**The blood-brain barrier in systemic infection and inflammation**

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**ABSTRACT**

The vascular blood-brain barrier is a highly regulated interface between blood and brain. Its primary function is to protect central neurons, while signalling the presence of systemic inflammation and infection to the brain to enable a protective sickness behavioural response. With increasing degrees and duration of systemic inflammation, the vascular blood-brain barrier becomes more permeable to solutes, undergoes an increase in lymphocyte traffic, is infiltrated by innate immune cells, and frank endothelial cell loss may occur. Perturbation of neuronal function results in the clinical features of encephalopathy. The molecular and cellular anatomy of the vascular blood-brain barrier is reviewed, first during health and secondly in a systemic inflammatory context. Distinct from the molecular and cellular mediators of the blood-brain barrier’s response to inflammation, influencing direction and magnitude, are several moderators at genetic, system, cellular and molecular levels. These include sex, genetic background, age, pre-existing brain pathology, systemic comorbidity and gut dysbiosis. Further progress is required to measure and define mediators and moderators of the blood-brain barrier’s response to systemic inflammation, to explain the heterogeneity observed in animal and human studies.

**INTRODUCTION**

The brain is a delicate organ because neurons require a highly specialised environment to function properly. Ionic homeostasis of brain interstitial fluid1 is essential to enable the maintenance and controlled modulation of transmembrane gradients and movements; these are necessary to generate accurate changes in electrical potential, locally in synapses, along axons, or in distributed networks. Interstitial protein concentration is kept low,1 to minimize cellular proliferation and protein binding of charged ions and neurotransmitters, and to optimize rheology of extracellular fluids at low pressures in the intracranial compartment. Leucocyte entry is highly regulated2, with complete suppression of innate immune cell infiltration to avoid acute inflammatory responses *in situ*, since if neuronal damage occurs, regeneration is slow and limited. On the other hand, a low level of T cell immunosurveillance is allowed to keep latent viruses under check with minimal inflammatory consequences.

The vascular blood-brain barrier (BBB) represents the brain’s main interface with its external environment at which these processes are mostly controlled.2,3 Although referred to as a “barrier”, this is a misnomer, since cells and substances can be exchanged bi-directionally.4,5 There are two main reasons why the barrier cannot be absolute. First, due to its high level of specialization the brain depends on the rest of the body for supply of nutrients and clearance of toxic by-products of metabolism. For instance the brain’s capacity for gluconeogenesis is limited6 and glucose supply is heavily reliant on its transport across the vascular BBB via the insulin-independent glucose transporter GLUT-1.7 Second, during systemic inflammation, a number of brain responses occur which have survival value, collectively referred to as sickness behaviour.8 This is a set of coordinated physiological and behavioural changes, orchestrated by the brain, which protects the individual from predators while they are ill (such as lethargy), enables them to fight the infection (such as fever and anorexia), and the wider species (such as anhedonia and social withdrawal). This permissive quality of the vascular BBB may sometimes be taken too far during sustained or overwhelming systemic inflammation, or in cases where there is already BBB damage due to neurological disease.9

This review focuses on current knowledge regarding the relationship between systemic inflammation and the vascular BBB, with three aims: (1) to describe the molecular and cellular components of the BBB which may respond to inflammation (2) to review how the BBB responds to systemic inflammation (3) to define those factors which are known to moderate this relationship. The literature search strategy employed is illustrated in **Figure 1**. In summary a multi-step approach was used. If the parent search revealed a molecule, cell, tissue or organ level factor linking the BBB and systemic inflammation, each of these factors were searched separately. Due to the vast number of publications in this area, it was impossible to acknowledge all publications in this review. Lack of mention is not a judgement of study quality, since examples were used to illustrate specific key concepts.

Although this review discusses in detail the vascular BBB during systemic inflammation, it is important to put the vascular BBB in context of the other brain interfaces with its external environment.5 These other interfaces arise since the central nervous system is highly compartmentalized. Their anatomical locations are listed in **Table 1**. While each of these other interfaces are likely to be important during systemic inflammation, they are less well studied than the vascular BBB in this setting and their precise contribution remains to be determined. The vascular BBB has the largest surface area amongst the various interfaces, corresponding to a total surface area of 15–25 square metres,10 and will be hereafter referred to simply as the BBB.

**BBB MOLECULAR AND CELLULAR ANATOMY**

The BBB is composed of several components: glycocalyx, endothelial cells, basement membrane containing pericytes, and astrocytic end-feet (**Figure 2**). Some components are entirely molecular (glycocalyx and basement membrane) while others are cellular (endothelial cells, pericytes and astrocytes). This section will lay out how the alternating molecular and cellular layers exert control over solute and cellular transport across the BBB in different ways, in the healthy state and during systemic inflammation.

**Glycocalyx**

The glycocalyx is secreted by endothelial cells and coats the luminal surface of blood vessels, consisting of a closely knitted network of glycosaminoglycans (heparan sulfate, chondroitin sulfate, keratan sulfate and hyaluronic acid) (**Figure 3**).11,12 It is tethered to the endothelial cells via membrane-associated CD44 and the proteoglycans syndecan and glypican (**Figure 3**).11,12 It has the appearance of a non-uniform thick bed of seaweed on the sea floor.11 Orosomucoid secreted by endothelial cells,13 imparts a negative charge to the glycocalyx, which has been demonstrated to inhibit BBB permeability to negatively charged plasma proteins.14 However its main barrier function appears to be that of a molecular sieve, with a measurable 50% drop in diffusion of molecular tracers of size 40 kilodaltons or above, across its thickness.15 The glycocalyx in the brain presents a more formidable barrier compared to the glycocalyx elsewhere in the body. One study showed 40% capillary luminal surface coverage in the brain, compared to 15% in the heart and 4% in the lung; thickness was also greatest in the brain (301nm, versus 136nm in the heart and 65nm in the lung).16 In addition, the glycocalyx in cerebral capillaries appears to be less permeable to 70 kilodalton dextran,17 in contrast to the systemic glycocalyx.18

