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Review

Interactions between macrophages and brain microvascular endothelial cells: role in pathogenesis of HIV-1 infection and blood–brain barrier function

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Monocytes have been shown to infiltrate in brain tissue during various neurological disorders including AIDS dementia complex. The presence of an excess of activated macrophages in brain tissue is accompanied by tissue damage resulting in a loss in neuronal function and viability. Therapeutic options against such neurological disorders could therefore be aimed at the prevention of monocyte infiltration across the blood–brain barrier. Therefore, a better understanding of these processes is needed. Recent insights in cellular processes between monocytes/macrophages and brain microvascular endothelial cells in the neuropathogenesis of HIV-1 infection demonstrate that monocytes roll on endothelial cells via the inducible endothelial adhesion molecule E-selectin. Binding of these cells are mainly mediated via the endothelial adhesion molecule vascular cell adhesion molecule-1. The transmigration through the blood–brain barrier is facilitated by both endothelial and monocyte/macrophage-derived nitric oxide and by the increased production of gelatinase B activity by HIV-infected monocytes/macrophages. Chemokines produced within the brain regulate the traffic of the infiltrating monocytes through the brain parenchyma. In addition, endothelial cells also produce monocyte attracting chemokines during their first interactions with HIV-infected monocytes/macrophages thus promoting additional influx of phagocytes into the brain. Furthermore, excessive infiltration of monocytes is accompanied by endothelial damage resulting in the loss of tight junctions. Thus, in toto, brain microvascular endothelial cells might contribute to the neuropathogenesis of HIV-1 infection.

Keywords: macrophages; blood–brain barrier; endothelial cells; HIV-1

Introduction

The brain can be viewed as an intensive network of cellular interactions between neurons, astrocytes, oligodendroglia and microvascular endothelial cells. The task of these cells are well defined in this network and are aimed at the creation and maintenance of optimal neuronal functioning. The neurons in the brain coordinate complex human processes such as learning and memory, behavior, language, and movement. Oligodendroglia produce the myelin sheet that is surrounding the axons of the neurons and thereby allowing electrical signaling between and within the neurons. Astrocytes create and maintain the extracellular milieu that is needed for optimal neuronal functioning, occupy lost space in the CNS after neuronal death, and, in contrast to other brain cells, even signal to neurons (Norenberg, 1994; Nedergaard, 1994; Parpura *et al*, 1994). Brain microvascular endothelial cells (BMEC) are the major component of the blood–brain barrier (BBB) and thus contribute to the maintenance of a stable extracellular milieu for the neurons. Microglia, the resident brain macrophages, form the defence against infectious diseases and remove dead neurons. Brain cells have a neuroectodermal origin, with the exception of microglia. Although the latter is still a matter of

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debate, the majority of studies suggest that microglia are derived from blood monocytes (Ling and Wong, 1993).

Whether or not monocytes are the precursor cells for microglia, an excess of infiltrating monocytes into the central nervous system (CNS) seems in the long term not beneficial for the brain. For instance, monocytes and/or macrophages play a pathological role in stroke, trauma and neurological diseases such as multiple sclerosis (Li *et al*, 1993), Parkinson's disease (McGeer *et al*, 1988a), Huntington's disease (McGeer *et al*, 1988b), Creutzfeldt-Jakob disease (Muhleisen *et al*, 1995) and AIDS dementia complex (Nottet and Gendelman, 1995a). In addition, microglial activation very likely also contributes to the neurological manifestations of Alzheimer's disease (Smits *et al*, 1999; van Muiswinkel *et al*, 1999). For these reasons, it is important to understand the cellular processes that underlie the mechanisms of monocyte trafficking into the CNS during physiological and especially during pathophysiological circumstances. This review will be focused on mechanisms of monocyte infiltration in AIDS dementia complex.

AIDS dementia complex is the most severe neurological manifestation of HIV infection of the brain. In the era before treatment with anti-HIV compounds 30% of the adults with AIDS and 50% of the pediatric AIDS cases were affected by HIV-induced neurological complications in the Western world. Interestingly, in the adult brain HIV only productively replicates in macrophages/microglia and not in neurons, astrocytes, oligodendrocytes and BMEC (Koenig *et al*, 1986; Wiley *et al*, 1986; Vazeux *et al*, 1987). Neuropathological features of HIV-1 encephalitis include productive HIV-1 infection of brain cells of monocyte/macrophage lineage, multinucleated giant cell formation, macrophage infiltration of the CNS, astrogliosis, and myelin pallor. Some neurons undergo dendritic pruning, simplification of synaptic contacts, and frank cell loss (Masliah *et al*, 1992). These neuronal changes are clinically manifested by cognitive and motor dysfunctions that affect many individuals with AIDS. The finding that neurological symptoms are a consequence of dendritic injury and synaptic loss (Masliah *et al*, 1997), and occur in absence of neuronal cell loss (Seilhean *et al*, 1993), suggest that treatment may retard the onset of cognitive and motor defects.

