Journal of NeuroVirology, 15: 111–122, 2009
© 2009 Journal of NeuroVirology
ISSN 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280902769764

informa
healthcare

Review

Acquired immunodeficiency syndrome and the blood-brain barrier

Nathan S Ivey, Andrew G MacLean, and Andrew A Lackner

The blood-brain barrier (BBB) plays a critical role in normal physiology of the central nervous system by regulating what reaches the brain from the periphery. The BBB also plays a major role in neurologic disease including neuropathologic sequelae associated with infection by human immunodeficiency virus (HIV) in humans and the closely related simian immunodeficiency virus (SIV) in macaques. In this review, we provide an overview of the function, structure, and components of the BBB, followed by a more detailed discussion of the subcellular structures and regulation of the tight junction. We then discuss the ways in which HIV/SIV affects the BBB, largely through infection of monocytes/macrophages, and how infected macrophages crossing the BBB ultimately results in breakdown of the barrier. *Journal of NeuroVirology* (2009) **15,** 111–122.

Keywords: HIV; macaque; neuroAIDS; SIV

Introduction

Since Paul Ehrlich recognized the compartmentalized and restrictive qualities of the central nervous system (Ehrlich, 1885), many researchers have sought to understand the mechanisms that govern passage of substances across the barrier from the blood into the brain. Understanding how the blood-brain barrier (BBB) functions as a selective barrier is an ongoing pursuit that should result in enhanced treatment of central nervous system (CNS) disease, targeted drug therapy, and improved surgical prognosis (Strbian *et al*, 2008).

The BBB is composed of closely packed unfenestrated brain microvascular endothelial cells (BMECs) situated between the bloodstream and the basement membrane (Figure 1). The basement membrane, composed largely of collagen IV and laminin, is an extracellular matrix that anchors BMECs to the underlying tissues. Surrounding the BMECs of the BBB and in contact with the basement membrane are perivascular macrophages and the foot processes of microglia and astrocytes (Graeber *et al*, 1992; Hickey and Kimura, 1988; Lassmann *et al*, 1991; Streit and Graeber, 1993). Astrocytes and microglia support the BBB biochemically to fulfill its purpose as a diffusion barrier and also play an immunoregulatory role (Williams *et al*, 2001a). Given the intimate juxtaposition among BMECs, astrocytes, perivascular macrophages, and parenchymal microglia, all are likely to encounter agents entering the CNS via the circulation.

Structural components of the BBB and BBB function

Brain microvascular endothelial cells

Brain microvascular endothelial cells are highly dynamic cells that form and regulate tight junctions (TJs), the critical structure responsible for the complex task of barrier regulation. TJs are composed of fibril networks of transmembrane homo- and heterodimeric proteins that can be modified and regulated to allow physiologic processes, such as replacement of perivascular macrophages by circulating monocytes. TJ regulation occurs by way of complex, rapid phosphorylation events that may be initiated in a polarized fashion, functioning differently based on whether the stimulus originates from the luminal or abluminal side of BMECs. The presence of adhesion molecules on the luminal surface of BMECs is very important for leukocyte extravasation into the CNS.

Address correspondence to Andrew A. Lackner, Tulane National Primate Research Center, 18703 Three Rivers Road, Covington, LA 70433, USA. E-mail: alackner@tulane.edu
This work was supported by Public Health Service grants RR000164, NS30769, RR20159 and MH077544. Nathan Ivey is supported by a teaching assistantship from the Tulane University School of Science and Engineering.
Received 28 November 2008; revised 7 January 2009; accepted 14 January 2009.

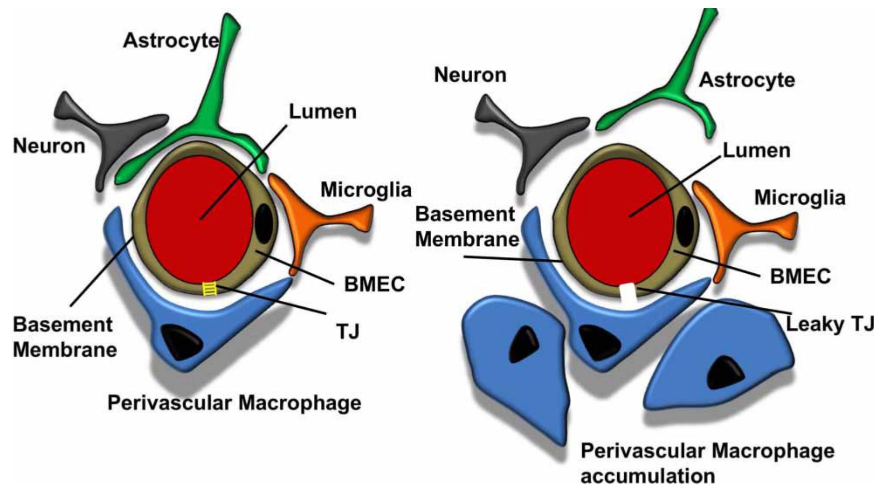

Figure 1 The normal blood-brain barrier (*left*) is composed of multiple cell types. The tightly apposed brain microvascular endothelial cells (BMECs) joined by tight junctions play a major role. The role of the BMECs is augmented by perivascular macrophages, and foot processes of astrocytes and microglia that envelope the BMEC. Following HIV/SIV neuroinvasion (*right*), perivascular macrophages accumulate and this is associated with increases in BBB permeability associated with leaky TJs.

Astrocytes

Astrocytes play a critical role in the formation and coordination of the BBB. It is known that the dense network of foot processes surrounding BMECs develops after birth in the rat (LeVine and Goldman, 1988). Although the BBB can form and function prior to gliogenesis in early prenatal development (Weidenfeller *et al*, 2007), the contributions of astrocytes to maintenance and signaling to BMECs in health and disease are well studied. While astrocyte foot processes cover over 90% of the surface of brain microvessels (Willis *et al*, 2004), the gap between astrocytic foot processes and BMECs is known to be at least 20 nm, a sufficiently large space to allow passage of many solutes normally restricted by the intact BBB. However, in coculture of astrocytes and BMECs, transendothelial electrical resistance is increased over the culture of BMECs alone (Rubin *et al*, 1991). Furthermore, damage or removal of astrocytes in vivo results in a transient increase in BBB permeability (Hamm *et al*, 2004; Krum *et al*, 1997). The prominent role of astrocytes in supporting a healthy BBB may be the result of trophic factors secreted by astrocytes that nourish and regulate BMECs (Igarashi *et al*, 1999).

Perivascular macrophages and parenchymal microglia

Perivascular macrophages and parenchymal microglia are both bone marrow–derived cells that are continuously replaced by monocytes. Bone marrow chimera studies in rodents and transplantation studies in humans show a fairly rapid turnover of perivascular macrophages (30% in 90 days) and much slower turnover of parenchymal microglia (less than 1% in 90 days) (Hickey and Kimura, 1988; Hickey *et al*, 1992; Lassmann *et al*, 1986; Matsumoto and Fujiwara, 1987; Unger *et al*, 1993). The normal turnover of perivascular macrophages may be exploited by pathogens such as human immunodeficiency virus (HIV) as discussed below.

In addition to differences in location, morphology, and turnover between perivascular macrophages and parenchymal microglia, the two cell types can be distinguished by the expression of various myeloid markers (Becher and Antel, 1996; Borda *et al*, 2008; Sedgwick *et al*, 1991; Ulvestad *et al*, 1994a,b; Williams *et al*, 1992). The differential expression of myeloid markers as well as other markers associated with antigen-presenting function and limited functional studies indicate that perivascular macrophages are the antigen-presenting cells of the CNS and sensors of brain injury (Williams *et al*, 2001a). Perivascular macrophages and parenchymal microglia can both be activated by inflammation of diverse causes that can modify the rate of cell turnover as well as the morphology and immunophenotype of the cells (Borda *et al*, 2008).