Systemic inflammation *in vivo* leads to cerebral endothelial glycocalyx damage with reduction in thickness and coverage, as shown by several studies after intravenous17 or intraperitoneal16 lipolysaccharide (LPS) challenge in mice – LPS is a pro-inflammatory component of bacterial cell walls used to model systemic inflammation in laboratory settings. Exposure to LPS, the cytokine tumour necrosis factor-α, or the procoagulant enzyme thrombin singly led to significant reductions in glycocalyx thickness of a human pulmonary microvascular endothelial cell line *in vitro*,19 so that several circulating molecules during systemic infection or inflammation can result in glycocalyx shedding. Also enzymes from the family of matrix metalloproteinases (MMPs) secreted by rolling leucocytes or endothelial cells20, may degrade glycocalyx components such as syndecan, as shown in human and mouse cell lines21 and rat mesenteric vessels.22 The glycocalyx also presents some obstruction to the interaction between leucocytes and the endothelial cell surface which is needed for rolling and adhesion. This physical distancing is not sufficiently mitigated by the combined length of leucocyte pseudopod projections, the leucocyte selection or integrin and their respective endothelial ligands (P-selectin glycoprotein 1 and intercellular adhesion molecules). Hence glycocalyx degradation enhances leucocyte immobilization, as shown in mouse cremaster venules23 which would facilitate entry into the tissue.

**Endothelial cells**

Microcerebrovascular endothelial cells are specialized when compared to their counterparts outside the brain.24 They lack fenestrae, have tight junctions, exhibit a low rate of macropinocytosis and caveolar transcytosis,25 express low levels of leucocyte adhesion molecules9 and possess a plethora of substrate-specific transport systems that control influx of polar nutrients into the brain (such as solute carrier, or SLC, family transporters)26 and efflux of unwanted substances into the blood (such as ATP-binding cassette, or ABC, family transporters e.g. P-glycoprotein).26 Several saturable transport systems are predicted, based on kinetic modelling of blood-to-brain transfer, yet their molecular identity remains unknown – an important example is the insulin transporter.27 Tight junctions are composed of a complex of three types of molecules: occludins, claudins and junctional adhesion molecules (**Figure 3).** These molecular complexes form a continuous band around the capillary, which is a formidable barrier to small molecules diffusing down concentration gradients across the paracellular route. The size selectivity of tight junctions was beautifully illustrated in claudin-5 knockout mice which, compared to wild-type mice, showed increased permeability to 562 dalton but not 1.9 kilodalton tracers.28 Tight junctions are attached to the endothelial cytoskeleton by zonula occludens proteins; they are not static structures, responding to the physiological state of the endothelial cell and its environment, including systemic inflammation, as discussed further below. Compared to endothelium elsewhere, microcerebrovascular endothelial cells express lower levels of molecules involved in transmigration of leucocytes into the brain; this includes E- and P-selectins which mediate rolling, and the integrin ligands ICAM-1, PECAM-1 and VCAM-1 which mediate firm adhesion.29

Microcerebrovacular endothelial cells express receptors which enable them to respond to systemic inflammation such as for the cytokines interleukin-1β (IL-1β),30-32 interleukin-6 ,33 tumour necrosis factor-α34 and the pro-inflammatory molecule cyclophilin.35 They also express toll-like receptors (TLR) e.g. TLR2, TLR3, TLR4 and TLR6, which are pattern recognition receptors for molecules derived from pathogens namely lipoteichoic acid from Gram-positive bacteria, double-stranded RNA from viruses, LPS from Gram-negative bacteria and diacyl lipopeptides from Mycoplasma.36 TLR4 requires the co-receptor CD14, which is also expressed by cerebral endothelial cells.37

**Basement membrane**

The basement membrane at the BBB is an extracellular amorphous but highly organized matrix of four main types of structural proteins: collagen IV family proteins, nidogens, heparan sulphate proteoglycans such as perlecan and laminins (**Figure 3**). It is a two-ply juxtaposition of endothelium and astrocyte (glial) derived layers which differ in composition, especially with respect to laminin content, such that laminin-411 and laminin-511 are found in the endothelial layer while the glial layer contains laminin-111 and laminin-211 (**Figure 3**).38 A potential perivascular space is present between these two layers which is where CD163-positive macrophages physiologically reside, and leucocytes sometimes congregate during inflammatory brain diseases. CD163-positive perivascular macrophages perform a scavenging function at the BBB,39 and express surface molecules that enable them to respond to systemic inflammation: scavenger receptor A Types I and II,39,40 mannose receptor,41 DC-SIGN,42 CD14,43 CD18/CD11b/c,44 and IL-1β.45 They also express antigen presentation molecules such as major histocompatibility complex type II, B7‐2 and CD40.42 While they play a negligible role in transducing an inflammatory message across the BBB compared to endothelial cells,46,47 they perform an important antigen-presenting role to lymphocytes crossing the BBB.48,49

The basement membrane plays an important role in determining BBB permeability to cells. Cellular migration occurs at capillaries and especially at postcapillary venules. The endothelial layer is characterized by areas of laminin 411 and 511 colocalization alternating with laminin 511-low areas, called “low expression regions”. These areas act as exit points for T cell extravasation in postcapillary venules,50 since lymphocyte surface integrin α6β1- and αvβ1-mediated interaction with laminin 511 would otherwise hold cells in check.51 After traversing the endothelial layer of the basement membrane, leucocytes tend to be held up in the perivascular space52 and may survey the perivascular space at length.53 They require an activation step in the perivascular space before they can proceed to cross the glial layer;48 this passage is enabled by selective cleavage of dystroglycan, a transmembrane receptor that anchors astrocyte endfeet to the glial basement membrane by focal MMP-2 and MMP-9 action.54 Hence the glial-derived layer of the basement membrane presents a more formidable barrier to infiltrating leucocytes, compared to its endothelial counterpart.