The blood–brain barrier

Characteristics of the blood–brain barrier

The BBB prevents the free diffusion of circulating molecules and cells into brain interstitial space. Electron microscopic studies show that the cardinal morphologic feature of the barrier is the presence of epithelial-like, high-resistance tight junctions that fuse brain capillary endothelia together into a continuous cellular layer separating blood and brain interstitial space (Pardridge, 1983). These junctions are quite tightly sealed, and therefore exchange between blood plasma and brain must pass through the two membranes and the cell cytoplasm of the brain endothelium. A scarcity of fenestrations and vesicular traffic in brain capillary endothelia furthermore restricts free flow between brain interstitium and blood.

The functional existence of a BBB is demonstrated by the restricted movement of ions from the blood to brain, the limited ability of macromolecules and polar organic molecules to cross the barrier, and the presence of very high electrical resistance across brain microvessels (Pardridge, 1983). In contrast, molecules smaller than about 30 kd cross capillaries in nonneural tissues freely. Rather than being determined solely by molecular size, their migration through the BBB is highly dependent on their chemical characteristics. Despite the barrier created by the continuous tight junctions and very low rate of pinocytosis of the endothelial cells, certain polar molecules rapidly cross the papillary wall. Indeed, the BBB has several saturable transport systems that present the brain with nutrients at a relative constant rate in the presence of shifting blood plasma levels (Goldstein and Betz, 1983). Movement of D-glucose, in contrast to L-glucose, and the essential large neutral L-amino acids are mediated by such carrier-mediated processes. Specific transport systems that mediate the sequential endocytosis at the luminal border of the barrier and exocytosis at the abluminal border have also been reported (Pardridge *et al*, 1985). In addition, undirectional transport across the BBB takes place due to asymmetric distribution of membrane-bound carriers. Furthermore, substrate conversing actions of cytoplasmic enzymes regulate entry into the brain interstitium. For instance, the alanine transporter has a polarized distribution with preferential localization to the abluminal surface (Betz and Goldstein, 1987). Thus, although the term 'blood–brain barrier' implies a general impermeability, the barrier is best considered as selectively permeable. It optimizes the brain interstitial space as an ultrastable subcompartment of the general extracellular fluid, thus creating a neuronal environment that allows optimal brain function.

Formation of the blood–brain barrier characteristics

The functional difference between brain capillary endothelia and capillary endothelia in other organs is likely orchestrated by factors secreted by the brain itself. The vasculature of the brain is derived from non-barrier vessels of mesodermal origin. Soon after these vessels come in contract with neural tissue their phenotype changes and as the development of the brain progresses, the vessels begin to express BBB characteristics (Stewart and

Wiley, 1981). The sharing of the abluminal basement membrane between brain endothelium and astrocytes or their foot processes (or both) in the mature brain suggest that astrocytes plan an important role in the induction and/or maintenance of the BBB. Indeed, the extensive tight junctions characteristic of the BBB are lost with increasing passages of these endothelial cells in culture but are rapidly restored on exposure of the cells to media conditioned by primary astrocytes (Arthur *et al*, 1987). Similarly, capillaries of non-neural origin become highly impermeable and exhibit extensive tight junctions after exposure to cultured astrocytes (Janzer and Raff, 1987). Other important barrier features in endothelial cells that are induced or maintained by astrocytes include the increased expression and/or polarized distribution of enzymes and transport systems (DeBault and Cancilla, 1980). In addition to endothelial damage, malfunctioning of astrocytes during disease can, therefore, also result in loss of BBB characteristics and create an imbalance in the homeostatic microenvironment for neurons.

Mononuclear phagocyte traffic across the blood–brain barrier

The BBB is present during embryogenesis in the mouse, but is more permeable to solutes than during the postnatal period. Shortly after birth, there is a very rapid maturation of the junctions, such that they attain their adult characteristics (Perry *et al*, 1997). It has been shown that monocytes can enter the developing brain not only when the BBB is immature, but also during this early postnatal period. The use of rat bone-marrow chimeras has served as a tool to study the relative rates of replacement and turnover of the different macrophage populations of the brain. In rats, the macrophages in the meninges and the perivascular macrophages have a relatively rapid turnover, while the microglial population is maintained by local division as well as by influx of circulating monocytes (Hickey *et al*, 1992; Lawson *et al*, 1992). These experiments show that only a small number of microglia are derived from the bone marrow over a period of several months, suggesting that the BBB plays a significant role in regulating their turnover and traffic. However, during the same period, large numbers of perivascular macrophages are donor derived, indicating that the BBB is not a major impediment to mononuclear phagocyte traffic (Perry *et al*, 1997). Although it is in general known that leucocytes first roll on the vessel wall, followed by their firm adhesion and diapedis, little is known about the molecular mechanisms that support leukocyte traffic across the BBB as part of normal physiology. However, molecular mechanisms that allow excessive macrophage infiltration into the brain during the pathophysiological response to HIV-1 infection have been studied in detail.

