

Neuroimmunity and the Blood–Brain Barrier: Molecular Regulation of Leukocyte Transmigration and Viral Entry into the Nervous System with a Focus on NeuroAIDS

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Abstract HIV infection of the central nervous system (CNS) can result in neurologic dysfunction with devastating consequences in a significant number of individuals with AIDS. Two main CNS complications in individuals with HIV are encephalitis and dementia, which are characterized by leukocyte infiltration into the CNS, microglia activation, aberrant chemokine expression, blood–brain barrier (BBB) disruption, and eventual damage and/or loss of neurons. One of the major mediators of NeuroAIDS is the transmigration of HIV-infected leukocytes across the BBB into the CNS. This review summarizes new key findings that support a critical role of the BBB in regulating leukocyte transmigration. In addition, we discuss studies on communication among cells of the immune system, BBB, and the CNS parenchyma, and suggest how these interactions contribute to the pathogenesis of NeuroAIDS. We also describe some of the animal models that have been used to study and characterize important mechanisms that have been proposed to be involved in HIV-induced CNS dysfunction. Finally, we review the pharmacologic interventions that address neuroinflammation, and the effect of substance abuse on HIV-1 related neuroimmunity.

Key words HIV · dementia · neuroimmunology · transmigration · animal models · therapeutics

Introduction

An early consequence of human immunodeficiency virus type 1 (HIV-1, which we will refer to as “HIV”) infection is entry of the virus into the central nervous system (CNS), resulting in several neurological sequelae. Neuropathological conditions that result from HIV entry into the CNS include HIV-associated encephalitis (HIVE), which is characterized by the formation of multinucleated giant cells, inflammation, microglial activation, astrogliosis, myelin pallor, loss of neurons and astrocytes, and the development of a syndrome of cognitive and motor dysfunction often leading to HIV-associated dementia (HAD) (Gendelman et al. 2005). Prior to the availability of highly active antiretroviral treatment (HAART), HAD was detected in approximately 20% of individuals with AIDS (McArthur et al. 1993). Initially, there was a drop in the incidence of dementia and other neurological manifestations as a result of HAART. However, the prevalence of neurologic disease in people with HIV has actually increased because individuals are living much longer, and therapeutic drugs are relatively ineffective in crossing the blood–brain barrier (BBB) (McArthur et al. 2003; Anthony et al. 2005). Thus infection and inflammation persist in the CNS despite HAART, suggesting that CNS infection and inflammation are independent of peripheral HIV status. The mechanism of HIV-induced CNS infection and inflammation is complex. In this review, we summarize several reports that provide an understanding of leukocyte transmigration across

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the BBB and viral entry into the CNS, and their role in the pathogenesis of cognitive impairment. Lastly, we describe some animal models that have contributed to our understanding of HIV entry into the CNS, therapeutics that could potentially improve or limit some of the neurological complications induced by HIV infection, and the contributions of substances of abuse to the pathogenesis of NeuroAIDS.

Mechanisms of viral entry across the BBB

The molecular mechanisms by which HIV enters the CNS and contributes to both acute and chronic inflammatory processes that may culminate in neurologic dysfunction are still unclear. The most accepted hypothesis on viral entry into the CNS is the “Trojan Horse” model, in which infected monocytes and lymphocytes transport the virus from the blood into the CNS. After entry into the CNS, these infected cells release toxic molecules, including viral proteins, cyto-

kines, and chemokines that recruit resident CNS cells, especially microglia and astrocytes, to areas of viral infiltration (Fig. 1). This recruitment allows for subsequent infection of these resident cells and the spread of HIV within the CNS. HIV infects perivascular macrophages and microglia productively, while astrocyte infection is restricted, resulting in the formation of viral reservoirs within CNS cells in which replication competent viral genomes persist in a stable state (Lavi et al. 1998). There is no evidence of direct HIV infection of neurons; therefore, the neuronal cell damage and death that occurs in HAD must be mediated by production and release of neurotoxic factors by other infected or uninfected cells within the CNS. In fact, neuronal dysfunction correlates more closely with inflammation and activated monocytes/microglia than with viral load (Sevigny et al. 2004).

A second proposed mechanism of HIV entry into the CNS is the direct infection of BBB cells by cell-free HIV, providing a source of infectious particles in close proximity to the CNS parenchyma, thus promot-

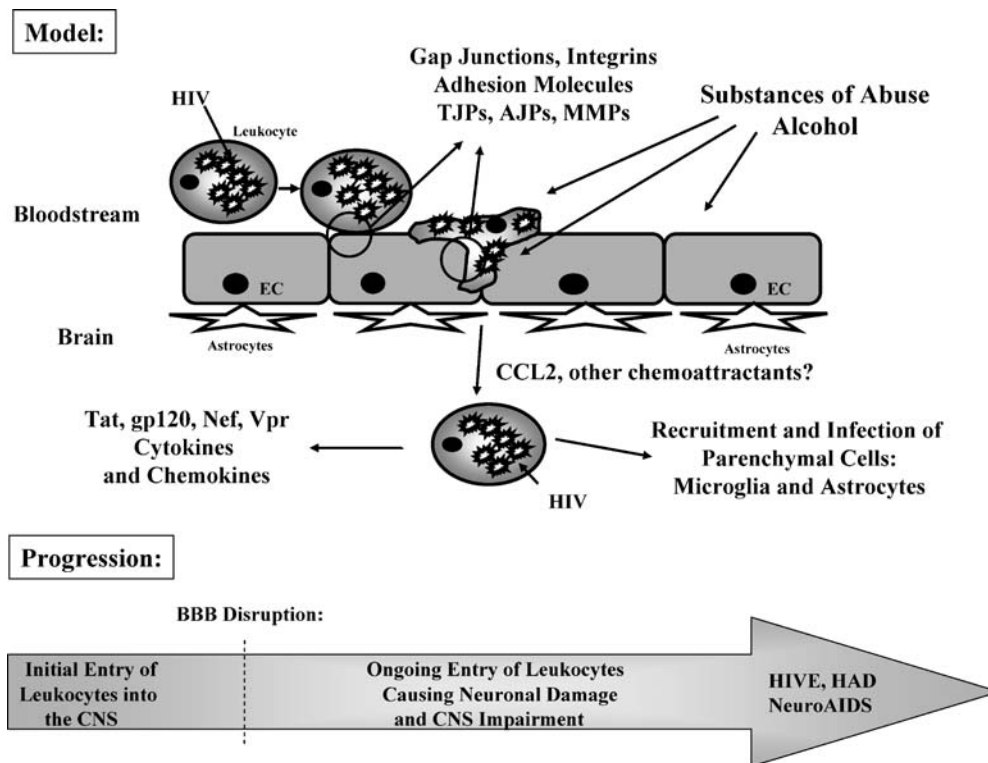


Fig. 1. Sequence of inflammatory events in NeuroAIDS. Gap junctions, integrins, adhesion molecules, tight junction proteins (TJPs), adherens junction proteins (AJPs), matrix metalloproteases (MMPs), and viral proteins such as Tat, gp120, Nef, and Vpr, participate in HIV-infected leukocyte adhesion to and transmigration across EC of the BBB. CCL2, and possibly other chemoattractants, significantly enhances this transmigration. Once within the CNS, the leukocytes produce soluble viral proteins, cytokines, chemo-

kines, and other factors, thus recruiting, activating, and facilitating the infection of microglia and astrocytes. HIV-associated cognitive impairment begins with the initial transmigration of infected leukocytes into the CNS, which leads to BBB disruption, causing acute inflammation. During the subsequent chronic phase of inflammation and infection, leukocyte transmigration is an ongoing process, resulting in NeuroAIDS. Substances of abuse and alcohol contribute to the progression of this neuropathogenesis.

ing viral infection of resident CNS cells as well as the further release free virus. However, this theory does not explain how the extracellular virus in the circulation survives long enough to infect BBB cells. Recent studies demonstrated that proteoglycans on the surface of endothelial cells (EC) bound and protected circulating HIV from degradation for a prolonged period of time, providing an opportunity for EC to become infected (Argyris et al. 2003; Bobardt et al. 2004) through a CD4 and galactosylceramide-independent mechanism (Moses et al. 1993; Mankowski et al. 1994). This mechanism, however, still does not address how HIV crosses the structural components of the BBB, which includes astrocyte foot processes and pericytes, after EC infection, nor does it distinguish between viral uptake and productive infection. Furthermore, the issue on whether EC are productively infected by HIV remains controversial.

