ANTIOXIDANTS & REDOX SIGNALING
Volume 15, Number 5, 2011

DOI: 10.1089/ars.2011.3929

FORUM REVIEW ARTICLE

Tight Junctions in Brain Barriers During Central Nervous System Inflammation

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Abstract

Homeostasis within the central nervous system (CNS) is a prerequisite to elicit proper neuronal function. The CNS is tightly sealed from the changeable milieu of the blood stream by the blood–brain barrier (BBB) and the blood–cerebrospinal fluid (CSF) barrier (BCSFB). Whereas the BBB is established by specialized endothelial cells of CNS microvessels, the BCSFB is formed by the epithelial cells of the choroid plexus. Both constitute physical barriers by a complex network of tight junctions (TJs) between adjacent cells. During many CNS inflammatory disorders, such as multiple sclerosis, human immunodeficiency virus infection, or Alzheimer's disease, production of pro-inflammatory cytokines, matrix metalloproteases, and reactive oxygen species are responsible for alterations of CNS barriers. Barrier dysfunction can contribute to neurological disorders in a passive way by vascular leakage of blood-borne molecules into the CNS and in an active way by guiding the migration of inflammatory cells into the CNS. Both ways may directly be linked to alterations in molecular composition, function, and dynamics of the TJ proteins. This review summarizes current knowledge on the cellular and molecular aspects of the functional and dysfunctional TJ complexes at the BBB and the BCSFB, with a particular emphasis on CNS inflammation and the role of reactive oxygen species. *Antioxid. Redox Signal*. 15, 1285–1303.

Introduction

Cerebral barriers: blood–brain barrier and blood–cerebrospinal fluid barrier—cellular composition and function

The blood–brain barrier (BBB) and the blood–cerebrospinal fluid (CSF) barrier (BCSFB) protect the central nervous system (CNS) from the changeable milieu of the blood stream to establish CNS homeostasis, which is a prerequisite for proper neuronal function. Whereas the BBB is localized at the level of highly specialized endothelial cells within CNS microvessels, the BCSFB is formed by the epithelial cells of the choroid plexus. BBB endothelial cells establish a physical barrier by an elaborate network of complex tight junctions (TJs) between adjacent endothelial cells combined with a very low pinocytotic activity and a lack of fenestrae thus preventing the paracellular and transcellular diffusion of water-soluble molecules across BBB endothelial cells, respectively (71). At the same time, however, BBB endothelium establishes a metabolic barrier by the expression of a number of permanently active transmembrane transport systems and cytoplasmatic enzymes, which ensure the transport of nutrients from the blood into the CNS and the rapid exclusion of toxic metabolites out of the CNS. In addition, the BBB is protected from oxidative stress by the presence of high levels of antioxidant enzymes such as peroxide detoxifying enzymes, glutathione reductase and peroxidase, which are essential for proper brain functions (92, 115). While the endothelial cells form the BBB proper, it has been well established that interaction with the adjacent cells and the extracellular matrix within the neurovascular unit are a prerequisite for complete barrier function (30). A high number of pericytes embedded into the endothelial basement membrane contribute to proper BBB function of CNS microvascular endothelial cells (79) as do polarized astrocytes, whose endfeet ensheath CNS microvessels. Astrocytic endfeet and the parenchymal basement membrane form the glia limitans (Fig. 1) (1). Thus, endothelial function and vascular morphology make the BBB unique and distinguishable from any other microvessels in the body.

In analogy to the endothelial BBB TJs, the morphological correlate of the BCSFB is found at the level of unique parallel apical TJs between the choroid plexus epithelial cells (23), which inhibit the paracellular diffusion of water-soluble molecules across this epithelial barrier (Fig. 3). The choroid plexus is a villous structure consisting of an extensive network of fenestrated capillaries that are enclosed by a single layer of cuboidal epithelium. It extends from the ventricular surface into the lumen of the ventricles. Its major known function is the secretion of CSF. Therefore, besides their barrier function,

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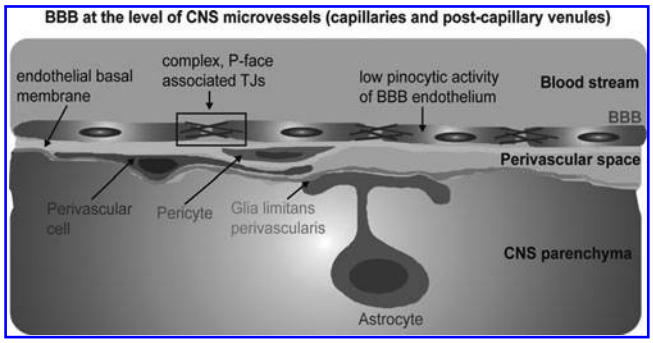

FIG. 1. Schematic view of the cellular and extracellular elements of the blood–brain barrier (BBB). BBB endothelial cells characterized by a low pinocytotic activity and complex tight junctions (TJs) form the barrier proper. Continuous cross-talk with pericytes embedded in the endothelial basement membrane as well as with astrocytes covering the abluminal aspect of the central nervous system (CNS) microvessels is required for BBB maintenance. Astrocytes lay down the parenchymal basement membrane, which together with the astrocytic end-feet establishes the glia limitans, a second barrier to the CNS parenchyma. At the postcapillary level the endothelial and parenchymal basement membrane border a perivascular space, in which rare perivascular antigen-presenting cells can be found. The junctional complex boxed is shown in detail in Figure 2.

choroid plexus epithelial cells are secretory active by producing the CSF. Again, similar to the BBB, the barrier and secretory function of the choroid plexus epithelial cells are maintained by the polarized expression of a number of specific transmembrane transport systems that allow for the directed transport of nutrients into the CSF and the removal of toxic agents out of the CSF. Additionally, high levels of glutathione-S-transferase and catalase activities have been described in choroid plexus epithelial cells ensuring proper brain functions (44, 105).

Here we will summarize our current knowledge on the cellular and molecular basis of the functional and dysfunctional blood–CNS barriers with focus on CNS inflammation including oxidative stress components.

BBB Tight Junctions

Ultrastructural and molecular composition

The junctional complexes between CNS microvascular endothelial cells include adherens junctions (AJs) and TJs (Fig. 2). In contrast to epithelial cells, where TJs are concentrated at the apical side of the intercellular cleft, in endothelial cells AJs and TJs are frequently found to be intermingled along the intercellular cleft (25, 133). Nevertheless, AJs and TJs have distinct functions at the BBB. In endothelial cells outside of the CNS, AJs are shown to initiate endothelial cell-to-cell contacts and promote their maturation and maintenance. However, in the CNS expression of vascular endothelial (VE)-cadherin declines with BBB maturity, suggesting that maintenance of BBB TJs in the adult may not require high amounts of VE-cadherin (11). TJs establish a barrier function by regulating the passage of solutes and ions through the paracellular cleft. Highly complex TJs between adjacent CNS microvascular endothelial cells are therefore primarily responsible for the unique restriction of the paracellular diffusion pathway between the endothelial cells, which establishes unlike in simple TJs, a high electrical resistance of about 1800–2000 $\Omega \cdot cm^2$ across the BBB (14, 21). In addition to the barrier function, TJs establish a fence function by limiting the free movement of lipids and proteins between the apical and the basolateral cell surface, thus establishing BBB endothelial cell polarity.

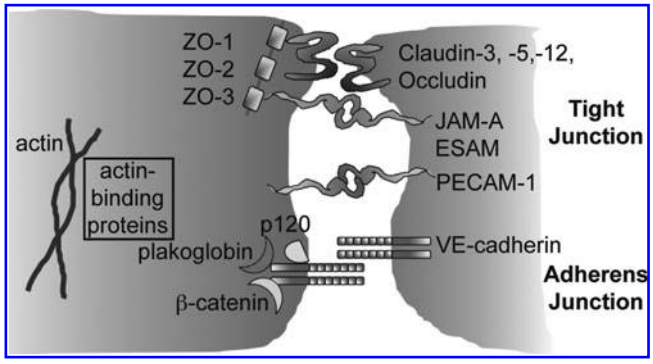

FIG. 2. Schematic view of the BBB junctions. Homophilic binding to the transmembrane protein vascular endothelial (VE)-cadherin establishes cell-to-cell adhesion at the level of the adherens junctions (AJs). p120, *β*-catenin and plakoglobin bind to the cytoplasmic tail of VE-cadherin and by interacting with a large number of actin-binding proteins link the AJ to the cytoskeleton. Occludin and claudins mediate in a homophilic and heterophilic manner the adhesion of adjacent outer membrane leaflets in TJs. PDZ domain containing proteins such as the ZO-proteins provide a scaffold for these transmembrane TJ proteins and link them to the cytoskeleton and to a large number of signaling molecules.

