and tissue remodelling in health and disease	An increased MMP9-to-TIMP1 ratio correlates with the presence of gadolinium-enhancing lesions on MRI in patients with relapsing-remitting and secondary progressive multiple sclerosis, whereas IFNβ treatment restores aberrant ratios in relapsing-remitting multiple sclerosis
Neurofilament light chain	Expressed by neurons of the CNS and PNS	Maintains neuronal calibre; might have a role in intracellular transport to axons and dendrites	Can be correlated with increased BBB permeability at multiple sclerosis onset; increased serum concentrations indicate neuroaxonal damage; correlated with relapses, new lesion formation (shown on MRI), multiple sclerosis severity, and poor prognosis
S100β	Astrocytes (CNS), certain neuronal populations (CNS), oligodendrocytes (CNS), Schwann cells (PNS), and myeloid-derived cells	Calcium-binding protein	Correlates with BBB permeability independent of neuronal damage; increased blood and CSF concentrations in relapsing-remitting multiple sclerosis at diagnosis compared with non-inflammatory or inflammatory neurological disease controls; correlates with disease severity and progression
PECAM1 (CD31)	Endothelial cells, platelets, monocytes, neutrophils, and some T cells	Leukocyte migration, angiogenesis, and integrin activation	Plasma concentrations of $CD31^+$ microparticles correlate with gadolinium-enhancing lesions in multiple sclerosis; serum PECAM-1 correlates with outcomes after rehabilitative exercise outcomes in patients with progressive multiple sclerosis
Monocyte chemoattractant protein-1 (CCL-2)	Endothelial cells, smooth muscle cells, fibroblasts, epithelial cells, mesangial cells, astrocytes, T cells, myeloid cells, and tumour cells	Attraction of monocytes and basophils; binds to CCR2 and CCR4	Significant inverse correlation with Q_{ALB} in paediatric onset and adult multiple sclerosis
CD40	B cells, activated T cells, dendritic cells, monocytes, platelets, macrophages, myofibroblasts, fibroblasts, epithelial cells, and endothelial cells	Member of the TNF-receptor superfamily; promotes BBB permeability, monocyte infiltration, and endothelial adherence	Significantly upregulated in serum and CSF from patients with multiple sclerosis, and correlates with Q_{ALB} concentrations
Osteopontin	Bone tissue, macrophages, endothelial cells, smooth muscle cells, and epithelial cells	Binds to vitronectin; linked to attachment of osteoclasts to the mineralised bone matrix; acts as a cytokine and upregulates expression of IFNγ and IL12	Concentrations in blood and CSF increase from clinically isolated syndrome to progressive forms to relapsing-remitting multiple sclerosis, and show highest concentrations in patients with active disease

Biomarkers are listed as either direct or indirect indicators of BBB disturbance and are sorted chronologically by date of discovery as a potential indicator of BBB status. As BBB disturbance changes with disease progression, a combination of several markers might aid in the diagnosis, classification of clinical course, and disease management in people with multiple sclerosis. References are in the appendix (pp 1, 2). BBB=blood–brain barrier. CCL= C-C motif ligand. CCR=C-C chemokine receptor type. CD=cluster of differentiation. EAE=experimental autoimmune encephalitis. EDSS=Expanded Disability Status Scale. IFNβ=interferon beta. IL-12=interleukin 12. MMP=matrix metalloproteinase. PNS=peripheral nervous system. Q_{ALB}=CSF: albumin index. S100β=S100 calcium binding protein B. PECAM-1= platelet and endothelial cell adhesion molecule-1. TIMP=tissue inhibitor of metalloproteinase. TNF=tumour necrosis factor.

Table 1: **Potential biomarkers of BBB status in people with multiple sclerosis**

and non-inflammatory neurological diseases, and its concentrations correlate with Q_{ALB}.[78] By promoting BBB permeability and monocyte infiltration and adherence, sCD40L could indirectly contribute to BBB breakdown.[78]

Similarly, osteopontin is a potential biomarker of BBB damage and a mediator of inflammation. Concentrations of osteopontin are increased in the CSF and blood of patients with multiple sclerosis compared with healthy

donors.[79] Concentrations of the protein are lowest in samples from people with clinically isolated syndrome, higher in people with progressive multiple sclerosis, further increased in people with relapsing-remitting disease, and highest in patients with active relapse. Although studies in ischaemic stroke mouse models suggest that osteopontin stabilises the BBB,[80] the protein is generally considered to promote inflammatory processes in multiple sclerosis and worsen EAE.[81]

