Published in final edited form as:

J Neuroimmune Pharmacol. 2013 March ; 8(1): 118–131. doi:10.1007/s11481-012-9353-4.

When Human Immunodeficiency Virus Meets Chemokines and Microglia: Neuroprotection or Neurodegeneration?

Italo Mocchetti¹, Lee A Campbell¹, G. Jean Harry², and Valeriya Avdoshina¹

¹Department of Neuroscience, Georgetown University Medical Center, Washington, DC 20057

²National Toxicology Program Laboratory, National Institute of Environmental Health Science, Research Triangle Park, NC 27709

Abstract

Chemokines are chemotactic cytokines that were originally discovered as promoters of leukocyte proliferation and mobility. In recent years, however, evidence has demonstrated constitutive expression of chemokines and chemokine receptors in a variety of cells in the central and peripheral nervous system and has proposed a role for chemokines in neurodegenerative diseases characterized by inflammation and microglia proliferation. In addition, chemokine receptors, and in particular CXCR4 and CCR5, mediate human immunodeficiency virus type 1 (HIV) infection of immunocompetent cells as well as microglia. Subsequently, HIV, through a variety of mechanisms, promotes synapto-dendritic alterations and neuronal loss that ultimately lead to motor and cognitive impairments. These events are accompanied by microglia activation. Nevertheless, a microglia-mediated mechanism of neuronal degeneration alone cannot fully explain some of the pathological features of HIV infected brain such as synaptic simplification. In this article, we present evidence that some of the microglia responses to HIV are beneficial and neuroprotective. These include the ability of microglia to release anti-inflammatory cytokines, to remove dying cells and to promote axonal sprouting.

Keywords

HIV; CCL5; CXCL12; dopamine; glutamate; cytokines

Introduction

Infection by human immunodeficiency virus type 1 (HIV) promotes apoptosis of immune cells, especially CD4⁺ T lymphocytes, that leads to acquired immune deficiency syndrome (AIDS) (Pantaleo and Fauci, 1995). Loss of immune cells can be reversed by antiretroviral therapy so that the immune system can recover. Nevertheless, despite the introduction of combination antiretroviral therapy or cART, a substantial proportion of HIV-positive subjects also develops neurocognitive abnormalities termed HIV-associated neurocognitive disorders (HAND) [rev. in (Ellis et al., 2007)]. The failure of cART to reduce such neurological deficits can be multifactorial and include ongoing viral replication due to drug resistance or inefficient penetration of drugs through the blood brain barrier (BBB), a barrier consisting of brain microvascular endothelial cells and end feet of astrocytes that is selectively permeable to immune cells. Each phenomenon results in an ineffective

Corresponding Author: Italo Mocchetti, Georgetown University Medical Center, Research Building, Room EP04 Box 571464, Washington, D.C. 20057, Phone: 202 687-1197, FAX: 202 687-0617, mocchetti@georgetown.edu.

The authors have no conflict of interest to declare.

elimination of the virus in the central nervous system (CNS). Either of these mechanisms can result in the CNS serving as a reservoir for HIV prior to the onset of neurological signs (Thompson et al., 2011). A further confounding factor is the co-morbidity of drug abuse in HIV patients presenting the possibility of an interaction between HIV and drugs to exacerbate neuronal injury or dysfunction. Indeed, the incidence and severity of neuropsychological deficiencies in HIV infected individuals is augmented in HIV positive subjects taking cocaine, methamphetamine (Nath et al., 2001; Rippeth et al., 2004) or other drugs of abuse (Cristiani et al., 2004; Cosenza-Nashat et al., 2011).