The blood–brain barrier during HIV-1 infection

HIV-1 infection of the brain

HIV-1 is a haematogenously spread virus that most likely gains entry into the brain within blood-derived macrophages (Nottet and Gendelman, 1995a). Indeed, HIV-1 is selectively localized within perivascular and infiltrated parenchymal blood-derived macrophages and microglia (Koenig *et al*, 1986; Wiley *et al*, 1986; Vazeux *et al*, 1987). Axonal spread of HIV as a mechanism to enter the CNS is ruled out, since direct neuronal infection has not been demonstrated in pathologic specimens of AIDS patients with HIV-1 encephalitis. Theoretically, cell-free HIV might infect brain microvascular endothelial cells and subsequent release of virus at the abluminal membrane of the BBB would result in early HIV entry into the brain parenchyma. Although direct *in-vitro* infection of brain endothelium by lymphotropic HIV-1 has been described, the relevance of that finding remains questionable (Moses *et al*, 1993). First, macrophagetropic HIV-1 is the predominant viral phenotype isolated during the acute seroconversion reaction, thus excluding a role for lymphotropic HIV-1 in early CNS infiltration (Zhu *et al*, 1993). Second, brain-derived viral strains are macrophagetropic rather than lymphotropic (Sharpless *et al*, 1992; Korber *et al*, 1994). Third, there is no *in- vivo* evidence for HIV infection of brain endothelial cells (Koenig *et al*, 1986; Wiley *et al*, 1986; Vazeux *et al*, 1987). In addition, several other investigators could find no *in-vitro* evidence for permissiveness of these cells to either lymphotropic or macrophagetropic HIV-1 (Gilles *et al*, 1995; Poland *et al*, 1995; Nottet *et al*, 1996). These findings and the ample evidence that only blood-derived macrophages and microglia support productive viral replication within the CNS suggest that HIV-1 gains entry into the brain within macrophages (Nottet and Dhawan, 1998).

The occurrence of aseptic meningitis during the acute seroconversion reaction in HIV-infected individuals provides clinical support for the haematogenously spread of HIV-1 to the CNS (Hollander and Stringari, 1987). Detection of virus and anti-HIV antibodies in CSF of individuals during acute HIV-1 infection suggest that early viral penetration of the CNS might underlie this neurologic complication (Ho *et al*, 1985; Goudsmit *et al*, 1986). That early entry might also involve the brain parenchyma is supported anecdotally (Davis *et al*, 1992). A case was reported on an individual who was mistakenly injected with HIV-1-infected white blood cells and in whom infected cells were identified in *post-mortem* brain days later (Davis *et al*, 1992). In addition, studies of the time course of simian immunodeficiency virus infection indicate that virus enters the brain early in the course of disease (Chakrabarti *et al*, 1991; Sharer *et al*, 1991).

Despite this early viral invasion of the CNS, neurologic manifestations of cognitive and motor impairment occur relatively late in disease. The latter might be indicative of suppressive factors, for instance produced by astrocytes (Nottet *et al*, 1995b), endogenously present within the CNS that either prevents rapid spread of HIV through the CNS and/or prevents neurotoxin formation by HIV-infected macrophages (Nottet *et al*, 1997). In addition, a unique subset of monocytes in demented AIDS patients as compared to non-demented AIDS patients has been described (Pulliam *et al*, 1997). Supernatants from these cells were found to be toxic for human brain cells. These cells might enter the brain during the later stages of HIV-1 infection, expose neural cells to toxic factors, and cause dementia (Pulliam *et al*, 1997).