Permeability properties of the tight junction

Tight junctions (TJs) between individual BMECs are the focal adhesion units responsible for the barrier properties of the BBB. The TJ is an intricate complex containing over 40 transmembrane proteins, anchorage proteins, and TJ-associated proteins in the membrane and cytosol of adjoining BMECs. The normal TJ is characterized as having high transendothelial electrical resistance values between 1000 and 1500 ω/cm^2 (Butt *et al*, 1990). TJs and the BBB in general impose restrictions upon what can pass from the blood into the CNS down to the level of small molecules. The TJ restricts solutes by size, charge, and lipophilicity; there is a direct correlation between lipophilicity and ease of diffusion through the BBB. It is widely accepted that lipophilic alcohols or gases like O_2 and CO_2 can pass

relatively unobstructed. L-Amino acids are rapidly absorbed via transporters (Oldendorf, 1973). Substances smaller than 60 Å can cross the BBB in a non–rate-limited manner (Strbian *et al*, 2008). Other substances necessary for CNS metabolism like glucose cross via adenosine triphosphate (ATP)-dependent influx transport mechanisms. Other diverse and specific efflux mechanisms that serve to rid the brain of metabolic toxins and xenobiotics are also present (Ohtsuki and Terasaki, 2007).

Tight junction–associated proteins

The function of the TJ is dependent on its component parts, including transmembrane proteins, anchorage proteins, and other associated proteins. Current research suggests that an intricate network of transmembrane proteins form a fibril network across the span of the TJ's 2-nm gap. These fibril networks can be clearly observed in freeze-fracture scanning electron micrographs. Numerous proteins have been isolated and identified in and around the TJ and the crystal and ribbon structures of many have been determined. These include occludin, claudins, junctional adhesion molecules (JAMs), endothelial-specific adhesion molecule (ESAM), and zonula occludens (zo) proteins (Figure 2).

The first TJ protein predicted to span the TJ was occludin (Furuse *et al*, 1993; Gumbiner, 1993). More recent data, however, questions whether occludin is a major restrictive element of the TJ. This conclusion is the result of studies using occludin knockout mice and in vitro research that show that TJs form and function well in the absence of occludin. Thus it is unclear what role this protein plays in instances of increased BBB permeability (Balda *et al*, 1996; Saitou *et al*, 1998, 2000).

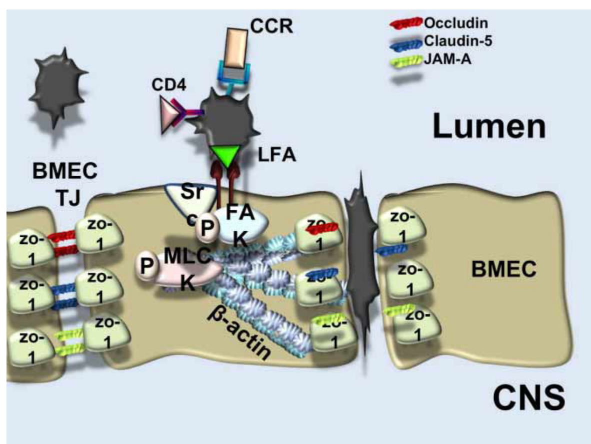

Figure 2 Occludin, claudin-5, JAM-A, and zo-1 are important tight junction–associated proteins. Occludin, claudin-5, and JAM-A span the intercellular gap of the tight junction and are anchored into the cell via zo-1, which in turn is bound to the actin cytoskeleton. Paracellular monocyte diapedesis involves traversing the tight junction via a series of interactions with integrin receptors at the luminal surface of a BMEC. This initiates highly regulated intracellular signaling, which results in temporary opening of the TJ to allow the monocyte to pass into the perivascular space.

The highly conserved claudin family of cell adhesion molecules was first isolated from the TJs of the BBB in 1998 (Furuse *et al*, 1998). Although this family contains 24 known members in humans, only two have been isolated and shown to have a possible role in BBB permeability changes in virus-host interaction. These are claudin-1 and claudin-5. Claudin-1 is ubiquitous, and can be found in normal mammary gland epithelial cells, testis sertoli cells, hair follicles, and many other cell types and is reviewed in more detail elsewhere (Turksen and Troy, 2004), whereas claudin-5 is unique to BMEC tight junction (Morita, 1999; Van Itallie and Anderson, 2006). Claudins can form homo- or heterodimeric pairs across the TJ, anchored in the cytoplasm by zo-1, -2, and -3 (Itoh *et al*, 1999). The role of claudins has not been fully determined; however, there is evidence that they may limit solutes by size and/or charge. Claudin-5 knockout mice have been created and although it is reported that these mice do not exhibit edema or gross morphological changes in blood vessels, there is a fundamental deficiency in the size restriction of the BBB that can be observed in tracer experiments (Nitta *et al*, 2003), as well as decreases in transendothelial electrical resistance (Yu *et al*, 2003).

The study of JAMs has so far yielded five dimeric transmembrane TJ protein members, including JAM-A (also known as JAM-1, F11 Receptor), JAM-B, JAM-C, JAM-4, and JAML (Weber *et al*, 2007). As a rule, JAMs are located at TJs, but can reorganize to the apical surface of the endothelium during inflammation or monocyte transmigration (Ozaki *et al*, 1999). All members of the JAM family participate in the process of monocyte extravasation during inflammation (Lamagna *et al*, 2005). JAM-A is highly enriched in the brain and is thought to be important in BBB regulation and was the first of these proteins to be identified. JAM-A has been shown on the surface of endothelial cells in the brain, circulating monocytes, macrophages, and dendritic cells. At endothelial TJs in the CNS, the cytoplasmic domain of JAM-A is bound in the endothelial cell cytoplasm by the zo-1 complex and associated scaffolding proteins. JAM-A is believed to be phosphorylated by protein kinase C. JAM-B and JAM-C have been localized to BMEC junctions and were demonstrated to have a role in monocyte transmigratory regulation in human umbilical vein endothelial cells in vitro. In a recent study, the blockade of JAM-B/-C dimers reduced the number of monocytes at sites of tissue inflammation (Bradfield *et al*, 2007). In short, JAMs are likely to be important players in BBB regulation and monocyte extravasation.

Zonula occluden-1, -2, and -3 (zo-1, -2, and -3) are TJ associated proteins that form complex plaques bound to the actin cytoskeleton on the cytoplasmic

side and transmembrane proteins (including occludin, claudins, JAMs) on the TJ side (Figure 2). Functionally, the zo proteins are believed to anchor fibrillary TJ dimers to the cytoskeletons of adjoining endothelial cells.

The blood-brain barrier and HIV/SIV infection of the CNS

Mechanisms of neuroinvasion: with an emphasis on HIV/SIV

A number of infectious agents cause CNS damage and employ strategies of various specificity to circumvent the defenses of the BBB. Some pathogens (e.g., *Esherichia coli, Streptococcus pneumoniae, Bacillus anthracis)* employ nonspecific toxin-mediated inflammation to directly breach BMECs (Bermpohl *et al*, 2005; Kirby, 2004; Tunkel *et al*, 1991). In contrast to this nonspecific approach, some viruses (e.g., rabies and reoviruses) and transmissible prions use retrograde neuronal transport to enter the CNS from the periphery (Kelly and Strick, 2000; Morrison *et al*, 1991).

Lentiviruses such as HIV and simian immunodeficiency virus (SIV) take advantage of the normal turnover of perivascular macrophages and use a ''Trojan horse'' strategy as first proposed by Narayan *et al* (Narayan *et al*, 1982; Peluso *et al*, 1985) (Figure 3). Specifically, lentiviruses are able to infect monocytes in the periphery but do not replicate efficiently until the monocyte has entered tissues (in this case the CNS) and differentiated into macrophages. For HIV and SIV, this process of neuroinvasion can occur as early as the initial 10 days post infection (Davis *et al*, 1992; Lackner *et al*, 1994) and is thought to involve HIV-infected circulating monocytes entering the brain during the course of routine immune surveillance and replacement of perivascular macrophages, a process reviewed elsewhere (Annunziata, 2003).

The process of replacement of perivascular macrophages by bone marrow–derived monocytes involves complex interactions between the monocyte and BMEC, mediated by adhesion molecules, chemokines, and cytokines that facilitate migration through the BBB without compromising the selectivity of the barrier. The tempo of this turnover can be greatly increased in inflammatory conditions such as neuroAIDS (Persidsky *et al*, 1999). Furthermore, HIV-infected monocytes have been shown to cross the BBB more efficiently than noninfected monocytes (Persidsky *et al*, 1999).