A third model of viral entry into the CNS is the internalization of the HIV virion by EC or astrocytic foot processes by macropinocytosis or endocytosis, with subsequent transfer of the virus to parenchymal CNS cells (Banks et al. 1998, 2001; Marechal et al. 2001; Liu et al. 2002a). Macropinocytosis in BBB EC is dependent on intact lipid rafts and MAPK signaling (Liu et al. 2002a). The possibility of macropinocytotic entry of HIV into CNS EC is supported by the presence of increased surface microvilli on EC, abundance of cytoplasmic vesicles, and inhibition of HIV entry by dimethylamiloride, an inhibitor of the Na^+/H^+ pump in membranes (Liu et al. 2002a). However, the presence of virions within EC or BBB cells may also be due to direct infection of these cell types, transcytosis of virions through the BBB, or the presence of HIV-infected leukocytes trapped in the BBB during the process of transmigration.

The last proposed mechanism for viral entry into the CNS is the nonspecific passage of HIV through the BBB and into the brain. This mechanism is a combination of the other mechanisms (previously described) that is dependent on the loss of BBB integrity due to exposure to cytokines, chemokines, viral proteins, or neurotransmitters, and/or the induction of apoptosis in EC and/or astrocytes. However, this theory is the least likely explanation, because it requires extensive BBB disruption early in the course of infection without repair. This seems unlikely given that HIV-infected individuals now live longer, in a chronic phase of infection, as a result of HAART.

In all of these proposed models, the integrity and function of the BBB is essential in regulating the passage of the virus into the CNS parenchyma. Alterations in the structural integrity of the BBB result in increased

EC permeability. The mechanisms involved in this increase in permeability are unknown, but there is a close correlation between elevated blood and cerebral spinal fluid (CSF) cytokine levels induced by injury and the breakdown of the BBB, again suggesting that inflammation plays an important role (Martiney et al. 1992; Hurwitz et al. 1994; Fiala et al. 1997, 1998; Dallasta et al. 1999; Rubin and Staddon 1999; Gloor et al. 2001; Strelow et al. 2001; Eugenin et al. 2006a).

Blood–brain barrier function

BBB is a physical and metabolic barrier that separates the CNS from the periphery (see Persidsky et al. in this issue for details on BBB biology). It is not rigid and is composed of dynamic vessels that are capable of responding to rapid changes in the brain or blood (Huber et al. 2001). BBB is mainly composed of EC in close contact with astrocytic end foot processes (Fig. 1). BBB cells express very specific systems of transport of metabolites, as well as tight junction proteins (TJP), which seal the intercellular gaps between EC–EC and EC–astrocytes, resulting in impermeability to most macromolecules and blood cells (Pardridge and Choi 1986; Goldstein 1988; Pardridge et al. 1990; Risau and Wolburg 1990; Risau 1991; Rubin and Staddon 1999).

The major functions of BBB are to transmit biochemical signals from the blood to the brain, to exclude leukocytes and soluble factors, and to select specific nutrients to transport into or out of the brain (Pardridge and Choi 1986; Pardridge et al. 1990; Risau and Wolburg 1990). The concept of the CNS as an “immune privileged” site requires that the cells of the BBB and CNS parenchyma be shielded from physical and chemical interactions with circulating cells of the immune system. However, cells of the BBB, CNS parenchyma, and circulating leukocytes all express many common cell surface receptors that respond to soluble factors released by these cell types. Many studies demonstrated crosstalk among BBB cells, cells within the CNS expressing immune cell receptors, and immune cells expressing receptors common to CNS cells, warranting the reevaluation of the extent to which the CNS is “immune privileged” (Fig. 2).

The role of BBB as an active participant in CNS function is still not completely understood. However, the recent findings that leukocytes and CNS cells share proteins originally believed to be expressed uniquely on one or the other, may enhance our understanding of viral entry into the CNS, as well as explain how the neurological complications seen in NeuroAIDS are

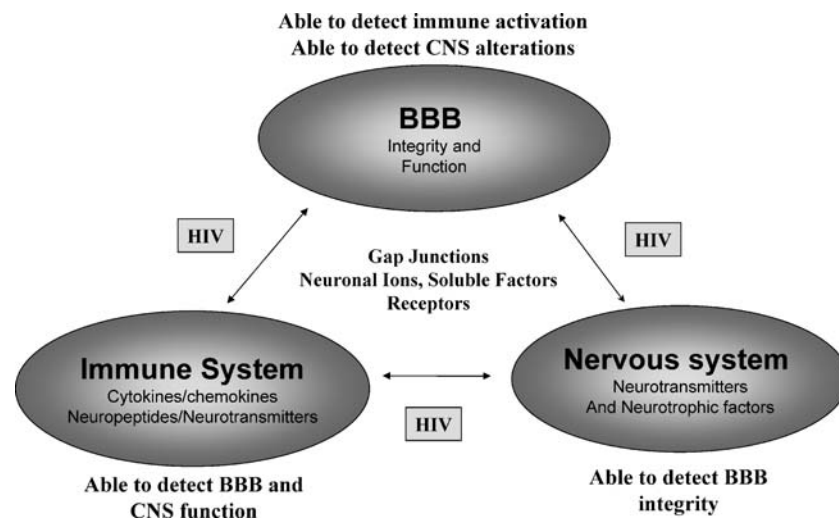


Fig. 2. Neuroimmune interactions in NeuroAIDS. A simplified representation of extensive interactions among BBB cells, and immune and central nervous systems. These interactions allow each system to detect changes in others. Most of the communication among these systems is between gap junctions, ions (sodium, potassium, and calcium), receptor–ligand interactions,

and soluble factors, including cytokines, chemokines, neurotransmitters, neurotrophic factors, and adhesion molecules, both intact on the cell surface and/or in soluble forms. HIV infection in both the CNS and the periphery can alter the ability of these systems to detect change.

continuing during the chronic phase of infection and become highly prominent during the late stages of HIV disease pathogenesis. Some of the newly described mechanisms by which BBB cells may have a proactive role in the modulation of CNS function are: (1) calcium waves in BBB cells, supported by ATP receptors and gap junctions; (2) neurotransmitters originating from the CNS or blood acting on BBB cells; (3) intercellular communication between BBB cells and leukocytes through gap junctions; (4) BBB activation of accessory cells, such as pericytes and perivascular macrophages.

Calcium waves

This new communication system was initially identified in liver. It is faster than chemical synapses and slower than electrical synapses (Gaspers and Thomas 2005). Calcium waves are inositol triphosphate (IP₃)-dependent and can involve large areas of tissue (mm) (Nathanson et al. 1995). One study demonstrated that BBB cells establish an active and coordinated communication system mediated by calcium waves that can be visualized by using confocal microscopy, multiphoton, and calcium imaging. This study also showed that the purinergic receptors P2Y(2) and P2Y(4) and gap junctions, mainly Connexin 43 (Cx43), are present in astrocytic end feet of the BBB and, once activated, trigger changes in intercellular calcium signaling, altering some of the biological characteristics of BBB EC (Simard et al. 2003). This suggests that ATP and gap junctions may contribute to the regulation of BBB functions, such as blood flow, metabolic trafficking, and

water homeostasis. ATP in the CNS is normally released by neurons and astrocytes; therefore, this finding also suggests that alterations in the CNS parenchyma can directly affect the functional properties of the BBB. It was recently suggested that signaling through P2X7 receptor (a member of the P2X family of purinergic receptors), which forms a pore in response to ligand stimulation and regulates cell permeability and cytokine release, may modulate the astrocytic response to inflammation in the CNS (Narcisse et al. 2005). Interestingly, ATP and gap junctions are the two pathways that propagate calcium waves in the CNS. These calcium waves are bidirectional (EC ↔ astrocyte) and involve IP₃ generation (Braet et al. 2001; Paemeleire 2002a,b).

