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REVIEW ARTICLE

Cellular Elements of the Blood-Brain Barrier

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Abstract The Blood-brain-barrier (BBB) provides both anatomical and physiological protection for the central nervous system (CNS), shielding the brain for toxic substances in the blood, supplying brain tissues with nutrients and filtering harmful compounds from the brain back to the bloodstream. The BBB is composed of four main cellular elements: endothelial cells (ECs), astrocyte end-feet, microglial cells, and perycites. Transport across the BBB is limited by both physical and metabolic barriers (enzymes, and different transport systems). Tight junctions (TJs) present between ECs form an important barrier against diffusion, excluding most blood-borne substances for entering the brain.

Keywords Blood brain barrier · Transport · Endothelial cells · Astrocytes · Perycites · Basement membrane matrix

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Introduction

The blood-brain barrier (BBB) establishes both a physical as well as a metabolic barrier isolating the Central Nervous System (CNS) from systemic circulation, creating a unique and stable environment for optimum neuronal activity [1]. It exerts bi-directional control over the passage of a large diversity of regulatory proteins, nutrients and electrolytes, as well as potential neurotoxins. This traffic is regulated through different transport mechanisms [2, 3]. In addition, the BBB exerts its defense of the CNS through the action of efflux transporters, blocking intravascular toxin entry and promoting toxic substance elimination to the bloodstream [4]. In certain regions of the CNS, classical BBB structure is replaced by a blood-cerebrospinal fluid (CSF) barrier, which, although more permeable than the BBB, still prevents free passage of serum proteins from the blood into CSF. This occurs in neurosecretory areas like the posterior pituitary, and in areas performing chemoreceptive functions like the pineal gland, the subfornical organ, the median eminence, the area postrema, the subcommissural organ and the organum vasculosum of the lamina terminalis [5]. In the blood-CSF barrier, choroid plexus blood vessels are fenestrated and form a non-restrictive barrier; however epithelial cells have apical tight junctions (TJs) restricting intercellular passage of molecules (Fig. 1a). The arachnoid barrier represents an additional barrier. It is the least studied and structurally most complex of all the brain barriers. The blood vessels of the dura are fenestrated and provide little barrier function; however, outer cells on the arachnoid membrane have TJs, and this cell layer is believed to form the physical barrier between the subarachnoid and overlaying structures. Blood vessels on arachnoid and pial surfaces have TJs similar to cerebral blood vessels, although lacking perycites and astrocytic end-feet [6] (Fig. 1b).

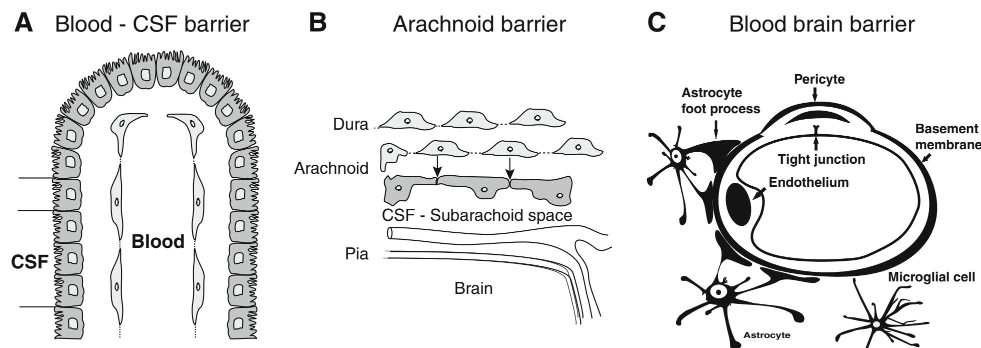

Fig. 1 Schematics of different barrier interfaces. **a** The blood-CSF barrier is a barrier between choroid plexus blood vessels and the CSF. The choroid plexus blood vessels are fenestrated and form a non-restrictive barrier; however, the epithelial cells have apical tight junctions that restrict intercellular molecule passage. **b** In the arachnoid barrier, blood vessels of the dura are characterized by multiple enlarged extracellular space and few intercellular contacts, implicating a lack of structural cohesion within this layer. In contrast, outer cells of the arachnoid have numerous tight junctions, without significant extracellular space. This cell layer is therefore considered an effective morphological and functional barrier between circulating blood in the dura and the CSF in the subarachnoid space. **c** The blood-brain-barrier is a barrier between the lumen of cerebral blood vessels and brain parenchyma. In the brain, endothelial cells form tight junctions at their margins, sealing off paracellular pathways between cells. Perycites are vascular cells adjacent to capillaries that share a common basement membrane with endothelial cells, and have many cytoplasmic processes encircling capillaries. Endfeet processes from astrocytes form a network surrounding capillaries, and microglial cells represent the immunocompetent cells of the brain

BBB Structure

Endothelial Cells

BBB structure is conformed by cerebral endothelial cells (ECs) forming brain and spinal cord capillaries, in association with various perivascular cells such as smooth muscle cells, perycites, microglial cells and astrocytes [3, 7] (Fig. 1c). Ultrastructural cytochemistry and immunocytochemistry revealed the morphology of a functional polarity of the brain microvascular endothelium. This polarity is evidenced by asymmetric distribution of the majority of the transport-related enzymes and carriers present in the luminal and abluminal ECs plasma membranes, indicating that apical and basolateral membranes of epithelial cells are functionally distinct [8, 9].

ECs are normally connected at a junctional complex by TJs, and adherent junctions (AJs). Electron microscopy studies of the BBB have identified distinct morphologic and metabolic characteristics of these particular ECs, different to those present in peripheral tissues. The most important of these include: (a) ECs forming TJs at their adjacent margins, produced by the interaction of several transmembrane proteins projecting into the paracellular space and effectively sealing it, thus confining penetration across brain endothelium to transcellular mechanisms [10, 11]; (b) Endothelial cytoplasm lacking fenestrations typically present in peripheral-tissue capillaries [12]; (c) Fewer pinocytotic vesicles compared to peripheral endothelial cells [13]; and (d) More mitochondria, suggesting important metabolic activity [14].

The molecular biology of TJs is quite complex [15], and their proteins, as well as their adaptor molecules, which link TJs to the cytoskeleton, are often affected during acute or chronic brain disease. Figure 2 shows the molecular organization of BBB TJs, critical determinants of BBB restrictiveness. They are composed of a network of intra-cellular and trans-membrane proteins linked to an active cytoskeleton base, causing TJs to form a seal while retaining capacity for rapid modulation and regulation. Two major trans-membrane protein components of TJs have now been identified, occludin [16] and claudins [17].