Interactions between monocytes in blood and the BMEC result in activation of integrin receptors and

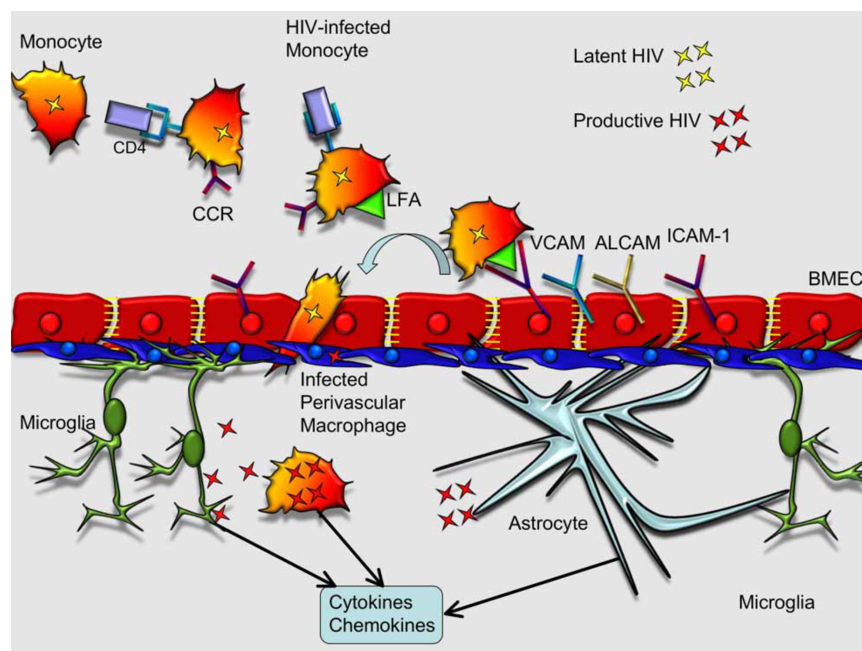

Figure 3 Infection of the central nervous system by SIV/HIV primarily involves cells of monocyte/macrophage lineage. HIV/SIV infection likely subverts the normal process of monocyte replacement of perivascular macrophages to cross the blood-brain barrier. Some of these cells will be infected and carry the virus into the brain within days of infection. As the monocytes differentiate into macrophages, they become a better substrate for viral replication and produce a variety of cytokines and chemokines. The cytokines and chemokines cause activation of various other cell types in the brain, including endothelial cells, microglia, and astrocytes. The endothelial cells up-regulate adhesion molecules, which along with increased chemokine production facilitates further recruitment of leukocyte (primarily monocyte/macrophages) into the brain, giving rise to perivascular cuffs. Most of the cells in these cuffs are not infected by HIV/SIV but likely serve as fodder for further rounds of viral replication.

adhesion molecules that initiate intracellular signaling cascades within BMECs. These signals may lead to the temporary opening of BMEC TJs and coordinate seamless passage of monocytes into the CNS (Sasaki *et al*, 2003). The intercellular signaling cascade involved in monocyte transmigration is believed to occur via highly regulated stepwise phosphorylation events, with sequential alterations in the conformation of fibril forming transmembrane junctional dimers (Figure 4).

The importance of the BBB and turnover of perivascular macrophages in the neuropathogenesis of acquired immunodeficiency syndrome (AIDS) is further illustrated by results of experimental models where the BBB is bypassed and SIV is directly inoculated into the brain (Hurtrel *et al*, 1991; Sharma *et al*, 1992).

The anticipated result of such studies was that SIV encephalitis would occur with increased frequency, but this was not the case. Furthermore, it is interesting to note that areas of the brain without a BBB, such as the area postrema, do not typically exhibit the neuropathologic lesions of SIV or HIV infection. These data suggest that simply gaining access to the CNS is not sufficient for the induction of HIV/SIV-induced neuroAIDS and that it is the result of an active, facilitated process. In other words, these studies and observations present a strong indication that lentiviruses such as HIV and SIV require active interaction between monocytes/macrophages and the BBB to initiate neuroinvasion and the development of encephalitis.

Monocyte diapedesis

There are two models by which circulating monocytes are thought to cross the BBB. The first is paracellular diapedesis, which occurs when monocytes roll, arrest, and extravasate between the TJs of BMECs in response to inflammatory stimuli in the CNS (Eugenin *et al*, 2006) (Figure 5). Paracellular diapedesis occurs when endothelial cells, activated by cytokines (such as interleukin [IL]-1 and tumor necrosis factor [TNF]-α) on the abluminal side, up-regulate E-selectin on their luminal surface. This cell adhesion molecule binds monocytes with a shear stress–dependent catch bond, causing monocytes to roll. The rolling of the monocytes along the endothelial cells then allows engagement between integrins such as leukocyte function antigen-1 (LFA-1; α1β2 integrin) and very late antigen-4 (VLA-4; α4β1 integrin) on the monocyte with their ligands, including immunoglobulin superfamily members intercellular adhesion molecule (ICAM)-1 (CD54) and vascular cell adhesion molecule (VCAM)-1 (CD106) on the luminal side of BMECs. Both ICAM-1 and VCAM-1 can be up-regulated in response to inflammation, and VCAM-1 has been shown to be rapidly up-regulated soon after SIV infection and is thought to facilitate monocyte recruitment (Nottet *et al*, 1996; Sasseville *et al*, 1994). The integrin-mediated interactions between monocytes and endothelial cells causes circulating monocytes to slow and arrest and yields a complex signaling cascade that ultimately leads to contractility and opening of the TJ via myosin light chain kinase (MLCK) and other mediators in BMECs (Haorah *et al*, 2005; Persidsky *et al*, 1999; Smith *et al*, 2003; Stamatovic *et al*, 2003) (Figures 2 and 4).

In addition to the trafficking and adhesion molecules discussed above, several proteins originally associated with TJs have been found to facilitate paracellular extravasation. Among these, JAM-A

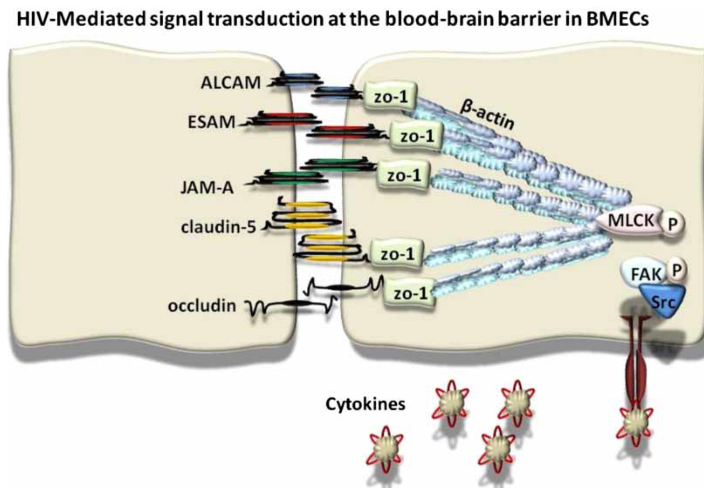

Figure 4 The tight junction between BMECs is composed of a matrix of dimeric transmembrane proteins including but not limited to occludin, claudin-5, and JAM-A that are likely to play a major role in the maintenance, regulation, and selectivity of the BBB. TJ proteins are anchored to the cytosol of BMECs by way of a protein complex comprised of zo proteins and others. This zo protein anchorage is bound to actin in the BMEC cytosol. When stimulated by proinflammatory cytokines, FAK, through MLCK and actin, causes redistribution of the zo-1 anchorage. Redistribution of zo-1 leads to tensile stress of the transmembrane proteins JAM, claudin-5, occluding, and others, which leads to opening of the tight junction and increased permeability of the BBB.