**Pericytes**

Pericytes are neural crest-derived cells embedded within the endothelial basement membrane. Although pericyte coverage of the endothelium is around 40%,55 there is an intimate association between pericytes and endothelial cells,56 such that each pericyte forms thousands of cytoplasmic extensions into endothelial cell invaginations – the molecular components and functional implications of this anatomical arrangement are currently unknown. The pericyte-to-endothelial cell ratio in the brain, the value of which varies between 1:157 and 1:3,58 is the highest such ratio in the body.

Pericytes play two major roles at the BBB which may affect the passage of solutes and cells into the brain. The first is maintenance of BBB integrity, by inhibition of transcytosis and by inducing endothelial cell expression of tight junction proteins59,60 and major facilitator domain-containing protein 2A (MFSD2a), a docosahexaenoic acid transporter.61 The second is regulation of capillary diameter and cerebral blood flow.62,63 The passage of substances into the brain parenchyma in terms of amount of substance per unit gram of brain tissue depends on two main factors: the permeability characteristic of the BBB and the surface area of the BBB available for exchange, which together form the permeability surface product.9 At the low levels of permeability at the BBB, changes in cerebral blood flow have a negligible impact on absolute amounts of circulating substance entering the brain, as predicted by the Cronin-Renkin equation,64,65 since permeability is the limiting factor.66 However if the substance is very BBB permeable by its nature or the BBB become highly permeable during pathology, cerebral blood flow starts to increasingly play a role. Hence by regulating the BBB surface area and blood flow, pericytes control barrier function in a region specific manner.

**Astrocytes**

Astrocytic foot processes form the innermost layer of the BBB. They interdigitate and overlap, leaving no slits between them, so that coverage is near total.55 The clefts between apposed astrocyte endfeet are not sealed by tight junctions and therefore provide no absolute barrier to markers as large as horseradish peroxidase (44 kilodaltons).67 However closer study reveals a heterogenous barrier, impeding molecules as small as a 500 dalton flourophore in some areas while allowing free passage in other areas,68 overall representing a resistance which is measurable, with a diffusion coefficient roughly one order of magnitude lower than in the brain parenchyma.15 Moreover, astrocytes control BBB integrity remotely. For example they produce angiotensinogen which after cleavage to angiotensin II, binds to type 1 angiotensin receptors on BBB endothelial cells; this triggers threonine-phosphorylation and organized recruitment of occludin at tight junctions.69 Astrocytes also produce angiopoietin-1, which acts at tie-2 receptors on endothelial cells,70 to promote tyrosine dephosphorylation of occludin, stabilising tight junctions.71 Yet another protein astrocytes produce is sonic hedgehog which interacts with its receptor Patched-1 on endothelial cells, to maintain a transcriptional programme including all three molecular components of tight junctions.72 The overall role of astrocytes at the BBB is controversial; for example their laser ablation in mice did not affect permeability to molecules in the region of 4-70 kilodaltons73; however this study only addressed a time window of a few hours before neighbouring astrocytes contributed new astrocytic endfeet.

Astrocytes at the BBB express aquaporin-4, a water channel which allows bidirectional water transport. Aquaporin-4 expression at the BBB changes during systemic inflammation74 and in neuropathologies which are highly responsive to systemic inflammation including conditions like Alzheimer’s disease and multiple sclerosis.75 The astrocytic pool of aquaporin-4 regulates water flux across the BBB, and the precise contribution depends on brain health status, mediating efflux of water in the healthy state and vasogenic oedema, and influx of water in cytotoxic oedema (see **Box 1**). During systemic inflammation, for instance in rats challenged with intraperitoneal LPS, an increase in astrocytic aquaporin-4 expression, and water permeability was observed in the absence of vasogenic oedema (the latter measured by brain water content).74 This is one illustration of the fact that an increase in BBB permeability does not necessarily equate to oedema (e.g. if drainage pathways are unimpeded during vasogenic oedema, or in the setting of cytotoxic oedema), though vasogenic oedema always implies a high BBB permeability – this explanation starts to provide some clarity to the controversy as to whether increased BBB water permeability and vasogenic oedema are one and the same thing. A higher flux of water across the BBB during states of increased permeability is not accounted for by current kinetic tracer modelling of BBB permeability, possibly leading to an underestimation of clearance parameters such as Ktrans; this requires further study.

In summary, while the role of endothelial cells in BBB function has long been established, the most important recent studies have highlighted the role pericytes in BBB development, maintenance and function.59-63 The glycocalyx is the least studied component of the BBB and further work is required, particularly around developing methods to measure its integrity *in vivo* such as sidestream dark-field imaging.76 There is some correlation between systemic glycocalyx thickness and plasma concentration of glycocalyx degradation markers.77 Comparative studies of brain and systemic glycocalyx are needed to identify specific molecular components of the BBB glycocalyx, the plasma concentration of which may serve as a marker of its integrity.

**BBB RESPONSES TO SYSTEMIC INFLAMMATION**

During systemic inflammation and/or infection, a plethora of circulating soluble inflammatory mediators may influence the BBB, as exemplified by the fact that serum from LPS‐treated mice compromised the integrity of *in vitro* BBB model (as measured by transendothelial electrical resistance of cultured primary mouse brain microvascular endothelial cells), compared to serum from vehicle‐treated mice.78 However, other important effects occur within the circulation in response to systemic inflammation, including increases in the number of circulating leucocytes and their migratory potential and circulatory physiological changes, as laid out in **Box 2**.

The BBB may react to systemic inflammation in several ways, though a limited number have been identified so far. In order of degree of structural disruption to the BBB, these include: changes in signalling, enhanced cellular traffic, an increase in solute permeability and direct damage (**Figure 4**), and will be discussed in this section.