Occludin

Occludin was the first integral membrane protein discovered in endothelial cell TJs, including the BBB. It is composed of four trans-membranous domains with carboxyl and amino-terminals oriented towards the cytoplasm and two extracellular loops spanning the intracellular cleft [18]. Multiple phosphorylation sites have been identified its serine and threonine residues, and the phosphorylation state of occludin regulates its association with both cell membrane and barrier permeability [19]. Recent data have shown that occludin phosphorylation regulates TJs permeability in a G-protein dependent or -independent manner, depending on the receptor involved, regardless of cytoskeleton changes [20]. Both external loops as well as trans-membrane and C terminal cytoplasmic occludin domains are important for para-cellular permeability regulation [21]. The N terminal cytoplasmic domain of occludin regulates trans-epithelial migration of neutrophils, and has an important role in maintaining TJs assembly and barrier function [22]. Deletion constructs lacking N terminus or extracellular domains exert a dramatic effect on TJs integrity [22]. In addition, recent studies have

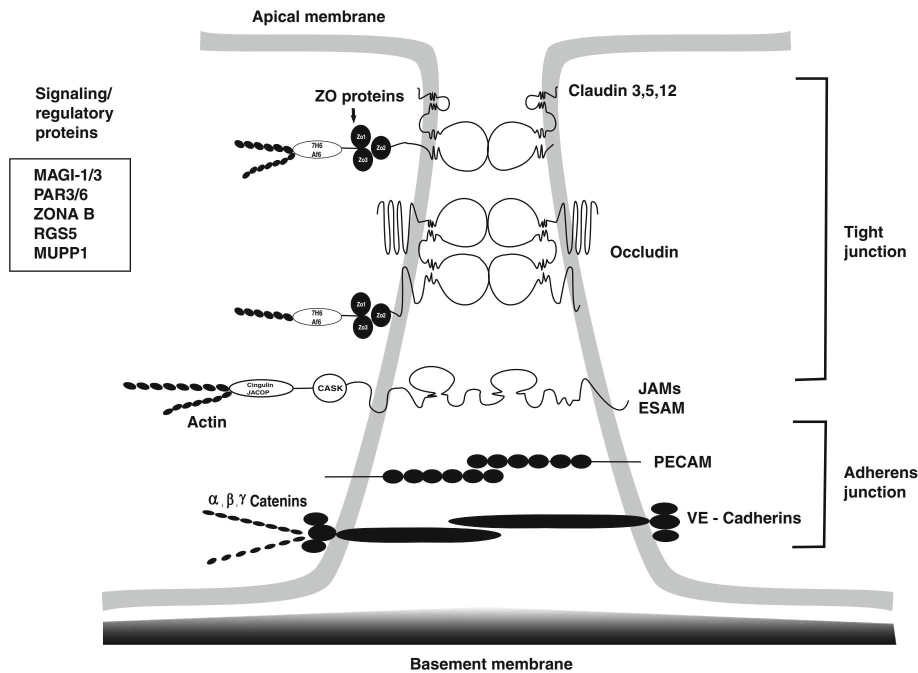

Fig. 2 Molecular organization of tight and adherent junctions. The main transmembrane molecules mediating cellular contact at the tight junction are occludin and the endothelial claudins. Other transmembrane molecules with leukocyte trafficking functions include junctional adhesion molecules (JAMs) and the endothelial selective adhesion molecule (ESAM), both members of the immunoglobulin superfamily. These molecules bind to the cytoskeleton via first-order (ZO-1-3 and CASK) and second-order (cingulin, and JACOP) adaptor molecules. Signaling and regulatory proteins include MAGI-1-3, PAR3-6, ZONA-B, RGS5, AF6, and MUPP1 (details on these proteins are provided in the text). These adaptor/signaling proteins control interaction between the membranous component and the cytoskeleton. Contact in adherent junctions is established mainly through vascular endothelial cadherin (VE-cadherin) and platelet-endothelial cell adhesion molecule (PECAM), coupled to the cytoskeleton via catenins and/or desmoplakin

demonstrated that occludin regulates epithelial cell differentiation [23].

Claudins

Claudins are a multigene family of more than 20 members forming TJ strands through homophilic claudin–claudin interactions, mediated by claudin′s second extracellular loop [24]. These proteins share all four trans-membrane domains of occludin, but do not contain any sequence homology to occludin. Claudins are believed to be the major trans-membrane proteins of tight junctions, as occludin knockout mice are still capable of forming these inter-endothelial connections, while claudin knockout mice are nonviable [25–28]. Claudin-3, -5 and -12 are localized on the BBB [29], whereas the presence of claudin-1 seems to vary among species, though this has not been fully clarified [30]. New findings point to a promoter function for claudin-5 in promatrix metalloproteinase (MMP)-2 activation through a membrane-type matrix metalloproteinase. This new function described for claudin may be important in angiogenesis, and in disease processes with increased vessel permeability [31].

Other Junction Proteins

A third group of TJ-associated molecules recently identified include junctional adhesion molecules (JAMs) [32], and the endothelial selective adhesion molecule (ESAM or 1G8 antigen) [33]. These belong to the immunoglobulin (Ig) gene superfamily, and mediate homophilic and probably heterophilic interactions. They have a single trans-membrane domain and the extracellular portion has two immunoglobulin-like loops that are formed by disulphide bonds. Three JAM-related proteins have been investigated in rodent brain sections. It has been observed that JAM-1 is expressed in endothelial and epithelial cells, whereas JAM-2 and JAM-3 are expressed in most vascular endothelial cells [34–36]. Investigators believe altered expression of JAMs, in addition to affecting tight junctional structure, may also affect leukocyte trafficking, with implications for immune status in CNS disease.

Carboxi-terminal cytoplasmic tails present in occludin, claudins and JAMs are linked to a number of first order cytoplasmic adaptor proteins such as zonula occludens -1 (ZO-1), ZO-2, ZO-3, and Ca^{2+}-dependent protein serine kinase (CASK) [37–39]. ZO-1, ZO-2, and ZO-3 share three

defined core regions: (a) a SH3 domain important in signal transduction and in anchoring transmembrane TJs to cytoskeleton, (b) a guanylate kinase-like domain that catalyses ATP-dependent transformation of GMP to GDP, and (c) a PDZ-domain mediating specific binding to carboxy-terminal cytoplasmic ends of transmembrane proteins. Second order adaptor molecules include cingulin, afadin (AF6) and function-associated coiled-coil protein (JACOP). These two groups of adaptor proteins have sequence similarity with each other and belong to the membrane-associated guanylate kinase (MAGUK) protein family. Importantly, actin, the primary cytoskeleton protein binds to the carboxi-terminal of ZO-1 and ZO-2, a complex cross-linking trans-membrane elements, and thus providing structural support to ECs [38]. Adaptor proteins also interact with different signaling and regulatory molecules such as MAGI-1, MAGI-2, and MAGI-3, the partitioning defective proteins PAR-3 and PAR-6, MUPP-1, the binding protein ZONA B, and the regulator of G-protein signaling (RGS5), all of which control interactions between the membranous component and the actin/vinculin-based cytoskeleton [40–42]. Thus, modulation of TJ-associated proteins and cytoskeletal organization may be controlled through local chemical signals, thereby providing BBB permeability regulation.

Cell–cell interactions in the functional zone are also stabilized by AJs, typically found intermingled with TJs (Fig. 2). These junctions are composed of membrane protein cadherin joined to actin cytoskeleton via intermediary proteins named catenins forming adhesive contact between cells.

VE-Cadherin (cadherin-5)

Among AJs, the endothelial-specific integral protein VE-cadherin is linked to cytoskeleton via catenins. At the BBB, the cytoplasmic domain of cadherins binds to the sub-membrane plaque protein β- or γ-catenin, which is linked to actin cytoskeleton via α-catenin. The role of catenins in AJs bears resemblance to that of zonula occludens proteins in TJs. Recently, a new p120 catenin family was identified whose role remains controversial [43]. High affinity binding of p120 catenin to VE-cadherin suggests it may be engaged in vascular permeability regulation, thus affecting BBB function in some way. Recent in vitro and in vivo studies show that VE-cadherin is required for endothelial integrity in quiescent vessels and in organization of new vessels [44]. VE-cadherin may regulate EC function through different mechanisms: (a) activation of signaling molecules with a role in cytoskeleton organization; (b) transcription factor regulation; (c) formation of complexes with growth factor receptors, and modulation of their signaling [45]. In contrast to VE-cadherin, immunoreaction for N-cadherin indicated that this molecule is located abluminally. Thus, presumably VE-cadherin, which is located in interendothelial junctions promotes interaction between ECs, whereas N-cadherin may be responsible for anchorage to other cell types such as perycites or vascular smooth muscle cells [46].