Neurotransmitters originating from the CNS or blood acting on BBB cells

This communication system of neurons and leukocytes has been extensively studied; however, only a few studies have examined the role of neurotransmitters in the regulation of BBB function. Despite conflicting reports, the possibility of active communication by the BBB in response to neurotransmitters under normal and pathological conditions merits consideration (Fig. 2). In this work, we review some representative studies.

Glutamate

Glutamate is the major excitatory neurotransmitter in the CNS. In excess, glutamate has also been found to be highly toxic to neuronal and glial cells. Most of the

glutamate present in the brain is synthesized de novo by astrocytes (Hertz et al. 1998). Some reports indicate that cerebral EC express NMDA receptors (NMDAR), and that exposure of these cells to excitotoxic levels of glutamate leads to a breakdown of the BBB through activation of NMDAR (Krizbai et al. 1998; Sharp et al. 2003, 2005a). Glutamate–glutamate receptor interactions can up-regulate P-glycoprotein (P-gp) expression in rat microvessel EC (Zhu and Liu 2004), suggesting that glutamate treatment results in the enhanced capability of the BBB to transport P-gp substrates. The most likely mechanism of glutamate modulation of BBB function is that high neuronal activity releases glutamate into the extracellular space in the CNS that acts on the BBB. However, it has also been demonstrated that neutrophil-derived glutamate regulates BBB integrity, suggesting that glutamate from the bloodstream may also be important (Collard et al. 2002). Increased glutamate was detected in the CSF and plasma of individuals with HAD. In addition, HIV-infected macrophages release quino-lic acid, a glutamate receptor agonist that can enhance glutamate toxicity in neurons (Heyes et al. 2001). These and other data suggest a pathogenic role for glutamate in HIV dementia, thereby supporting the use of glutamate receptor antagonists as an alternative therapeutic treatment to maintain BBB integrity.

Dopamine

Dopamine is a neurotransmitter that is synthesized both peripherally and in the brain. T cells express dopamine receptors (Ilani et al. 2004). A subset of T cells, highly activated T cells (termed blast cells), expresses dopamine receptors and has been shown to cross the BBB (Owens et al. 1998; Ilani et al. 2004). Thus, these cells can encounter and bind dopamine in the brain. Studies investigating substances of abuse and HIV infection suggest that drugs accelerate neuronal loss in HIV infection, and that dopamine, in addition to its typical neurotransmission role, can alter BBB integrity and promote leukocyte activation (see [Substance abuse, HIV, and the BBB](#) for a more comprehensive discussion of dopamine) (Reyes et al. 1991; Bennett et al. 1995; Herz 1995; Czub et al. 2004; Wang et al. 2004).

Substance P

The neuropeptide substance P (SP) is a critical link between the nervous and immune systems (Ho and Douglas 2004). Several lines of evidence demonstrate that SP may play an important role in the pathophysiology of neuropsychiatric disorders, including stress

and depression, in HIV-infected individuals. SP can activate and damage brain EC in the presence of pro-inflammatory cytokines that have been found to be elevated in the CSF of HIV-infected individuals (Annunziata et al. 2002). Monocyte-derived macrophages and lymphocytes from both placental cord blood and adult peripheral blood were demonstrated to express SP, which was significantly increased by HIV infection, suggesting that HIV enhances SP expression in immune cells (Ho et al. 2002). In addition, SP was shown to enhance HIV replication in a latently infected promonocytic cell line and in a T lymphocyte cell line (Li et al. 2001). In conjunction with gp120, an HIV protein, SP can compromise the BBB (Annunziata et al. 1998). SP binds to neurokinin-1 receptor, a G-protein coupled receptor in neurons and immune cells (Ho and Douglas 2004). Understanding the mechanisms of SP activity and its interaction with its receptor may provide a novel approach to the treatment of CNS disorders induced by HIV infection.

Neuropeptide Y

Neuropeptide Y (NPY) is one of the most abundant peptides in the central and peripheral nervous systems, where it plays a role in the regulation of cardiovascular, metabolic, endocrine, immunological, and cognitive functions (Malessa et al. 1996). NPY is a trophic/angiogenic factor at concentrations below those required for vasoconstriction (Zukowska-Grojec et al. 1998). In addition, NPY alters the release of neurotransmitters, such as norepinephrine (Bitran et al. 1999). These data suggest that NPY may play a role in angiogenesis during development and pathological conditions. Increased NPY-like immunoreactivity in the CSF and plasma of HIV positive individuals has been detected and positively correlated with the degree of HIV encephalopathy, suggesting its role in HIV neuropathogenesis (Malessa et al. 1996).

Neurotrophic factors

Neurotrophic factors such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3) belong to the neurotrophin family of growth factors. They are produced by neurons and glial cells to promote neuronal survival and growth (Encinas et al. 2000). Fibroblast growth factor-2 (FGF2) and vascular endothelial growth factor (VEGF) are angiogenic factors produced by astrocytes and EC, promoting survival, proliferation, and differentiation of brain microvascular cells (Sobue et al. 1999). In HIV infection, BDNF is neuroprotective. It activates NF- κ B,

thereby inducing expression of the antiapoptotic Bcl-2 gene, which protects neurons from the proapoptotic effects of HIV Tat (Ramirez et al. 2001). In addition, BDNF reduces gp120 neurotoxicity (Nosheny et al. 2005). Another study showed that FGF levels are increased in tissue sections obtained from individuals with HIV who exhibited little neuronal cell death, and that FGF protects primary cultured neurons from the neurotoxic effects of gp120 (Everall et al. 2001). These factors therefore provide a mechanism whereby bidirectional communication between the blood and CNS cells may be facilitated by the BBB. The direct or indirect mechanisms by which these factors regulate BBB integrity require further study.

Intercellular communication between BBB cells and leukocytes through gap junctions

This new avenue of investigation does not involve soluble factors or indirect mechanisms of cellular communication. We previously reported that leukocytes express functional gap junction channels during the process of transmigration across BBB cells (Eugenin et al. 2003a). Gap junctions provide an additional mechanism for neighboring cells to communicate. The gap junction protein Cx43 is expressed homotypically and heterotypically between EC and astrocyte foot processes in the BBB (Virgintino et al. 2001; Simard et al. 2003). Interactions through gap junctions may facilitate the bidirectional communication between peripheral blood leukocytes and BBB cells, as well as between BBB and CNS cells, thus participating in the BBB function of selective transport of metabolites (Braet et al. 2001). We demonstrated that tumor necrosis factor alpha (TNF- α) plus interferon gamma (IFN- γ) induced gap junctional communication between human monocytes/macrophages mediated by Cx43. During transmigration, Cx43 was also detected at cellular interfaces between monocytes and EC in our *in vitro* model of the BBB, and blocking these channels with chemical inhibitors resulted in reduced monocyte transmigration across the BBB. Therefore, we proposed that transient expression of Cx43 by monocytes/macrophages allows for the formation of gap junction channels that mediate intercellular communication required for different cellular responses and functions during inflammation (Eugenin et al. 2003a).