Neurological alterations in HIV positive individuals can be asymptomatic, mild, or severe. In the severe form, termed HIV-associated dementia (HAD), patients exhibit motor, cognitive, and behavior impairments. It has now become apparent that many of these patients are suffering from protracted forms of HIV encephalitis (HIVE), a neuro-inflammatory condition characterized by the presence of activated microglia, formation of microglial nodules, multinucleated giant cells, astrogliosis and myelin loss (Wiley and Achim, 1994; Davies et al., 1997; Anderson et al., 2002; Everall et al., 2005). Moreover, the brains of these individuals exhibit extensive neurodegeneration in various brain areas. For instance, studies using postmortem or imaging techniques have revealed cerebral atrophy and cell death in the subcortical regions of the basal ganglia and hippocampus, as well as thinning of the cerebral cortex (Albright et al., 2003; McArthur, 2004; Everall et al., 2005). Abnormalities of the basal ganglia include neuronal loss in the putamen (Everall et al., 1995) and globus pallidus (Fox et al., 1997), loss of nigro-striatal dopamine neurons (Reyes et al., 1991; Itoh et al., 2000), and dysfunctional dopaminergic transport (Wang et al., 2004). HAD patients exhibit decreased dopamine and homovanillic acid levels in the caudate nucleus (Sardar et al., 1996) as well as in cerebrospinal fluid (Berger et al., 1994; di Rocco et al., 2000) and are more likely than other HIV positive individuals to develop extrapyramidal side effects from neuroleptics blocking striatal dopamine neurotransmission (Edelstein and Knight, 1987; Hriso et al., 1991). Given the similarity of dopaminergic neuronal death, it is not uncommon to find clinical features of HAD resembling Parkinson's disease, such as loss of postural stability, involuntary movements, bradykinesia, and impairment in fine motor skills (Berger and Arendt, 2000; Koutsilieri et al., 2002; McArthur, 2004; Nath and Berger, 2004).

The underlying causes of pathological alterations observed in these patients are not understood and are still under investigation. Morphologically, neurons exhibit synapto-dendritic atrophy (Masliah et al., 1997) accompanied by shrinkage and retraction of neurites and loss of dendritic spines. Thus, it appears that the process of neuronal degeneration by HIV first occurs in axons and dendrites and then "spreads" in a retrograde manner to the neuronal cell body, resulting in apoptosis and cell loss. While there is agreement about the clinical and pathological scenario for HAD, the underlying molecular and cellular mechanisms of this disease remain unknown. One proposal put forth is related to the immune-mediated activities of the disease and the concurrent activation of microglia and astrocytes leading to an inflammatory environment. The purpose of this review is to present old and new hypotheses that may help define the neurobiology of HIV and the role of chemokines and microglia and their interaction with viral proteins.

HIV and chemokines

HIV infects various CD4⁺ cells of the immune system including T lymphocytes, monocytes and macrophages, with macrophages being among the first cells infected with HIV following sexual transmission (Zhu et al., 1993). Nevertheless, HAND does not develop in HIV-infected individuals until many years later, usually in association with the depletion of CD4⁺ T cells and the resultant development of immunodeficiency (Persidsky and

Gendelman, 2003). HIV enters immune cells using a dual receptor system comprised of CD4 as a primary receptor and the mandatory co-receptors of the chemokine family. Chemokines (short for chemoattractant cytokines) belong to a superfamily of immunomodulatory, secreted proteins that were initially identified according to their ability to modulate the hematopoietic/lymphopoietic system and regulate leukocyte trafficking during inflammation. Chemokines are small peptides (8–14 kDa) subdivided over 4 subfamilies encompassing 46 members. They are classified based on their primary structure containing cysteines and by the number and spacing of these cysteines. For instance, α -chemokines have the first two cysteines separated by one amino acid, hence the name “CXC” chemokines, whereas β -chemokines are named CC because there are no amino acids separating their cysteines. The γ -chemokines (C or XC), have only a single cysteine whereas the δ chemokine (CX3C), or fractalkine, has 3 amino acids between the two cysteines (Rossi and Zlotnik, 2000). A new nomenclature has recently been adopted to be more consistent with their genomic organization (Zlotnik et al., 2006). The nomenclature contains the letter “L” for ligand or “R” for receptor and is followed by the numbering system that designates the genes encoding each chemokine. The human proteins are indicated using capital letters, whereas rodent molecules are in lower cases. Thus, CCL5 (formerly known as regulated upon activation-normal T-cell expressed and secreted, or RANTES) and Ccl5 are the human and mouse molecules, respectively, that bind to CCR5. CXCL12 (formerly known as stromal-cell derived factor-1) is a ligand for CXCR4. One receptor can be activated by more than one chemokine and a single chemokine may activate only one receptor. Such examples include: CCL5, CCL3 (macrophage inflammatory protein, or MIP-1 α) and CCL4 (MIP-1 β) that all bind to CCR5; whereas CXCL12 and CX3CL1 bind to CXCR4 and CX3CR1 receptors, respectively (Table 1).