PECAM-1

Platelet-endothelial cell adhesion molecule (PECAM), also known as CD31, is concentrated at the apical domain of the intercellular junction, and is not structurally associated with TJs [47]. PECAM-1 is involved in cell–cell adhesion through either homophilic interactions with other PECAM molecules or heterophilic interactions with other proteins, such as integrin $\alpha_v\beta_3$ [48]. Recently, altered vascular permeability has been observed in PECAM-1 deficient mice [49], and PECAM-1 has shown an important role in monocyte transmigration through CNS endothelia [50]. During pathological insults, the BBB is capable of modulating its own cyto-architecture, increasing permeability while retaining structural integrity, and hence protecting the brain and maintaining homeostasis; however, this function may be lost under extreme conditions, causing TJs along the BBB to dissociate, which in turn leads to edema, decreased neuronal function and brain damage.

BBB Transport Systems

In addition to structural elements assuring BBB tightness, drug- and nutrients-metabolizing enzymes as well as transport systems provide additional barriers. These enzymes include γ-glutamyl transpeptidase (γ-GT), alkaline phosphatase (AP) aromatic acid decarboxylase, and several cytochrome 450 enzymes. They metabolize neuroactive bloodborne solutes and their concentration is significantly higher in cerebral microvessels compared to non-neuronal capillaries, endowing this dynamic interface with metabolic activity [1].

Nutrients and water-soluble compounds such as ions, amino acids, vitamins and proteins necessary for brain function possess specific transport systems embedded in BBB plasma membranes that allow entry into the brain. Various physiological transport processes are present at the BBB level [45, 51].

Carrier-Mediated Transport

Relies on molecular carriers present at both the apical (blood) and basolateral (brain) membranes of the BBB. These carriers tend to be highly stereospecific, and function in the selective transport of small molecules, such as hexoses, amino-acids, monocarboxylic acids, nucleosides,

amines and vitamins [1]. Nutrient concentration gradients generally follow a direction from blood to brain, regulated according to CNS metabolic needs as well as by substrate concentration in plasma.

Ion Transporters

The sodium pump is localized in the abluminal membrane [52] and regulates sodium influx into the brain interstitial space in exchange for potassium. This pump maintains a high Na^+ gradient at the BBB, allowing Na^+ dependent transport to occur. The sodium–potassium-two chloride co transporter resides predominantly in the luminal BBB membrane [53]. Finally, the sodium-hydrogen exchanger is expressed in the luminal membrane, whereas the chloride-bicarbonate exchanger is expressed on each side. These two transporters play critical roles in regulating the intracellular pH of the endothelium.

Active Efflux

Efflux of molecules from ECs can be initiated at the luminal membrane, as in the case of ATP-binding cassette (ABC) transporters [54]. The multidrug resistance transporter P-glycoportein (P-gp) is an ATP-dependent efflux pump which prevents the passage of drugs and toxins across the BBB into the brain and may facilitate their transport from brain to blood [55]. First described in certain tumor cells that are protected against anticancer agents as a result of P-glycoprotein overexpression, it is now established that P-glycoprotein is expressed constitutively in many normal non-tumorous tissues [56, 57]. In addition to Pgp, several mutidrug resistance-associated proteins (MDRs) are expressed in brain microvessels, and are responsible for reducing the penetration of different drugs into the brain and increasing their efflux from the brain. Recent studies in rat and human brain tissue have shown that Pgp is expressed on both luminal and abluminal membranes as well as in perycites, microglia, and astrocytes [58]. The second efflux transport subfamily, which belongs to the ABC protein superfamily and can confer multidrug resistance is the MRP family. Thus far, the mammalian MRP family consists of nine proteins [59], and most cells appear to express multiple MRP family members. MRP1 and MRP5 appear to be ubiquitous and are both expressed at the BBB [60]. Similar to P-gp, MRP6 is also expressed on BBB endothelium and upregulated in response to glial signals [61].

Receptor Mediated Transport

BBB ECs express several transport systems for neuroactive peptides such as arginine-vasopressin, enkephalins, luteinizing-hormone-releasing hormone, and some cytokines and chemokines. Influx of large proteins into the brain, such as transferrin, low density lipoprotein (LDL), IgG, insulin and insulin growth factor occurs through a transcellular receptor-mediated transport mechanism known as transcytosis [1]. A circulating ligand interacts with a specific receptor at the apical plasma membrane of the ECs. Once bound to ligand, the process of endocytosis is initiated, with receptor-ligand complexes forming intracellular vesicles [62]. During transcytosis these vesicles travel to the basolateral side of the polarized endothelial cell, where they are released.

Caveolae are vesicular invaginations of the plasma membrane 50- to 100-nm in size involved in molecular transport, cell adhesion, and signal transduction [63]. Caveolin, a 21- to 24-kDa protein, is the principal structural component of caveolae, and three caveolin genes (caveolin-1, -2, and -3) have been identified. Endothelial cells are known to express the highest levels of caveolin-1 [64]. Moreover, caveolin-1 and -2 are found in rat microvessels, and caveolin-1 is expressed in the human BBB [65]. Transcytosis is one of the first functional roles proposed for caveolae. Molecules such as albumin and insulin are known to undergo endothelial transcytosis in peripheral tissue, perhaps through different subsets of caveolae [64]. Caveolar membranes contain receptors for transferring, insulin, albumin, ceruloplasmin, RAGE, LDL, HDL, IL-1 and vesicle-associated membrane protein-2 [15].

In order to exploit endogenous receptor mediated transport systems at the BBB for drug delivery, different molecules that normally do not cross the BBB can be conjugated to a molecule with capability of targeting receptor mediated transport. These molecules could be either natural or artificial ligands, such as monoclonal antibodies or peptides [51], which can in turn be used to carry conjugated neuro-therapeutic substances across the BBB [66]. Classical examples of receptors involved in receptor mediated trancytosis are: the insulin receptor, the transferrin receptor, and the transporters for low-density lipoprotein and insulin-like growth factors [51, 67, 68].

Absorptive Mediated Endocytosis

While receptor-mediated transcytosis systems require binding of a ligand to a specific receptor, absorptive mediated endocytosis is initiated by polycathionic molecules binding to negative charges on the plasma membrane. This process lacks specific targeting and may lead to widespread absorption [69].

Astrocytes

Among the glial cells of the CNS, the role of astrocytes remained enigmatic. In the late 1980s, experiments

demonstrating astrocytes expressed voltage-gated channels and neurotransmitter receptors generated interest in these cells as potential participants in intercellular communication. However, only recently have certain astrocyte functions been revealed, these include the control of cerebral vascular tone and of synapse formation and function, and adult neurogenesis. Astrocytes show a number of different morphologies, depending on their location and association with other cell types. Of the 11 distinct phenotypes distinguished, 8 involved specific interactions with blood vessels. Evidence from cell culture studies indicate astrocytes upregulate many BBB features, leading to tighter TJs, expression as well as polarized distribution of transporters, and of specialized enzyme systems [70–74].