Serum fibrinogen, and the fibrinogen-to-albumin ratio, in patients with relapsing-remitting multiple sclerosis were significantly higher during a relapse than in healthy controls,[82] and plasma fibrinogen concentrations were elevated in people with multiple sclerosis who exhibited active lesions on MRI compared with people with no active lesions.[83] However, fibrinogen concentrations did not vary among patients with different disease duration or scores on the extended disability status scale.[82,83] Therefore, fibrinogen concentration in the blood might be an objective marker of multiple sclerosis relapse and associated BBB breakdown, which could aid in guiding decisions to initiate corticosteroids for suspected acute relapses. After fibrinogen enters the CNS, it is rapidly converted to fibrin, which is proteolytically degraded to D-dimer. Correspondingly, patients with relapsing-remitting multiple sclerosis show significantly higher quotients of CSF and plasma D-dimer normalised to Q_{ALB} compared with disease controls.[84] Absolute CSF D-dimer values were significantly higher in patients with multiple sclerosis who had signs of BBB disturbance on contrast-enhanced MRI, compared with patients without BBB impairment.[84]

Drugs affecting the BBB

As brain endothelial cells are in direct contact with molecules and cells in the periphery, they represent an important therapeutic target. However, the BBB restricts the capacity of most drugs to reach adequate concentrations in the CNS beyond areas surrounding active lesions. Disease-modifying therapies currently available for multiple sclerosis are immunomodulatory or immunosuppressive drugs that deplete specific immune cell subsets or restrict proliferation, activation, or trafficking of immune cells. All approved therapies are associated with a reduction in new gadolinium-enhancing lesions, indicating that they have beneficial properties on BBB function through direct or indirect effects (table 2).

Current multiple sclerosis therapies that directly modulate the BBB

Glucocorticosteroids are still prescribed for acute treatment of multiple sclerosis relapses. Being lipophilic, glucocorticoids show a high ability to reach the CNS under physiological conditions and significantly enhance BBB function through upregulation of tight junctions and adherens junctions, and downregulation of inflammation-induced endothelial CAMs in vitro. Although glucocorticoids exert systemic anti-inflammatory properties and restore BBB function in patients with multiple sclerosis,[85] adverse effects restrict their long-term use.

The first approved disease-modifying therapy, IFNβ, enhances BBB integrity, reduces trans-endothelial migration of proinflammatory $CD4^+$ Th1 cells, and restores aberrant MMP9-to-TIMP-1 ratios (an indicator for BBB breakdown and increased immune cell infiltration into the deep brain parenchyma) in patients with relapsing-remitting multiple sclerosis.[85] IFNβ is available as subcutaneous or intramuscular injections for patients with active disease, but rodent studies indicate that intranasal application could improve passage across the BBB (appendix p 6), and therefore might be more effective in patients with no signs of increased BBB permeability.

Natalizumab, a monoclonal antibody against α4β1 integrin, the cognate ligand of VCAM-1, directly interferes with trans-endothelial migration of immune cells, which reduces further disruption and inflammation of the BBB. Natalizumab was also shown to reduce MMP-to-TIMP ratios in mice with EAE.[86] Other approved disease-modifying therapies for multiple sclerosis mainly reduce immune-mediated BBB damage indirectly by restricting the activation, proliferation, or circulation of proinflammatory immune cells in the periphery (table 2).[87–90] Therapies able to reach the CNS could indirectly improve BBB dysfunction by reducing the activation of glia within that compartment.

Emerging therapies that affect the BBB

Bruton's tyrosine kinase (BTK) inhibitors are currently under investigation in phase 2 and 3 clinical trials for active relapsing-remitting and primary progressive multiple sclerosis.[91] In addition to their ability to cross the BBB and their promising role in modulating B-cell and myeloid cell biology, BTK inhibitors can interfere with inflammasomes induced by nucleotide-binding oligomerisation domain, leucine-rich repeat, and pyrin domain-containing 3 (NLRP3).[92] This pathway is involved in trans-endothelial migration of $CD4^+$ T cells and has been implicated in the pathology of multiple sclerosis. BTK inhibitors might also prevent NLRP3-induced activation of brain endothelial CAMs, but its direct effect on BBB integrity needs further exploration.