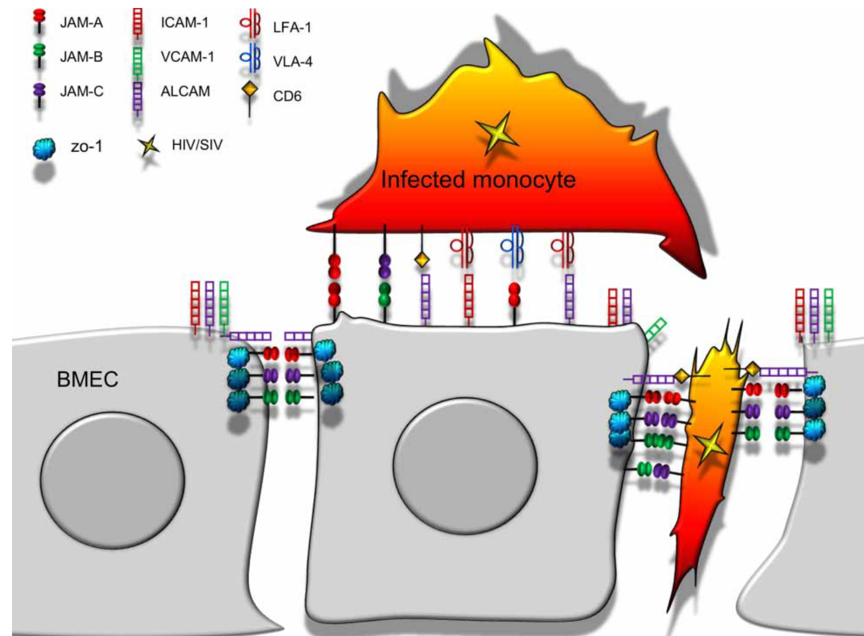

Figure 5 Paracellular diapedesis occurs when a circulating monocyte is bound by adhesion molecules (primarily ICAM-1 and ALCAM), and moves along a haptotactic adhesion gradient through the TJ. Occludin and claudin-5 (not shown) are also likely to have a role in extravasation.

and activated leukocyte cell adhesion molecule (ALCAM) are typically located at endothelial tight junctions, but can translocate to the luminal BMEC membrane, forming a haptotactic gradient during inflammation. JAM-A has a role in the recruitment and extravasation of monocytes via interaction with LFA-1 (Ostermann *et al*, 2002). ALCAM has been demonstrated at the luminal surface of BMECs colocalized with ICAM-1 (Cayrol *et al*, 2008), and is believed to play a role in monocyte trafficking through self-association (low affinity) or binding of CD6 (high affinity) on $CD4^+$ monocytes (Lee and Imhof, 2008). Both of these adhesion molecules are up-regulated during inflammatory processes and our understanding of the roles of JAM-1 and ALCAM are expanding (Figure 5).

In the second model, transcellular diapedesis (Marchesi and Gowans, 1964), integrins on rolling monocytes bind to the endothelial surface via ICAM-1 in response to inflammatory stimuli. Adhesion molecules concentrate on the BMEC and form a transmigratory cup that guides extended monocyte 'podosomes' through the cytosol of the BMEC (Carman *et al*, 2007; Carman and Springer, 2008; Dejana, 2006). The TJ is not involved in transcellular diapedesis and thus the tight junction remains intact. Although transcellular diapedesis is likely to have a role in neuroinvasion by HIV/SIV and other agents, current research suggests that paracellular diapedesis is much more common, occurring about 90% of the time for monocytes (Carman and Springer, 2004).

In addition to the TJ-associated molecules discussed above for which there is evidence of involvement in the neuropathogenesis of AIDS, a large number of additional molecules are likely to be involved; as many as 900 proteins and over 6000 protein-protein interactions are thought to play a role in leukocyte adhesion and diapedesis (Ley *et al*, 2007).

Neuroinflammation, tight junction dysregulation, and BBB permeability

Following extravasation, infected monocytes/perivascular macrophages begin to actively produce and release HIV/SIV as well as a variety of proinflammatory mediators, which impacts the function of surrounding cells and enhances further monocyte recruitment (Figure 3) (Williams *et al*, 2001b; Orandle *et al*, 2002b; Roberts *et al*, 2004; Toborek *et al*, 2005; Town *et al*, 2005; Librizzi *et al*, 2006). This occurs concurrently with dysregulation of tight junctions.

In vivo, several studies have shown that the loss of transmembrane TJ proteins and perijunctional proteins in microvessels is associated with increased permeability of the BBB following HIV/SIV neuroinvasion (MacLean *et al*, 2005). Fiala *et al* have

described changes in zo-1 patterning in brains of patients with HIV encephalitis, with a complete lack of zo-1 staining in cortical vessels of one patient (Fiala *et al*, 1997). Similarly, loss of occludin and claudin-5 was observed in brains of HIV-infected patients and correlated with monocyte migration in to the brain (Persidsky *et al*, 2006). It is clear that occludin and zo-1 are affected by HIV encephalitis (Dallasta *et al*, 1999), and it is likely that JAMs are similarly affected (Dobrogowska and Vorbrodt, 2004; Yeung *et al*, 2008). As in HIV-infected humans, decreases in quantity or changes in distribution (repatterning) of occludin have been observed in the brain of SIV-infected macaques and correlated with events leading to microvascular leakage (Luabeya *et al*, 2000).

Whereas the presence of proinflammatory cytokines following HIV/SIV neuroinvasion has been shown, the signal transduction events that occur in BMECs that lead to TJ dysregulation are still being investigated. It is likely that parallel pathways are activated within BMECs that both attract monocytes and cause TJ dysregulation. One possible pathway under investigation involves cytokine signaling via receptors on BMECs that leads to the phosphorylation of MLCK and focal adhesion kinase (FAK), which would increase permeability and facilitate monocyte binding (Figures 2 and 4) (McKenzie and Ridley, 2007; Mon *et al*, 2006; Webb *et al*, 2004).

Production of HIV-induced cytokines and chemokines such as TNF-α (Orandle *et al*, 2002a), CCL2 (monocyte chemoattractant protein [MCP]-1) (Eugenin *et al*, 2003), CCL3 (macrophage inflammatory protein [MIP]-1α), CCL4 (MIP-1β), and CCL5 (RANTES) in the CNS adjacent to BMECs can yield clustering of integrin receptors (facilitating monocyte binding) on vessels and immediate phosphorylation of FAK on tyrosine 397 (Tyr-397) in BMECs. Recent data from our laboratory shows a colocalization between MLCK and FAK Tyr-397 in SIV treated ex vivo microvessels (unpublished data) that have been immunostained. *Rho* (Persidsky *et al*, 2006) and MLCK can function in sequence through cytoskeletal organization pathways, resulting in translocation of zo-1 toward the cytosol.

Our data and the data of others suggest that the process of BBB alteration is directly impacted by the translocation of the zo-1 anchorage apparatus. Decreases in zo-1 have been correlated with increased permeability of the tight junction, and BBB disruption in general (Anathbandhu *et al*, 2007; Kebir *et al*, 2007). Luabeya *et al* demonstrated that in ex vivo cerebral vessels from macaques with SIV encephalitis, BMECs often stained in a punctate pattern for zo-1 rather than the characteristic ''zipper'' pattern of normal vessels (Luabeya *et al*, 2000). Because zo-1 plays a major role in the anchorage of TJs, it is likely that the degradation or reorganization of zo-1 results in tight junction dysfunction, in part by separating the transmembrane TJ dimers occludin, JAM-A, and claudin-5 (McKenzie and Ridley, 2007). A model like this may explain how BBB TJs are impacted by inflammatory cytokine activity.

Potential role of Tat/gp120 proteins: effects on the BBB

An alternate paradigm for HIV-mediated disruption of the BBB centers on Tat and/or gp120. Tat and gp120 are regulatory and envelope proteins of HIV respectively. These proteins, when isolated and applied in vitro to porcine BMECs or in vivo in mice (Toborek *et al*, 2003), are neurotoxic and capable of inducing dose-dependent oxidative stress. More recently, these viral proteins have been shown to have effects on BBB integrity.