Ninety percent of the abluminal surface of cerebral microvasculature endothelium is ensheathed by astrocytic end-feet, which play an essential role in determining different BBB features. These specialized structures show high density of purinergic P2Y receptors, of potassium channel Kir4.1, and of water-channel protein aquaporin-4 (AQP4), indicating key roles in gliovascular signaling and in regulation of brain water and electrolyte metabolism under normal and pathological conditions [75].

AQP4 is a type III transmembrane protein regulating water entry to and from multiple types of tissue epithelia, but it also has a critical role when expressed by astrocytes, which regulate water and ion movement in the brain. AQP4 mRNA riboprobe revealed a remarkable prevalence of AQP4 in multiple periventricular areas. These include the dorsal hypothalamic area, the dorsomedial hypothalamic nucleus, and the suprachiasmatic nucleus lining the third ventricle, the paraventricular thalamic nucleus and subfornical organ lining the dorsal third ventricle. In addition, strong AQP4 hybridization signal was found in the most dorsal part of the lateral ventricle, in the dorsal raphe contacting the aqueduct, and in the choroid plexus of the lateral ventricles [76, 77]. Interestingly, the strongest hybridization signal was observed at the pial surface, where the brain is in contact with the CSF in the subarachnoid space. This preferential location of AQP4 mRNA-expressing cells in periventricular areas is reminiscent of that seen for the atrial natriuretic peptide, suggesting that the presence of AQP4 channels in these nuclei may be of critical importance in detecting alterations in CSF homeostasis preceding neurosecretory processes [77]. In addition, it is worthy to note the co-localization of AQP4 with the inward-transporting potassium channel Kir4.1 in astrocytes at the BBB interface, suggesting a functional relationship between both proteins (Fig. 3). It has been suggested that the presence of a water channel could facilitate excess K+ clearance, generated during high neuronal activity [78, 79]. Supporting this hypothesis, mice with reduced perivascular expression of AQP4, show

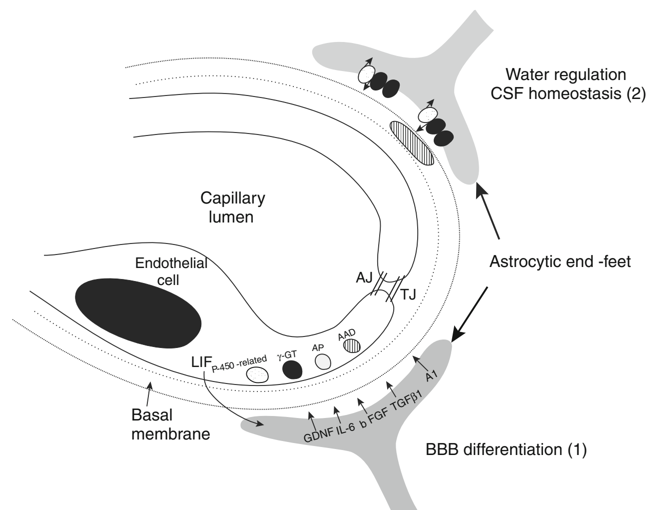

Fig. 3 Astroglial-endothelial signaling interactions. The BBB is formed by endothelial cells surrounded by basal membrane, and astrocytic perivascular endfeet. Astrocytes are strongly implicated in induction of certain BBB characteristics such as tighter TJs, specialized enzyme systems, and polarized transporter localization (1). In addition they also play key roles in gliovascular signaling and in the regulation of brain water and electrolyte metabolism under normal and pathological conditions (2). *GDNF* Glial cell line-derived neurotrophic factor, *IL-6* Interleukin-6, *bFGF* basic fibroblast growth factor, *TGF-β1* transforming growth factor $\beta 1$, *A1* angiopoietin, *LIF* Leukemia inhibitory factor, *γGT* γ-glutamil transpeptidase, *AAD* aromatic acid decarboxylase. *AP* alkaline phosphatase; [symbol] Aquaporin-4; [symbol] Agrin; [symbol] potassium channel Kir4.1

alterations in K+ homeostasis with delayed clearance after neuronal stimulation [80].

Astrocytes are also strongly implicated in induction of other BBB characteristics such as tighter TJs, specialized enzyme systems, and polarized localization of transporters [72, 73, 81–83] (Fig. 3). Using heterologous culture, and allowing only astrocytic end-feet to contact umbilical vein endothelial cells, Hayashi and co-workers showed that a number of specific BBB markers such as glucose transporters, transferrin receptor (TfR), γ-glutamil transpeptidase, and P-glycoprotein undergo transcription upregulation [72]. Interestingly, astrocytic end-feet membranes show unique aggregates of intramembrane particles packed in orthogonal arrays (OAPs) developed in parallel with endothelial TJs [84]. When the BBB breaks down, this high number of orthogonal particles in end-feet membranes is greatly reduced or even absent [85]. Such observations suggest a specific role for these particles in astrocyte/endothelium interactions, particularly during BBB development. Interestingly, OAPs and AQP4 correlate with the expression of agrin, a heparin sulfate proteoglycan of the basal lamina [86, 87]. Agrin accumulates in brain microvessels at the time of BBB tightening, and is required for the segregation of AQP4 to perivascular astrocytic end-feet, a process mediated by agrin binding to α-dystroglycan [75].

Moreover, other culture systems have shown that transplanted astrocytes induce BBB properties in non-neural vascular ECs, indicating that astrocytes represent a major source of the neural tissue inductive influence [88]. Finally, selective elimination of reactive astrocytes using suicide gene strategies causes BBB disruption, inhibiting its repair [89]. There is still debate about the factors involved in this differentiation, but it is likely they are multiple, involving some that are soluble and others depending on cell-to-cell contact [90]. Several molecules, e.g., transforming growth factor-β1 (TGFβ1), glial cell line-derived neurotrophic factor (GDNF), basic fibroblast growth factor (bFGF) and interleukin-6 (IL-6), and angiopoietin 1 (Ang-1), have all been associated with the regulation of different aspects of BBB phenotype. For example, studies in mice lacking or over-expressing ang-1 have revealed that ang-1 is responsible for recruiting and sustaining periendothelial support cells and for contributing to the impermeability of blood vessels [91]. The secretion of ang-1 from perivascular astrocytes can bind its receptor Tie-2 onto endothelial cells. On the other hand, endothelial cells can also modulate astrocyte phenotype by secreting growth factors, such as the leukemia inhibitory factor [71]. In addition, endothelial cells, pericytes and astrocytes further contribute to extracellular matrix structure, which in turn influences EC differentiation [71].