BBB activation of accessory cells, such as pericytes and perivascular macrophages

In addition to the direct effects of cell contact and soluble factors, the presence of other cell types, not often

considered a part of the BBB, is also essential in regulating BBB permeability. The activation of pericytes and perivascular macrophages triggers BBB disruption by their elaboration of factors that compromise BBB integrity. Pericytes wrap around the EC in the BBB. These cells provide structural support and regulate the microvasculature. Pericytes express contractile proteins that help regulate capillary flow (Bandopadhyay et al. 2001). In hypoxia and traumatic brain injury, pericytes migrate away from the BBB, resulting in increased BBB permeability (Dore-Duffy et al. 2000; Gonul et al. 2002). Pericytes have roles in aneurysm formation in PDGF-B-deficient mice (Lindahl et al. 1997), retinal microaneurysm formation in diabetes mellitus (Kern and Engerman 1996), hereditary cerebral hemorrhage with amyloidosis, and Alzheimer's disease (Verbeek et al. 1997). In addition, pericyte-derived angiopoietin can induce endothelial expression of occludin, a major constituent of BBB tight junctions (Hori et al. 2004). Taken together, these data suggest that pericytes can induce/maintain BBB properties. Pericytes and perivascular macrophages express a number of immune and CNS receptors and mediators, including catecholamines, angiotensin, VIP, ET-1, MHC class I and II, CD4, Fc receptor, CR3 complement receptor, and vasopressin (van Zwieten et al. 1988; Elfont et al. 1989; Healy and Wilk 1993; Benagiano et al. 1996; Dehouck et al. 1997; Thomas 1999). Thus, these cells can sense CNS and immune alterations. The activation of these cells is similar to that of cells of the macrophage lineage, causing the release of cytokines/chemokines and other factors that can alter BBB integrity.

Transmigration of HIV-infected leukocytes across the BBB

Leukocyte transmigration across the BBB during normal immune surveillance is an active process not only for the leukocyte but also for the BBB cells (Fig. 1). It has been characterized as a dynamic multistep process involving the initial "rolling" of cells on vessel endothelium in response to locally produced proinflammatory mediators, and subsequent firm adhesion to, and diapedesis across the vasculature. The rolling of leukocytes along the endothelial surface is mediated by weak interactions of selectin molecules and their corresponding glycoprotein ligands, expressed by activated EC and leukocytes. Rolling leukocytes are then stimulated by chemokines and other chemotactic molecules, resulting in activation of the β 1 integrin, VLA-4, and the β 2 integrin, LFA-1. Ultimately,

leukocyte arrest occurs, mediated by strong interactions between LFA-1 and its EC counter receptor, intercellular adhesion molecule-1 (ICAM-1), and between VLA-4 and its EC counter receptor, vascular cell adhesion molecule-1 (VCAM-1) (Muller 2003; Liu et al. 2004; van Buul and Hordijk 2004). Leukocyte diapedesis across the blood vessel endothelium is a process mediated, in part, by homophilic and heterophilic binding molecules including junctional adhesion molecules (JAM), platelet endothelial cell adhesion molecule 1 (PECAM-1), and CD99 (Martin-Padura et al. 1998; Del Maschio et al. 1999; Ostermann et al. 2002; Schenkel et al. 2002; Ebnet et al. 2004). Although other adhesion proteins and integrins are involved in leukocyte transmigration, data suggest that those mentioned above may be associated with the development of HIV CNS pathology (Seilhean et al. 1997; Eugenin et al. 2006b).

The BBB excludes most circulating leukocytes, although there is baseline trafficking of lymphocytes and monocytes. Therefore regulatory mechanisms must exist to minimize the deleterious effects of inflammatory events in the CNS. There are a few studies that address how HIV infection alters the dynamic interaction between leukocytes and EC. An immunohistochemical study of HIVE tissue showed increased expression of adhesion proteins on astrocytes (Seilhean et al. 1997). HIV infection of human monocytes increased their expression of LFA-1 (Stent and Crowe 1997), and another study showed that the adhesion of HIV-infected monocytes to EC induced the expression of E selectin and VCAM-1 (Nottet et al. 1996). Recently, we demonstrated that in brain tissue from individuals with HIVE there is an accumulation of cleaved, soluble forms of the extracellular region of PECAM-1 (sPECAM-1) (Eugenin et al. 2006b). In addition, we assayed sera from individuals with HIV for sPECAM, and found elevated levels. These findings suggest that this sPECAM-1 production by HIV-infected cells can alter PECAM interactions between EC–EC and EC–leukocytes and thus contribute to enhanced transmigration of HIV-infected leukocytes into the CNS and changes in BBB permeability during the pathogenesis of NeuroAIDS.

There is evidence in end-stage HIV disease of abnormal BBB structure and altered expression of the TJP ZO-1 and occludin on sites where leukocyte infiltration and HIV infection were detected (Dallasta et al. 1999; Boven et al. 2000). Using an *in vitro* model of the BBB and blood-derived primary monocytes infected with the R5 HIV strain, HIV-1_{ADA}, it was shown that the state of immune activation of cells that constituted the BBB dictated the ability of monocytes

to traverse the BBB (Persidsky et al. 1997). Using a similar *in vitro* model of the BBB, we demonstrated increased BBB permeability and a loss of TJP when HIV-infected monocytes transmigrated across the BBB in response to the chemokine CCL2. The mechanism of BBB disruption and enhanced transmigration of HIV-infected cells was specifically CCL2-dependent, because other chemokines did not elicit the same effect (Eugenin et al. 2006a). We also found that BBB disruption was associated with enhanced expression of matrix metalloprotease-2 (MMP-2) and matrix metalloprotease-9 (MMP-9) (Eugenin et al. 2006a). We propose that during acute infection, HIV-infected cells disrupt the BBB as they gain access to the CNS *in vivo*. This transient disruption facilitates ongoing inflammation within the CNS. The advent of HAART has created a chronic phase of CNS infection/inflammation in which this ongoing inflammation is subtle, but continues to cause CNS damage over an extended time period. In late-stage disease, extensive inflammation occurs, and may result in overt dementia. Future studies will investigate the role of CCL2 in HIV entry into the CNS by characterizing the transmigration of HIV-infected monocytes in the absence and presence of CCL2, and by examining the contribution of BBB cells and HIV-infected peripheral blood mononuclear cells to CCL2 enhanced transmigration.

Cytokines/chemokines that contribute to neuropathogenicity of HIV

Blood–brain barrier dysfunction during HIV infection may be attributable to many host factors. Cytokines/chemokines, excitatory amino acids, neuropeptides, and other factors have been shown to cause BBB disruption, increased leukocyte transmigration, and EC and neuron apoptosis. Cytokines are a large and diverse group of small polypeptides that have a broad range of effects on different cell types. Chemokines are a subclass of cytokines that chemoattract and activate immune and nonimmune cells both *in vivo* and *in vitro*. Neurons, astrocytes, microglia, and oligodendrocytes produce many of these molecules, and all cell types in the brain express an array of corresponding receptors that provide responsiveness to a variety of different cytokines/chemokines (Cartier et al. 2005). Cytokines including TNF- α , IFN- γ , interleukin (IL)-1 β and IL-6, and the chemokine CCL2 have been shown to be increased in sera, brain tissue, and CSF of individuals with HIVE and to modulate the BBB.

