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AIDS and the blood-brain barrier

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Abstract

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.

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 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. The TJs are composed of fibril networks of transmembrane homo- and hetero-dimeric 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,

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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.

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 pre-natal 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 co-culture 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 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; Ulvestad *et al*, 1994b; 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 which 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 (TJ) 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 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; Saitou *et al*, 2000).

The highly conserved claudin family of cell adhesion molecules was first isolated from the TJs of the BBB in 1998 (Furuse *et al*, 1998). While 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 hetero- dimeric 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 while 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 transmigration 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 occludens -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. *E. coli*, *S. pneumoniae*, *B. anthracis*) employ nonspecific toxin-mediated inflammation to directly breach BMECs (Berpohl *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 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 non-infected monocytes (Persidsky *et al*, 1999).

Interactions between monocytes in blood and the BMEC result in activation of integrin receptors and 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 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 IL-1 and TNF- α) on the abluminal side, upregulate 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, α 1 β 2 integrin) and very late antigen-4 (VLA-4, α 4 β 1 integrin) on the monocyte with their ligands including immunoglobulin superfamily members ICAM-1 (CD54) and VCAM-1 (CD106) on the luminal side of BMECs. Both ICAM-1 and VCAM-1 can be upregulated in response to inflammation and VCAM-1 has been shown to be rapidly upregulated 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 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 upregulated 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. While 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 were 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 (re patterning) of occludin have been observed in the brain of SIV-infected macaques and correlated with events leading to microvascular leakage (Luabeya *et al*, 2000).

While the presence of pro-inflammatory 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 like TNF- α (Orandle *et al*, 2002a), CCL2 (MCP-1) (Eugenin *et al*, 2003), CCL3 (MIP-1 α), CCL4 (MIP-1 β), 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 lab 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 (Ananthbandhu *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). Since 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; Andras *et al*, 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 claudins -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 co-receptors including CCR5 and CXCR4 immediately *ex vivo* which 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 pro-inflammatory cytokines in proximity to microvessels when infected or activated by SIV/HIV. The release of cytokines such as IL-1 β , 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 Tat₁₋₇₂ 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 TEER 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. While 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 diapedesis however the relative contribution of each pathway is unknown. While 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 BMEC or the action of inflammatory mediators and chemoattractants that facilitate leukocyte recruitment to the CNS appear promising avenues to further our understanding of HIV/SIV neuroinvasion and the role of the BBB in neuroAIDS.

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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.

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 upregulate 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.

HIV-Mediated signal transduction at the blood-brain barrier in BMECs

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 pro-inflammatory 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, occludin 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.