Perycites

Perycites are vascular cells adjacent to capillaries that share a common basement membrane with endothelial cells, and have many cytoplasmic processes encircling capillaries. The lineage and identity of perycites is still not fully characterized. These cells seem to be morphologically, biochemically and physiologically heterogeneous [92]. They express non-muscle actins and they also contain α-smooth muscle actin which is characteristic of vascular smooth muscle cells. Perycite to endothelia ratio in the brain is 30-fold higher compared to that of striated muscles [93]. Through long cytoplasmic processes that extend over and encircle the endothelial tube, perycites make focal contact with ECs through specialized junctions [94]. It is believed that recruitment and further interaction of perycites along the microvascular endothelial wall are essential for the formation, maturation, and maintenance of normal microvascular structure and function. In the CNS perycites contribute to microvessel stability covering a major part of the abluminal endothelial surface [94]. In addition, they influence vessel stability through matrix deposition and activating signals promoting ECs differentiation [95]. Their distribution and degree of coverage of the vascular endothelium vary among different vessel types. During embryonic angiogenesis, PDGF-β produced by ECs appears to be critical in recruitment perycites to the forming blood vessels. Thus, PDGF-β deficient mutant mice develop defective capillary endothelial structures. These abnormalities contribute to structural instability of the capillary wall leading to microvascular hemorrhage and other vascular damage [96, 97]. On the other hand, perycites release different growth factors and angiogenic molecules which regulate microvascular permeability, remodeling and angiogenesis (e.g., TGF-β, ang-1 and 2, PDGF, and sphingosine-1 phosphate; [98]). Thus, the active form of TGF-β1 secreted from perycites appears to at least partially mediate perycite effects on actin expression and organization on ECs [99, 100]. Perycites migrate away from brain microvessels in response to hypoxia [101] or brain trauma [102], conditions associated with increased BBB permeability and disorganization of actin filaments in ECs. A lack of perycites results in endothelial hyperplasia and abnormal vascular morphogenesis in the brain. Moreover, rich expression of the contractile protein α-smooth muscle actin in perycites associated with brain capillaries suggests perycites may also control blood flow [92, 103], functioning similarly to vascular smooth muscle cells in arterioles and small pial arteries in the brain, which regulate cerebral blood flow responses [104]. Some BBB perycites might be of macrophage lineage, possessing capacity to phagocytize exogenous proteins and present antigens [105].

Basement Membrane Matrix

The basement membrane separates ECs from neighboring cells, namely perycites and astrocytes. It is composed of different extracellular matrix (ECM) structural proteins such as collagen, fibronectin, heparan sulfate, proteoglycans, chondroitin sulfate proteoglycans and laminin. Matrix adhesion receptors are expressed on vascular cells, neurons, and supporting glial cells (i.e., astrocyte end feet and microglia; [106]). Integrins play a key role in mediating endothelial signaling, cell migration and brain capillary formation during angiogenesis [107]. Growth factors such as vascular endothelial growth factor are bound to ECM proteins and can be activated in situ by MMPs [108]. Disruption of the ECM is strongly associated with increased BBB permeability in pathological states, such as brain tumors and cerebral ischemia [109, 110].

Microglia Cells

In addition to astrocytes and perycites, the development and maintenance of the BBB probably involves other cell populations. Another cell type in close contact with cerebral blood vessels is the cerebral perivascular macrophage, also referred to as perivascular cell, or perivascular microglia. This cell occupies a strategic position in the

BBB allowing control of innate and adaptive immune responses in the brain. Microglial cells derive from leptomeningeal mesenchymal cells, which transform into microglia on entry into the brain [111]. Circulating monocytes provide another important source of microglia in the brain [112]. Studies in rodents and humans have shown that these cells are bone marrow derived and regularly replaced by monocytic precursor cells, therefore representing tissue macrophages of the brain [113]. Indeed, perivascular macrophages are recognized by markers for peripheral macrophages, confirming their origin from monocyte lineage [114]. Recent studies have shown that non-contact culture of confluent brain capillary ECs with human blood-derived macrophages, substantially decreases paracellular permeability, indicating an active role of these cells in BBB physiology [115]. In addition, perivascular microglia in the human brain express molecules involved in antigen recognition, antigen presentation and co-stimulation, supporting a possible role for these cells in perivascular inflammation regulation in the human CNS [116]. This link between microglia in the brain, circulating monocytes, and bone marrow cells has changed our preconception of the brain as an immune privileged site.

Concluding Remarks and Future Perspectives

Knowledge of BBB biology has advanced significantly in recent years. Application of modern molecular and cell biology techniques, together with traditional structural studies, enables us to appreciate the BBB not only as a static anatomical barrier, but also as a highly complex and metabolically active bi-directional interface. Nevertheless, several key questions related both to physiology and pathology of the BBB remain unanswered. Challenges for the future include, understanding crosstalk between glial cells, vessel cells (such as endothelial cells and perycites) and neurons. Better knowledge of human BBB transport systems is essential in order to translate findings from animal models or tissue culture systems to humans. Also, better ways of imaging and monitoring functions of in vivo barrier activity will be need in the future. Finally, better understanding of cellular proteomics and metabolism will have to be devised before BBB-targeted therapies can be developed. Over the coming years, emerging information may help to understand the role of the BBB in the pathogenesis of different neurological diseases. Early treatment of barrier dysfunction could reduce neuropathological symptom severity and facilitate recovery. Future studies should therefore explore the translational potential of different approaches currently at the preclinical development level. Overall, understanding the structure and physiology of the BBB will open new future directions for diagnosis and therapy of several neurological disorders.

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References

1. Pardridge WM (2005) Molecular biology of the blood-brain-barrier. Mol Biotechnol 30:57–70
2. Abbott NJ, Romero IA (1996) Transporting therapeutics across the blood-brain barrier. Mol Med Today 2:106–111
3. Begley D, Brightman MW (2003) Structural and functional aspects of the blood-brain-barrier. In: Prokai L, Prokai-Tatrai K (eds) Peptide transport and delivery into the central nervous system. Progress in drug research. Birkhauser Verlag, Basel, pp 39–78
4. Begley DJ (2004) Efflux mechanisms in the central nervous system: a powerful influence on drug distribution within the brain. In: Sharma HS, Westman J (eds) Blood-spinal cord and brain barriers in health and disease. Elsevier, San Diego, pp 83–97
5. Prestcott L, Brightman MW (1998) Circunventricular organs of the brain. In: Pardridge WM (ed) Introduction to the blood-brain barrier: methodology, biology and pathology. Cambridge University Press, Cambridge, pp 270–276
6. Saunders NR, Ek CJ, Habgood MD, Dziegielewska KM (2008) Barriers in the brain: a renaissance. Trends Neurosci 31:279–286
7. Pardridge WM (1999) A morphological approach to the analysis of blood-brain-barrier transport function. In: Paulson O, Knudsen G, Moos T (eds) Brain barrier systems. Munkgaard, Copenhagen, pp 39–78
8. Farell CL, Pardridge WM (1991) Blood-brain-barrier glucose transporter is symmetrically distributed on brain capillary endothelial luminal and abluminal membranes: an electronic microscopic immunogold study. Proc Natl Acad Sci USA 88: 5779–5783
9. Vorbrodt AW (1993) Morphological evidence of the functional polarization of brain microvascular endothelium. In: Pardridge WM (ed) The blood-brain-barrier. Raven, New York, pp 137–164
10. Brightman MW, Reese TS (1969) Junctions between intimately apposed cell membranes in the vertebrate brain. J Cell Biol 40: 648–667
11. Lane NJ, Reese TJ, Kacher B (1992) Structural domains of the tight junctional intramembrane fibrils. Tissue Cell 24:291–300
12. Bearer EL, Orci L (1985) Endothelial fenestral diaphragms: a quick-freeze, deep-etch study. J Cell Biol 100:418–428
13. Dorovini-Zis K, Prameya R, Bowman PD (1991) Culture and characterization of microvascular endothelial cells derived from human brain. Lab Invest 64:425–436
14. Villegas JC, Broadwell RD (1993) Transcytosis of protein through the mammalian cerebral epithelium and endothelium. II. Adsorptive transcytosis of WGA-HRP and the blood-brain and blood-brain barriers. J Neurocytol 22:67–80
15. Wolburg H (2006) The endothelial frontier. In: Dermietzel R, Spray DC, Nedergaard M (eds) From ontogeny to artificial barriers. Willey-VCH, Weinheim, pp 77–109
16. Furuse M, Hirase T, Itoh A, Nagafuchi A, Yomemura S, Tsukita S, Tsukita S (1993) Occludin: a novel integral membrane protein localizing at tight junctions. J Cell Biol 123:1777–1788
17. Furuse M, Fujita K, Hiiragi T, Fujimoto K, Tsukita S (1998) Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. J Cell Biol 141:1539–1550