Although the signals that mediate the early transmigration of HIV-infected leukocytes into the brain

are unknown, it has been proposed by our group and others that chemokines, especially CCL2, play a critical role during this process. Chemokines and their receptors are more highly expressed in HIV and simian immunodeficiency virus (SIV) encephalitic brain tissue as compared to normal brain, as well as in cultured microglia, astrocytes, and neurons in the presence of HIV (Sasseville et al. 1996; Conant et al. 1998; Gabuzda et al. 1998; Kelder et al. 1998; Lavi et al. 1998; Sanders et al. 1998; Albright et al. 1999; McManus et al. 2000). Experiments in transgenic mice and humans indicated that CCL2 is an essential chemokine for monocyte transmigration into the brain parenchyma (Fuentes et al. 1995; Gonzalez et al. 2002). Moreover, it has been demonstrated that HIV-infected individuals homozygous for the MCP-1-2578G allele have elevated levels of MCP-1/CCL2 in the CSF, and a 4.5-fold increased risk of developing HAD (Gonzalez et al. 2002; Letendre et al. 2004). In addition, elevated levels of CCL2 have been detected in the CSF of individuals with HAD and HIVE (Conant et al. 1998; Kelder et al. 1998). In SIV, an animal model of HIV (see later sections for more details), CSF levels of CCL2 are biphasic, rising early after infection, and again later during the course of CNS disease (Zink et al. 2001; Mankowski et al. 2004). When minocycline, an antibiotic with potent anti-inflammatory and neuroprotective properties, was given beginning 21 days postinoculation, there was reduced severity of encephalitis, suppressed viral load in the brain, and decreased expression of CNS inflammatory markers (Zink et al. 2005). These SIV studies have shown that the second phase of increased CCL2 CSF levels may correlate with the severity of injury. As noted above, we demonstrated the enhanced transmigration of HIV-infected leukocytes across the BBB, and BBB disruption, in response to CCL2. The expression by EC of CCR2, the CCL2 receptor, may also play a role in this process (Dzenko et al. 2005).

Interestingly, CCL2 may also have a novel role as a protective factor in human neurons and astrocytes from NMDA and HIV-Tat induced apoptosis (Eugenin et al. 2003b). Thus, CCL2 has a complex pivotal role in the pathogenesis of NeuroAIDS. For a detailed discussion on cytokine and chemokine expression induced or modulated by HIV in the CNS, see Speth et al. (2005).

Viral factors and the BBB

Many laboratories examined the direct effects of HIV-specific proteins on the human BBB and CNS cells. Viral proteins in the brain are released or secreted

from free viral particles and HIV-infected cells. These proteins induce functional changes in their host cells that further contribute to neuronal injury and BBB disruption (Fig. 1).

gp120

The viral protein gp120 is a part of the envelope protein of HIV that binds the coreceptors, CCR5 and/or CXCR4, facilitating entry of the virus into cells. Chemokine receptors on the surface of neurons interact with gp120, causing an increase in intracellular Ca^{2+} concentration, which induces neuronal apoptosis (see review Martin-Garcia et al. 2002). Macrophages/microglia are activated by gp120, causing them to secrete TNF α and chemokines that cause neuronal injury (see Yi et al. 2004; D'Aversa et al. 2005). Treatment of EC with gp120 induces cell death, as well as increases the permeability of endothelial monolayers by disrupting and down-regulating TJP important to the BBB, such as ZO-1, ZO-2, and occludin (Kanmogne et al. 2002, 2005). Exposure of astrocytes to gp120 decreases their capacity to uptake glutamate (Wang et al. 2003b), and alters gene expression patterns (Galey et al. 2003).

Tat

Many studies examined the effects of Tat on different cell types of the CNS. Tat is released by HIV-infected cells and can be taken up by uninfected cells, including neurons (Liu et al. 2000). In human neurons, Tat can induce an increase in the levels of intracellular calcium and the generation of reactive oxygen species that eventually causes cell death through a mechanism that is attributable, in part, to the activation of NMDA and non-NMDA receptors (reviewed by Peruzzi et al. 2005). We propose that Tat induces apoptosis in neurons through the formation of a plasma membrane complex that includes Tat, low-density lipoprotein receptor-related protein (LRP), PSD-95, and NMDAR channels (Eugenin and Berman, unpublished data; King et al. 2006). Tat has also been demonstrated to affect chemokine production and function in the CNS. We demonstrated that Tat facilitates microglia migration by inducing autocrine CCL2 release (Eugenin et al. 2005), and induces CCL2 expression in astrocytes (Weiss et al. 1999). We also demonstrated that Tat induces chemokine expression by microglia, which may be involved in inflammatory cell influx into the CNS (D'Aversa et al. 2004). Exposure of brain microvascular EC (BMVEC) to Tat resulted in the disruption of TJP (Andras et al. 2003; Buckner and Berman, unpublished data). Tat treatment

of astrocytes induced inducible nitric oxide synthase (iNOS) and increased production of NO (Liu et al. 2002b). Tat facilitated the infiltration of monocytes into the brain through its up-regulation of VCAM-1 and ICAM-1 production by astrocytes and EC (Woodman et al. 1999; Pu et al. 2003). Thus, Tat may play a central role in viral entry into the CNS and disruption of the BBB. (For a detailed review of Tat and its neurotoxic effects in the CNS, see King et al. 2006).

Nef and Vpr

Nef and Vpr are viral proteins that induce the release of proinflammatory cytokines and cause cell death in the CNS. Nef downmodulates host immune cell receptors, thereby enabling the virus to escape the host defense and increase viral infectivity. It also enhances viral replication and infection through a combination of different effector functions (Joseph et al. 2005). Nef has been detected in supernatants of HIV-infected cell cultures and in the sera of individuals with AIDS (Fujii et al. 1996). Extracellular Nef may interfere directly with neuronal survival or functions, as recombinant Nef treatment of cultured neuronal cells was toxic (Trillo-Pazos et al. 2000). Exposure of human monocytes/macrophages to Nef induced the release of proinflammatory factors (Olivetta et al. 2003), and supernatants from Nef-expressing macrophages induced both the chemotaxis and activation of resting T lymphocytes, facilitating productive HIV infection. This suggests a role for Nef in lymphocyte recruitment and activation at sites of viral replication (Swingler et al. 1999). Vpr has been implicated in the regulation of cellular functions including apoptosis, cell cycle arrest, differentiation, and immune suppression (Muthumani et al. 2005). In the CNS, it has been shown to induce apoptosis in neuronal cells (Patel et al. 2000), as well as to be involved in the induction of CCL5 in microglial cells (Si et al. 2002). CCL5 is a chemokine that has been shown to limit HIV infection by binding to CCR5, a coreceptor for HIV, thereby blocking viral entry into the cell (Cocchi et al. 1995).

Stress and HIV

There have been several studies examining the effects of depression and stress on AIDS progression. Although there are conflicting data due to differences in research design, duration of follow-up, number and quality of control variables, and measurement of depression or study outcome, evidence suggests that

depression and stress may be predictive of, or contribute to, HIV disease progression (Burack et al. 1993; Lyketsos et al. 1993; Leserman et al. 1997, 1999, 2000; Ickovics et al. 2001; Leserman et al. 2002). Many studies demonstrated the negative effects of stress and depression on cells of the immune system that are also affected by HIV (Evans et al. 1989; Weisse 1992). Comorbid clinical depression and some symptoms of AIDS are similar, such as poor appetite, weight loss, and cognitive impairment. It has been shown that inflammatory cytokines can contribute to these symptoms (Dantzer and Kelley 1989; Clerici et al. 1997).

Inflammation within the CNS results in the elaboration of cytokines that may act on neurons and other cells within the CNS to cause changes in neuronal function, thereby contributing to cognitive impairment or dementia. HAART may not be effective in reducing neuroinflammation (Anthony et al. 2005), so psychiatric disorders may increase. In addition, the hypothalamic–pituitary–adrenal (HPA) axis and sympathetic nervous system (SNS) have been implicated in mediating the relationship among stress, depression, and HIV. It is proposed that hormones of the HPA axis and SNS are affected by stress and depression, and that dysregulation of these systems may, in turn, have negative immunologic consequences in HIV infection and contribute to disease progression (Leserman 2003). Further research is needed to address these hypotheses as well as to determine if treatment of depression, in addition to enhancing the mental health of individuals with HIV, may also limit disease progression and mortality.