Avraham and colleagues have shown that Tat can induce phosphorylation of focal adhesion kinase (FAK), leading to increased endothelial permeability by focal adhesion assembly. When FAK was inhibited, BMEC assembly and migration were inhibited (Avraham *et al*, 2004). In addition to Tat, gp120 from HIV has been shown to cause disruption of TJs and TJ proteins (Andras *et al*, 2003, 2005; Avraham *et al*, 2004; Kanmogne *et al*, 2005), leading to vascular dysfunction (Lohmann *et al*, 2004; Price *et al*, 2005; Toborek *et al*, 2003). Whether or not Tat is free in the extracellular space is still a matter of speculation. Further complicating this issue, Tat has been shown to have no effect on zo-1 or occludin in an in vitro model, but does decrease zo-2 and claudin-1 and -5 (Andras *et al*, 2003). HIV envelope proteins can also be linked to neuronal and astrocyte apoptosis (Agrawal *et al*, 2007; Eugenin *et al*, 2007).

The possibility that viral proteins such as Tat and gp120 may directly affect the integrity of the BBB in vitro is intriguing but leaves several unanswered questions. Key among these is whether viral proteins would be present in sufficient, metabolically active quantities in proximity to BMECs to induce permeability changes of the BBB in vivo.

Regardless, HIV-mediated BBB permeability changes are probably driven by multiple intracellular and intercellular events involving multiple cell types. One cell type that is likely to be involved because of its role in neuroinflammation is the astrocyte.

Astrocytes HIV and the BBB

In vitro studies with astrocytes and in vivo studies of HIV-infected infants have shown that astrocytes can be infected by HIV (Brack-Werner, 1999; Conant *et al*, 1994; Eugenin and Berman, 2007). These studies suggest that the normal function of astrocytes could be impaired, which would have significant consequences for the normal function of the BBB. In this context, it is important to note that astrocytes have functional HIV coreceptors, including CCR5 and CXCR4, immediately ex vivo that could facilitate infection (Klein *et al*, 1999). Even in the absence of infection, engagement of these

receptors by chemokine ligands such as CCL3 and CCL5 that are increased in the brain of HIV-infected humans and SIV-infected macaques could alter astrocyte function (Klein *et al*, 1999).

It is also known that astrocytes release proinflammatory cytokines in proximity to microvessels when infected or activated by SIV/HIV. The release of cytokines such as IL-1β, IL-6, and TNF-α by astrocytes and microglia is likely to result in paracrine signaling as well as direct regulatory effects on BMECs (Deli *et al*, 1995; Didier *et al*, 2003; Schwaninger *et al*, 1999; Xing *et al*, 2008). Receptors on the abluminal side of BMECs receive and respond to signaling molecules released from microglia, perivascular macrophages, and astrocytes. Ultimately, the response of astrocytes to HIV/SIV in the CNS is likely to increase BBB permeability via previously discussed signal transduction pathways in BMECs.

Astrocytes are also known for the overproduction of chemokines in response to SIV/HIV or their envelope proteins. For example, CCL2 (MCP-1) provides a gradient for peripheral monocyte recruitment (Gendelman *et al*, 2008) and exhibits up to 80-fold increases in mRNA expression when mice receive Tat_{1-72} injections in the hippocampus (Pu *et al*, 2003). The release of CCL2 by astrocytes may have a neuroprotective effect, inhibiting apoptosis in astrocytes and neurons against the toxic effects of Tat or glutamate (Eugenin *et al*, 2003). However, its protective effects may not extend to the tight junctions, as demonstrated by decreased transendothelial electrical resistance values and decreased protein expression of occludin, claudin-5, zo-1 and zo-2 (Stamatovic *et al*, 2005). In short, chronic release of large quantities of CCL2 by astrocytes in response to purified Tat protein has been shown to dysregulate tight junctions and effect BBB permeability. However, in SIVE, no increase in MCP-1 protein was demonstrated in the parenchyma at any stage following infection (Sasseville *et al*, 1996).

Conclusions and future directions

Our understanding of the role of the BBB in the neuropathogenesis of AIDS is a developing one. Although it is clear that HIV and SIV enter the CNS and that breakdown of the BBB occurs, exactly how this happens is unclear. Both HIV and SIV enter the CNS very quickly and repeatedly after infection, most likely by infecting monocytes in the periphery that traffic to the CNS as part of the normal turnover of perivascular macrophages. The infected cells cross the BBB either by paracellular or transcellular diapdedesis; however, the relative contribution of each pathway is unknown. Although paracellular diapedesis involves opening and closing of the TJs, this is part of the normal process of cell trafficking and would not be expected to cause abnormal BBB permeability. Nevertheless, breakdown of the BBB does occur; however, we don't know when it begins, what the specific mechanisms are, or how it contributes to the neuropathogenesis of AIDS. Recent work by us and others suggests that inflammatory mediators induced by the infection are responsible for initiating signal transduction pathways that leads to breakdown of the TJ. A better understanding of the mechanisms of TJ breakdown in neuroAIDS should lead to new therapeutic targets such as compounds that block the action of FAK. Such a pharmacologic approach to maintain BBB integrity during SIV infection may result in a better understanding of the significance of BBB breakdown in the neuropathogenesis of AIDS. In addition, other approaches that block adhesion molecules on BMECs or the action of inflammatory mediators and chemoattractants that facilitate leukocyte recruitment to the CNS appear to be promising avenues to further our understanding of HIV/SIV neuroinvasion and the role of the BBB in neuroAIDS.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Agrawal L, Louboutin JP, Strayer DS (2007). Preventing HIV-1 Tat-induced neuronal apoptosis using antioxidant enzymes: mechanistic and therapeutic implications. *Virology* **363:** 462–472.

Anathbandhu C, Yang B, Gendelman HE, Persidsky Y, Kanmogne GD (2007). STAT1 signaling modulates HIV-1-induced inflammatory responses and leukocyte transmigration across the blood-brain barrier. *Blood* **111:** 2062–2072.

Andras IE, Pu H, Deli MA, Nath A, Hennig B, Toborek M (2003). HIV-1 Tat protein alters tight junction protein expression and distribution in cultured brain endothelial cells. *J Neurosci Res* **74:** 255–265.

Andras IE, Pu H, Tian J, Deli MA, Nath A, Hennig B, Toborek M (2005). Signaling mechanisms of HIV-1 Tat-induced alterations of claudin-5 expression in brain endothelial cells. *J Cereb Blood Flow Metab* **25:** 1159–1170.

Annunziata P (2003). Blood-brain barrier changes during invasion of the central nervous system by HIV-1. Old and new insights into the mechanism. *J Neurol* **250:** 901–906.

Avraham HK, Jiang S, Lee TH, Prakash O, Avraham S (2004). HIV-1 Tat-mediated effects on focal adhesion assembly and permeability in brain microvascular endothelial cells. *J Immunol* **173:** 6228–6233.

Balda MS, Whitney JA, Flores C, Gonzalez S, Cereijido M, Matter K (1996). Functional dissociation of paracellular permeability and transepithelial electrical resistance and disruption of the apical-basolateral intramembrane diffusion barrier by expression of a mutant tight junction membrane protein. *J Cell Biol* **134:** 1031–1049.

Becher B, Antel JP (1996). Comparison of phenotypic and functional properties of immediately ex vivo and cultured human adult microglia. *Glia* **18:** 1–10.

Bermpohl D, Halle A, Freyer D, Dagand E, Braun JS, Bechmann I, Schroder NW, Weber JR (2005). Bacterial programmed cell death of cerebral endothelial cells involves dual death pathways. *J Clin Invest* **115:** 1607–1615.

Borda JT, Alvarez X, Mohan M, Hasegawa A, Bernardino A, Jean S, Aye P, Lackner AA (2008). CD163, a marker of perivascular macrophages, is up-regulated by microglia in simian immunodeficiency virus encephalitis after haptoglobin-hemoglobin complex stimulation and is suggestive of breakdown of the blood-brain barrier. *Am J Pathol* **172:** 725–737.

Brack-Werner R (1999). Astrocytes: HIV cellular reservoirs and important participants in neuropathogenesis. *AIDS* **13:** 1–22.