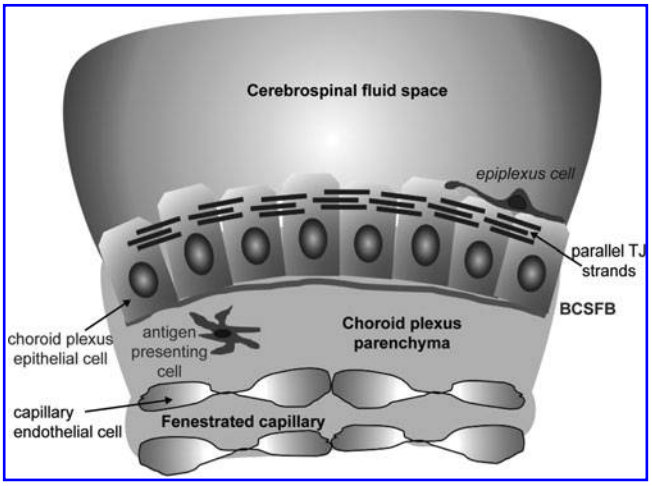

FIG. 3. Schematic view of the blood-cerebrospinal fluid barrier (BCSFB). In the choroid plexus fenestrated capillaries lie within the choroid plexus stroma that is surrounded by choroid plexus epithelial cells establishing the BCSFB. TJs between the choroid plexus epithelial cells are unique with expression of claudin-1 or −3, claudin-2, and −11, with the expression of claudin-11 inducing parallel non anastomosing TJ strands. In analogy to the BBB right behind the barrier rare antigen presenting cells, the epiplexus or Kolmer cells, can be found.

At the ultrastructural level, BBB TJ morphology closely resembles that of epithelial cells rather than that of endothelial cells of other vascular beds. In ultrathin section electron micrographs, BBB TJs appear as a chain of fusion points—kisses—of the outer plasma membrane leaflet of the adjacent CNS endothelial cells (32). Additionally, TJs between the endothelial cells of the brain microvessels have been analyzed in elaborate qualitative freeze-fracture electron microscopy studies. These studies have demonstrated that in mammalian BBB endothelial cells TJs are more complex than TJs in other endothelial cells in the body by establishing a continuous network of parallel and highly interconnected strands that circumscribe the apex of lateral membranes of adjacent endothelial cells (71, 103). In addition to the complexity of TJ strands it was suggested that the association of the TJ particles with the protoplasmic leaflet (P-face) or the exocytoplasmic leaflet of the cell membrane (E-face) is another criterion to correlate morphology and physiology of TJs (163). Further freeze-fracture replica electron microscopy studies have demonstrated that the TJ particles from BBB endothelial cells are preferentially associated with the P-face rather than the E-face (163), thus resembling TJs of epithelial cells. The P-face association was shown to correlate with the sealed barrier function of the BBB endothelium in mammals *in vivo* and *in vitro* (163). The concept of a correlation of P-face-associated TJ particles with BBB function is also consistent with the observation that in TJs of peripheral, nonbarrier forming endothelial cells, P-face-associated TJ particles are rarely found and E-face-associated TJ particles clearly predominate (100, 134). It has been hypothesized that there is an important functional role for the P-face association, and thus presumably the cytoplasmic anchoring of the TJ particles, particularly for BBB function (163). This is further supported by the finding that the transmembrane proteins of the TJs are localized to these particles (40) and that a molecular component, which was identified to associate with the P-face, claudin-3, is predominantly incorporated into BBB endothelial cell TJs (see below).

In both AJs and TJs, the adhesion between adjacent endothelial cells is mediated by transmembrane proteins that promote homophilic and, at least in TJs, probably also heterophilic interactions that establish a pericellular ziplock-like adhesive structure sealing the intercellular cleft. In endothelial cells including those of the BBB, AJs are largely composed of VE-cadherin, an endothelium-specific member of the cadherin family of adhesion proteins (20). With its cytoplasmic domain VE-cadherin binds to several protein partners, including β-catenin and plakoglobin, members of the family of armadillo proteins (156) as well as to p120. Junctional association of these proteins is crucial for the functional state of AJs (26). The association of a large set of actin-binding proteins such as α-catenin, vinculin, and α-actinin to AJs promotes the anchoring of AJs to actin filaments. Anchoring of AJs to the cytoskeleton is thought to be a prerequisite for junctional stabilization, but probably also for dynamic opening and closing of the AJs. Additional signaling components and enzymes associated with AJs and reviewed elsewhere in great detail (26) further contribute to the dynamic regulation of endothelial AJs. In addition to VE-cadherin, expression of the classical type II cadherin–cadherin 10 has been described to be specifically expressed in BBB endothelium in human and mouse brain but not in the fenestrated endothelial cells of the choroid plexus (161).

TJs of all endothelial cells, including those of the BBB, are composed of claudin-5, an endothelial-specific member of the claudin family of transmembrane TJ proteins (97, 107). The claudins represent a gene family of integral membrane TJ proteins with 24 members (42) [for review see Overgaard *et al.* (107a)]. Mammalian claudins range from 22 to 27 kDa and have four transmembrane helices, a short internal amino-terminal sequence (two to six amino acids), two extracellular loops with the first loop being larger than the second and a long variable cytoplasmic tail (Fig. 2). In addition to claudin-5, expression of claudin-3 and claudin-12 mRNA, but not of claudin-1 mRNA, is detectable in BBB endothelium (Lyck *et al.*, unpublished observations from our laboratory). At the protein level, however, only the localization of claudin-3 and claudin-5 to BBB TJs could be successfully demonstrated to date (53, 107, 157, 166) (Figs. 4 and 5). Previous studies have described expression of claudin-1 at the protein level in BBB endothelium (77). Those studies that specifically excluded cross-reactivity of the anti-claudin-1 antibody with claudin-3 failed to detect any expression of claudin-1 in CNS parenchymal microvessels or in primary mouse or human brain endothelial cells (19, 53, 160, 166). Taken together, BBB TJs seem to be specifically assembled by the endothelial cell-specific claudin-5 together with claudin-3 and claudin-12.

Occludin, the first transmembrane component of TJs to be identified (39), localizes to BBB TJ strands [for review see Blasig *et al.* (8a)]. In fact expression levels of occludin have been reported to increase during brain angiogenesis and are significantly higher in TJs of the mature BBB than in TJs of endothelial cells outside the CNS (56, 157). Occludin is an approximately 60-kDa tetraspan membrane protein with two extracellular loops, a short intracellular turn, an N-terminal, and a very long C-terminal cytoplasmic domain (146) and, therefore, at a first glance resembles claudins. Occludin does, however, not share any sequence homologies with the claudins. In immunoreplica electon microscopy anti-occludin antibodies exclusively label TJ strands, indicating that occludin directly incorporates into the TJ strands (128).

In addition, the immunoglobulin (Ig)-supergene family members, junctional adhesion molecule (JAM)-A (91), and endothelial cell-selective adhesion molecule (ESAM) (106) are localized to TJs including those of the BBB (157). JAM-A is a 32-kDa type I transmembrane protein, with two extracellular V-type Ig-domains, a single membrane-spanning domain and a cytoplasmic tail (28) [for review see Bazzoni *et al.* (7a)]. ESAM is a 55-kDa type I transmembrane protein containing a V-type and a C2-type extracellular Ig domain, a single membrane-spanning domain, reminiscent of JAM-A, but displays a much longer 120-amino acid cytoplasmic domain (106). JAM-A has been demonstrated to physically interact with zonula occludens-1 (ZO-1) and cingulin, indicating that JAM-A is a component of the multiprotein complex of TJs (7) [for review see Bazzoni *et al.* (7a)]. As both proteins, JAM-A and ESAM, mediate homophilic interactions *via* their extracellular domains, they may contribute to the ziplock-like adhesive contacts at the BBB TJs (7, 72, 86) (see Tables 1 and 2 for summary).

The integral membrane proteins of the BBB TJs are linked to the endothelial cytoskeleton by TJ-associated cytoplasmic peripheral membrane proteins of the membrane associated with a guanylyl kinase-like domain (MAGUK) family, such as ZO-1, ZO-2, and ZO-3 (147, 162) [for review see