Animal models of NeuroAIDS

Macaque, mouse, and feline animal models have advanced the study of the progression and pathology of NeuroAIDS. In this section, we discuss recent findings using these models, placing emphasis on their contributions to the understanding of neuroimmunity and the BBB in NeuroAIDS, with the recognition that none of these models fully replicate the disease condition exhibited in humans.

The macaque model

The macaque is a major animal model of HIV infection and NeuroAIDS, allowing studies of the CNS and CSF that cannot be performed in living humans. SIV, like HIV, causes fatal immunodeficiency syndrome in rhesus macaques. SIV crossed species and diverged to establish both HIV-1 and HIV-2 (see review by Sharp et al. 2005b). SIV is a well-accepted

alternative virus for the study of HIV because SIV and HIV share morphologic and antigenic similarities, phylogenetic proximity, and parallel neuropathogenic disease progression (Desrosiers et al. 1989; Murray et al. 1992; Zink et al. 1998; Apetrei et al. 2004; Weed and Steward 2005). HIV and SIV affect several of the same cells of the CNS and elicit similar immunological responses (Clements et al. 1994; Lackner et al. 1994; Mankowski et al. 1994; Fox et al. 1997; Zink et al. 1998). Both SIV and HIV predominantly utilize CD4 for entry (Clapham et al. 1989, 1991). Unlike HIV, which can switch tropism from R5 to X4, SIV is an R5 virus. Some strains of SIV use other coreceptors, such as GPR15 (Bob) and STRL33 (Bonzo) (Marx and Chen 1998; Gabuzda and Wang 1999; Unutmaz et al. 2000). Here, we review applications of various macaque models, each of which has made its own unique contribution to the understanding of the role of neuroinflammation in NeuroAIDS.

One of the most common strains of study is SIV_{mac251}; its injection causes SIV encephalitis in rhesus macaques. Studies using this viral strain determined that perivascular macrophages are one of the primary infected cells in SIV neuropathogenesis (Williams et al. 2001), and that SIV infection causes disruption of the BBB (Maclean et al. 2005). RT-PCR was used to quantify IL-1 β , TNF- α , and IFN- γ during acute and chronic infection in this model. IL-1 β was found in both infected and uninfected macaques. TNF- α and IFN- γ were detected in several areas of the brain in infected macaques within 1–2 weeks of SIV injection as well as at terminal infection (Orandle et al. 2002), but were not present in uninfected animals.

Two SIV strains are used simultaneously to model NeuroAIDS in pigtailed macaques. Infection with both the neurovirulent SIV/17E-Fr strain and the immunosuppressive SIV/deltaB670 strain recapitulates the acute phase of NeuroAIDS (Zink and Clements 2000, 2002). The neurovirulent strain quickly causes SIV encephalitis (Edinger et al. 1997), and 90% of infected macaques show CNS symptoms and pathology consistent with NeuroAIDS. The severity of encephalitis in the infected macaques correlates with high CNS viral load, not plasma viral load (Zink et al. 1999). The role of chemokines and inflammation in the CNS associated with NeuroAIDS has been examined in this model. Because monocyte and lymphocyte activity in the model is similar to that in NeuroAIDS, the CNS/plasma ratio of CCL2, a potent monocyte chemotactic factor that also recruits activated T cells, was investigated. Encephalitic macaques had a higher CNS/plasma ratio of CCL2 than those that had mild to no encephalitis, or were uninfected controls (Zink et al.

2001). Thus, in this model, CCL2 is a predictor of SIV encephalitis. CCL3, CCL4, and CCL5 cause T cell and monocyte chemotaxis as well. When their role in SIV encephalitis was examined, no significant differences between infected and uninfected macaques were found (Edinger et al. 1998).

This two-virus model of NeuroAIDS also facilitates the study of HAART. HAART has not been effective in reducing the prevalence of HIV-related neurodegenerative disease (see review by Perry et al. 2005), suggesting the existence of a viral reservoir in the CNS. This was found in the two-virus model of NeuroAIDS; infected macaques treated with a compound incapable of crossing the BBB were found to have higher CNS viral loads than those that received a compound that could (Clements et al. 2005). The finding that the CNS is a reservoir for SIV in this model underscores its ability to measure the effectiveness of current and future clinical interventions, such as minocycline, which will be described later.

Another macaque model achieves infection by using an anti-CD8 antibody at the time of infection (with SIV_{mac251}), depleting the animal of CD8-mediated immunity and allowing rapid infection (Schmitz et al. 1999). This method can mimic acute infection, showing effects on IL-6, IFN α , and CCL2 production (Madden et al. 2004). This model has been used to identify novel amino acids of interest in neurovirulent strains of SIV required for CNS infectivity (Gaskill et al. 2005), and was also used for microarray analysis of frontal lobes of macaques exhibiting signs of SIV encephalitis (Roberts et al. 2003). Increased cyclin D3, tissue transglutaminase, α 1-anti-chymotrypsin, and STAT1 were found—all of which are involved in pathways promoting migration of macrophages to the brain (Roberts et al. 2003). A similar study showed that IL-6 and other inflammatory factors were up-regulated in both the acute and long-term phases of infection (Roberts et al. 2004). Further investigation using this model may clarify whether the activities of IL-6 are neuroprotective or damaging in chronic infection.

A chimera of SIV and HIV termed SHIV is also used for the study of HIV-1 and HIV-2 infection in macaque models. SHIV is generated by the substitution of HIV genes with the similar genes from SIV. The HIV-1 *env* gene was the first gene replaced to study its protein product in nonhuman primates (Shibata et al. 1991; Shibata and Adachi 1992). Many strains of SHIV have been developed; the highly neuropathic SHIV-KU2 strain was produced by passaging an earlier neurovirulent strain (Raghavan et al. 1997; Liu et al. 1999). Another study examined the role of PDGF, which induces CCL2, in SHIV-KU2-infected

cell lines and encephalitic macaques (Potula et al. 2004). This study showed that up-regulation of PDGF was associated with encephalitis.

Microarray analysis of total RNA extracted from basal ganglia of SHIV_{89,6}P-infected encephalitic macaques showed significant up-regulation of a number of inflammatory mediators, including PDGF-B, CCL2, and IL-4 (Sui et al. 2003; see review Buch et al. 2004). Another study, using the pathogenic SHIV_{50OLNV} strain, showed that BBB disruption in the SHIV model was transient. Zona Occludens-1, a TJP, was absent in brain vascular EC during the early acute phase of infection, but was restored at the end stage of infection (Stephens et al. 2003). The results of these studies support the hypothesis that dysregulation of cytokines and chemokines during SHIV infection may be responsible for disruption of the BBB as well as for infiltration of monocytes.

The rodent model

Rodent models utilizing transgenic mice, specialized viral chimeras, and severe combined immunodeficient (SCID) mice have also contributed to the understanding of neurological disease associated with HIV infection (for a comprehensive review, see Persidsky et al. 2005). One mouse model was developed by using SCID mice that are injected with infected human primary macrophages to mimic the acute phase of NeuroAIDS (Tyor et al. 1993; Persidsky et al. 1996) as well as chronic infection, causing both behavioral and cognitive deficits (Avgeropoulos et al. 1998; Griffin et al. 2004). This model was used in conjunction with an *in vitro* cell culture BBB model, and postmortem histopathology of human tissue from individuals with HIVE, to investigate the role of chemokines in monocyte migration across the BBB in HAD. This approach demonstrated that production of the chemokines CCL2, CCL3, CCL4, and CCL5 by microglia, astrocytes, and macrophages was responsible for migration of monocytes across the BBB (Persidsky et al. 1999). Additionally, the neuropathology in this model was attributed to viral infection of the macrophages and their subsequent production of inflammatory mediators including TNF α and IL-6 (Nukuna et al. 2004).

Another mouse model of HIV infection and AIDS was developed by constructing a JR-CSF transgenic mouse line and crossing it with a SCID mouse line. The JR-CSF line itself was produced by microinjection of a full molecular clone of HIV-1_{JR-CSF}, under its natural LTR promoter, into FVB \times C57/B6 fertilized embryos (Browning Paul et al. 2000; Wang et al. 2002).