Bradfield PF, Scheiermann C, Nourshargh S, Ody C, Luscinskas FW, Rainger GE, Nash GB, Miljkovic-Licina M, Aurrand-Lions M, Imhof BA (2007). JAM-C regulates unidirectional monocyte transendothelial migration in inflammation. *Blood* **110:** 2545–2555.

Butt AM, Jones HC, Abbott NJ (1990). Electrical resistance across the blood-brain barrier in anaesthetized rats: a developmental study. *J Physiol* **429:** 47–62.

Carman CV, Sage PT, Sciuto TE, de la Fuente MA, Geha RS, Ochs HD, Dvorak HF, Dvorak AM, Springer TA (2007). Transcellular diapedesis is initiated by invasive podosomes. *Immunity* **26:** 784–797.

Carman CV, Springer TA (2004). A transmigratory cup in leukocyte diapedesis both through individual vascular endothelial cells and between them. *J Cell Biol* **167:** 377–388.

Carman CV, Springer TA (2008). Trans-cellular migration: cell-cell contacts get intimate. *Curr Opin Cell Biol* **20:** 533–540.

Cayrol R, Wosik K, Berard JL, Dodelet-Devillers A, Ifergan I, Kebir H, Haqqani AS, Kreymborg K, Krug S, Moumdjian R, Bouthillier A, Becher B, Arbour N, David S, Stanimirovic D, Prat A (2008). Activated leukocyte cell adhesion molecule promotes leukocyte trafficking into the central nervous system. *Nat Immunol* **9:** 137–145.

Conant K, Tornatore C, Atwood W, Meyers K, Traub R, Major EO (1994). In vivo and in vitro infection of the astrocyte by HIV-1. *Adv Neuroimmunol* **4:** 287–289.

Dallasta LM, Pisarov LA, Esplen JE, Werley JV, Moses AV, Nelson JA, Achim CL (1999). Blood-brain barrier tight junction disruption in human immunodeficiency virus-1 encephalitis. *Am J Pathol* **155:** 1915–1927.

Davis LE, Hjelle BL, Miller VE, Palmer DL, Llewellyn AL, Merlin TL, Young SA, Mills RG, Wachsman W, Wiley CA (1992). Early viral brain invasion in iatrogenic human immunodeficiency virus infection. *Neurology* **42:** 1736–1739.

Dejana E (2006). The transcellular railway: insights into leukocyte diapedesis. *Nat Cell Biol* **8:** 105–107.

Deli MA, Descamps L, Dehouck MP, Cecchelli R, Joo F, Abraham CS, Torpier G (1995). Exposure of tumor necrosis factor-alpha to luminal membrane of bovine brain capillary endothelial cells cocultured with astrocytes induces a delayed increase of permeability and cytoplasmic stress fiber formation of actin. *J Neurosci Res* **41:** 717–726.

Didier N, Romero IA, Creminon C, Wijkhuisen A, Grassi J, Mabondzo A (2003). Secretion of interleukin-1beta by astrocytes mediates endothelin-1 and tumour necrosis factor-alpha effects on human brain microvascular endothelial cell permeability. *J Neurochem* **86:** 246–254.

Dobrogowska DH, Vorbrodt AW (2004). Immunogold localization of tight junctional proteins in normal and osmotically-affected rat blood-brain barrier. *J Mol Histol* **35:** 529–539.

Eugenin EA, Berman JW (2007). Gap junctions mediate human immunodeficiency virus-bystander killing in astrocytes. *J Neurosci* **27:** 12844–12850.

Eugenin EA, D'Aversa TG, Lopez L, Calderon TM, Berman JW (2003). MCP-1 (CCL2) protects human neurons and astrocytes from NMDA or HIV-tat-induced apoptosis. *J Neurochem* **85:** 1299–1311.

Eugenin EA, King JE, Nath A, Calderon TM, Zukin RS, Bennett MV, Berman JW (2007). HIV-tat induces formation of an LRP-PSD-95–NMDAR-nNOS complex that promotes apoptosis in neurons and astrocytes. *Proc Natl Acad Sci U S A* **104:** 3438–3443.

Eugenin EA, Osiecki K, Lopez L, Goldstein H, Calderon TM, Berman JW (2006). CCL2/monocyte chemoattractant protein-1 mediates enhanced transmigration of human immunodeficiency virus (HIV)-infected leukocytes across the blood-brain barrier: a potential mechanism of HIV-CNS invasion and NeuroAIDS. *J Neurosci* **26:** 1098–1106.

Fiala M, Looney DJ, Stins M, Way DD, Zhang L, Gan X, Chiappelli F, Schweitzer ES, Shapshak P, Weinand M, Graves MC, Witte M, Kim KS (1997). TNF-alpha opens a paracellular route for HIV-1 invasion across the blood-brain barrier. *Mol Med* **3:** 553–564.

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.

Furuse M, Hirase T, Itoh M, Nagafuchi A, Yonemura S, Tsukita S (1993). Occludin: a novel integral membrane protein localizing at tight junctions. *J Cell Biol* **123:** 1777–1788.

Gendelman HE, Ding S, Gong N, Liu J, Ramirez SH, Persidsky Y, Mosley RL, Wang T, Volsky DJ, Xiong H (2009). Monocyte chemotactic protein-1 regulates voltage-gated K+ channels and macrophage transmigration. *J Neuroimmune Pharmacol* **4**:47–59.

Graeber MB, Streit WJ, Buringer D, Sparks DL, Kreutzberg GW (1992). Ultrastructural location of major histocompatibility complex (MHC) class II positive perivascular cells in histologically normal human brain. *J Neuropathol Exp Neurol* **51:** 303–311.

Gumbiner BM (1993). Breaking through the tight junction barrier. *J Cell Biol* **123:** 1631–1633.

Hamm S, Dehouck B, Kraus J, Wolburg-Buchholz K, Wolburg H, Risau W, Cecchelli R, Engelhardt B, Dehouck MP (2004). Astrocyte mediated modulation of blood-brain barrier permeability does not correlate with a loss of tight junction proteins from the cellular contacts. *Cell Tissue Res* **315:** 157–166.

Haorah J, Heilman D, Knipe B, Chrastil J, Leibhart J, Ghorpade A, Miller DW, Persidsky Y (2005). Ethanol-induced activation of myosin light chain kinase leads to dysfunction of tight junctions and blood-brain barrier compromise. *Alcohol Clin Exp Res* **29:** 999–1009.

Hickey WF, Kimura H (1988). Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. *Science* **239:** 290–292.

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.

Hurtrel B, Chakrabarti L, Hurtrel M, Maire MA, Dormont D, Montagnier L (1991). Early SIV encephalopathy. *J Med Primatol* **20:** 159–166.

Igarashi Y, Utsumi H, Chiba H, Yamada-Sasamori Y, Tobioka H, Kamimura Y, Furuuchi K, Kokai Y, Nakagawa T, Mori M, Sawada N (1999). Glial cell line-derived neurotrophic factor induces barrier function of endothelial cells forming the blood-brain barrier. *Biochem Biophys Res Commun* **261:** 108–112.

Itoh M, Furuse M, Morita K, Kubota K, Saitou M, Tsukita S (1999). Direct binding of three tight junction-associated MAGUKs, ZO-1, ZO-2, and ZO-3, with the COOH termini of claudins. *J Cell Biol* **147:** 1351–1363.

Kanmogne GD, Primeaux C, Grammas P (2005). HIV-1 gp120 proteins alter tight junction protein expression and brain endothelial cell permeability: implications for the pathogenesis of HIV-associated dementia. *J Neuropathol Exp Neurol* **64:** 498–505.

Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M, Giuliani F, Arbour N, Becher B, Prat A (2007). Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat Med* **13:** 1173–1175.

Kelly RM, Strick PL (2000). Rabies as a transneuronal tracer of circuits in the central nervous system. *J Neurosci Methods* **103:** 63–71.

Kirby JE (2004). Anthrax lethal toxin induces human endothelial cell apoptosis. *Infect Immun* **72:** 430–439.