**Changes in signalling**

The normal physiological response of the cerebral endothelium to systemic inflammation is non-disruptive, achieving immune-to-brain signalling without invoking changes in permeability or cell traffic across the BBB. It consists of a well-choreographed sequence of molecular events which transduce the systemic inflammatory message to changes in neuronal activity leading to sickness behaviour and protection of the host. Intravenous IL-1β challenge triggers expression of the master transcription factor c-fos in cerebral endothelial cells indicating their activation, preceding that of neighbouring brain parenchyma,79 demonstrating that BBB activation is an intermediate step. Intraperitoneal injection of LPS results in upregulation of the cytokine responsive nuclear transcription factor IκBα mRNA in endothelium and activation of neurons as indicated by c-fos mRNA; only the latter was inhibited by indomethacin suggesting that a first prostaglandin-independent step is followed by a second prostaglandin-dependent step.80 Further investigation showed that endothelial-specific knockdown of IL-1R1 in mice blocked cyclooxygenase-2 expression in brain endothelial cells, neuronal activation as assessed by c-fos, fever and locomotor hypoactivity, all usually induced by circulating IL-1β.81 A similar picture was observed with endothelial-specific knockout of key molecules in the signalling cascade: myeloid differentiation factor 88 (MyD88), an essential component of the intracellular IL-1R1 signal transduction cascade leading to activation of a transcriptional programme driven by the transcription factor NF-κB,47 and two prostaglandin E2 synthesizing-enzymes cyclooxygenase-2 and microsomal prostaglandin E synthase 1.82 Based on these and other studies demonstrating this phenomenon, it is widely held that endothelial cells can signal an inflammatory message across the BBB, in the absence of measurable changes in solute permeability, by detecting IL-1β in the blood and synthesizing prostaglandin E2 which then diffuses into the parenchyma to engage with cognate prostaglandin receptors on neurons and glia, to induce sickness behaviour.

Another example of immune-brain signalling across the BBB which is dissociated from BBB permeability relates to insulin. Systemic inflammation induced by intraperitoneal LPS increased the uptake of insulin into the brain independent of BBB disruption,83 through a mechanism involving post-translational modulation of endothelial and inducible nitric oxide synthase enzyme activities.84 High insulin levels result in decreased feeding, loss of weight and change in cognition,85 reminiscent of sickness behaviour.

P-glycoprotein is a transporter at the BBB, known for a long time to mediate brain-to-blood efflux of drugs and xenobiotics, but recently found to regulate brain endogenous steroids level such that loss of P-glycoprotein results in higher brain aldosterone and anxiety-type behaviour.86 Since P-glycoprotein expression is reduced during systemic inflammation,87 so this is another way by which changes in signalling at the BBB can mediate behavioural change.

**Enhanced cellular traffic**

Even in the absence of pathogen neuroinvasion or other neuropathology, systemic inflammation stimulates leucocyte passage into the brain. A low level of T cell immunosurveillance occurs in the healthy brain2 which excludes B cells and innate immune cells.88 However systemic inflammation increases lymphocytic traffic,89 and with higher degrees of inflammation one starts seeing influx of natural killer cells,90 neutrophils91 and monocytes.92 Leucocyte entry into the brain is a highly coordinated process involving multiple sequential steps: rolling, firm adhesion, and diapedesis (**Figure 4).** As discussed above, complete diapedesis is a two step process, first involving transmigration across the endothelium and its basement membrane into the perivascular space (second inter-endothelial junction, lower panel, **Figure 3**), followed by a second step during which the glial basement membrane is traversed (third inter-glial junction, lower panel, **Figure 3**). Intravital microscopy studies in mice after caecal ligation and perforation to induce sepsis have captured an increase in rolling and adhesion of leucocytes to brain endothelium, which is then followed by infiltration.93 Several changes at the BBB occur to enhance this process during systemic inflammation including degradation of the glycocalyx19,21-23 and endothelial upregulation of E/P-selectins94-96 (which mediate rolling) and chemokines such as CCL297 and integrin ligands such as ICAM-191 (which mediate adhesion). Under inflammatory conditions, the cytokines tumour necrosis factor-α, IFN-γ, and interleukin-17 (IL-17) induce focal MMP-2 and MMP-9 activity at the BBB which promotes chemokine-induced leucocyte migration through the basement membranes, especially the glial limitans.54,98 Endothelial sphingosine-1-phosphate receptor 2 also plays an important part, since experiments with knockout mice and pharmacological agonism demonstrated that signalling at the receptor increases BBB solute permeability and promotes neutrophil infiltration via endothelial E-selectin expression.99

**Solute permeability**

There is emerging evidence that there are compensatory changes at the BBB during the initial stages of systemic inflammation which prevent BBB disruption such as increased solute permeability. In mice treated for seven days with intraperitoneal LPS, microglial dynamics were visualized daily with *in vivo* two-photon imaging and immuno-electron microscopy. Microglia were seen to migrate towards blood vessels in response to the release of the chemokine CCL5 from endothelial cells. They started producing claudin-5 (a tight junction protein), infiltrated their processes through the basement membrane and made claudin-5 immunoreactive contacts with endothelial cells. Early obliteration of vessel-associated microglia through well-timed inducible genetic expression of diphtheria toxin was associated with an increased BBB permeability to 10 kilodalton dextran. However once inflammation was sustained, this initial protective response was followed by a transformation of the vessel-associated microglia to a CD68-positive phagocytic phenotype with loss of BBB integrity.100

A number of studies have shown that solute permeability at the BBB is increased during systemic inflammation or infection. A systematic review has found that this is not a universal finding, with 60% of studies able to demonstrate an effect.9 This controversy may be rationalized by considering the molecular size of the marker used to measure BBB permeability, the severity of the inflammatory stimulus and the presence of pre-existing brain pathology, amongst a number of other factors. A commonly used surrogate marker of permeability is the ratio of albumin (67 kilodaltons) in cerebrospinal fluid (CSF) to serum, expressed as a quotient. The CSF/serum quotient of albumin increased during systemic inflammation, but only in the presence of CSF abnormality101; it is possible that leakage was not seen in the absence of neurological disease because albumin is too large a molecule. Fibrinogen is an even larger molecule (340 kilodaltons), and while its concentration in *post mortem* human brain tissue homogenate was increased during systemic inflammation, the latter was more severe, since the patients had died of sepsis.102 Markers with smaller molecular weight should be more sensitive to changes in BBB permeability103 and would emulate the passage of cytokines (5-20 kilodaltons) better, yet these studies are yet to be performed in the context of systemic inflammation.