18. Furuse M, Hata M, Furuse K, Yoshida Y, Harakate A, Sugitani Y, Noda T, Kubo A, Tsukita S (2002) Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. J Cell Biol 156:1099–1111
19. Sakakibara A, Furuse M, Saitou M, Ando-Akatsuka Y, Tsukita S (1997) Possible involvement of phosphorylation of occludin in tight junction formation. J Cell Biol 137:1391–1401
20. Hirase T, Kawashima S, Wong EYM, Ueyama T, Rikitake Y, Tsukita S, Yokoyama M, Staddon JM (2001) Regulation of tight junction permeability and occludin phosphorylation by RhoA-p160 ROCK-dependent and independent mechanisms. J Biol Chem 276:10423–10431
21. Balda MS, Matter K (2000) Transmembrane proteins of tight junctions. Semin Cell Dev Biol 11:281–289
22. Bamforth SD, Kniesel U, Wolburg H, Engelhardt B, Risau W (1999) A dominant mutant occludin disrupts tight junction structure and function. J Cell Sci 112:1879–1888
23. Schulzke JD, Gitter AH, Mankertz J, Spiegel S, Seidler U, Amasheh S, Saitou M, Tsukita S, Fromm M (2005) Epithelial transport and barrier function in occludin-deficient mice. Biochim Biophys Acta 1669:34–42
24. Piontek J, Winkler L, Wolburg H, Muller SL, Zuleger N, Piehl C, Wiesner B, Krause G, Blasig IE (2008) Formation of tight junction: determinants of homophilic interactions between classic claudins. FASEB J 22:146–158
25. Saitou M, Furuse M, Sasaki H, Schulzke JD, Fromm M, Takano H, Noda T, Tsukita S (2000) Complex phenotype of mice lacking occluding, a component of tight junctions strands. Mol Biol Cell 11:4131–4142
26. Tsukita S, Furuse M (2000) Pores in the wall: claudins constitute tight junction strands containing aqueous pores. J Cell Biol 149: 13–16
27. Gow A, Southwood CM, Li JS, Pariali M, Riordan GP, Brodie SE, Danias J, Bronstein JM, Kachar B, Lazzarini RA (1999) CNS myelin and sertoli cell tight junction strands are absent in Osp/claudin-11 null mice. Cell 99:649–659
28. Tsukita S, Furuse M (1999) Occludin and claudins in tight-junction strands: leading or supporting players? Trends Cell Biol 9:268–273
29. Nitta T, Hata M, Gotoh S, Seo Y, Sasaki H, Hashimoto N, Furuse M, Tsukita S (2003) Size-selective loosening of the blood-brain-barrier in claudin-5-deficient mice. J Cell Biol 161:653–660
30. Lee SW, Kim WJ, Choi YK, Song HS, Son MJ, Gelman IH, Kim YJ, Kim KW (2003) SSeCKS regulates angiogenesis and tight junction formation in blood-brain-barrier. Nat Med 9:900–906
31. Miyamori H, Takino T, Kobayashi Y, Tokai H, Itoh Y, Seiki M, Sato H (2001) Claudin promotes activation of Pro-MMP-2 mediated by membrane-type matrix metalloproteinases. J Biol Chem 276:28204–28211
32. Bazzoni G, Tonetti P, Manzi L, Cera MR, Balconi G, Dejama E (2005) Expression of junctional adhesion molecule-A prevents spontaneous and random motility. J Cell Sci 118:623–632
33. Nasdala I, Wolburg-Bucholz K, Wolburg H, Kuhn A, Ebnet K, Brachtendorf G, Samulowitz U, Kuster B, Engelhardt B, Westweber D, Butz S (2002) A transmembrane tight junction protein selectively expressed on endothelial cells and platelets. J Biol Chem 277:16294–16303
34. Martin-Padura I, Lostaglio S, Schneemann M, Willimas L, Romano M, Fruscella P, Panzeri C, Stoppacciaro A, Ruco L, Villa A, Simmons D, Dejana E (1998) Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration. J Cell Biol 142:117–127
35. Aurronds-Lions M, Duncan L, Ballestrem C, Imhof BA (2001) JAM-2, a novel immunoglobulin superfamily molecules, expressed by endothelial and lymphatic cells. J Cell Chem 276:2733–2741
36. Palmeri D, van Zante A, Huang CC, Hemmerich S, Rosen SD (2000) Vascular endothelial junctions-associated molecule, a novel member of the immunoglobulin superfamily, is localized to intercellular boundaries of endothelial cells. J Biol Chem 275: 19139–19145
37. Fanning AS, Jameson BJ, Jesaitis LA, Anderson JM (1998) The tight junction protein ZO-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton. J Biol Chem 273:29745–29753
38. Haskins J, Gu L, Wittchen ES, Hibbard J, Stevenson BR (1998) ZO-3, a novel member of the MAGUK protein family found at the tight junctions, interacts with ZO-1 and occludin. J Cell Biol 141:199–208
39. Ebnet K, Schulz CU, Meyer ZU, Brickwedde MK, Pendl GG, Westweber D (2000) Junctional adhesion molecule interacts with the PDZ domain-containing proteins AF-6 and ZO-1. J Biol Chem 275:27979–27988
40. Citi S, Sabanay H, Kendrick-Jones J, Geiger B (1989) Cingulin: characterization and localization. J Cell Sci 93:107–122
41. Zhong Y, Saitoh T, Minase T, Sawada N, Enomoto K, Mori M (1993) Monoclonal antibody 7H6 reacts with a novel tight junction-associated protein distinct from ZO-1, cingulin and ZO-2. J Cell Biol 120:477–483
42. Ebnet K, Suzuki A, Ohno S, Westweber D (2004) Junctional adhesion molecules (JAMS): more molecules with dual functions? J Cell Sci 117:19–29
43. Anastasiadis PZ, Reynolds AB (2000) The p120 catenin family: complex roles in adhesion, signaling and cancer. J Cell Sci 113: 1319–1334
44. Lampugnani MG, Dejana E (2007) Adherens junctions in endothelial cells regulate vessel maintenance and angiogenesis. Thromb Res 120:S1–S6
45. Zlokovic BV (2008) The blood-brain-barrier in health and chronic neurodegenerative disorders. Neuron 57:178–201
46. Gerhardt H, Liebner S, Redies C, Wolburg H (1999) N-cadherin expression in endothelial cells during early angiogenesis in the eye and brain of the chicken: relation to blood-retina and blood-brain barrier development. Eur J Neurosci 11:1191–1201
47. Ayalon O, Sabanai H, Lampugnani MG, Dejana E, Geiger B (1994) Spatial and temporal relationship between cadherins and PECAM-1 in cell–cell junctions of human endothelial cells. J Cell Biol 126:247–258
48. Buckley CD, Doyonnas R, Newton JP, Blystone SD, Brown EJ, Watt SM, Simmons DL (1996) Identification of alpha v beta 3 as a heterotypic ligand for CD31/PECAM-1. J Cell Sci 109: 437–445
49. Graesser D, Solowiej A, Bruckner M, Osterweil E, Juedes A, Davis S, Ruddle NH, Engelhardt B, Madri JA (2002) Altered vascular permeability and early onset of experimental allergic encephalomyelitis in PECAM-1 deficient mice. J Clin Invest 109: 383–392
50. Floris S, Ruuls SR, Wierinckx A, van der Pol SM, Dopp E, van der Meide PH, Dijkstra CD, De Vries HE (2002) Interferon-beta directly influences monocyte infiltration into the central nervous system. J Neuroimmunol 127:69–79
51. Jones AR, Shusta EV (2007) Blood-brain-barrier transport of therapeutics via receptor-mediation. Pharm Res 24:1759–1771
52. Vorbrodt AW (1988) Ultrastructural cytochemistry of blood-brain-barrier endothelia. Prog Histochem Cytochem 18:1–99
53. OíDonnell ME, Lam TI, Tran LQ (2006) Estradiol reduces activity of the blood-brain-barrier Na-K-Cl contransporter and decreases edema formation in permanent middle cerebral artery occlusion. J Cereb Blood Flow Metab 26:1234–1249
54. Hernan DM, Basetti CL (2007) Indications of ATP-binding cassette transporters for brain pharmacotherapies. Trends Pharmacol Sci 28:128–134