Different strains of HIV can use either CCR5 or CXCR4 to enter cells. The infection begins when the HIV envelope glycoprotein 120 (gp120) binds sequentially to CD4 and either CCR5 or CXCR4 on the surface of immunocompetent cells. Such binding activates cell-virus membrane fusion mediated by the gp41 subunit of the envelope protein which facilitates HIV entry into cells (Berson et al., 1996; Feng et al., 1996). A nomenclature has been developed wherein viral strains were designated R5 or X4 depending upon the co-receptor usage. R5 indicates a strain of HIV that uses CCR5 to infect and replicate efficiently in macrophages (Alkhatib et al., 1996; Deng et al., 1996; Dragic et al., 1996; Weissman et al., 1997), whereas X4 uses CXCR4 to enter and replicate in T lymphocytes (Dittmar et al., 1997; Scarlatti et al., 1997). Dual tropism viruses that use both co-receptors are designated R5X4 (Doranz et al., 1996). The presence of R5 or X4 strains varies during the course of the infection. R5 tropic viruses predominate during the initial phase of infection and are transmitted with greater efficiency (Gendelman et al., 1990; Tuttle et al., 2002), whereas the X4 viruses emerge later during the disease and are associated with rapid progression to AIDS (Hu et al., 2000; Raymond et al., 2010).

The importance of CCR5 and CXCR4 in HIV infection is supported by a number of evidence. First, genetic mutation of CCR5 confers resistance to HIV infection (Dean et al., 1996; Paxton et al., 1996). Second, the CCR5 ligand CCL5 or the CCR5 antagonist maraviroc (Kondru et al., 2008) prevents R5 viral entry. Similarly, the CXCR4 antagonist AMD3100 (Donzella et al., 1998) blocks X4 infection of lymphocytes. When AMD3100 and maraviroc are used together, they block dual-tropic HIV (Gouwy et al., 2011). Agonists or antagonists inhibit the viral entry from the cell surface, and thereby viral infection, through either an allosteric block of the binding interaction between gp120 and co-receptors CCR5 and CXCR4, or by promoting receptor internalization (Aramori et al., 1997; Tarasova et al., 1998; Escola et al., 2010). Based upon these properties, these and similar antagonists have been proposed in a prophylactic strategy for individuals at risk for HIV infection, including drug abusers (De Clercq, 2003). While these compounds may serve to minimize

infection, their limited entry into the brain due to poor penetration across the BBB limits their ability to reduce HAND.

HIV and microglia

Preventing the development of HIVE requires an understanding of how the virus accesses the CNS. There are many theories about how HIV crosses the BBB, the most compelling being the ‘Trojan Horse’ hypothesis in which infected monocytes and T cells are trafficked to the CNS parenchyma [reviewed in (Gonzalez-Scarano and Martin-Garcia, 2005)]. Here the role of chemokines, and especially CCL2, is to promote transmigration of these cells (Buckner et al., 2011). Once within the CNS, HIV infected monocytes can differentiate into macrophages and initiate infection and/or activation of cells that express markers for macrophage/microglia lineage. Thus, transmigration of infected monocytes across the BBB plays a major role in HIV infection and neuropathogenesis. Additional *in vitro* data suggest that HIV enters the CNS by transcytosis of endothelial cells (Bomsel, 1997; Liu et al., 2002) or by direct infection (Argyris et al., 2003; Bobardt et al., 2004). Regardless of which cellular mechanism(s) is used by HIV to infect the CNS, it has been widely accepted that HIV penetrates into the CNS a few weeks after systemic infection, where the virus mostly targets and resides in perivascular macrophages (Koenig et al., 1986) and perivascular microglial cells [reviewed in (Kramer-Hammerle et al., 2005)]. Nevertheless, recent data have demonstrated that parenchymal microglia are capable of productive infection (Thompson et al., 2011). Thus, infected parenchymal microglia, by the time of HIVE, may well represent a CNS viral reservoir. Nevertheless, infection of microglia has been reported to coincide with microglia proliferation and dystrophy characterized by increased branching, cytoplasmic distension, and nodules (Budka, 1991; Masliah et al., 1996).