The BBB leakage that occurs during systemic infection may be exclusively mediated through systemic inflammatory effects, without direct infection of cerebral endothelium or brain parenchyma. For example, an increase in solute permeability is commonly observed in studies during which systemic inflammation is modelled by non-infective inflammatory challenges, such as the bacterial cell wall component LPS,104 the double-stranded RNA molecule poly (I:C) to simulate viruses105,106 and the cytokine tumour necrosis factor-α.107 In patients dying from COVID-19, magnetic resonance microscopy and immunofluorescence clearly showed areas of microvascular injury and fibrinogen leakage in the brain, in association with endotheliitis.108 In this series, none of the tissue samples had detectable SARS-CoV-2 demonstrating that if severe enough, systemic inflammation may cause damage to the BBB. Some systemic autoimmune inflammatory diseases have also been associated with increased solute permeability at the BBB. One example where this has been clearly shown in humans using dynamic contrast enhanced magnetic resonance imaging is systemic lupus erythematosus,109 but it is unclear whether systemic inflammation causes the BBB leakage or a parallel factor such as autoimmunity against cerebral endothelium or neuronal antigens, is implicated in these situations. In a mouse model of rheumatoid arthritis, using immunization with collagen II, sustained increased BBB permeability to sodium fluorescein was seen throughout the time course studied, up to 100 days after immunization, suggesting a link with the inflammatory arthritis rather than the initial immunization.110

A number of mechanisms link systemic inflammation with tight junction disruption to explain solute leakage across the BBB. An important pathway is the disruption of tight junctions via MMPs induced by inflammation. An immunohistochemical study of brain tissue from patients dying with sepsis showed decreased or absent tight junction molecules occludin, ZO-1, and claudin-5.111 Tight junction disruption in human cerebral microvascular endothelial cells can be mediated by MMPs after LPS exposure *in vitro*.112 After caecal ligation and perforation in rats, a model of sepsis, Evans Blue leakage was MMP2 and MMP9 dependent such that inhibition of these two enzymes could reverse the clinical features of sepsis-associated encephalopathy.113 Other important pathway is a decrease in endothelial sphingosine 1–phosphate receptor 1 signalling due to lower circulating levels of sphingosine 1–phosphate 1 during sepsis.114 Sphingosine 1–phosphate receptor 1 maintains the BBB by regulating the proper localization of tight junction proteins.115 Electron microscopy studies following the fate of circulating colloidal iron oxide after systemic LPS challenge,116 and horseradish peroxidase after caecal ligation and perforation,117 found evidence of increased macropinocytotis.

Increased BBB solute permeability during sepsis has clinical repercussions. Sepsis-associated encephalopathy manifests clinically as delirium and in a large study, elevated circulating markers of endothelial activation (plasminogen activator inhibitor-1 and E-selectin), and blood-brain barrier / neuropathology (S100B) during critical illness were associated with delirium.118 However, although sepsis-associated encephalopathy may be associated with solute leakage across the BBB, the latter is not essential. In one sets of experiments in rats with sepsis after caecal ligation and perforation, behavioural changes in keeping with sepsis-associated encephalopathy were observed in association with cortical hypoperfusion and microglial/astrocytic activation – but this occurred in the absence of immunoglobulin G leakage.119 This underlines the multifactorial nature of encephalopathy, encompassing physiological circulatory disturbances as well as circulating pro-inflammatory changes, and how the contribution of these factors may be variable, even in animal studies.

One important systemic factor present during systemic sepsis is hypoxia, which may itself effect BBB function. An *in vitro* study using primary bovine cerebral microvascular endothelial cells showed that hypoxia (1% oxygen for one day) induced a 2.6-fold increase in sucrose permeability, accompanied by alterations in occludin, ZO-1 and ZO-2 protein localization suggesting a perturbation of tight junction complexes.120 A similar phenomenon was observed in mice exposed to hypoxia (8% oxygen) for four days.121 Exposure of primary human cerebral microvascular pericytes to hypoxic culture conditions (1% oxygen for 48 hours) resulted in a 4.3-fold elevation of the shedded soluble form of platelet-derived growth factor receptor β in the culture medium.122 Interestingly microglia protect against hypoxia-induced BBB disruption by aggregating around leaky vessels; their pharmacological depletion with the colony stimulating factor-1 receptor inhibitor PLX5622 markedly increased hypoxia-induced BBB leakage.121

**Direct damage**

While changes in signalling, cellular traffic and solute permeability are commonly observed during systemic inflammation or infection, direct damage to endothelium may occasionally occur. Systemic inflammation or infection may result in direct damage to endothelium. One example is COVID-19, caused by SARS-CoV-2. Electron microscopy of post-mortem tissue from COVID-19 patients demonstrated viral inclusion structures in systemic endothelial cells, and histological assessment showed inflammatory cell infiltration close to the endothelium and endothelial apoptotis.123 Another example is anti-CD19 chimeric antigen receptor T cell immunotherapy for refractory B cell malignancies. This treatment can cause serious neurotoxicity with a delayed onset of five days, known as immune effector cell-associated neurotoxicity syndrome, and characterized by headache, delirium, aphasia, seizures, and other neurological deficits, progressing to cerebral oedema, coma and sometimes death.124 There is evidence of BBB leakage to albumin and cells, as well as endothelial cell destruction.125 Anti-CD19 chimeric antigen receptor T cells recognize and destroy CD19-expressing pericytes, an unforeseen off-target effect, leading to BBB leakage.126

In summary, the most important advance in recent years has been the increasing recognition that systemic inflammation is associated with increased BBB permeability, not only in animals, but also in humans.101,109 Important next questions to address include: (1) regional susceptibility in terms of neuroanatomical areas, and (2) whether this increased BBB permeability causes any transient or lasting clinically relevant pathology, rather than being purely associative, making this pathway a therapeutic target.