55. Fricker G, Miller DS (2004) Modulation of drug transporters at the blood-brain-barrier. Pharmacology 70:169–176
56. Juliano RL, Ling V (1976) A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 455:152–162
57. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC (1987) Cellular localization of the multi-drug-resistance gene product P-glycoproetin in normal human tissues. Proc Natl Acad Sci USA 84:7735–7738
58. Bendayan R, Ronaldson PT, Gingras D, Bendayan M (2006) In situ localization of P-glycoprotein (ABCB1) in human and rat brain. J Histochem Cytochem 54:1159–1167
59. Schinkel AH, Jonker J (2003) Mammalian drug efflux transporters of ATP-binding cassette (ABC) family: an overview. Adv Drug Deliv 55:3–29
60. Zhang Y, Schueltz JD, Elmquist WF, Miller DW (2004) Plasma membrane localization of multidrug resistance-associated protein homologies in brain capillary endothelial cells. J Pharmacol Exp Ther 311:449–455
61. Fenart L, BuÈe-Scherrer V, Descamps L, Duhem C, Poullain MG, Cecchelli R, Dehouck MP (1998) Inhibition of P-glycoprotein: rapid assessment of its implication in blood-brain-barrier integrity and drug transport to the brain by an in vitro model of the blood-brain-barrier. Pharm Res 15:993–1000
62. Brown VI, Greene MI (1991) Molecular and cellular mechanisms of receptor-mediated endocytosis. DNA Cell Biol 10:399–409
63. Parton RG, Richards AA (2003) Lipid rafts and caveolae as portals for endocytosis: new insights and common mechanisms. Traffic 4:724–738
64. Frank PG, Woodman SE, Park DS, Lisanti MP (2003) Caveolin, caveolae, and endothelial cell function. Arterioscler Thromb Vasc Biol 23:1161–1168
65. Virgintino D, Robertson D, Erreded M, Benagiano V, Tauer U, Roncali L, Bertossi M (2002) Expression of caveolin-1 in human brain microvessels. Neuroscience 115:145–152
66. Pardridge WM (2007) Blood-brain-barrier delivery. Drug Discov Today 12:54–61
67. Boer AG, Gaillard PJ (2007) Strategies to improve drug delivery across the blood-brain barrier. Clin Pharmacokinet 46:553–576
68. Cornford EM, Cornford ME (2002) New systems for delivery drugs to the brain in neurological diseases. Lancet Neurol 1: 306–315
69. Bickel U, Yoshikawa T, Pardridge WM (2001) Delivery of peptides and proteins through the blood-brain-barrier. Adv Drug Deliv Rev 46:247–279
70. Rubin LL, Hall DE, Porter S, Barbu K, Cannon C, Horner HC, Jantpour N, Liaw CW, Manning K, Morales J, Tanner LI, Tomaselli KJ, Bard F (1991) A cell culture model of the blood-brain-barrier. J Cell Biol 115:1725–1735
71. Abbott NJ, Revest PA, Romero IA (1992) Astrocyte–endothelial interaction: physiology and pathology. Neuropathol Appl Neurobiol 18:424–433
72. Hayashi Y, Nombra M, Yamagishi S, Harada S, Yamashita J, Yamamoto H (1997) Induction of various blood-brain-barrier properties in non-neuronal endothelial cells by close apposition to co-cultured astrocytes. Glia 19:13–26
73. Sobue K, Yamamoto N, Yoneda K, Hodgson ME, Yamashiro K, Tsuruoka N, Tsuda T, Katsuya H, Miura Y, Asai K, Kato T (1999) Induction of blood-brain barrier properties in immortalized bovine brain endothelial cells by astrocytic factors. Neurosci Res 35:155–164
74. McAllister MS, Krizanac-Bengez L, Macchia F, Naftalin RJ, Pedley KC, Mayberg MR, Marroni M, Leaman S, Stannes KA, Janigro D (2001) Mechanism of glucose transport at the blood brain barrier: an in vitro study. Brain Res 409:20–30
75. Abbott NJ, Ronnback L, Hansson E (2006) Astrocyte–endothelial interactions at the blood-brain-barrier. Nat Rev Neurosci 7:41–53
76. Nielsen S, Nagelhus EA, Amiry-Moghaddam M, Bourque C, Agre P, Ottersen OP (1997) Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. J Neurosci 17:171–180
77. Venero JL, Vizuete ML, Ilundain AA, Machado A, Echevarria M, Cano J (1999) Detailed localization of aquaporin-4 messenger RNA in the CNS: preferential expression in periventricular organs. Neuroscience 94:239–250
78. Nagelhus EA, Horio Y, Inanobe A, Fujita A, Haug FM, Nielsen S, Kurachi Y, Ottersen OP (1999) Immunogold evidence suggests that coupling of K+ siphoning and water transport in rat retinal Muller cells is mediated by a coenrichment of Kri 4.1 and AQP4 in specific membrane domains. Glia 26:47–54
79. Connors NC, Kofuji P (2002) Dystrophin DP71 is critical for the clustered localization of potassium channels in retinal glial cells. J Neurosci 22:4321–4327
80. Amyry-Moghaddam M, Williamson A, Palomba M, Eid T, de Lanerolle NC, Nagelhus EA, Adams ME, Froehner SC, Agre P, Ottersen OP (2003) Delyed K+ clearance associated with aquaporin-4 mislocalization: phenotypic defects in brains of alpha-syntrophin-null mice. Proc Natl Acad Sci USA 100:13615–13620
81. Raub TJ (1996) Signal transduction and glial cell modulation of cultured brain microvessel endothelial cell tight junctions. Am J Physiol 271:C495–C503
82. Deahouck B, Dehouck MP, Fruchard JC, Cechelli R (1994) Upregulation of the low density lipoprotein receptor at the blood-brain-barrier: intercommunications between brain capillary endothelial cells and astrocytes. J Cell Biol 126:465–473
83. DeBault LE, Cancilla PA (1980) Gamma-Glutamiltranspeptidase in isolated brain endothelial cells: induction by glial cells in vitro. Science 207:653–655
84. Nico B, Cantino D, Sassoe Pognetto M, Bertossi M, Ribatti D, Roncali L (1994) Orthogonal arrays of particles (OAPs) in perivascular astrocytes and tight junctions in endothelial cells. A comparative study in developing and adult brain microvessels. J Submicrosc Cytol Pathol 26:103–109
85. Wolburg H, Lippoldt A (2002) Tight junctions of the blood-brain-barrier: development, composition and regulation. Vasc Pharmacol 38:323–337
86. Wolburg H, Neuhaus J, Pettmann B, Labourdette G, Sensenbrenner M (1986) Decrease in the density of orthogonal arrays particles in membranes of cultured rat astroglial cells by the brain fibroblast growth factor. Neurosci Lett 72:25–30
87. Verkman AS (2002) Aquaporin water channels and endothelial cell function. J Anat 200:617–627
88. Janzer RC, Raff MC (1987) Astrocytes induce blood-brain barrier properties in endothelial cells. Nature 325:253–257
89. Bush TG, Puvanachandra N, Horner CH, Polito A, Ostenfeld T, Svendensen CN, Mucke L, Jhonson MH, Sofroniew MV (1999) Leukocyte infiltration, neuronal degeneration, and neurite outgrowth after ablation of scarforming, reactive astrocytes, in adult transgenic mice. Neuron 23:297–308
90. Bauer HC, Bauer H (2000) Neural induction of the blood-brain barrier: still an enigma. Cell Mol Neurobiol 20:13–28
91. Thurston G, Suri C, Smith K, McClain J, Sato TN, Yancopoulos GD, McDonald DM (1999) Leakage-resistant blood vessels in mice transgenically overexpressing angiopoietin-1. Science 286: 2511–2514
92. Shepro D, Morel MM (1993) Perycite physiology. FASEB 7: 1031–1038
93. Allt G, Lawrenson JG (2001) Perycites: cell biology and pathology. Cells Tissues Organs 169:1–11