Klein RS, Williams KC, Alvarez-Hernandez X, Westmoreland S, Force T, Lackner AA, Luster AD (1999). Chemokine receptor expression and signaling in macaque and human fetal neurons and astrocytes: implications for the neuropathogenesis of AIDS. *J Immunol* **163:** 1636–1646.

Krum JM, Kenyon KL, Rosenstein JM (1997). Expression of blood-brain barrier characteristics following neuronal loss and astroglial damage after administration of anti-Thy-1 immunotoxin. *Exp Neurol* **146:** 33–45.

Lackner AA, Vogel P, Ramos RA, Kluge JD, Marthas M (1994). Early events in tissues during infection with pathogenic (SIVmac239) and nonpathogenic (SIVmac1A11) molecular clones of simian immunodeficiency virus. *Am J Pathol* **145:** 428–439.

Lamagna C, Meda P, Mandicourt G, Brown J, Gilbert RJ, Jones EY, Kiefer F, Ruga P, Imhof BA, Aurrand-Lions M (2005). Dual interaction of JAM-C with JAM-B and alpha(M)beta2 integrin: function in junctional complexes and leukocyte adhesion. *Mol Biol Cell* **16:** 4992–5003.

Lassmann H, Vass K, Brunner C, Wisniewski HM (1986). Peripheral nervous system lesions in experimental allergic encephalomyelitis. Ultrastructural distribution of T cells and Ia-antigen. *Acta Neuropathol* **69:** 193–204.

Lassmann H, Zimprich F, Vass K, Hickey WF (1991). Microglial cells are a component of the perivascular glia limitans. *J Neurosci Res* **28:** 236–243.

Lee BP, Imhof BA (2008). Lymphocyte transmigration in the brain: a new way of thinking. *Nat Immunol* **9:** 117–118.

LeVine SM, Goldman JE (1988). Embryonic divergence of oligodendrocyte and astrocyte lineages in developing rat cerebrum. *J Neurosci* **8:** 3992–4006.

Ley K, Laudanna C, Cybulsky MI, Nourshargh S (2007). Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol* **7:** 678–89.

Librizzi L, Mazzetti S, Pastori C, Frigerio S, Salmaggi A, Buccellati C, Di Gennaro A, Folco G, Vitellaro-Zuccarello L, de Curtis M (2006). Activation of cerebral endothelium is required for mononuclear cell recruitment in a novel in vitro model of brain inflammation. *Neuroscience* **137:** 1211–1219.

Lohmann C, Krischke M, Wegener J, Galla HJ (2004). Tyrosine phosphatase inhibition induces loss of blood-brain barrier integrity by matrix metalloproteinase-dependent and -independent pathways. *Brain Res* **995:** 184–196.

Luabeya MK, Dallasta LM, Achim CL, Pauza CD, Hamilton RL (2000). Blood-brain barrier disruption in simian immunodeficiency virus encephalitis. *Neuropathol Appl Neurobiol* **26:** 454–462.

MacLean AG, Belenchia GE, Bieniemy DN, Moroney-Rasmussen TA, Lackner AA (2005). Simian immunodeficiency virus disrupts extended lengths of the blood--brain barrier. *J Med Primatol* **34:** 237–242.

Marchesi VT, Gowans JL (1964). The migration of lymphocytes through the endothelium of venules in lymph nodes: an electron microscope study. *Proc R Soc Lond B Biol Sci* **159:** 283–290.

Matsumoto Y, Fujiwara M (1987). Absence of donor-type major histocompatibility complex class I antigen-bearing microglia in the rat central nervous system of radiation bone marrow chimeras. *J Neuroimmunol* **17:** 71–82.

McKenzie JA, Ridley AJ (2007). Roles of Rho/ROCK and MLCK in TNF-alpha-induced changes in endothelial morphology and permeability. *J Cell Physiol* **213:** 221–228.

Mon NN, Hasegawa H, Thant AA, Huang P, Tanimura Y, Senga T, Hamaguchi M (2006). A role for focal adhesion kinase signaling in tumor necrosis factor-alpha-dependent matrix metalloproteinase-9 production in a cholangiocarcinoma cell line, CCKS1. *Cancer Res* **66:** 6778–6784.

Morita K, Sasaki H, Furuse M, Tsukita S (1999). Endothelial claudin: claudin-5/TMVCF constitues tight junction strands in endothelial cells. *J Cell biol* **147:** 158–194.

Morrison LA, Sidman RL, Fields BN (1991). Direct spread of reovirus from the intestinal lumen to the central nervous system through vagal autonomic nerve fibers. *Proc Natl Acad Sci U S A* **88:** 3852–3856.

Narayan O, Wolinsky JS, Clements JE, Strandberg JD, Griffin DE, Cork LC (1982). Slow virus replication: the role of macrophages in the persistence and expression of visna viruses of sheep and goats. *J Gen Virol* **59:** 345–356.

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.

Nottet HS, Persidsky Y, Sasseville VG, Nukuna AN, Bock P, Zhai QH, Sharer LR, McComb RD, Swindells S, Soderland C, Gendelman HE (1996). Mechanisms for the transendothelial migration of HIV-1-infected monocytes into brain. *J Immunol* **156:** 1284–1295.

Ohtsuki S, Terasaki T (2007). Contribution of carrier-mediated transport systems to the blood-brain barrier as a supporting and protecting interface for the brain; importance for CNS drug discovery and development. *Pharm Res* **24:** 1745–1758.

Oldendorf WH (1973). Stereospecificity of blood-brain barrier permeability to amino acids. *Am J Physiol* **224:** 967–969.

Orandle MS, MacLean AG, Sasseville VG, Alvarez X, Lackner AA (2002a). Enhanced expression of proinflammatory cytokines in the central nervous system is associated with neuroinvasion by simian immunodeficiency virus and the development of encephalitis. *J Virol* **76:** 5797–5802.

Orandle MS, MacLean AG, Sasseville VG, Alvarez X, Lackner AA (2002b). Enhanced expression of proinflammatory cytokines in the central nervous system is associated with neuroinvasion by simian immunodeficiency virus and the development of encephalitis. *J Virol* **76:** 5797–5802.

Ostermann G, Weber KS, Zernecke A, Schroder A, Weber C (2002). JAM-1 is a ligand of the beta(2) integrin LFA-1 involved in transendothelial migration of leukocytes. *Nat Immunol* **3:** 151–158.

Ozaki H, Ishii K, Horiuchi H, Arai H, Kawamoto T, Okawa K, Iwamatsu A, Kita T (1999). Cutting edge: combined treatment of TNF-alpha and IFN-gamma causes redistribution of junctional adhesion molecule in human endothelial cells. *J Immunol* **163:** 553–557.

Peluso R, Haase A, Stowring L, Edwards M, Ventura P (1985). A Trojan Horse mechanism for the spread of visna virus in monocytes. *Virology* **147:** 231–236.

Persidsky Y, Ghorpade A, Rasmussen J, Limoges J, Liu XJ, Stins M, Fiala M, Way D, Kim KS, Witte MH, Weinand M, Carhart L, Gendelman HE (1999). Microglial and astrocyte chemokines regulate monocyte migration through the blood-brain barrier in human immunodeficiency virus-1 encephalitis. *Am J Pathol* **155:** 1599–1611.

Persidsky Y, Heilman D, Haorah J, Zelivyanskaya M, Persidsky R, Weber GA, Shimokawa H, Kaibuchi K, Ikezu T (2006). Rho-mediated regulation of tight junctions during monocyte migration across the blood-brain barrier in HIV-1 encephalitis (HIVE). *Blood* **107:** 4770–4780.

Price TO, Ercal N, Nakaoke R, Banks WA (2005). HIV-1 viral proteins gp120 and Tat induce oxidative stress in brain endothelial cells. *Brain Res* **1045:** 57–63.

Pu H, Tian J, Flora G, Lee YW, Nath A, Hennig B, Toborek M (2003). HIV-1 Tat protein upregulates inflammatory mediators and induces monocyte invasion into the brain. *Mol Cell Neurosci* **24:** 224–237.

Roberts ES, Masliah E, Fox HS (2004). CD163 identifies a unique population of ramified microglia in HIV encephalitis (HIVE). *J Neuropathol Exp Neurol* **63:** 1255–1264.