**MODERATORS OF THE BBB RESPONSE**

The above section discussed in detail the cellular and molecular *mediators* of the BBB response to systemic inflammation. The causal relationship between the mediators and the BBB response may vary depending on the level or magnitude of *moderators*, including genetics, age, pre-existing brain pathology, comorbidity and time. This is important since moderators require inclusion in statistical analyses, as additional covariates in regression analyses or factors in analyses of covariance, with interaction effects (**Figure 5**).

**Genetics**

Genetics, including sex, plays an important role in determining baseline BBB permeability; this is intuitive since variations in the genes coding for BBB proteins may result in changes in BBB structure and function. There are several rare genetic mutations causing neurological disease which on further investigation have been found to cause a constitutively leakier BBB. One example is major facilitator superfamily domain containing 2a (Mfsd2a), which causes a lethal microcephaly syndrome127 and is a docosahexaenoic acid transporter which suppresses caveolae-mediated transcytosis in microcerebrovascular endothelial cells.61 Another is platelet-derived growth factor receptor β (PDGFR-β) which causes Fahr’s disease128 and is essential for pericyte recruitment and maintenance at the BBB59. More relevant to this review are common mutations which may explain individual variability in the BBB response to systemic inflammation. One example of a common protein regulating BBB permeability is apolipoprotein E (APOE), a cholesterol and lipid carrier. The *APOE* gene is polymorphic, with combinations of two single nucleotide polymorphisms resulting in three alleles (ε2, ε3 and ε4). In cognitively normal carriers of the *APOE* ε4 allele, BBB permeability as measured by the CSF/serum albumin quotient129 and dynamic-contrast enhanced magnetic resonance imaging130 was high compared to *APOE* ε2 or *APOE* ε3 carriers. One mechanism underlying this phenomenon is thought to be a loss of constitutive suppression of the cyclophilin A–nuclear factor-κB–matrix-metalloproteinase-9 pathway within pericytes, leading to MMP9-mediated degradation of capillary basement membrane and tight-junction proteins and BBB leakage.131 In keeping with this explanation, a high correlation between the CSF/serum albumin quotient, cyclophilin A and active MMP9 was observed in CSF in humans, with higher levels of all three in *APOE* ε4 carriers.129 Other mechanisms may be involved. APOE ε4 expression by pericytes was associated with a higher permeability and lower levels of basement membrane collagen IV, using an *in vitro* BBB primary mouse pericyte/endothelial cell co-culture model.132

There is evidence that genetics moderates the BBB response to systemic inflammation. For example, C57 versus CD1 and female versus male mice had higher degrees of BBB disruption (as measured by brain uptake of circulating radiotracer) after LPS challenge; some but not all of the gender effect could be mediated via a higher cytokine response in females.133 In another example, a sexually dimorphic and strain specific role for sphingosine-1-phosphate receptor 2 is evident at the BBB with higher expression in female SJL mice compared with male SJL mice and C57BL/6 mice of both sexes.134 Signalling at the sphingosine-1-phosphate receptor 2 was associated with increased fluorescein permeability, luminal expression of the chemokine CXCL12 and diminished inflammatory cell infiltration.134 Mice deficient in endothelial podocalyxin showed normal BBB permeability, but when stimulated with intraperitoneal LPS, there was a marked increase in 70 kilodalton dextran leakage compared to wild-type mice.135 Of relevance to what we know about BBB genetics in humans, mice expressing human APOE ε4, compared to APOE ε3, exhibited greater BBB leakiness to sodium fluorescein in the cortex after intraperitoneal challenge with LPS.136

**Age**

Numerous studies have confirmed that BBB permeability increases with age, with techniques such as the CSF/serum albumin quotient137 and dynamic contrast enhanced magnetic resonance imaging.138 It is important to note that these techniques utilize tracers which do not require receptor-mediated transporters, the expression of which is decreased with age.24,139 A recent study in healthy male mice has demonstrated a shift from receptor-mediated transport for plasma proteins (which decreases with age) to caveolar transcytosis (which increases with age),140 thus confirming that the age-related increase in BBB permeability occurs for substances which use non-specific pathways of transport. Structural changes such as in tight junction expression, basement membrane thickness or pericyte coverage are not consistently seen to increase with age when considered singly,25 however their combination in various degrees is likely to be at least partially explanatory. Aging cells undergo senescence and acquire a senescence-associated secretory phenotype. This is linked to a transcriptional programme geared towards production of extracellular matrix proteases, cytokines, chemokines and growth factors which stimulate leucocyte migration, activation and infiltration.141 Single-cell RNA-seq technology identified the unique transcriptional signature characteristic of cellular senescence in 10% of microvascular endothelial cells in mouse brain at a biological age equivalent to 75 years in humans.142 Cerebrovascular endothelial senescence is associated with BBB permeability *in vitro* using primary mouse brain endothelial cell and pericyte co-cultures143 and one mechanism is a decline in Sirtuin-1 expression as shown in mice *in vivo*.144 Brain complement 3 expression increases with age which stimulates the receptor for the active signalling peptide of complement (C3aR) in the basolateral compartment of cerebral endothelial cells with effects on cellular and solute permeability, upregulation of the integrin ligand VCAM-1 enhancing lymphocyte entry and reduction of tight junctional protein expression with consequent increased solute permeability to a 65–85 kilodalton tracer in mice.145