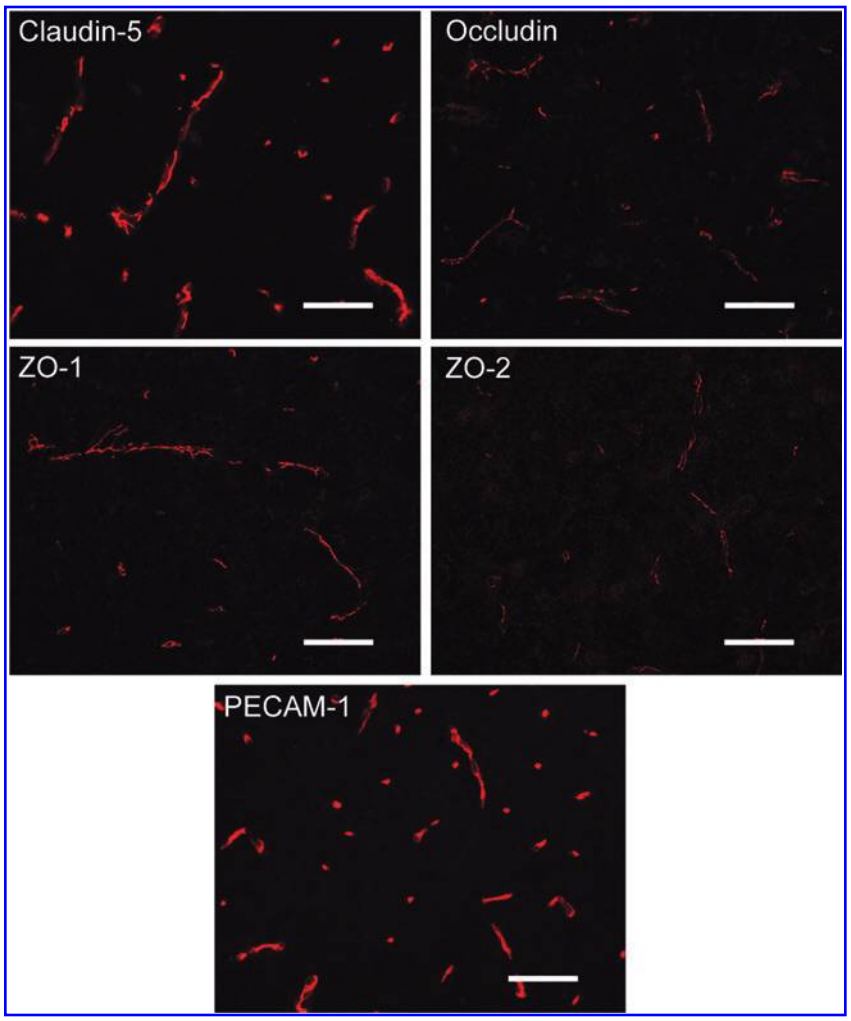

FIG. 4. Expression of junctional proteins in mouse brain parenchymal microvasculature. Female C57BL/6 wild-type mice were anesthetized and perfused with cold PBS through the left heart ventricle. Brains were removed, embedded in OCT™ Compound, and frozen (Sakura Finetek). Sections were cut at 6 μm on a cryostat and air-dried overnight before staining and fixation. Tissue sections were stained with rabbit anti-mouse claudin-5 (from H. Wolburg/H. Kalbacher), rabbit anti-mouse Occludin (71–1500), rabbit anti-mouse ZO-1 (61–7300), rabbit anti-mouse ZO-2 (71–1400) (all from Zymed), and rat anti-mouse platelet endothelial cell adhesion molecule (PECAM-1) (Mec13.3, BD PharMingen). Cyanine Cy3 goat anti-rat and cyanine Cy3 goat anti-rabbit (Jackson Immunoresearch) were used as secondary antibody. Samples were analyzed using a Nikon Eclipse E600 microscope connected to a Nikon Digital Camera DXM1200F with the Nikon NIS-Elements BR3.10 software (Nikon) (scale bars = 50 μm). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

Gonzales-Mariscal *et al.* (47a)]. The ZO proteins share sequence homologies and besides a common GUK domain, three PDZ domains and one SH3 domain. The PDZ domains bind to diverse four amino acid sequences ending in Val. Interestingly, most claudins, with the exception to claudin-12, display a Val in their carboxyl terminus and were in fact shown to specifically bind the PDZ1 domains of the ZO proteins [summarized in Ref. (148)] [for review see Overgaard *et al.* (107a)]. In contrast, the GUK domain of the ZO proteins mediates binding to the carboxyl terminus of occludin [for review see Blasig *et al.* (8a)]. In addition, the SH3-GUK module in association with its flanking regulatory motifs (Unique-5 and Unique-6) was reported to play a predominant role in the scaffolding properties of ZO-1, and more specifically, in the modulation of the binding of occludin to ZO-1, through the Unique-5 domain, and for targeting ZO-1 to the TJs (33). Further, JAM-A but not ESAM was shown to bind to ZO-1 and to other PDZ-containing proteins such as isotype-specific interacting protein (ASIP)/partitioning defective-3 (PAR-3) and afadin-6 (AF-6) (27), whereas ESAM-1 binds to MAGUK inverted protein-1 (MAGI-1) (67, 158) [for review see Bazzoni *et al.* (7a)]. The large number of PDZ-containing proteins at the BBB TJs is therefore thought to provide a scaffold of adaptor proteins, allowing to recruit cytoskeletal and signaling molecules to the TJs. Finally, outside of the organized AJs and TJs, the Ig-superfamily member platelet endothelial cell adhesion molecule-1 (PECAM-1) is localized to endothelial cell-to-cell junctions, including the BBB (51) (see Tables 1 and 2 for summary).

Function of BBB TJ proteins

Claudins have been demonstrated to be sufficient for the formation of TJ strands (97). Upon transfection into fibroblasts the endothelial cell-specific claudin-5 induced E-face-associated TJs (99), whereas transfection of claudin-3, which is also expressed in BBB TJs, induced P-face-associated TJs (41). This observation shows that different claudins induce structurally different TJ strands and suggests that at the BBB claudin-3 and claudin-5 may be specifically responsible for the presence of P-face *versus* E-face-associated TJs, respectively. The specific expression of claudin-3 in TJs of CNS, but not other endothelial cells, suggests a brain-specific function. This is further supported by the recent observations that Wnt/β-catenin signaling is specifically required for brain angiogenesis and induction of BBB characteristics in endothelial cells, including the expression of claudin-3 (78). Despite this evidence, the specific function of claudin-3 in BBB TJs remains unknown to date. In contrast, deletion of claudin-5 in mice allowed to define that claudin-5 is not required for the development and

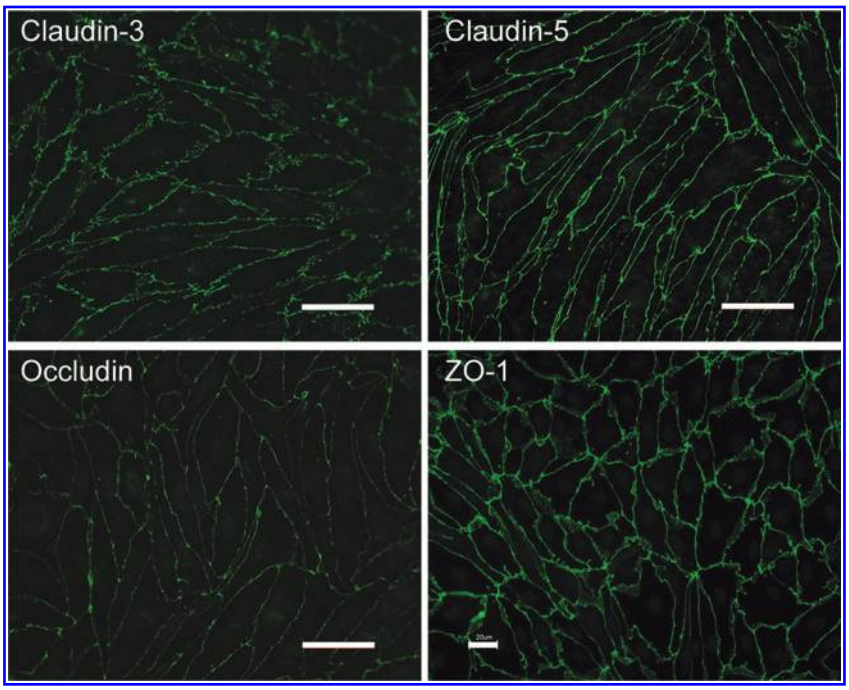

FIG. 5. Expression and localization of TJs and TJ-associated proteins in primary mouse brain microvascular endothelial cells (pMBMECs). Confluent pMBMEC monolayers were prepared from 6-week-old C57BL/6 wild-type mice as described by Coisne *et al.* (19). Cells were fixed with ice-cold methanol and stained for rabbit anti-mouse claudin-3 (34–1700), rabbit anti-mouse claudin-5 (34–1600), rabbit anti-mouse Occludin (71–1500), and rabbit anti-mouse ZO-1 (61–7300) (all from Invitrogen AG, former Zymed). Alexa Fluor 488 goat anti-rabbit (Molecular Probes) was used as secondary antibody. Samples were analyzed using a Nikon Eclipse E600 microscope connected to a Nikon Digital Camera DXM1200F with the Nikon NIS-Elements BR3.10 software (Nikon) (scale bars = 50 μm for claudin-3, claudin-5 and occludin, scale bars = 20 μm for ZO-1). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

morphology of blood vessels, but rather establishes a size selective barrier of BBB TJs for molecules smaller than 800 Da (107) [for review see Overgaard *et al.* (107a)]. On the molecular level little is known on how claudin–claudin interactions may establish such a size-selective barrier function at BBB TJs. Both extracellular loops and possibly the transmembrane domains of claudins have been suggested to be involved in adhesive claudin–claudin interactions and assembly of TJ strands (73). Recently, it could be demonstrated that a highly conserved aromatic binding core within the second extracellular loop of claudins mediates homophilic or heterophilic adhesive interactions of claudins in trans (114). Thus, the combined homophilic and heterophilic interactions between claudin-3 and claudin-5, and probably also claudin-12, at the BBB seem to be responsible for the unique barrier function of the BBB TJs (73).