Rubin LL, Hall DE, Porter S, Barbu K, Cannon C, Horner HC, Janatpour M, Liaw CW, Manning K, Morales J, *et al.* (1991). A cell culture model of the blood-brain barrier. *J Cell Biol* **115:** 1725–1735.

Saitou M, Fujimoto K, Doi Y, Itoh M, Fujimoto T, Furuse M, Takano H, Noda T, Tsukita S (1998). Occludin-deficient embryonic stem cells can differentiate into polarized epithelial cells bearing tight junctions. *J Cell Biol* **141:** 397–408.

Saitou M, Furuse M, Sasaki H, Schulzke JD, Fromm M, Takano H, Noda T, Tsukita S (2000). Complex phenotype of mice lacking occludin, a component of tight junction strands. *Mol Biol Cell* **11:** 4131–4142.

Sasaki H, Matsui C, Furuse K, Mimori-Kiyosue Y, Furuse M, Tsukita S (2003). Dynamic behavior of paired claudin strands within apposing plasma membranes. *Proc Natl Acad Sci U S A* **100:** 3971–3976.

Sasseville VG, Newman W, Brodie SJ, Hesterberg P, Pauley D, Ringler DJ (1994). Monocyte adhesion to endothelium in simian immunodeficiency virus- induced AIDS encephalitis is mediated by vascular cell adhesion molecule-1/alpha 4 beta 1 integrin interactions. *Am J Pathol* **144:** 27–40.

Sasseville VG, Smith MM, Mackay CR, Pauley DR, Mansfield KG, Ringler DJ, Lackner AA (1996). Chemokine expression in simian immunodeficiency virus-induced AIDS encephalitis. *Am J Pathol* **149:** 1459–1467.

Schwaninger M, Sallmann S, Petersen N, Schneider A, Prinz S, Libermann TA, Spranger M (1999). Bradykinin induces interleukin-6 expression in astrocytes through activation of nuclear factor-kappaB. *J Neurochem* **73:** 1461–1466.

Sedgwick JD, Schwender S, Imrich H, Dorries R, Butcher GW, ter Meulen V (1991). Isolation and direct characterization of resident microglial cells from the normal and inflamed central nervous system. *Proc Natl Acad Sci U S A* **88:** 7438–7442.

Sharma DP, Zink MC, Anderson M, Adams R, Clements JE, Joag SV, Narayan O (1992). Derivation of neurotropic simian immunodeficiency virus from exclusively lymphocytetropic parental virus: pathogenesis of infection in macaques. *J Virol* **66:** 3550–3556.

Smith A, Bracke M, Leitinger B, Porter JC, Hogg N (2003). LFA-1-induced T cell migration on ICAM-1 involves regulation of MLCK-mediated attachment and ROCK-dependent detachment. *J Cell Sci* **116:** 3123–3133.

Stamatovic SM, Keep RF, Kunkel SL, Andjelkovic AV (2003). Potential role of MCP-1 in endothelial cell tight junction 'opening': signaling via Rho and Rho kinase. *J Cell Sci* **116:** 4615–4628.

Stamatovic SM, Shakui P, Keep RF, Moore BB, Kunkel SL, Van Rooijen N, Andjelkovic AV (2005). Monocyte chemoattractant protein-1 regulation of blood-brain barrier permeability. *J Cereb Blood Flow Metab* **25:** 593–606.

Strbian D, Durukan A, Pitkonen M, Marinkovic I, Tatlisumak E, Pedrono E, Abo-Ramadan U, Tatlisumak T (2008). The blood-brain barrier is continuously open for several weeks following transient focal cerebral ischemia. *Neuroscience* **153:** 175–181.

Streit WJ, Graeber MB (1993). Heterogeneity of microglial and perivascular cell populations: insights gained from the facial nucleus paradigm. *Glia* **7:** 68–74.

Toborek M, Lee YW, Flora G, Pu H, Andras IE, Wylegala E, Hennig B, Nath A (2005). Mechanisms of the blood-brain barrier disruption in HIV-1 infection. *Cell Mol Neurobiol* **25:** 181–199.

Toborek M, Lee YW, Pu H, Malecki A, Flora G, Garrido R, Hennig B, Bauer HC, Nath A (2003). HIV-Tat protein

induces oxidative and inflammatory pathways in brain endothelium. *J Neurochem* **84:** 169–179.
Town T, Nikolic V, Tan J (2005). The microglial ''activation'' continuum: from innate to adaptive responses. *J Neuroinflamm* **2:** 24.
Tunkel AR, Rosser SW, Hansen EJ, Scheld WM (1991). Blood-brain barrier alterations in bacterial meningitis: development of an in vitro model and observations on the effects of lipopolysaccharide. *In Vitro Cell Dev Biol* **27A:** 113–120.
Turksen K, Troy TC (2004). Barriers built on claudins. *J Cell Sci* **117:** 2435–2447.
Ulvestad E, Williams K, Bjerkvig R, Tiekotter K, Antel J, Matre R (1994a). Human microglial cells have phenotypic and functional characteristics in common with both macrophages and dendritic antigen-presenting cells. *J Leukoc Biol* **56:** 732–740.
Ulvestad E, Williams K, Mork S, Antel J, Nyland H (1994b). Phenotypic differences between human monocytes/macrophages and microglial cells studied in situ and in vitro. *J Neuropathol Exp Neurol* **53:** 492–501.
Unger ER, Sung JH, Manivel JC, Chenggis ML, Blazar BR, Krivit W (1993). Male donor-derived cells in the brains of female sex-mismatched bone marrow transplant recipients: a Y-chromosome specific in situ hybridization study. *J Neuropathol Exp Neurol* **52:** 460–470.
Van Itallie CM, Anderson JM (2006). Claudins and epithelial paracellular transport. *Annu Rev Physiol* **68:** 403–429.
Webb DJ, Donais K, Whitmore LA, Thomas SM, Turner CE, Parsons JT, Horwitz AF (2004). FAK-Src signalling through paxillin, ERK and MLCK regulates adhesion disassembly. *Nat Cell Biol* **6:** 154–161.
Weber C, Fraemohs L, Dejana E (2007). The role of junctional adhesion molecules in vascular inflammation. *Nat Rev Immunol* **7:** 467–477.
Weidenfeller C, Svendsen CN, Shusta EV (2007). Differentiating embryonic neural progenitor cells induce blood-brain barrier properties. *J Neurochem* **101:** 555–565.
Williams K, Alvarez X, Lackner AA (2001a). Central nervous system perivascular cells are immunoregulatory cells that connect the CNS with the peripheral immune system. *Glia* **36:** 156–164.
Williams K, Bar-Or A, Ulvestad E, Olivier A, Antel JP, Yong VW (1992). Biology of adult human microglia in culture: comparisons with peripheral blood monocytes and astrocytes. *J Neuropathol Exp Neurol* **51:** 538–549.
Williams KC, Corey S, Westmoreland SV, Pauley D, Knight H, deBakker C, Alvarez X, Lackner AA (2001b). 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:** 905–916.
Willis CL, Nolan CC, Reith SN, Lister T, Prior MJ, Guerin CJ, Mavroudis G, Ray DE (2004). Focal astrocyte loss is followed by microvascular damage, with subsequent repair of the blood-brain barrier in the apparent absence of direct astrocytic contact. *Glia* **45:** 325–337.
Xing HQ, Moritoyo T, Mori K, Sugimoto C, Ono F, Izumo S (2009). Expression of proinflammatory cytokines and its relationship with virus infection in the brain of macaques inoculated with macrophage-tropic simian immunodeficiency virus. *Neuropathol* **29:** 13–19.
Yeung D, Manias JL, Stewart DJ, Nag S (2008). Decreased junctional adhesion molecule-A expression during blood-brain barrier breakdown. *Acta Neuropathol* **115:** 635–642.
Yu AS, Enck AH, Lencer WI, Schneeberger EE (2003). Claudin-8 expression in Madin-Darby canine kidney cells augments the paracellular barrier to cation permeation. *J Biol Chem* **278:** 17350–17359.

This paper was first published online on iFirst on 22 March 2009.