There is evidence that age moderates the BBB response to systemic inflammation. Aged male Wistar rats rendered septic by the caecal ligation and perforation procedure demonstrated more profound BBB impairment compared to younger rats, and this affected both solute (Evans blue) and cellular (neutrophil) influx.146 Higher order interactions between moderators are evident, for instance between sex and age, in mice challenged with intraperitoneal LPS or vehicle; the increase in Evans blue BBB leakage with systemic inflammation was present in males only at a young age, but present in both sexes at an older age.147 This was shown to be related to oestrogen, by combinations of ovariectomy and estradiol replacement.147

**Brain pathology**

Pre-existing neuropathology may render the BBB more sensitive to systemic inflammation. In mice expressing human-type amyloid-β precursor protein as a model of Alzheimer’s disease, BBB permeability to fluorescein isothiocyanate labelled albumin after intraperitoneal LPS injection was higher compared to wild-type controls.148 Similar observations were made in animal models of ischemic stroke149 and multiple sclerosis150,151, namely that the effect of systemic inflammation on BBB permeability is more pronounced in the presence of central nervous system pathology. Alzheimer’s disease like pathology (modelled by intracereboventricular injection of oligomeric β-amyloid peptide) together with cerebrovascular ischemia (modelled by striatal injection of endothelin-1) in rats caused a greater vascular disruption than the same single challenges in isolation.152

A similar observation is seen in humans. In a large study of 1273 human lumbar punctures in a general hospital setting, systemic inflammation as measured by C-reactive protein significantly predicted the CSF/serum albumin quotient in individuals with abnormal CSF findings but not in those without.101 In a human post-mortem study, fibrinogen measured by ELISA in brain tissue homogenate was used as a surrogate marker for BBB permeability; it was found to be higher in patients who were septic at the time of death, versus those who were not septic; this effect was most marked in patients with Alzheimer’s disease or vascular dementia, compared to non-dementia controls.102

**Systemic comorbidity**

A number of systemic comorbidities may affect BBB permeability such as diet, alcohol consumption and diabetes. A high fat diet rendered the BBB more susceptible to the effect of age on immunoglobulin extravasation into the parenchyma, demonstrating an interaction between diet and age.153 In primary human brain microvascular endothelial cell cultures, ethanol‐mediated oxidative stress led to decreased BBB integrity and facilitated monocyte transmigration.154 In humans with type II diabetes, BBB permeability is increased as measured by signal intensity change during contrast-enhanced magnetic resonance imaging;155 evidence for causation was established in rats with streptozotocin-induced diabetes.156,157 Whether alcohol intake or diabetes moderate the effect of systemic inflammation on the BBB remains to be shown. Multiple co-existent systemic inflammatory stimuli, such as co-infections, may interact with each other; for example Evans blue leakage into the brain in mice inoculated intranasally with LPS and influenza A virus was much higher than the increases in BBB permeability seen with the single challenges.158

**Gut microbiome**

A firm link has been established between the gut microbiome and BBB integrity. After it was noticed that germ-free mice have a leakier BBB, investigations showed that short‐chain fatty acids such as butyrate induce tight junction formation at the BBB.159 Butyrate and other short‐chain fatty acids including propionate and acetate, are produced during fermentation of complex plant-based polysaccharides by gut commensal bacteria. In rhesus monkeys, BBB permeability as measured by the CSF/serum albumin quotient and dynamic contrast enhanced magnetic resonance imaging, showed a clear increase after oral antibiotic treatment, associated with a decrease in gut bacterial diversity and decreased short‐chain fatty acids in faecal samples.160 This pathway is being mapped out in humans. Human cerebral endothelium expresses free fatty acid receptor 3, which is the receptor for proprionate.161 In a human microcerebrovascular endothelial cell line model of the BBB, propionate moderated the permeability-inducing effect of LPS.161

**Time**

The effect of systemic inflammation or infection on BBB integrity may vary as a function of time. Some of the BBB responses mentioned above may occur with a delay after the onset of the systemic challenge, especially if transcription and/or translation is required. The duration of systemic inflammation is important, since if sustained this is more likely to overcome perivascular microglial protective capacity and loss of BBB integrity100 and innate immune cell infiltration into the parenchyma may occur.92 Changes to water permeability with cerebral oedema may be complex depending on temporal sequencing of cytotoxic and vasogenic components; cytotoxic oedema is not associated with increased BBB permeability but if endothelial cells undergo swelling or there is bystander damage to the vascular wall, BBB integrity will be compromised and vasogenic oedema may develop.162

In summary, important discoveries have been made in recent years relating to factors which moderate the BBB response to systemic inflammation. Perhaps the most important emerging factor is genetic background,129,130 since this will allow stratification in clinical studies of BBB permeability. So far studies have taken a candidate gene approach – unbiased studies are needed to determine the genetic determinants of BBB permeability since these may modulate the BBB response to systemic inflammation. More studies are needed to determine whether, in progressive neurological diseases such as Alzheimer’s disease148 and multiple sclerosis,150,151 the increased susceptibility to BBB leakage during systemic inflammation contributes to neurodegeneration – if this is the case this pathway becomes a therapeutic target, to break the vicious cycle and slow progression.

**CONCLUSIONS**

The BBB is a highly regulated interface between blood and brain with a primary function to protect central neurons, while signalling the presence of systemic inflammation and infection to the brain to enable a protective sickness behavioural response. Progress is being made in understanding the molecular and cellular determinants of the BBB in the healthy state and during the physiological response to systemic inflammation. With worsening degrees and duration of systemic inflammation, supra-physiological responses occur, and the BBB undergoes an increase in solute permeability and lymphocyte traffic, innate immune cell influx occurs, and frank endothelial cell loss may occur. With these pathological changes at the BBB, encephalopathy becomes clinically manifest. Several moderators may influence the direction and magnitude of the BBB response to systemic inflammation, including sex, genetic background, age, pre-existing brain pathology, systemic comorbidity and gut dysbiosis. Further study is required to improve tools to measure the BBB response to systemic inflammation, and advance our understanding of the genetic, system level, cellular and molecular mediators and moderators of the BBB response. This will help explain the heterogeneity observed in animal and human studies.