Mice carrying a null mutation in the occludin gene are viable and develop morphologically normal TJs in different tissues, including the BBB (129). This demonstrates that occludin is not essential for TJ formation [for review see Blasig *et al.* (8a)]. However, introduction of either C-terminally or N-terminally truncated occludin mutants into epithelial cells resulted in disturbance of TJ integrity, proving that occludin is involved in TJ functions (6). Expression of occludin splice variants has been reported and these may regulate TJ function in distinct manners [summarized in Ref. (35)]. To what degree this applies to BBB TJs function *in vivo* will need to be determined. In any case, the specifically high expression levels of occludin in BBB TJs suggest a prominent regulatory function of occludin in these TJs. Interestingly, recruitment of occludin to cell junctions can be achieved by JAM-A probably *via* interaction with ZO-1 (7) [for review see Gonzales-Mariscal *et al.* (47a)].

In epithelium, JAM-A interacting with PAR-3 could directly adjust its subcellular localization, suggesting a role for JAM-A in regulating the formation of TJs and cell polarity in epithelial cells (61, 125, 142). At the blood–retinal barrier, which shares some common barrier features with the BBB, the monoclonal antibody blockade of JAM-A resulted in an increased permeability of the rabbit corneal endothelium *in vitro*. This suggests a direct implication of JAM-A in the maintenance of TJ integrity in this barrier endothelium (85) [for review see Frey and Antonetti (37a) and Bazzoni (7a)]. The precise roles of JAM-A and ESAM in BBB TJs remain, however, to be determined as neither JAM-A nor ESAM-deficient mice display any overt BBB phenotype (16, 159) (see Tables 1 and 2 for summary).

Dynamics of TJs

Beginning to understand the individual functions of the specific TJ proteins allows to better understand that BBB TJs are dynamic structures, which are sensitive to ambient factors. Already under physiological conditions, the diapedesis of immune cells during immunosurveillance or apoptosis of barrier cells during barrier renewal require dynamic remodeling of TJs. In this context it has been shown that the function of occludin depends on its phosphorylation stage, which can be influenced by small GTPases, Rho kinases, oxidative stress, and angiogenic factors (35, 141, 169) [for review see Lehner *et al.* (76a)]. Oxidative stress responses are mediated by various reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), nitric oxide (NO), and peroxynitrite. H_2O_2-induced *in vitro* BBB permeability was shown to correlate with higher expression levels of occludin but not ZO-1 and their rearrangement at the TJs (75). Altered occludin phosphorylation has further been shown following low-dose exposure of *in vitro* BBB models to H_2O_2 (80). Phosphorylation and ubiquitination of occludin upon vascular endothelial growth factor stimulation has recently been described to promote internalization of TJ proteins at the blood–retinal barrier endothelium, leading to increased permeability (101). In addition to alterations of the functional state of individual TJ proteins, their expression levels in brain endothelium can be influenced by growth factors [for review see

Table 1. Comparative Expression and Known Functions of Claudins at the Blood–Brain Barrier and Blood–Cerebrospinal Fluid Barrier

	BBB		*BCSFB*	
	Expression	*Functions*	*Expression*	*Functions*
Claudin-1	Protein detected when antibody cross-reactivity with claudin-3 was not addressed (21) Protein not detected when antibody cross-reactivity with claudin-3 was excluded (15)	n.a.	Protein detected but antibody cross-reactivity with claudin-3 was not excluded (44, 46) TJ strand localization (11, 25)	Unknown at BCSFB Induces P-face associated TJs in mouse L fibroblasts (11) Establishes size-selective barrier for small water-soluble tracers (600 Da) in epidermis TJs (9)
Claudin-2	Not expressed (mRNA, protein) (44, 46)	n.a.	Expressed (mRNA, protein) TJ strand localization (11, 25)	Unknown at BCSFB but forms cation-channels (29), and paracellular water channels in MDCK cells (32)
Claudin-3	Expressed in brain endothelial cells at mRNA and protein level (4, 13, 15, 42, 45) Expression regulated by canonical wnt-signaling (20) Specifically localized within TJ strands (25)	Induces P-face associated TJs in mouse L fibroblasts (12) Loss of claudin-3 at the BBB is associated with neurological disorders (45) but specific function at BBB is unknown	Positive immunostaining described (44, 46) mRNA expression not investigated	Unknown at BCSFB–see BBB
Claudin-5	Expressed at the mRNA and protein level (13, 31, 35) Specific TJ strand localization (25)	Induces E-face associated TJs in mouse L-fibroblasts (27) Establishes size-selective barrier at the level of the BBB ($M_w < 800$ Da) (31) Lack of claudin-5 at the BBB is lethal (31)	Not expressed (44) (Fig. 5)	n.a.
Claudin-11	Not expressed	n.a.	Expressed at the mRNA and protein level TJ strand localization (14, 44, 46)	Forms unique parallel TJ strands in mouse L fibroblasts (26) Claudin-11$^{-/-}$ mice are viable but lack TJs in CNS myelin and Sertoli cells Claudin$^{-/-}$ TJs in BCSFB have not been investigated (14)
Claudin-12	Expressed at mRNA level Protein expression not described (31)	Unknown	Not expressed	n.a.

n.a., not applicable; BCSFB, blood–cerebrospinal fluid barrier; TJ, tight junction; MDCK, Madin-Darby canine kidney cells; BBB, blood–brain barrier; CNS, central nervous system.

Table 2. Comparative Expression and Known Functions of Occludin, Zonula Occludens-1, Junctional Adhesion Molecule-A, and Endothelial Cell-Selective Adhesion Molecule at the Blood–Brain Barrier and Blood–Cerebrospinal Fluid Barrier

	BBB		*BCSFB*	
	Expression	*Functions*	*Expression*	*Functions*
Occludin	Highly expressed at mRNA and protein level (16, 40) TJ strand localization (10, 33, 37)	Occludin is not necessary for TJ formation as occludin$^{-/-}$ mice are viable and have an intact BBB (34) Function regulated by phosphorylation/ ubiquitination (1, 17, 28, 36, 47) Tightens and regulates TJs (1, 8) Localizes to cell junction through JAM-A *via* ZO-1 in CHO cells (2)	Expressed (mRNA and protein level) TJ strand localization (10, 33, 37, 46)	Occludin$^{-/-}$ mice have an apparent intact BCSFB (34) No specific functions for BCSFB described See BBB
ZO-1	Expressed (mRNA, protein) TJ-associated cytoplasmic protein Binds to claudins (through PDZ domain) in L fibroblasts, to occludin (through SH3-GUK) in MDCK, to JAM-A in CHO cells (2, 5, 7, 39)	Intracellular scaffolding protein of TJs Links transmembrane TJ proteins to cytoskeleton Recruits signaling molecules to TJs (38, 43)	Expressed at mRNA and protein level TJ-associated intracellular scaffolding protein Binds to claudins (via PDZ domain) and to occludin and JAM-A (via SH3-GUK) domain (5, 7, 39)	Functions similar to BBB
JAM-A	Expressed at mRNA and protein level Localizes outside of TJ strands as detected by freeze-fracture microscopy (6, 24, 40)	Unknown for BBB as JAM-A$^{-/-}$ mice have an intact BBB (3) Interacts with ZO-1, ASIP/ PAR-3 and AF-6 in bEnd.3 cells (5) and with cingulin and ZO-1 in CHO cells (2) May contribute to barrier function by adhesive homophilic interactions at cell-cell junctions (2, 19, 22, 23)	Expressed at mRNA and protein level (24, 40)	Not specifically known for BCSFB See BBB
ESAM	Expressed at mRNA and protein level Localized outside TJ strands as detected by freeze-fracture microscopy (30, 40)	Not specifically known for BBB Contributes to barrier function by adhesive homophilic interactions at cell–cell junctions in CHO cells (30) Interacts with MAGI-1 in CHO cells (18,41)	Not expressed (44) (Fig. 5)	n.a.

AF-6, afadin; ASIP, isotype-specific interacting protein; ESAM, endothelial cell-selective adhesion molecule; GUK, guanylyl kinase-like domain; JAM, junctional adhesion molecule; PAR-3, partitioning defective-3; ZO, zonula occludens; CHO: Chinese hamster ovary, n.a.: not applicable.

Frey and Antonetti (37a), in this review at the blood–retinal barrier]. Pericyte derived angiopoietin-1 was shown by inducing the phosphorylation of its Tie-2 receptor to trigger a downstream signaling cascade, finally leading to increased expression of occludin in immortalized rat brain endothelial cells (59). Taken together, BBB TJs are highly dynamic molecular complexes that will change in response to neuroinflammatory insults generating pro-inflammatory cytokines, chemokines, matrix metalloproteases (MMPs), and ROS.