Mechanisms of early brain infiltration by HIV-infected macrophages

As mentioned before, the first step in the transmigration of leukocytes into tissue involves the rolling on the blood vessel wall. This rolling is mediated by the inducible endothelial adhesion molecule E-selectin and by sialylated and sulfated lactosamine oligosaccharides that are present on monocytes (Varki, 1994). Importantly, *in-vitro* HIV-infected monocytes/macrophages (M/M) induce E-selectin expression on BMEC thereby thus facilitating the rolling of these virus-infected cells on brain endothelium. In addition, the expression of endothelial E-selectin in brain tissue of demented AIDS patients was dramatically increased as compared to non-demented AIDS patients (Nottet *et al*, 1996). Since recombinant tat induces E-selectin expression on other endothelial cells, the induction of this adhesion molecule is most likely mediated by the HIV-1 tat molecule that is secreted by HIV-infected M/M (Hofman *et al*, 1993). Furthermore, HIV-1 infection of M/M induces the production of low levels of nitric oxide (NO), a potent vasoactive molecule (Bukrinsky *et al*, 1995; Boven *et al*, 1999a). Since NO can slow down the speed of blood cells through the blood vessel the change that rolling on endothelium via E-selectin might increase (Figure 1). Furthermore, treatment of monocytes with tat protein increased their adhesion to endothelial cell monolayers, and adhesion was

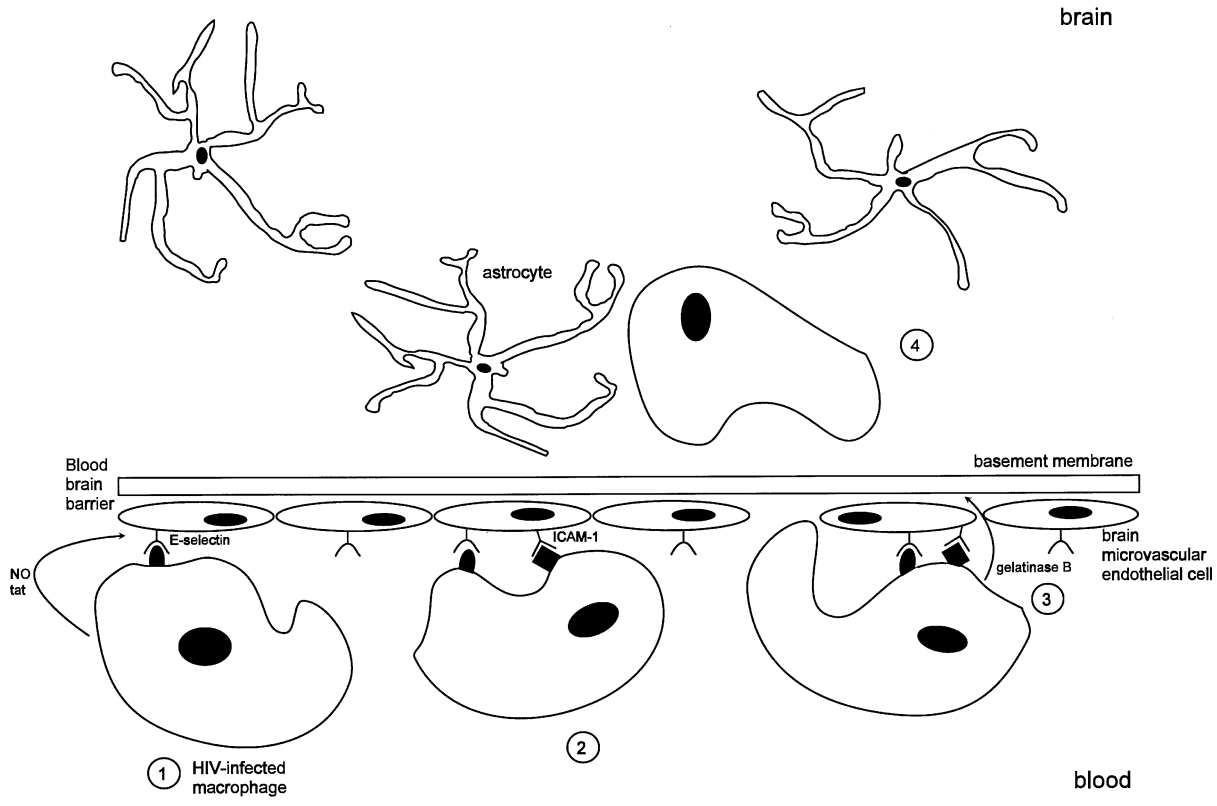

Figure 1 Mechanisms for early entry into brain. (1) HIV-infected monocytes/macrophages (M/M) selectively induce endothelial expression of E-selectin, most likely by a tat-dependent mechanism, that mediates their rolling on brain endothelium. The change that these cells indeed roll on brain endothelium via E-selectin might increase due to the secretion of NO, a potent vasodilator, by HIV-infected macrophages. (2) Their binding is mediated via ICAM-1 molecules on the BMEC. (3) In addition, HIV-induced gelatinase B activity results in degradation of abluminal extracellular matrix proteins. (4) These molecular events eventually allow M/M infiltration into the brain.