**ABBREVIATIONS**

APOE: apolipoprotein E

BBB: blood-brain barrier

CSF: cerebrospinal fluid

IL-1: interleukin-1

LPS: lipopolysaccharide

MMP: matrix metalloproteinase

TLR: toll-like receptor

These abbreviations were not spelt out at first mention since they are common:

COVID-19

ELISA

mRNA

RNA

SARS-CoV-2

|  |  |
| --- | --- |
| **Blood-brain interface** | **Anatomical locations** |
| Blood - brain barrier4 | *brain capillary walls* |
| Blood - cerebrospinal fluid (CSF) interface172 | *choroid plexus, arachnoid granulations and leptomeningeal blood vessel walls* |
| Circumventricular organ - brain interface173 | *tanycytes at the junctions between circumventricular organs and the rest of the brain parenchyma* |
| Systemic lymph - brain interface174,175 | *peri-arterial mural drainage pathway, cranial and spinal nerves, cribriform plate and meninges* |
| Viscera - brain interface176 | *neural pathways such as the vagus nerve linking the central nervous system with the viscera* |

**Table 1.** The blood-brain barrier in context. Blood-brain interfaces and their neuroanatomical locations

**Healthy state**

In the absence of neuropathology, aquaporin-4 appears to predominantly mediate efflux of water. Mice with a glial-conditional aquaporin-4 deletion have a higher brain water content compared to wild-type littermates;163 brain water content may be intracellular or extracellular. α-syntrophin knockout mice whose aquaporin-4 cannot be anchored to the membrane, display swollen astrocytic endfeet164 and aquaporin-4-deficient mice have increased extracellular space;165 hence aquaporin-4 has a physiological role in the control of water homeostasis in both astrocytic intracellular and extracellular compartments.

**Pathological state: cytotoxic oedema**

In pathological states accompanied by cytotoxic oedema, water accumulates in the intracellular compartment, driven osmotically by reduced plasma osmolality in water intoxication, and by increased intracellular osmolality due to Na+/K+ pump failure in ischemic stroke, hypoxia, and predominantly ischemic traumatic central nervous system injury. In these situations, aquaporin-4 has the opposite effect to the healthy state, mediating influx of water into astrocytes, since its knockout166,167 or pharmacological relocalization away from the cell membrane168 improves or prevents oedema. The mice with the glial-conditional aquaporin-4 deletion mentioned above demonstrated less oedema during water intoxication, compared to wild-type littermates,163 demonstrating how the direction of water flux changes between health and disease.

**Pathological state: vasogenic oedema**

Vasogenic oedema is accompanied by increased BBB permeability (although increased BBB permeability does not necessarily result in vasogenic oedema – see text). Compared to cytotoxic oedema, aquaporin-4 has an opposite protective role, mediating water efflux during conditions associated with vasogenic oedema such as central nervous system injury or contusion,169 brain infection170 and tumour.171

**Box 1**. Water permeability at the BBB: opposing roles of aquaporin-4 in different situations

**Changes in circulation and circulating mediators during inflammation with potential to affect blood-brain barrier function**

**Physiological circulatory parameters**

* Hypotension with decrease in cerebral blood flow
* Loss of cerebral autoregulation
* Hypoxia

**Immune cells**

* Natural killer cells
* Neutrophils
* Monocytes
* Lymphocytes

**Proinflammatory substances**

* Pathogen associated molecular pattern molecules (e.g. LPS, viral nucleic acids)
* Cytokines (e.g. TNF-α, IL-1β, IL-6, IFN-γ)
* High mobility group box‐1
* Cyclophilin A
* Sphingosine
* Complement components
* Kinins
* Prostaglandins
* Serotonin
* Histamine
* Arachidonic acid

**Box 2.** Changes in physiological circulatory parameters and circulating mediators during inflammation

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**FIGURE LEGENDS**

**Figure 1.** **Review methodology.** A stepwise semi-automated approach was taken: an automated parent search was followed by a manual step, which then led to multiple daughter automated searches. Searches were last updated on 18th July 2021.

**Figure 2.** **The vascular blood-brain barrier.** Alternating layers of molecular (glycocalyx, basement membrane) and cellular (endothelial cells, pericytes, astrocytes) components form the blood-brain barrier. The five components are in bold typeface. The basement membrane is a single structure, with a two-ply composition, since it is secreted by endothelial cells and astrocytes on either side.

**Figure 3. Molecular components of the BBB.** The glycocalyx coats the luminal surface of cerebral endothelial cells, protruding into the lumen in a frond-like manner. The basement membrane is laid down by the endothelial cells and astrocytes; while the two types of extracellular matrix are biochemically distinct, they are indistinguishable and fused into one entity during health, only to be separated when perivascular spaces develop as a result of accumulation of cells or fluid. JAM: junctional adhesion molecule.

**Figure 4.** **The vascular blood-brain barrier in health (upper panel) and during systemic inflammation (lower panel).** The figure is divided into four vertical sections corresponding to the four types of BBB response to increasing levels of systemic inflammation described in the text. In the first vertical section on the left, changes in signalling are exemplified by up and down regulation of carriers and receptors. This is followed by increased cell and solute traffic across the BBB, with enhanced transendothelial vesicular transport and tight junction breakdown, in the second and third vertical sections. A rolling lymphocyte has adhered to the endothelium followed by diapedesis into the potential perivascular space where it can crawl (step 1) or penetrate the glia limitans (step 2) to enter the brain parenchyma. The fourth vertical section illustrates structural damage to various components of the BBB including the glycocalyx, basement membrane, endothelial cells, pericytes and astrocytic end feet.

**Figure 5.** **Moderators of the blood-brain barrier response to systemic inflammation and infection.** Moderators are highlighted in light orange and have coloured arrows. Stratifying on these moderators or accounting for them as covariates will become important in future experimental or observational studies of BBB permeability.