BCSFB Tight Junctions

Ultrastructural and molecular composition

The TJs between the choroid plexus epithelial cells form the morphological correlate of the BCSFB. Freeze-fracture

electron microscopy studies have shown that the particles of mouse choroid plexus TJs strongly associated with the P-face (167) and that choroid plexus epithelial cell TJs form unusual TJs composed of parallel in contrast to anastomosing strands of particles [summarized in Refs. (31, 164)]. Therefore, choroid plexus epithelial TJs resemble TJs between Sertoli cells in the testis building the blood–testis barrier and TJs between the myelin sheaths of oligodendrocytes. These TJs are exclusively constituted of claudin-11 previously called oligodendrocyte-specific protein (OSP) (50, 98). In choroid plexus epithelial TJs, presence of claudin-11 TJs is accompanied by the localization of claudin-3, claudin-2, occludin, and ZO-1 and maybe of claudin-1 (164, 167) (Fig. 6). As already described above, many studies describing expression of claudin-1 in the CNS, including those performed by us (167) did not exclude cross-reactivity of the respective antibodies with claudin-3. Therefore, presence of claudin-1 *versus* claudin-3 in BCSFB TJs still needs further clarification (see Tables 1 and 2 for summary).

Functions of BCSFB TJ proteins

The specific functions of the individual TJ proteins expressed in choroid plexus epithelial cells for BCSFB integrity has not been addressed in detail to date. Mice lacking claudin-11 develop no CNS myelin and Sertoli cell TJ strands leading to neurological and reproductive deficits, respectively (49). Unfortunately, the BCSFB TJs have not been analyzed in detail in claudin-11 null mice. Claudin-2 has been classified as cation-channel forming TJ protein in a number of studies (102). Transfection of claudin-2 into epithelial cells demonstrated that claudin-2 forms paracellular water channels (126), suggesting that expression of claudin-2 in BCSFB TJs regulates paracellular transport of water across the CSF producing choroid plexus epithelial cells. Interestingly, claudin-1-deficient mice were shown to die within 1 day of birth due to excessive dehydration from the skin (38). Epidermal TJs lacking claudin-1 display a leakiness for small ($\sim$600 Da) water-soluble tracers, demonstrating that claudin-1 establishes a specific diffusion barrier for these molecules. Therefore, if claudin-1 is present in BCSFB TJs, it is tempting to speculate that a balanced function of claudin-1 and claudin-2 may be relevant for paracellular balancing of the functions of BCSFB and CSF production at the level of choroid plexus epithelial cells. A function for claudin-3 still needs to be determined (see Tables 1 and 2 for summary).

The BBB TJs in the Inflamed CNS

The endothelial cells of the BBB and probably also the epithelial cells of the BCSFB are involved in the pathogenesis

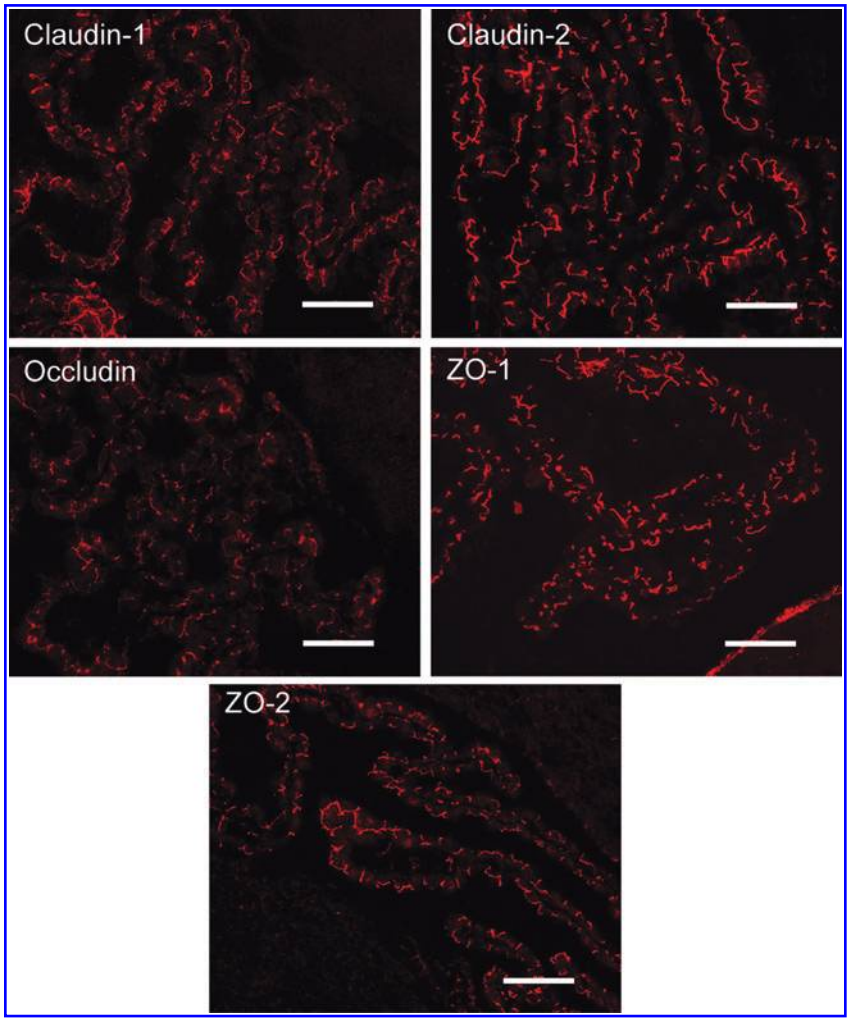

FIG. 6. Expression and localization of junctional proteins in mouse choroid plexus epithelium. Female C57BL/6 wild-type mice were anesthetized and perfused with cold PBS through the left heart ventricle. Brains were removed, embedded in OCT™ Compound, and frozen (Sakura Finetek). Sections were cut at 6 μm on a cryostat and air-dried overnight before staining and fixation. Tissue sections were stained with rabbit anti-mouse claudin-1 (51–9000), rabbit anti-mouse claudin-2 (51–6100), rabbit anti-mouse Occludin (71–1500), rabbit anti-mouse ZO-1 (61–7300), and rabbit anti-mouse ZO-2 (71–1400) (all from Zymed). Cyanine Cy3 goat anti-rat and cyanine Cy3 goat anti-rabbit (Jackson Immunoresearch) were used as secondary antibody. Samples were analyzed using a Nikon Eclipse E600 microscope connected to a Nikon Digital Camera DXM1200F with the Nikon NIS-Elements BR3.10 software (Nikon) (scale bars = 50 μm). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

of a wide range of inflammatory CNS disorders, including multiple sclerosis (MS) or its animal model experimental autoimmune encephalomyelitis (EAE), infectious diseases (human immunodeficiency virus [HIV]), as well as neurodegenerative diseases (Alzheimer's disease [AD]). In this context, the contribution of both CNS barriers can be considered twofold, a passive role characterized by vascular leakage of blood-borne molecules into the CNS and an active role by guiding inflammatory cell migration into the CNS. Both roles may directly be linked to alterations in the molecular composition of the TJ complexes or functional states of TJ proteins based on their phosphorylation state. Many studies focused on investigating junctional alterations of the BBB in the context of neuroinflammatory diseases. In general, most studies rather focused on the expression of occludin and ZO-1, although only selected studies have investigated the expression pattern of the complete array of TJ proteins known to be expressed at the BBB to date. Here, we summarize our present knowledge on BBB TJ alterations as described in MS, HIV, and AD as examples for inflammatory demyelinating diseases of the CNS, infectious diseases, and neurodegenerative diseases, respectively. These diseases have been associated with increased free radical production and chronic oxidative stress (108, 118, 119), suggesting a crucial role of ROS mediators in triggering endothelial dysfunctions that accompany neuroinflammatory disorders. Vascular leakage of blood-borne molecules and inflammatory cell migration across the BBB represent two major phenomena following BBB disruption. Both may directly be linked to alterations in the molecular composition or dynamics of the TJ proteins. However, while TJ alteration can easily be associated with increased paracellular flux of solutes across the BBB (independently of increased transcellular fluid-phase processes), the induction of immune cell infiltration into the CNS represents a complex phenomenon that implies several features. It necessitates the contribution of various inflammatory mediators present in the CNS environment that directly affect the motility of immune cells toward the site of inflammation (by chemoattractants) and then within the CNS (by chemoattractants and MMPs) as well as the endothelial integrity *per se* (by pro-inflammatory cytokines and oxidative stress) [for review see Lehner *et al.* (76a)]. In addition, altered TJ integrity may facilitate immune cell migration across the BBB, but not necessarily, as immune cells may rather use the paracellular route to cross the BBB (15, 165).