94. von Tell D, Armulik A, Betsholtz C (2006) Perycites and vascular stability. Exp Cell Res 312:623–629
95. Armulik A, Abramsson A, Bestsholtz C (2005) Endothelial/perycite interactions. Circ Res 97:512–523
96. Hellstrom M, Kalen M, Lindahl P, Abramsson A, Betsholtz C (1999) Role of PDGF-B and PDGF-beta in recruitment of vascular smooth muscle cells and perycites during embryonic blood vessel formation in the mouse. Development 126:3047–3055
97. Lindahl BR, Johansson P, Leveen P, Betsholtz C (1997) Perycites loss and microaneurysms formation in PDGF-B-deficient mice. Science 277:242–245
98. Dore-Duffy P, LaManna JC (2007) Physiologic angiodynamics in the brain. Antioxid Redox Signal 9:2449–2452
99. Papetti M, Shujath J, Riley KN, Herman IM (2003) FGF-2 antagonizes the TFG-beta1-mediated induction of perycite alpha-smooth muscle actin expression: a role of myf-5 and Smad-mediated signaling pathways. Invest Ophtalmol Vis Sci 44:4994–5005
100. Clements RT, Minnear FL, Singer HA, Keller RS, Vincent PA (2005) RhoA and Rho-kinase dependent and independent signals mediate TGF-beta-induced pulmonary endothelial cytoskeletal reorganization and permeability. Am J Physiol Lung Cell Mol Physiol 288:L294–L306
101. Gonul E, Duz B, Kahraman S, Kayali H, Kubar A, Timurkaynak E (2002) Early pericyte response to brain hypoxia in cats: an ultrastructural study. Microvasc Res 64:116–119
102. Dore-Duffy P, Owen C, Balabanov R, Murphy S, Beaumont T, Rafols JA (2000) Perycite migration from the vascular wall in response to traumatic brain injury. Microvasc Res 60:55–69
103. Rucker HK, Wynder HJ, Thomas WE (2000) Cellular mechanisms of CNS perycites. Brain Res Bull 51:363–369
104. Chow N, Bell RD, Deane R, Streb JW, Chen J, Brooks A, Van Nostrand W, Miano JM, Zlokovic BV (2007) Serum response factor and myocardin mediate cerebral arterial hypercontractility and blood flow dysregulation in Alzheimerís phenotype. Proc Natl Acad Sci USA 104:823–828
105. Williams KC, Corey S, Westmoreland SV, Pauley D, Knight H, deBakker C, Alvarez X, Lackner AA (2001) Perivascular macrophages are the primary cell type productively infected by simian immunodeficiency virus in the brains of macaques: implications for the neuropathogenesis of AIDS. J Exp Med 193:905–915
106. del Zoppo GJ, Millner R, Mabuchi T, Hung S, Wang X, Koziol JA (2006) Vascular matrix adhesion and the blood-brain-barrier. Biochem Soc Trans 34:1261–1266
107. del Zoppo GJ, Millner R (2006) Integrin–matrix interactions in the cerebral microvasculature. Artherioscler Thromb Vasc Biol 26:1966–1975
108. Zlokovic BV (2006) Remodeling after stroke. Nat Med 12:390–391
109. Rascher G, Fishmann A, Kroger S, Duffner F, Grote EH, Wolburg H (2002) Extracellular matrix and the blood-brain-barrier in glioblastoma multiforme: spatial segregation of tenascin and agrin. Acta Neuropathol (Berlin) 104:85–91
110. Jian Liu K, Rosenberg GA (2005) Matrix metalloproteinases and free radicals in cerebral ischemia. Free Radic Biol Med 39: 71–80
111. Bechmann I, Galea I, Perry VH (2007) What is the blood-brain-barrier (not)? Trends Immunol 28:5–11
112. Bechmann I, Goldmann J, Kovac AD, Kwidzinski E, Simburger E, Naftoli F, Dirnagl U, Nitsch R, Priller J (2005) Circulating monocytic cells infiltrate layers of anterograde axonal degeneration where they transform into microglia. FASEB J 19:647–649
113. Hickey WF, Kimura H (1988) Perivascular microglial cells of the CNS are bone-marrow derived and present antigen in vivo. Science 239:290–292
114. Hickey WF, Vass K, Lassmann H (1992) Bone-marrow derived elements in the central nervous system: an immunohistochemical and ultrastructural survey of rat chimeras. J Neuropathol Exp Neurol 51: 246–256
115. Zenker D, Begley D, Bratze H, Rubsamen-Waigmann H, von Briesen H (2003) Human blood-derived macrophages enhance barrier function of cultured primary bovine and human brain capillary endothelial cells. J Physiol 551:1023–1032
116. Fabriek BO, van Haastert ES, Galea I, Polfliet MMJ, Döpp ED, van den Heuvel MM, van den Berg TK, De Groot CJA, van der Valk P, Dijstra CD (2005) CD163-positive perivascular macrophages in the human CNS express molecules for antigen recognition and presentation. Glia 51:297–305