In this model, HIV was detected in all organs tested, including the spleen, thymus, lymph node, bone marrow, and brain (Browning Paul et al. 2000). This model has been used to study the pathways by which inflammatory responses, such as the production of GM-CSF, can affect latently infected monocytes, causing them to produce virus (Osiecki et al. 2005). This model was also used to examine infected microglia (Wang et al. 2003a), and demonstrated that when stimulated by LPS, JR-CSF transgenic microglia not only expressed a greater level of CCL2, but also produced significantly more viruses. Recently, these mice were crossed with hu-cycT1 mice, to allow expression of the human cyclin T1 gene under a CD4+ promoter (Sun et al. 2006). The T lymphocytes, monocytes, and macrophages of JR-CSF/hu-cycT1 mice expressed cyclin T1, facilitating Tat-mediated transactivation. These mice exhibited even greater inflammatory responses, and more closely paralleled aspects of NeuroAIDS when challenged with LPS. Thus, this model provides a transgenic mouse line through which the interplay of inflammatory cells in the periphery and neurological inflammation has a role in productive HIV-infection and its associated neurodegeneration and pathologies.

Another informative mouse model of HIV-related disease progression and pathology uses a specialized chimera of HIV-1/NL4 and ecotropic murine leukemia virus (termed EcoHIV). This model allows for infection of adult immunocompetent mice, mimicking HIV infection of humans (Potash et al. 2005). EcoHIV contains an element of gp80 from MLV that substitutes for a portion of gp120, bypassing the normal receptor requirements of HIV. This pseudotyped virus produced inflammatory and immunological responses observed in archetypal human HIV infection; the virus was detectable in the spleens, macrophages, and brains of infected mice (Potash et al. 2005). The authors also showed that a similarly produced chimeric virus (EcoNDK) caused the expression of IL-1 β , CCL2, and STAT-1 in the brains of infected mice. These pseudotyped chimeric viruses produced markers of neuroinflammation, therefore providing a useful mouse model for NeuroAIDS.

The feline model

FIV reproduces a number of significant aspects of HIV infection as well as its associated neurological disease (see Fox and Phillips 2002). Its potential contribution is underscored by the findings that FIV is capable of infecting microglia, macrophages, and astrocytes in its natural host (Poli et al. 1999; Hein et al. 2001). Cats

have been used to study a number of immunological aspects of retroviral infection and neurodegeneration, including the role of PBMCs/monocytes, *N*-acetylaspartyl glutamate, and CD40 ligand (see Fox and Phillips 2002).

Pharmacology of HIV infection and the BBB

The pharmacological challenge: crossing the blood–brain barrier

Highly active antiretroviral therapy, although successful in maintaining low viral load in the periphery, may not effectively eradicate hidden viral reservoirs. This dichotomy is most striking in the increased prevalence of HAD since the advent of HAART (McArthur et al. 2003). While infected individuals are living longer, the prevalence of HAD suggests that drugs used in combination therapies are ineffective in the CNS. Zidovudine (AZT) has been found to be effective in treating symptoms of NeuroAIDS; however, this effect may be short-lived (Siddis et al. 1993; Baldeweg et al. 1998). Various approaches have been developed to address these challenges. Examination of the compartmentalization of drug resistance mutations in HIV to specific regions of the brain suggested that subpopulations of drug resistant virus may reseed, and spread through the circulation, thus disseminating drug resistant virus (Smit et al. 2004). One clinical study indicated that HAART was able to improve neurocognitive function in spite of its poor ability to penetrate the BBB (Robertson et al. 2004).

Other studies emphasized the importance of characterizing drug transport into, and efflux from, the CNS itself as a primary mode of addressing poor penetration by therapeutic agents and the prevalence of neurocognitive disorders during the course of HIV infection. P-glycoprotein was identified as one of the transporters capable of preventing antiretroviral therapies from accessing the brain (Strazielle et al. 2004; Loscher and Potschka 2005). In one study, postmortem tissue samples from encephalitic brains of HIV-infected individuals were immunostained for P-gp. Results showed that individuals with HIVE had more EC P-gp staining than those without HIVE (Langford et al. 2004). Those with HIVE also showed significantly enhanced staining of astrocytes and microglia, thus identifying these cells as primary participants in the exclusion of antiretroviral treatments in individuals with HIVE (Langford et al. 2004). These data may lead to the development of methods that improve the transport of NeuroAIDS therapies across the BBB.

Adjunctive therapy

Treatment of HIV-related neurodegenerative diseases requires combating the virus and its direct effects, as well as controlling the inflammatory response that mediates much of the CNS pathology. Therefore, the pharmaceutical focus should expand to include adjunctive therapies that may prevent HIV infection of immune cells that infiltrate the BBB, decrease neurocognitive impairment through neuronal protection, and target and control neurotoxic mediators of CNS inflammation (Lane et al. 1996; Nuovo and Alfieri 1996; Gartner 2000; Diesing et al. 2002; Perry et al. 2005).

Receptor antagonists

Chemokine antagonists. The identification of CXCR4 and CCR5 as coreceptors for HIV infection led to the study of the ligands of these receptors for their ability to prevent viral binding to, and infection of, CXCR4 and CCR5 positive cells. However, the finding that these ligands could also activate infected cells to produce virus precluded the use of these proteins as an antiviral therapeutic strategy (for comprehensive review, see Princen and Schols 2005). Thus, the focus of future studies centered on the identification of chemokine receptor antagonists. However, their ability to cross the BBB is not well characterized. AMD3465, a monomacrocyclic compound related to the X4 antagonist AMD3100, can strongly inhibit HIV entry through the CXCR4 receptor (Hatse et al. 2005). The structure of AMD3465 may translate into oral delivery and potential clinical use (Hatse et al. 2005). Another receptor antagonist, AMD3451, was shown to inhibit both X4 and R5 strains; however, more studies are needed to determine its therapeutic potential (Princen et al. 2004). A number of CCR5 antagonists are in clinical trials (Julg and Goebel 2005; Princen and Schols 2005). By preventing entry of the virus into cells that have the potential to cross the BBB, it may be possible to decrease incidence of NeuroAIDS.

NMDAR antagonists: memantine. NMDAR antagonists are a proposed therapy for HAD (Lipton 1994, 1998). Memantine, an NMDAR antagonist, has been successfully used for the treatment of Alzheimer's disease, a neurodegenerative disorder with several clinical manifestations similar to HAD (Reisberg et al. 2003). It is also prescribed for long-term treatment of Parkinson's disease (Lipton 1998). This drug was studied in the SCID mouse model of HIVE described above (Tyor et al. 1993; Persidsky et al. 1996; Anderson et al. 2004). Using monocyte-

derived macrophages infected with HIV-1_{ADA}, a well-characterized R5 strain, this model replicates the neurotoxicity and neuroinflammation associated with HIVE. The testing of long-term potentiation and other cognitive indicators, as well as histopathology, showed that the memantine-treated HIVE mice had a lesser degree of synaptic impairment than the HIVE mice that were not treated with memantine (Anderson et al. 2004). Thus, the authors conclude that this drug may be a candidate adjunctive therapy, capable of decreasing synaptic impairment in infected individuals by maintaining neuronal integrity in the face of the inflammatory response that is characteristic of NeuroAIDS.