The anti-inflammatory SCFA butyrate, which is released by the gut microbiome, or its derivative 4-phenyl butyrate, which has been approved by the US Food and Drug Administration for urea cycle disorders and progressive familial intrahepatic cholestasis type 2, can enhance BBB integrity in vitro and in vivo and can ameliorate the course of EAE.[93] Sodium butyrate is a potent histone deacetylase (HDAC) inhibitor that mainly interferes with the activity of class I HDAC enzymes, resulting in reversible epigenetic modulations. Sodium butyrate and other HDAC inhibitors exert anti-inflam-

	Indication or function	BBB modulation	Ability to cross the BBB
Approved therapies			
Glucocorticosteroids	Treatment of acute multiple sclerosis relapses; exert systemic anti-inflammatory properties	Enhance BBB function; upregulate TIMP1; protect against serum-derived deleterious soluble factors	Able to cross the BBB due to lipophilic properties and minor interaction with BBB efflux pumps
IFNβ	Cytokine and immunomodulator; reduction of new gadolinium-enhancing lesions in multiple sclerosis	Enhances BBB integrity; reduces trans-endothelial migration of pro-inflammatory $CD4^+$ Th1 cells; upregulates CD73 ecto-5′-nucleotidase activity and the anti-inflammatory and neuroprotective purine adenosine in vitro; restores aberrant MMP9-to-TIMP-1 ratios in relapsing-remitting multiple sclerosis	Minor; increased BBB trafficking when administered intranasally compared with intravenously in rats
Glatiramer acetate	Treatment for relapsing-remitting multiple sclerosis; copolymer and immunomodulator; might act as a decoy for myelin-attacking immune cells; induction of anti-inflammatory T cells; reduction of new gadolinium-enhancing lesions in multiple sclerosis	Alters inflammatory processes; might indirectly exert beneficial effects on BBB	None
Natalizumab	Monoclonal antibody against α4β1 integrin; reduction of new gadolinium-enhancing lesions in multiple sclerosis	Interferes with trans-endothelial migration of immune cells; has beneficial effects on MMP-to-TIMP ratios in an EAE mouse model; restricts immunosurveillance of the CNS	None
Fingolimod	Sphingosine 1-phosphate receptor modulator; reduces lymphocyte infiltration into the CNS; might protect against demyelination by supporting myelin integrity in animal models	Might indirectly modulate the BBB by reducing infiltrating immune cells and acting on CNS-resident cells; decreases vascular permeability	Exhibits lipophilic properties and can cross the BBB; might accumulate within myelin sheaths
Dimethyl fumarate	Derivatives of fumaric acid; activates Nrf2	Stabilises and increases BBB integrity possibly through the Nrf2 signalling pathway, which is crucial for the antioxidant response; decreases expression of CAMs (eg, VCAM and ICAM-1)	Rapidly hydrolysed after absorption to monomethyl fumarate, which can cross the BBB
Alemtuzumab	Monoclonal anti-CD52 antibody; depletes B and T lymphocytes; reduces in vitro trans-endothelial migration of $CD4^+$ effector memory and $CD8^+$ central memory T cells; reduces annual relapse rate	Around 40% of patients maintained no evidence of disease activity 3 (NEDA-3) status 2 years after treatment initiation measured by MRI, and might therefore provide beneficial long-term effects on the BBB	None
Monoclonal antibodies targeting CD20 (ocrelizumab, ublituximab, ofatumumab)	Ocrelizumab is approved for relapsing-remitting and primary progressive multiple sclerosis; ublituximab and ofatumumab are approved for relapsing multiple sclerosis; all drugs of this type deplete $CD20^+$ B cells (and a rare subset of $CD20^+$ T cells); suppress clinical and radiological disease activity; rituximab, another CD20-depleting drug, is used off-label for patients with multiple sclerosis	Might indirectly improve BBB integrity by reducing proinflammatory cytokine secretion, by interfering with antigen presentation to T cells, or by reducing complement activation and auto-antibody production by B cells (or by a combination of these mechanisms)	None
Cladribine	Adenosine analogue; potentially reduces the number of recruited lymphocytes and circulating lymphocytes, reduces in vitro trans-endothelial migration of $CD4^+$ effector memory and $CD8^+$ central memory T cells; inhibits microglial function	Might indirectly modulate the BBB by downregulating CXCL8 and CCL5 secretion by immune cells; reduces MMP2, MMP9, and ICAM-1 expression	Can cross the BBB
Potential therapies in development			
Monoclonal antibodies targeting CAMs	Specific blockade of interactions between pathogenic immune cells and brain endothelial cells and trans-endothelial migration into CNS; significantly reduce demyelinating lesions in EAE mouse models and promote amelioration of disease course	Blockade of specific CAMs associated with proinflammatory status of brain endothelium in EAE mouse models and in-vitro human BBB models	Untested
HDAC inhibitors	Epigenetic modifier drugs; increase histone acetylation; exhibit immunomodulatory function; might exert different function depending on specificity of various HDAC classes or their enzymes	4-PBA significantly restores BBB integrity in germ-free mice; 4-PBA enhances BBB integrity in vitro and in vivo; 4-PBA, belinostat, and others were shown to ameliorate EAE disease course	Short-chain fatty acids such as 4-PBA can cross the BBB via monocarboxylate transporters; belinostat was shown to cross BBB in vivo; other HDAC inhibitors might have different properties
Sirtuin-1 agonists	Epigenetic modifier drugs; enhance mitochondrial metabolism; act as antioxidants; exhibit pro-remyelinative function; only negligible immunomodulatory function	Resveratrol restores claudin-5 expression in a mouse model for ageing; might indirectly modulate BBB by counteracting ageing processes; boosts mitochondrial metabolism which might aid in vascular repair	Can cross the BBB but have general poor bioavailability; however, a pharmaceutical compound with enhanced absorption has been tested in mouse models of Huntington's disease
HSC transplantation	Re-establishment of haematopoietic compartment; aids in rebooting the immune system in aggressive multiple sclerosis	Might indirectly modulate BBB integrity by engraftment of immune cell compartment or cross correction (ie, the ability of lysosomal enzyme-expressing cells to correct other enzyme-deficient cells) of CNS-resident cells; halts inflammatory demyelination and prevents gadolinium-enhanced lesions	HSC or HSC-derived precursor cells might penetrate the BBB and differentiate into microglia-like cells that enable further cross-correction in the CNS