inhibited by inclusion of anti-β2 and anti-ICAM-1 antibodies (Lafrenie *et al*, 1996). The HIV-induced upregulation of adhesive receptors was associated with enhanced binding of monocytes to brain endothelium (Dhawan *et al*, 1992). These findings suggest that HIV-infected M/M, in addition to an advantage in rolling on the blood vessel wall, have also an advantage in the subsequent step in the process of extravasation into the brain, namely the binding to brain endothelium (Figure 1). Furthermore, HIV-infected M/M also produce higher levels of gelatinase B as compared to uninfected M/M. These cells therefore have an advantage in the process of diapedesis since gelatinase B degrades the extracellular matrix proteins that underlie the endothelial cell monolayer and in such a way increases the permeability of the BBB (Dhawan *et al*, 1992).

These HIV-induced alterations in M/M effector functions might underlie the early viral penetration of the CNS during the acute HIV-1 infection (Figure 1) and explain how HIV-infected macrophages can serve as viral reservoirs inside tissue compartments (Gendelman *et al*, 1989). Although the early transmigration of HIV-infected M/M into the CNS might result in meningitis, the occurrence of severe neurological symptoms including dementia occurs relatively late. In these stages the immune system is deteriorated and activated and as a consequence the mechanisms involved in the brain infiltration by HIV-infected M/M are much more complex as outlined hereafter.

The blood–brain barrier during AIDS dementia complex

Rolling and binding of HIV-infected macrophages
During the later symptomatic stages of HIV-1 infection elevated levels of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) have been detected in serum of AIDS patients and in macrophages isolated from HIV-infected individuals (Lahdevirta *et al*, 1988; Buhl *et al*, 1993). Both cytokines are mediators of meningitis and regulate BBB permeability in a reversible manner. Indeed, intracisternal injection of TNF-α and IL-1β into rats increased BBB permeability for systemically administered radioactive tracers (Quagliarello *et al*, 1991). In addition, these cytokines induce the expression of endothelial adhesion molecules that facilitate rolling and binding of leukocytes. Indeed, activated HIV-infected M/M induce the highest levels of E-selectin and vascular cell adhesion molecule-1 (VCAM-1) on brain microvascular endothelial cells (Nottet *et al*, 1996) as compared to unactivated HIV-infected cells and activated uninfected M/M. Since E-selectin mediates rolling and VCAM-1 mediates binding of leukocytes to endothelium, HIV-infected cells have an advantage in these processes when compared to normal M/M (Figure 2). The *in-vitro* studies are supported by the observation that endothelial expression of E-selectin, and to a lesser degree VCAM-1, was the highest in brain tissue of demented AIDS patients when compared to non-demented AIDS patients. In addition, monocyte binding to encephalitic brain could be prevented by blocking monoclonal antibodies directed against both adhesion molecules (Nottet *et al*, 1996). Cocultivation of HIV-infected M/M with BMEC resulted in an increased permeability of the endothelium, measured as ^{125}I-albumin passage through endothelial cell monolayers in transwell systems (Dhawan *et al*, 1995). In addition, immune-activated M/M placed on BMEC cultured on the upper chamber of a transwell migrated in larger numbers through the artificial BBB than unactivated cells (Persidsky *et al*, 1997).

Transmigration through the blood–brain barrier
However, besides these subtle changes between M/M and endothelial cells there is also evidence of endothelial damage that might underlie the excessive monocyte infiltration into the brain and even contribute to the severe stages of AIDS dementia complex. For instance, *in-vitro* HIV-1 infection of M/M resulted in superoxide anion production (Boven *et al*, 1999a) and endothelial NO synthase was induced in cocultures of M/M and endothelial cells (Boven *et al*, unpublished data). Since superoxide anion and NO form the highly toxic molecule peroxynitrite, immunohistochemical staining for nitrotyrosine, the footprint of peroxynitrite, was performed and showed extensive immunoreactivity in perivascular areas of brain tissue of demented AIDS patients. Furthermore, the architecture of many blood vessels was damaged as shown by immunohistochemical staining for zonula occludens-1, a tight junction protein (Boven *et al*, submitted). Thus, during the intimate contact with HIV-infected M/M endothelial cells may contribute to their own damage by the production of NO. These findings might explain the increased permeability of artificial BBB for radioactive albumin when put in contact with HIV-infected M/M (Dhawan *et al*, 1995).