Multiple sclerosis

Ultrastructural and molecular alterations. MS is an inflammatory demyelinating disease of the CNS with an assumed autoimmune cause. EAE is an animal model for the inflammatory phase of MS and reflects various aspects of MS, including reversibility of the neurological disabilities, inflammatory CNS lesion, and BBB disruption (66, 90). In both MS and EAE, inflammatory cells composed mostly of T cells and macrophages accumulate within the CNS parenchyma but also within perivascular spaces. CNS inflammation associated with MS and EAE has been described to involve a wide range of cytokines, chemokines, MMPs, and ROS produced by infiltrating immune cells and also CNS resident cells (46, 93). Besides the infiltrating immune cells, pro-inflammatory and oxidative stress mediators are considered to have direct impacts on BBB function in neuroinflammation. RNA profiling in MS brain tissues, using cDNA microarrays, revealed differential gene expression in normal appearing white matter (NAWM) of MS *versus* healthy tissues, indicating that beside inflammatory CNS lesion, inflammation, and degeneration take place throughout the MS brains (52, 68). Several alterations observed in MS tissues were characteristic of neuroprotective mechanisms against oxidative stress, identical to the ones induced following hypoxic damages, including upregulation of hypoxia-inducible factor-1α and its downstream partners (52, 68). In addition, higher levels of antioxidants such as peroxiredoxin V were reported in NAWM in acute and chronic lesions from MS patients and higher levels of superoxide dismutase (SOD) 1/2, and heme oxygenase 1 were found in active demyelinating MS lesions (58, 153). Peroxynitrites were found to be produced in astrocytes in acute and chronic MS lesions as well as in early stage of EAE, where it was associated with disease activity (22, 81, 152). Immunostainings of brain tissue biopsies from patients with various forms of MS showed in 20%–40% of CNS microvessel abnormalities in immunostaining for ZO-1 and occludin, characterized as discontinuous staining patterns in contrast to the continuous staining observed in normal brain tissue (76, 116). Interestingly, discontinuous immunostaining was observed in both active and inactive MS plaques, but also, however, to a lower extent in the NAWM, indicating that lesion activity may not be a prerequisite for TJ alterations in MS (76, 116). Furthermore, changes observed for ZO-1 and occludin immunostaining in MS brains were found to be independent of the vascular segment, with TJ abnormalities observed at the capillary level as well as in larger diameter microvessels to the same extent (69). Similarly, disrupted immunostaining for JAM-A has been described in CNS microvessels in brain biopsy tissues from MS patients (109). In EAE, molecular alterations of BBB TJs observed seem to be in apparent contrast to the observations made in MS tissues. Here, immunofluorescence stainings demonstrated a selective loss of claudin-3 immunostaining specifically at those postcapillary venules, which were surrounded by inflammatory cuffs, but not in inflamed CNS microvessels, where no perivascular inflammatory cells were present (166). The immunofluorescence staining for occludin, ZO-1, and claudin-5 in inflamed CNS microvessels during EAE was found to be unchanged, irrespective of the presence or absence of surrounding inflammatory cuffs (166). This apparent discrepancy to the observations made in MS tissues may be explained by different interpretations of the respective findings. Ultrastructural studies performed on brain tissues from EAE mice demonstrated dramatic alterations in BBB TJ morphology with an extended upfolding of membranes at the level of the cell–cell contacts (165). These upfolded junctional membranes might lead to the appearance of an interrupted immunostaining for junctional proteins at the light microscopy level. Also, the presence of inflammatory cells localized within the vascular wall during their diapedesis across the BBB may lead to apparently interrupted junctional staining patterns. Functional changes of BBB TJs might additionally be a consequence of the altered functional state of individual TJ proteins, induced by phosphorylation or dephosphorylation. This has been specifically demonstrated for occludin, which was found to be dephosphorylated in inflamed spinal cord microvessels during EAE in a rat model (95). In addition,

infiltrating immune cells and their cytokine production could alter BBB tightness. TH17 cells were reported to impair BBB integrity through the combined production of interleukin (IL)-17A and IL-22 that downregulated occludin and to a lesser extend ZO-1 (65). Recently, the mechanism underlying BBB alteration through IL-17A was associated with the production of ROS by NADPH oxidase and xanthine-oxidase in bEnd.3 cells (60). Taken together, molecular alterations of BBB TJs occur in MS/EAE in response to a wide range of inflammatory and oxidative stress mediators; however, their precise correlation with leukocyte infiltration and BBB leakiness needs to be further investigated.

Role of TJs in BBB leakiness and leukocyte trafficking across the BBB. BBB leakiness was demonstrated by vascular leakage of antemortem fibrinogen in association with TJ alterations in active and inactive lesions in autopsy brain tissues from patients with primary and secondary progressive MS. This supports the notion that BBB leakiness contributes to the pathogenesis of the disease (76, 116).

In the early phase of MS lesion formation, ROS are crucial in mediating the infiltration of monocytes into the CNS and in impairing BBB integrity (151) but also contribute to the persistence of MS lesion in favoring the phagocytosis and degradation of the myelin by macrophages as well as by inducing axonal damage (54, 150). *In vitro*, the adhesion of monocytes to the BBB endothelium was observed to induce the production of ROS, which increased BBB permeability. This effect was attenuated by pretreatment of the endothelium with the ROS scavenger, α-lipoic acid (131). This antioxidant has already demonstrated its therapeutic efficacy *in vivo* in preventing the development of clinical signs of EAE as well as decreasing the recruitment of monocytes and T cells into the CNS (89, 96, 131). Another antioxidant, S-nitrosoglutathione, was also effective in reducing cellular infiltration into the CNS and in improving EAE symptoms in the Lewis rat model (117). S-nitrosoglutathione downregulated adhesion molecule expression on the BBB *in vitro*, through the S-nitrosylation of p65, which subsequently inhibited nuclear factor-κB (NF-κB) activation in endothelial cells (117).

In a Theiler's virus-induced model of MS, CD8 T cells migrating into the CNS across the BBB were also shown to directly cause increased vascular permeability through a nonapoptotic perforin-dependent mechanism (140), underlining the notion that diapedesis of immune cells across the BBB may cause molecular changes in BBB TJs. However, ultrastructural studies of brain tissues from mice with EAE demonstrated that leakiness of the BBB, as identified by the perivascular deposition of fibrin, is observed in the presence and in the absence of perivascular inflammatory cells, demonstrating that leakiness of the BBB was not strictly dependent on prior immune cell diapedesis and the presence of perivascular inflammatory cells. Therefore, soluble mediators like cytokines, chemokines, ROS, and growth factors may rather be responsible for BBB dysfunction (165). As serial section conventional electron microscopy in the same EAE study demonstrated that mononuclear cells traverse cerebral microvessels by a transcellular pathway leaving the BBB TJs morphologically intact, it is tempting to speculate that a strict correlation of altered TJ integrity and the presence of cellular infiltrates is only observed with specific immune cell populations traversing the BBB *via* a paracellular route through the endothelial TJs. Whether different immune cells traverse the BBB *via* a transcellular or paracellular pathway and how this may affect BBB TJ integrity and further immune cell recruitment into the CNS is still a matter of debate and may depend on the CNS inflammatory context. Studying EAE in PECAM-1-deficient mice has shown that impaired integrity of BBB cell-to-cell contacts in the absence of PECAM-1 leads to increased inflammatory cell recruitment into the CNS and increased severity of EAE (51), suggesting that altered vascular permeability at the level of endothelial junctions favors paracellular immune cell diapedesis.

Human immunodeficiency virus

Ultrastructural and molecular alterations. AIDS patients can develop neurological disorders, such as HIV-1-associated encephalitis (HIVE) or dementia, when HIV enters the CNS (123). Immunohistochemical studies on brain tissue of HIVE also demonstrated a fragmentation or even absence of immunoreactivity for occludin and ZO-1 within vessels from subcortical white matter, basal ganglia, and, to a lesser extent, cortical gray matter (24) [for review see Gonzales-Mariscal *et al.* (47a)]. These alterations were found to be tightly associated with the accumulation of activated, HIV-1-infected brain macrophages and fibrinogen leakage, correlating TJ alterations with the presence of inflammatory cells and BBB leakiness. Similar observations were made in the brains of patients with (HIV)-1-associated dementia, where disrupted immunoreactivity for occludin and ZO-1 was observed in CNS microvessels surrounded with CD68-positive macrophages (10), further supporting the notion that HIV-infected monocytes may directly affect the expression and function of TJ proteins by producing inflammatory mediators, including cytokines, ROS, and metalloproteases (111). Indeed, HIV-1 infection has been shown to be accompanied by endothelial dysfunction resulting from an overproduction of free radicals that cause chronic oxidative stress (70, 108). HIV-1-positive patients have been reported to exhibit higher plasma levels of hydroperoxides compared with noninfected individuals, as an indicator of enhanced free radical production and lipid peroxidation (34), and lower levels of circulating antioxidants such as vitamin C, cysteine, and glutathione (13, 34, 113, 137). Infected monocytes or microglial cells can release viral proteins such as gp120, which was shown to directly alter the permeability of the BBB *in vivo* (17) and the integrity of the TJs as shown in *in vitro* models of the BBB by triggering proteasome-dependent degradation of ZO-1 and ZO-2 and loss of occludin (63, 104). Interestingly, whereas expression of claudins remains unaffected by gp120, exposure of brain microvascular endothelial cells *in vitro* to Tat, the principal transactivator for HIV-1 replication actively secreted by infected cells (130) leads to decreased protein expression of claudin-1, claudin-5, and ZO-2, whereas levels of ZO-1 and occludin remained unchanged (3). Tat-induced alterations in the expression of TJ proteins in CNS microvessels were confirmed *in vivo*, where Tat injection into the hippocampus of mice resulted in decreased expression of claudin-5 (3). This observation is, however, in apparent contrast to the studies by Pu and colleagues observing the decreased expression of occludin and ZO-1 and upregulated expression of COX-2 following intravenous exposure of Tat in mice (120). ZO-1 alteration was linked to the modification of the redox