(Table 2 continues on next page)

	Indication or function	BBB modulation	Ability to cross the BBB
(Continued from previous page)			
Mesenchymal stromal cells	Regulate tissue regeneration at local and systemic levels by secreting a plethora of molecules	Restore damaged BBB by improving angiogenesis, stabilising cerebral vessels, and upregulating cerebral occludin in an MCAO rat model; results in upregulation of cerebral tight junction molecules in EAE	Can cross BBB when administered intravenously
EPCs	Circulating cells that express cell surface markers similar to those expressed by vascular endothelial cells; adhere to endothelium at sites of hypoxia or ischaemia; participate in new vessel formation	Possibly by promoting mitochondrial metabolism; exposure of EPC particles promotes angiogenesis and enhanced BBB integrity in vitro	Might incorporate into vessels of choroid plexus in mice

Currently available therapies are listed chronologically by approval date and potential therapies in development are ordered by drug class (monoclonal antibodies, epigenetic modifier drugs, and stem cell or progenitor cell therapy). Bruton's tyrosine kinase inhibitors are not included in the table as studies assessing direct effects on the BBB have not yet been reported. References are in the appendix (pp 3, 4). 4-PBA=4-Phenyl sodium butyrate. CAM=cell adhesion molecule. CCL=chemokine (C-C motif) ligand. CD=cluster of differentiation. CXCL8=CXC motif chemokine ligand 8. EAE=experimental autoimmune encephalomyelitis. EPCs=endothelial progenitor cells. HDAC=histone deacetylase inhibitor. HSC=haematopoietic stem cell. ICAM-1=intercellular cell adhesion molecule 1. MCAO=middle cerebral artery occlusion. MMP=matrix metalloproteinase. Nrf2=nuclear factor erythroid 2-related factor 2. TIMP=tissue inhibitor of metalloproteinase. VCAM=vascular cell adhesion molecule.

Table 2: **Approved and potential drugs that might affect BBB status**

matory and neuroprotective effects,[94] and belinostat has beneficial effects on acute EAE.[95] However, other HDAC inhibitors (eg, panobinostat, givinostat, or entinostat) do not interfere with disease progression in a mouse model of progressive multiple sclerosis.[96] In contrast to classical HDAC enzymes (ie, classes I, IIa, IIb, and IV), overexpression of Sirtuin-1 restores BBB integrity through upregulation of claudin-5, as shown in a mouse model of ageing.[21] Resveratrol, a potent Sirtuin-1 agonist, is linked to increased mitochondrial metabolism, can cross the BBB, and is available as a pharmaceutical formulation with enhanced absorption (SRT501) to improve bioavailability. Resveratrol had pro-remyelinative effects in a cuprizone-induced demyelinating mouse model of multiple sclerosis,[97] and might be an attractive candidate therapeutic approach for patients with progressive multiple sclerosis.