After their migration through the endothelial cell layer, the monocytes will encounter the basement membrane that surrounds the albuminal side of the BBB. As described above, the capacity of HIV-infected M/M to digest and invade basement membrane gel matrix was significantly greater than that of uninfected control cells. Interestingly, incubation of HIV-infected M/M with tissue inhibitor of metalloproteinase-1 (TIMP-1) and TIMP-2 partially inhibited the increased permeability of endothelial cell monolayers to ^{125}I-albumin. These proteinase inhibitors antagonize the effects of matrix metalloproteinases, such as the HIV-induced gelatinase B activity (Dhawan *et al*, 1992). Im-

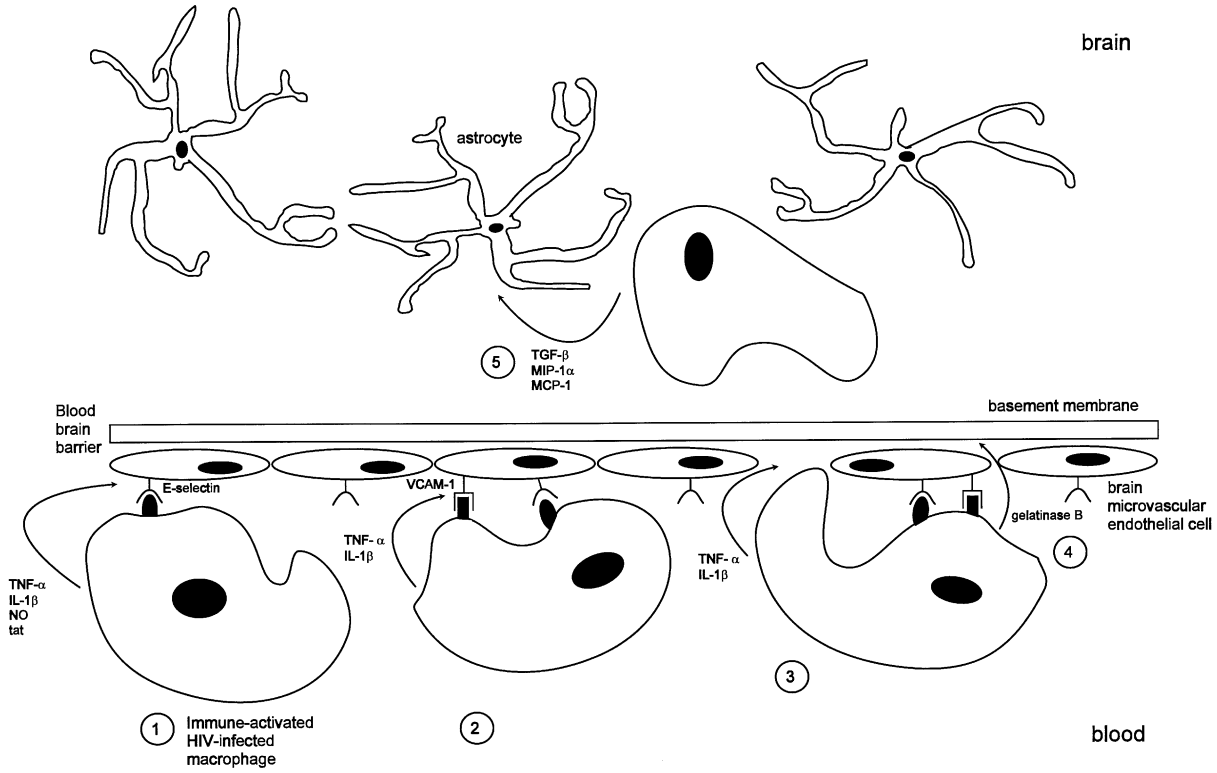

Figure 2 Mechanisms for HIV-1 entry into brain during neurologic disease. (1) During the later symptomatic stages of HIV-1 infection elevated levels of proinflammatory cytokines such as TNF-α and IL-1β are secreted by perivascular HIV-1-infected M/M. Both TNF-α, IL-1β and HIV-1 tat induce the expression of E-selectin on BMEC. The change that these cells indeed roll on brain endothelium via E-selectin might increase due to the secretion of NO, a potent vasodilator, by HIV-infected macrophages. E-selectin then mediates rolling of HIV-infected M/M on the vessel wall. (2) TNF-α and IL-1β also induce the expression of VCAM-1 on BMEC. The induction of this adhesion molecule allows binding of HIV-infected M/M to brain endothelium. (3) The overexpression of the proinflammatory mediators TNF-α and IL-1β, by HIV-infected M/M will result in an increase in blood–brain barrier (BBB) permeability. (4) The overexpression of gelatinase B by HIV-infected M/M will result in degradation of abluminal extracellular matrix proteins. All these factors will lead to the transmigration of HIV-infected M/M across the BBB. (5) Intercellular interactions between astrocytes and infiltrated HIV-infected M/M within the brain parenchyma will result in the induction of several chemokines, TGF-β, MIP-1α and MCP-1. These chemokines will attract more HIV-infected and uninfected M/M into the brain parenchyma resulting in an expansion of the viral load within the CNS.