signaling pathway of extracellular signal-regulated kinase (ERK1/2) upon intra-hippocampal injection of Tat in mice, as the pretreatment with the antioxidant N-acetylcysteine, a precursor of glutathione, significantly reduced the alteration of ZO-1 and the activation of ERK1/2 (121). The Tat-induced decrease in claudin-5 expression was correlated with the activation of multiple redox-regulated signaling pathways, including AKT/PI3K/NF-κB as well as Ras/ERK1/2 (4). Additionally, brain microvascular endothelial cells exposed to increasing doses of Tat *in vitro* resulted in an increment of the cellular oxidative stress and the activation of redox-responsive transcription factors such as the NF-κB and the activator protein-1 (AP-1) and a decrease in GSH levels (145). In addition, HIV gp120-induced leakiness of the BBB has been associated with oxidative stress in rats in response to an increased production of MMP-2 and MMP-9, following injection of gp120 into the caudate-putamen (82). Increment of lipid peroxidation in brain vascular endothelium and in neurons has been reported, whereas prior administration of antioxidants, such as glutathione peroxidase or Cu/Zn SOD, was protective against gp120-induced BBB leakiness (82, 83).

Role of TJs in BBB leakiness and leukocyte trafficking across the BBB. Studies investigating the diapedesis of HIV-1-infected monocytes across a human *in vitro* BBB model have shown increased Rho kinase mediated-phosphorylation of occludin and claudin-5, linking immune cell diapedesis to TJ alterations (112). However, thus, soluble mediators may directly influence the molecular composition of BBB TJs and subsequently facilitate paracellular inflammatory cell diapedesis and contribute to chronic pathology. This is supported by the observation that injection of Tat-protein into the hippocampus of mice induced expression of inflammatory mediators (monocyte chemoattractant protein-1 [MCP-1], tumor necrosis factor-α [TNF-α]) and of adhesion molecules (vascular cell adhesion molecule-1 [VCAM-1] and intercellular adhesion molecule-1 [ICAM-1]) (122). These alterations were associated with a marked infiltration of monocytes into brain tissue of Tat-treated mice. In addition, cellular exposure to Tat was demonstrated to activate BMECs and induce expression of E-selectin, which can facilitate leukocyte interaction with the BBB (57). Administration of Tat into the hippocampus of mice was found to induce endothelial expression of MCP-1, a chemokine expression of which is dependent on the redox-responsive transcription factors NF-κB and AP-1 (145). Similarly, intra-hippocampal injection of Tat in mice was reported to result in the redox-mediated alteration of expression of ZO-1, associated with the accumulation of inflammatory cells in the brain (121). These data indicate that Tat can induce redox-related inflammatory responses in the brain that may directly lead to disruption of the BBB in HIV-infected patients.

Alzheimer's disease

Ultrastructural and molecular alterations. Dysfunction of the BBB has also been suggested to be critically involved in AD and cerebrovascular dysfunction was suggested to precede cognitive decline and onset of neurodegenerative changes in AD and AD models (8). In AD, β-amyloid (Aβ) deposition within the CNS parenchyma activates microglial cells and astrocytes to produce proinflammatory mediators such as IL-1 β, TNF-α, and ROS. Both proinflammatory and oxidative stress mediators may cause alterations in BBB function. In addition, circulating Aβ aggregates may alter BBB integrity and contribute to the neuropathological sequelae of AD. Ultrastructural analysis of CNS microvessels during AD demonstrated decreased mitochondrial content and increased pinocytotic vesicles within the brain endothelial cells (18). An accumulation of collagen in the vascular basement membrane was a further indication of BBB dysfunction. However, major alterations in BBB TJs were not observed in this study. Reduced immunostaining of the endothelial marker CD34 and the junctional molecule PECAM-1 was, however, observed in AD brains, further supporting that there is degeneration of the endothelium during the disease progression (62). To what degree molecular alterations of BBB TJs develop in AD *in vivo* remains to be investigated. *In vitro*, slightly enhanced (possibly pathological) concentrations of Aβ peptides were reported to increase paracellular permeability of bovine brain capillary endothelial cell monolayers (139). Aβ-initiated endothelial signaling events mediated through JNK and p38MAPK, which are downstream partners of the signaling transduction pathway of ROS, have been demonstrated to trigger increased permeability associated with a decrease in occludin mRNA and protein expression in the human brain endothelial cell line hCMEC/D3 (144). Interestingly, expression of claudin-5 and ZO-1 remained unaffected pointing out that changes in expression levels of individual TJ proteins at the BBB are accompanied with alterations in barrier function. Oxidative stress has been directly implicated in the pathogenesis of AD (88, 119), as demonstrated by increased levels of lipid peroxidation, protein oxidation, and advanced glycosylation end products found in some areas of postmortem brain biopsies (55, 84, 87), accompanied by changes in antioxidant activities of catalase, SOD, gluthatione peroxidase and gluthatione reductase (87, 170). Aβ protein has been reported to exhibit its neurotoxic effects through the release of free radicals *in vitro* and *in vivo* in a transgenic mouse model of AD (110). Therefore, the oxidative stress response may directly contribute to BBB alteration observed in AD.

Taken together, these findings suggest that diverse neurological disorders are associated with alterations in the molecular architecture of TJ complexes, as the result of the action of both pro-inflammatory and oxidative stress compounds, and can contribute to disease pathogenesis.

Role of TJs in BBB leakiness and leukocyte trafficking across the BBB. 1–40 amino acid Aβ peptide (Aβ_{1-40}) represents the major form of circulating Aβ that may interact with the BBB endothelium (127, 171) and be responsible for the cerebrovascular alteration, possibly through the production of ROS in BBB endothelial cells (110), in parallel to Aβ depositions observed in the CNS parenchyma during AD (43, 149). Therefore, various *in vitro* studies have focused on evaluating the effect of such Aβ peptide on the BBB integrity, rather than the effect of proinflammatory mediators and ROS produced by activated microglial cells and astrocytes following Aβ deposition within the CNS parenchyma that represent another cause for the alterations in BBB function. The exposure of human brain microvascular endothelial cells to Aβ_{1-40} aggregates was shown to decrease transendothelial electrical resistance across the endothelial monolayer and to enhance adhesion and subsequent transmigration of monocytes across the Aβ_{1-40} treated BBB endothelium (48).

Diapedesis of monocytes across the *in vitro* BBB model was found to be further enhanced when Aβ_{1-40} was added to the basolateral side, suggesting that Aβ_{1-40} aggregates initiate endothelial signaling processes enhancing monocyte diapedesis across the BBB. A role for the putative Aβ receptor, receptor for advanced glycation end products, and for PECAM-1 has been proposed in this process (45). The direct involvement of oxidative stress in the recruitment of infiltrating immune cells was reported in AD brains, where larger areas of COX-2- and inducible NO synthase-positive macrophage infiltration were observed compared to healthy control brains (36).

In these neuroinflammatory disorders, the disruption of TJ proteins is quite exclusively associated with increased permeability to blood-borne molecules. However, whether this phenomenon is or is not associated with immune cell migration across the BBB is more complex, as in the context of MS/EAE morphologically intact TJs were seen in close vicinity of immune cells penetrating the BBB *via* a transcellular route (165). Recruitment of specific immune cell populations across the BBB greatly depends on the specific inflammatory stimulus or ROS, which regulate the expression of adhesion molecules on the BBB that govern immune cell trafficking into the CNS. Additionally, the maturation stage of the BBB may have an important impact on the role of TJ integrity in BBB function and neuroinflammation. Injection of IL-1 into the striatum of juvenile rats was shown to lead to neutrophil recruitment into the CNS and BBB dysfunction characterized by the loss of ZO-1 and occludin from those cerebral microvessels associated with neutrophil infiltrates (9). In contrast, injection of IL-1 into the striatum of adult rats did not cause increased BBB leakiness or neutrophil recruitment (5). Thus, the maturation stage of the BBB has an influence on its response to cytokines and the immature BBB seems to be more susceptible to cytokine-induced alterations of TJ architecture and immune cell diapedesis, whether this holds true for the effect of oxidative stress on the integrity of the BBB during maturation still needs to be elucidated.