Neuroprotective lithium

The neuroprotective potential of lithium and its pro- and antiapoptotic effects have been studied in a number of neurological diseases, including Alzheimer's disease. Lithium affects many neurological pathways through protective mechanisms, and is linked to each of the following apoptotic, signaling, and transduction molecules and pathways: phosphatidylinositol 3 (PI3)-kinase (PI3-K), Akt, MAPK, glycogen synthase kinase-3 β (GSK-3 β), Tau, β -catenin, heat shock factor 1, activator protein 1, cAMP, Bcl-2, BDNF, NMDAR activation, and calcium (see Dou et al. 2005). Immunohistochemical and long-term potentiation studies were used to investigate the neuroprotective potential of lithium in the previously described monocyte derived macrophage rodent model of HIVE (Dou et al. 2005). Histopathology showed that lithium was protective in the neurodegeneration associated with HIV infection, yet it had no specific antiretroviral effect. Thus, the authors theorize that lithium may protect neurons against neurotoxins associated with HIV. Further understanding of the effects of lithium on neuroinflammation are required.

Minocycline

Minocycline, a derivative of tetracycline, has been shown to have both antiretroviral and neuroprotective effects in macaques, dually infected with SIV (Zink et al. 2005). Minocycline-treated animals showed no or mild SIV encephalitis, whereas untreated macaques showed moderate to severe SIV encephalitis. The untreated macaques showed significantly decreased expression in the brain of the following markers of inflammation: major histocompatibility class II antigens, macrophage marker CD68, T-cell intracytoplasmic antigen I, and β -amyloid precursor protein. Activated p38 mitogen-activated protein kinase

(MAPK) had lower expression in HIV-infected minocycline-treated T lymphocytes as well. After 28 days (during the second phase of increased CCL2 in the CSF, discussed earlier), minocycline-treated, SIV-infected macaques showed little CCL2 in CSF and brain samples, as compared to untreated, infected animals. These results show a correlation between minocycline treatment and a diminished inflammatory response (Zink et al. 2005).

In addition to its potent anti-inflammatory effects, minocycline inhibits the release of both R5 and X4R5 viruses in cultured human primary microglia (Si et al. 2004). Minocycline was found to affect LTR promoter activity in U38 cells, as well as NF- κ B nuclear binding activity in cultured microglia (Si et al. 2004). Minocycline is a candidate for treatment of NeuroAIDS because it can cross the BBB, decrease viral release, and decrease CNS inflammation (Tikka et al. 2001; Colovic and Caccia 2003). Limiting neuroinflammation may positively affect the integrity of the BBB during the chronic HIV infection. A treatment strategy combining the ability of minocycline to reduce neuroinflammation with both antiretroviral and neuroprotective therapies may be most effective against the effects of NeuroAIDS.

Substance abuse, HIV, and the BBB

There is significant comorbidity in HIV-infected individuals who use substances of abuse. Thus, the effects of HIV infection and drug abuse on the BBB and viral entry into the CNS are a target of study (Fig. 1). (For a more comprehensive review, see Nath et al. 2002.)

Cocaine

The decline of neurocognitive function associated with cocaine abuse has similar properties to that associated with HAD (see Goodkin et al. 1998 for review of neuropathology). Cocaine was shown to induce inflammatory responses in the CNS through the up-regulation of cytokines (Fiala et al. 1996), and to alter the migration of monocytes across the BBB (Fiala et al. 1998). Thus, the role of cocaine in inflammation and monocyte trafficking into the CNS was significant and its effects on HIV infection were investigated. One study showed that cocaine increased the permeability of the BBB to the HIV-1_{JR-FL} R5 strain (Zhang et al. 1998). Additionally, when BMVEC were treated with cocaine, there was increased production of IL-8/CXCL8, CCL2, CCL3, TNF- α , and interferon-inducible protein-10 (IP-10/CXCL10) (Zhang et al.

1998). Another report demonstrated that HIV binds BMVEC in a manner not yet identified, causing the formation of gaps in cell junctions (Fiala et al. 2005). Cocaine-treated BMVEC showed disruption of the actin cytoskeleton. The data further suggested that this disruption facilitated the aggregation of HIV into “cytoplasmic lakes,” altering cytoplasmic trafficking of the virus (Fiala et al. 2005). Changes in BBB permeability due to the toxic effects of cocaine, combined with the response of HIV-infected monocytes to inflammatory mediators, may explain some of the accelerated HAD and neuronal pathology described clinically in HIV-infected individuals who use substances of abuse, as well as in animal studies (Reyes et al. 1991; Nath et al. 2001, 2002).

Methamphetamine

Like cocaine, methamphetamine (METH) damages neurological function. A number of studies examined the effects of METH abuse in HIV-infected individuals; one study showed changes in expression of genes that are induced by interferon (Everall et al. 2005). Other studies showed that the presence of Tat and METH induced changes in expression of TNF- α , IL-1 β , and ICAM-1 in the brain (Flora et al. 2003) as well as changes in dopaminergic activity (Maragos et al. 2002) and neuronal death (Turchan et al. 2001). Individuals who abuse METH have a higher plasma viral load, suggesting that the effectiveness of HAART is decreased with the use of this substance (Ellis et al. 2003). Thus, METH has a significant effect upon HIV-induced inflammation and progression of NeuroAIDS.

Morphine

The μ -opioid class of receptors (MOR) is associated with the effects of heroin, and its major metabolite, morphine (Matthes et al. 1996). Morphine increased the expression of the CXCR4 and CCR5 HIV coreceptors in T lymphocytes and monocytes, and the CCR5 receptor in primary human astrocytes (Mahajan et al. 2002; Steele et al. 2003). These findings suggest that morphine may increase both HIV binding to and infection of PBMCs and may be astrocytes (Mahajan et al. 2002; Steele et al. 2003). Studies of SIV-infected macaques that received morphine demonstrated increased CCR5 expression on PBMCs, as well as increased viral replication in the CNS (Suzuki et al. 2002; Kumar et al. 2004).

Morphine also affects chemokine production in astrocytes. When treated with Tat and morphine, human

and mouse astrocytes increased their production of CCL2, which caused chemotaxis of microglia (El-Hage et al. 2005, 2006). PBMCs treated with DAMGO ([D-ala², N-Me-Phe⁴, Gly-ol⁵] enkephalin), a ligand of MOR, produce CCL2, CCL5, and CXCL10 proinflammatory chemokines (Wetzel et al. 2000). When treated with 2-5A^{N6B}, an inhibitor of morphine-potentiated HIV replication, PBMCs showed elevated expression of CCL2 and CCL5 (Homan et al. 2002). (For a more comprehensive review on opioid receptors and inflammation, see Rogers and Peterson 2003). These results support a mechanism by which inflammatory mediators of NeuroAIDS are significantly affected by morphine.

Alcohol

Alcohol abuse has been shown to cause neurological dysfunction and disruption of the BBB (for review, see Tyor and Middaugh 1999; Mann et al. 2001; Meyerhoff 2001). Its metabolism generated reactive oxygen species that caused phosphorylation of myosin light chain and TJP. This affected immune function, decreased the integrity of the BBB, and increased monocyte migration across a BBB model (Haorah et al. 2004, 2005a,b). HIV infection compounded these effects. Ethanol and Tat treatment caused a synergistic increase in CCL2 production in rat hippocampus and corpus striatum (Flora et al. 2005). Alcohol was also shown to increase CXCR4 expression in peripheral blood lymphocytes, and CCR5 in monocyte derived macrophages, suggesting increased susceptibility of these cells to infection and chemotactic signals (Wang et al. 2002; Liu et al. 2003).

Conclusion

Alterations in signaling between components of the BBB with either HIV proteins or factors produced in response to HIV infection, or with HIV-infected cells, disrupt BBB integrity and result in compromise, thereby promoting continued transmigration of activated monocytes and/or HIV-infected cells into the brain. A more complete understanding of the interactions among the BBB, nervous system, and immune system is critical to identification of the mechanisms that mediate NeuroAIDS. The use of animal models will facilitate this understanding as well as assist in the development of therapeutic strategies that will limit CNS dysfunction. Clarification of the contribution of substances of abuse to BBB disruption will enhance our understanding of the effects of HIV and drugs on neuroinflammation and comorbidity.

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