portantly, matrix metalloproteinase-9 activity in the cerebrospinal fluid of HIV-infected patients was significantly higher in patients with neurological deficits than in patients without neurological deficits (Sporer *et al*, 1998). This finding suggest that, in addition to the effects of HIV-infected M/M on endothelial cells, interactions between HIV-infected M/M and the extracellular matrix also affect BBB permeability (Figure 2).

Migration through the brain parenchyma

The distribution of infected macrophages in brain is archetypal, with viral predilection for cerebral white matter, deep gray matter (basal ganglia and thalamus), and the brainstem. Although the exact mechanisms that underlie this distribution remain unknown, temporal expression of adhesion molecules and chemokines in the brain has been suggested to guide specific trafficking of HIV-infected M/M to these brain areas (Nottet *et al*, 1997). In any event, chemoattractants have been detected in brain tissue of AIDS patients with HIV-1 encephalitis, and might, therefore, play a role both in infiltration and subsequent migration of HIV-infected M/M into brain. For instance, transforming growth factor-β (TGF-β), a cytokine that induces the migration of monocytes at femtomolar concentrations, was clearly identified within AIDS brain tissues in association with macrophages and astrocytes, but not in control brain tissue (Wahl *et al*, 1991). More recently, the expression of the β chemokine peptides macrophage inflammatory protein-(MIP-1α) and MIP-1β was found to be elevated in brain tissue of demented AIDS patients as compared to non-demented AIDS patients (Schmidtmayerova *et al*, 1996; Sanders *et al*, 1998). Cells expressing these chemokines were identified morphologically as brain macrophages and astrocytes. Interestingly, MIP-1β levels were much more prevalent that the MIP-1α levels (Schmidtmayerova *et al*, 1996; Sanders *et al*, 1998). Since the monocyte chemotactic activity of

MIP-1α is much more potent than that of MIP-1β, the elevated levels of MIP-1α in HIV-infected brain tissue might result in further monocyte infiltration of brain tissue and subsequent spread of viral CNS infection. In addition, monocyte chemoattractant protein-1 (MCP-1), another chemokine with relatively selective chemoattractant properties for monocytes, was found to be elevated in astrocytes in brain tissue of demented AIDS patients (Conant *et al*, 1998; Sanders *et al*, 1998 (Figure 2)). Indeed, using a coculture of human endothelial cells and astrocytes to model the BBB, it was recently demonstrated that astrocyte derived MCP-1 directs the transmigration of monocytes (Weiss *et al*, 1998). And, finally, microglial expression of RANTES was found to be elevated in brain tissue of demented AIDS patients as compared to non-demented AIDS patients (Sanders *et al*, 1998).

Interestingly, endothelial cells themselves might also play a role in the recruitment of monocytes into the CNS by the production of chemokines. For instance, these cells elicit the production of several chemokines by uninfected as well as HIV-infected M/M (Boven *et al*, submitted). The levels produced by the HIV-infected cocultures are significantly higher than the levels produced by the uninfected cocultures. Importantly, endothelial expression of MCP-1 was readily detected in brain tissue of AIDS patients with AIDS dementia complex when compared to tissue of non-demented AIDS patients (Boven *et al*, submitted).

Treatment modalities for ADC

Anti-retroviral therapy

Several *in-vitro* studies have shown that antiretroviral drugs targeted at HIV-1 reverse transcriptase can prevent viral infection of macrophages (Perno *et al*, 1992). However, the effectiveness of these drugs to inhibit viral replication in macrophages *in vivo* might be hampered by the localization of these cells in the CNS and the emergence of drug-resistant viral strains. For instance, zidovudine, in sufficient doses, can produce clinical improvement in mental function, at least transiently, for weeks to months following its administration (Sidtis *et al*, 1993). However, didanosine does not reduce the incidence of neurological impairments among adult AIDS patients (Portegies *et al*, 1994), presumably because it does not enter the CNS (Balis *et al*, 1992). However, despite relatively poor CNS penetration, didanosine has been shown to be beneficial in children with neurological dysfunction (Butler *et al*, 1991). Besides, antiretroviral monotherapy has resulted in the emergence of resistant HIV-1, in some cases even after 3 weeks of therapy (Wei *et al*, 1995). Current and ongoing highly active antiretroviral therapy (HAART) drug studies show that the viral load in plasma of HIV-infected individuals can be decreased to very low and even undetectable levels, resulting in an increased expected life time after HIV-1 infection. However, if one or more of these drugs does not enter the CNS the incidence of HIV-associated cognitive/motor complex might increase among HIV-infected individuals. The latter should be taken seriously as illustrated by a recent study that showed that the HIV-1 protease inhibitors ritonavir, saquinavir, and indinavir, are substrates of the human multidrug transporter (or P glycoprotein) that is present in large quantities of BMEC (Lee *et al*, 1998). Indeed, studies using knockout mice deficient for this transporter molecule revealed that P glycoprotein limits the oral bioavailability and penetration of this class of anti-HIV drugs (Kim *et al*, 1998). Since protease inhibitors are part of all new triple anti-HIV therapy treatment regimens these data suggest that at least one of the drugs will poorly penetrate into the brain and therefore limit therapeutic efficacy in CNS infection.