The BCSFB TJs in the Inflamed CNS

Expression and function of BCSFB TJs in the inflamed CNS has been poorly investigated so far and if mostly in the context of MS and EAE, although the involvement of the choroid plexus in CNS inflammation has been suggested by the observations that immune cell counts increase in the CSF in many inflammatory disorders of the CNS. In addition, impaired mitochondrial functions and increased levels of NO and ROS were reported in choroid plexus from AD patients as well as in transgenic animal models of AD, suggesting a modification of the choroid plexus epithelium integrity upon oxidative stress (154). However, no study has specifically addressed ROS-induced TJ modifications at the level of the BCSFB. The same holds true for MS pathology, although detrimental effects of oxidative stress have been extensively reported in MS brain tissues (22, 81, 152). In MS, claudin-11 has been identified as a putative target autoantigen, because increased levels of anti-claudin-11 antibodies are found in the CSF of MS patients (12) and EAE can be induced by immunization with claudin-11 in susceptible mice (64, 138). OSP/claudin-11 was originally considered as a specific myelin autoantigen and its presence in the CSF was taken as a measure for myelin degradation. However, presence of claudin-11 in the CSF may well reflect a modulation of the BCSFB localized at the choroid plexus epithelium (167).

Ultrastructural and molecular alterations

During EAE, massive ultrastructural changes of the choroid plexus can be observed and seem to increase with disease severity. The most prominent changes seem to affect the epithelium rather than the fenestrated endothelium, with electron-dense or dark epithelial cells and electron-light epithelial cells lacking normal microvilli appearing adjacent to epithelial cells with normal morphology [summarized in Ref. (31)]. Interestingly, at the morphological level BCSFB TJs seemed to remain unchanged during EAE (31). However, molecular alterations of BCSFB TJs during EAE cannot be excluded as the comparison of the immunoreactivities for occludin, ZO-1, claudin-1, claudin-2, and claudin-11 in the choroid plexus of healthy mice and EAE- mice demonstrated subtle differences in immunoreactivity for the respective TJ proteins with interrupted immunostaining reported for claudin-1 and claudin-2 and weaker immunoreactivity for claudin-11 (167). To enter the CNS from the periphery *via* the choroid plexus, immune cells need appropriate trafficking cues. In this context it has been found that expression of ICAM-1 and VCAM-1 is upregulated and expression of mucosal addressin cell adhesion molecule (MAdCAM)-1 is induced by choroid plexus epithelial cells during EAE (135). However, these adhesion molecules are targeted to the apical membrane of choroid plexus epithelial cells and therefore not available for basolateral to apical immune cell diapedesis across the BCSFB (168).

Role of TJs in BCSFB leakiness and leukocyte trafficking across the BCSFB

The CSF of healthy individuals contains very few cells, the majority of which are central-memory CD4+ T cells, indicating that these cells routinely penetrate the choroid plexus epithelium [summarized in Ref. (29)]. By demonstrating that choroid plexus epithelial cells express CCL20, which mediates the migration of CCR6+ Th17 cells across the BCSFB into the CNS during initiation of EAE, the first molecular mechanism for T cell migration across the choroid plexus could be defined and underlined the particular role of the BCSFB in mediating immunosurveillance of the CNS (124). Many neurological disorders are characterized by the presence of increased numbers of immune cells in the CSF, suggesting that during inflammation trafficking mechanism across the BCSFB are upregulated. In MS brains, expression of VCAM-1 was found to be induced in the fenestrated choroid plexus microvasculature and T cells were found within the choroid plexus stroma, suggesting that these T cells enter the choroid plexus parenchyma *via* an α4-integrin/VCAM-1-dependent mechanism (155). Which molecules are involved in T cell diapedesis across the inflamed BCSFB remains to be investigated as T cell entry into the CNS during EAE is independent of CCL20. After traumatic brain injury neutrophils have been shown to enter the CNS *via* the choroid plexus (143). In this study enhanced release of neutrophil chemoattractants to the basolateral and apical side of choroid plexus epithelium was suggested to be involved in neutrophil diapedesis across the BCSFB, which was described to occur paracellularly through

the BCSFB TJs rather than transcellularly through the epithelial cells proper.

Upregulated expression of ICAM-1, VCAM-1, and MAdCAM-1 on the apical surface of choroid plexus epithelial cells might therefore be rather important for the adhesion and migration of Kolmer (epiplexus) cells on the choroid plexus surface and the execution of immune functions such as antigen presentation at the BCSFB than for the diapedesis of immune cells into the CSF space (31).

Conclusion: Outlook

Although our knowledge on the molecular composition of BBB and BCSFB TJs has significantly increased, we still lack knowledge about the precise function of most TJ proteins in these barrier locations and therefore do not yet understand the necessity of the combined expression of these molecules at the BBB and BCSFB TJs to regulate barrier integrity at both sides. As most *in vitro* BBB models do not truthfully reproduce *in vivo* expression of all junctional proteins and *in vitro* models of the BCSFB are extremely rare, analysis of mice with tissue-specific deletion of the respective junctional molecules combined with powerful techniques such as analysis of the transcriptome and proteome of BBB endothelium under different conditions may be useful to delineate the individual functions of the TJ proteins at these barrier tissues *in vivo*.

Production of free radicals that contribute to the development and the exacerbation of many inflammatory CNS disorders was shown to be detrimental on TJ integrity. However, in parallel antioxidative mechanisms are set up to counteract such excessive oxidative stress to protect CNS functions and homeostasis, including the maintenance of BBB TJ functions. The production of antioxidant enzymes such as SOD, catalase, gluthatione peroxidase, on one hand, and of the redox-sensitive nuclear factor E2-related factor 2 and the antioxidant response element, on the other hand, are currently considered for their therapeutic potentials as an alternative or in combination to other anti-inflammatory treatments. Many attempts using antioxidative treatments based on the modulation of ROS production, of endogenous antioxidant production, as well as supplementation with exogenous antioxidants, represent new approaches in treating inflammatory-mediated CNS disorders. Preclinical trials in animal models have demonstrated encouraging benefits and some have been translated to clinical trials. These therapeutical attempts have been reviewed in detail for EAE/MS (47, 94, 132), for HIV-1 (37, 136) and for AD (2, 74).

Furthermore, most studies investigating the regulation of TJ protein expression have focused on individual TJ protein; therefore, we do not yet have a general understanding on how neuroinflammatory mediators may influence the dynamics of the molecular architecture of the entire TJ complex. Considering that each TJ protein may have a unique and specific function at the brain barriers *in vivo*, studies may be relevant to develop concepts on how altered TJ architecture may impact on CNS neuroinflammation. Altered TJ architecture may not only lead to the failure to maintain a diffusion barrier to water-soluble mediators but may have an additional influence on the pathways available for immune cell entry across the brain barriers and therefore ultimately on the composition of immune cell infiltrates in the CNS during neuroinflammation. Thus, further investigations to understand the molecular signaling mechanisms involved in the expression of individual TJ components in brain endothelium and choroid plexus epithelium are necessary to capitalize unrealized opportunities on the therapeutic targeting of brain barrier TJs with the aim to specifically stabilize brain barrier function and/or to reduce inflammatory infiltration into the CNS parenchyma.

Acknowledgments

This work has been supported by the EU FP7-funded collaborative project JUSTBRAIN (grant no. 241 86). The authors thank Dr. Friederike Pfeiffer and Therese Périnat for assistance in immunofluorescence stainings of mouse brain tissue.

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Date of first submission to ARS Central, February 10, 2011; date of acceptance, February 19, 2011.

Abbreviations Used

Aβ = β-Amyloid
AD = Alzheimer's disease
AF-6 = afadin
AJ = adherens junction
AP-1 = activator protein-1
ASIP = isotype-specific interacting protein
BBB = blood–brain barrier
BCSFB = blood–cerebrospinal fluid barrier
CCL = CC chemokine ligand
CCR = CC chemokine receptor
CHO = Chinese hamster ovary
CNS = central nervous system
CSF = cerebrospinal fluid
EAE = experimental autoimmune encephalomyelitis
ERK1/2 = extracellular signal-regulated kinase
ESAM = endothelial cell-selective adhesion molecule
GUK = guanylyl kinase-like domain
H_2O_2 = hydrogen peroxide
hCMEC/D3 = human cerebral microvascular endothelial cells/D3
HIV = human immunodeficiency virus
HIVE = human immunodeficiency virus-1-associated encephalitis
ICAM = intercellular adhesion molecule
Ig = immunoglobulin
IL = interleukin
JAM = junctional adhesion molecule
MAdCAM = mucosal addressin cell adhesion molecule
MAGI = MAGUK inverted protein
MAGUK = membrane-associated with a guanylyl kinase-like domain
MCP-1 = monocyte chemoattractant protein-1
MDCK cells = Madin-Darby canine kidney cells
MMP = matrix metalloproteases
MS = multiple sclerosis
NAWM = normal appearing white matter
NF-κB = nuclear factor-κB
NO = nitric oxide
OSP = oligodendrocyte-specific protein
PAR-3 = partitioning defective-3
PDZ = postsynaptic density protein (PSD95), *Drosophila* disc large tumor suppressor (DlgA), Zonula occludens-1
PECAM-1 = platelet endothelial cell adhesion molecule
PI3K = phosphoinositide 3-kinase
pMBMECs = primary mouse brain microvascular endothelial cells
Ras = rat sarcoma
ROS = reactive oxygen species
SOD = superoxide dismutase
TJ = tight junction
TNF-α = tumor necrosis factor-α
VCAM-1 = vascular cell adhesion molecule
VE-cadherin = vascular endothelial cadherin
ZO = zonula occludens

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