Haematopoietic stem cell transplantation can halt inflammatory demyelination in aggressive multiple sclerosis.[98] This transplantation reboots the immune response and might indirectly affect BBB integrity. Other progenitors, such as multipotent mesenchymal stromal cells, appear to have a role in regeneration of the brain microvasculature and therefore could mediate BBB repair. In a middle cerebral artery occlusion rat model, mesenchymal stromal cell transplantation restored damaged BBB by improving angiogenesis, stabilising cerebral vessels, and upregulating occludin. Mesenchymal stromal cell treatment also upregulates cerebral tight junction molecules in EAE.

Vesicles derived from mesenchymal stromal cells are a promising cell-free therapeutic option in the context of neurodegenerative diseases.[99] Similar to nanotechnology-based therapies, these naturally occurring microvesicles can be used as drug delivery vehicles by modifying their surface with brain-ligand specific molecules and loading them with therapeutic RNAs or proteins. An in-vitro study found that extracellular mitochondria derived from endothelial progenitor cells are taken up and incorporated by damaged brain endothelial cells, as shown by increased mitochondrial protein TOM40, mitochondrial DNA copy number, and intracellular ATP.[100] Furthermore, exposure to endothelial progenitor cell particles (ie, cell-free fraction containing mitochondria) promoted angiogenesis and enhanced BBB integrity in vitro. Advances based on particles 1–100 nm in diameter could enable trans-endothelial trafficking to deliver therapeutics into the CNS.[101]

Conclusions and future directions

BBB breakdown is an early pathological event in multiple sclerosis, observed prior to lesion formation and in normal-appearing white matter, and accompanied by pathogenic immune cell infiltration. Both BBB impairment and proinflammatory immune cell invasion promotes a hostile environment for neuronal activity and CNS tissue repair. Although the effects on the BBB of genetic and environmental factors associated with multiple sclerosis (eg, dietary habits, the gut microbiome, vitamin D concentrations, and EBV infection) are often overlooked, these factors might contribute to brain endothelial cell dysfunction. Transcriptional profiling of brain endothelial cells in acute EAE has uncovered a role for these cells in antigen presentation and found downregulation of genes linked to angiogenesis and repair, while transcriptional changes were similar to their counterparts in the lung. Prospective studies further analysing the mechanisms underlying these observations might pave the way for novel treatments that directly target BBB function. Although BBB disturbance is less prominent in progressive multiple sclerosis than in relapsing-remitting multiple sclerosis, patients show dysfunctional neurovascular coupling that correlates with grey matter atrophy and cognitive decline, which increases with disease progression. The association between dysfunction of neurovascular coupling and grey matter atrophy highlights that BBB functionality is of interest not only in the early stages of multiple sclerosis, but also in the development of therapies for its progressive forms.

Search strategy and selection criteria

We searched PubMed and Google Scholar for peer-reviewed articles written in English and published between Jan 1, 2017, and March 31, 2023. We used the search terms "multiple sclerosis AND blood–brain barrier", "multiple sclerosis AND environmental risk factors AND blood–brain barrier", "multiple sclerosis AND genetic polymorphism AND blood–brain barrier", "multiple sclerosis AND biomarker AND blood-brain barrier ", and "multiple sclerosis AND drugs AND blood–brain barrier". All authors agreed on the final list of references, which was selected on the basis of originality, impact, and topical relevance. For some sections, further references are available in the appendix (pp 7–10).

Contributors

BZ searched the literature, wrote the manuscript, and prepared the figures. CL and AP reviewed and edited the manuscript.

Declaration of interests

BZ was supported by a joint Fonds de recherche Québec—Santé (FRQS)–Multiple Sclerosis Society of Canada fellowship. CL is an FRQS Clinicien–Chercheur Junior 2 Scholar; has served on scientific advisory boards for FIND Therapeutics, Novartis, Biogen, Sanofi, Bristol Myers Squibb, and Actelion; has served as a speaker for Novartis, Biogen, Sanofi, Roche, and EMD Serono; and has received a grant for Multiple Sclerosis Innovation from Merck–EMD Serono. AP holds the Senior Canada Research Chair in Multiple Sclerosis and active patents WO2016095046A1, US20110014183A1, and US20100310568A1.

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