Anti-inflammatory treatment

Therapeutic strategies aimed at preventing CNS dysfunction might be an effective adjunct for future therapy. The use of factors that promote neuronal survival, such as nerve growth factor, as suggested for Alzheimer's disease is most likely not the way to go as the endogenous production of these factors have been found to be elevated in brain tissue of demented AIDS patients (Soontornniyomkij *et al*, 1998; Boven *et al*, 1999b). Instead, these strategies should be directed against the instigator of HIV-induced neurological disease, the macrophage/microglia. To reach these goals one should prevent immune activation of these cells and prevent excessive monocyte infiltration of the brain. As discussed in the introduction, macrophage activation occurs in a variety of neurological disorders including Alzheimer's disease. Importantly, clinical and epidemiological studies not only argue for a detrimental role of inflammatory processes in the pathogenesis of Alzheimer's disease but also illustrate that anti-inflammatory drugs might retard the onset of this neurological disease (Rogers *et al*, 1993; Breitner *et al*, 1995; Breitner, 1996). This holds tremendous potential for the development and clinical use of drugs that are specifically targeted against individual or classes of HIV-induced macrophage-derived neurotoxins. Indeed, a recent study reported on the successful treatment of HIV-associated dementia using a combination of HAART and ibuprofen in a HIV-infected individual (Gendelman *et al*, 1998). In addition, treatment with anti-oxidants such as vitamin E, as has been suggested for Alzheimer's disease, might be worth pursuing since markers indicative of oxidative stress have very recently been detected in brain tissue of demented AIDS patients (Bukrinsky *et al*, 1995; Boven *et al*, 1999a). Indeed, a small clinical trial with a lipophilic antioxidant that acts to

scavenge superoxide anion radicals has recently been conducted in demented AIDS patients resulting in trends toward improvement in the cognitive tests scores (Kieburtz *et al*, 1997). As discussed above, endothelial and astrocytic MCP-1 expression mediate brain monocyte infiltration in other CNS disorders (Takeshima *et al*, 1994; Sato *et al*, 1995; Kim *et al*, 1995). Since high-dose methylprednisolone treatment reduced both endothelial MCP-1 expression and monocyte infiltration in the ischemic brain (Kim *et al*, 1995), and since abundant endothelial MCP-1 expression has been detected in cocultures of HIV-infected M/M and BMEC and in brain tissue of demented AIDS patients (Boven *et al*, unpublished data), such treatment might make sense in the context of AIDS dementia complex. Although clinical trials with the steroid prednisone are being conducted in Alzheimer patients, a recent study with the steroid dexamethasone in an animal model for HIV-1 encephalitis showed adverse effects of steroid treatment (Limoges *et al*, 1997).

Conclusions

HIV-1 enters the brain in a heamatogenously fashion early during HIV-1 infection. It gains entry via the 'Trojan horse' model by modulating both monocyte/macrophage and endothelial functions. HIV takes advantage of cellular machineries involved in rolling and binding processes between both cells and also increases the ability for monocytes to transmigrate into the CNS. Clinical signs are aseptic meningitis but these signs disappear soon after seroconversion. Apparently the brain is able to limit extensive HIV-1 replication within the CNS or is able to suppress inflammatory processes. However, when the immune system is deteriorated, neurological complications start to occur and will eventually lead to dementia. During these events macrophages are immune-activated leading to enhanced production of molecules that increase BBB permeability, endothelial adhesion molecules expression, and chemokine expression. Eventually, when there is an excess of infiltrating monocytes into the brain the BBB is damaged with concomitant loss of tight junctions. At these stages, the BMEC might, in addition to the HIV-infected monocyte/macrophages, also function as instigators of the BBB loss and also contribute to the neurological complications.

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