When compared to microglia, astrocytes are capable of a limited productive infection (Conant et al., 1994; Tornatore et al., 1994a)); however, neurons and oligodendrocytes are rarely directly infected by HIV. The cellular mechanism of this selective neurotropism appears to be due to the fact that microglia/macrophages but not the other brain cells express CD4 (Jordan et al., 1991) and several chemokine co-receptors, including CCR5, CXCR4 and CCR3 (Albright et al., 1999). While CD4 is a primary mechanism for HIV entry into the brain, a CD4-independent viral entry into astrocytes mediated via mannose receptors has been reported (Liu et al., 2004). Furthermore, propagation of HIV infection may also occur through a gap junction mechanism (Eugenin et al., 2011). Moreover, microglia express lower levels of CD4 than T cells; thus, HIV appears to exhibit a reduced dependence on CD4 in cells from the CNS (Thomas et al., 2007). Most likely, microglia could be infected because the HIV envelope protein consisting of gp120 and gp41 has reduced dependence to CD4 and/or CCR5 levels as well as an increased “fusion activity” with CD4 and co-receptors. Consistent with this notion, studies have shown a more efficient receptor interaction of gp120 requiring lower levels of expression of CCR5 and CD4 to mediate cell-to-cell fusion (Gorry et al., 2002). Moreover, mutated HIV envelope proteins with reduced dependence on CD4 levels are more frequent in the brain as compared with lymphoid tissue from the same patients (Dunfee et al., 2006b)). These data underscore an important role of microglia in HIV infection and suggest that HAD may be caused by HIV with an enhanced tropism for macrophages and microglia.

Which co-receptor is crucial for HIV infection of microglia? *In vitro* studies have shown that CCR5 and CCR3 but not CXCR4 mediate HIV infection of microglia (He et al., 1997; Gabuzda et al., 1998) implicating microglia as a primary R5 strain producing cells in the brain (Watkins et al., 1990). On the other hand, some R5 strains use CXCR4 for entry into microglia and macrophages (Simmons et al., 1998; Gorry et al., 2001; Koning et al., 2001; Singh et al., 2001; Naif et al., 2002). This may explain why X4 isolates or isolates that use

either R5 or X4 (dual-tropic) are identified in the brain or cerebral spinal fluid (Dunfee et al., 2006a)). Moreover, X4 infection of microglia may actually be facilitated following the antiretroviral therapy with CCR5 antagonists. In fact, *in vivo* data have shown that CXCR4 using viruses undergo specific adaptations in the late stage, thus facilitating the evolution to CXCR4 usage in the presence of low levels of CCR5 (Edo-Matas et al., 2011). These considerations have led to the belief that microglia are more efficiently infected by macrophages-tropic and dual-tropic strains than by T-tropic strains (Ioannidis et al., 1995) or alternatively, that microglia provide support for infection of X4 strains only at a later time (McCarthy et al., 2002). Thus, regardless of their exact function, microglia can become a reservoir for different viral strains serving to sequester them from systemic immune surveillance. Such cellular reservoirs strongly support the need for key research priorities for HIV eradication that include the development of targeted strategies that deplete viruses from these cells and eliminate a long-lasting source within the brain.

CXCR4, CCR5 and their ligands in the adult CNS

Chemokine receptors directly influence HIV neuropathogenesis by allowing the initiation of CNS infection or inducing microglia proliferation. For instance, the fractalkine CX3CL1, also known as neurotactin (Bazan et al., 1997), promotes microglia proliferation and migration via activation of its receptor CX3CR1 (Maciejewski-Lenoir et al., 1999; Hatori et al., 2002); thus, it may play a role in spreading HIV infection of the CNS. However, chemokines can also promote brain abnormalities through other mechanisms. In fact, an aspect of CXCR4 and CCR5 relevant to the neuropathology of HIV, aside from mediating microglia infection, is their role in neuronal degeneration. To better comprehend this issue, a brief overview of the expression of these receptors and their ligands is necessary. A comprehensive review describing all CNS cells expressing chemokines has been published (Cartier et al., 2005). It is crucial to point out that some of the initial discoveries of CXCR4 or CCR5 expression in the brain were obtained by looking at pathological conditions characterized by inflammation (Klein et al., 1999; Petit et al., 2001). From this work, it was subsequently suggested that chemokines, and their receptors, are present in the brain to participate in the inflammatory component of neurodegeneration. However, in more recent years, a number of studies have confirmed that CXCR4, CCR5, and their ligands are constitutively expressed in the adult uninjured brain in different cell types. Intriguingly, there appears to be heterogeneity across the neuronal populations in the level of expression of these chemokines. For instance, dopaminergic neurons of the *substantia nigra* as well as nigro-striatal fibers exhibit abundant CXCR4 immunoreactivity in both rats (Banisadr et al., 2002) and humans (van der Meer et al., 2000; Shimoji et al., 2009). Cholinergic neurons of the basal forebrain (Banisadr et al., 2003; Trecki et al., 2010) and retinal neurons (Ahmed et al., 2009) are also positive for CXCR4. Several neuronal layers of the hippocampal formation express CXCR4 mRNA. These include the molecular layer, the granular and polymorphic layers, and the *stratum lacunosum moleculare* (Stumm et al., 2002). CCR5 is expressed by microglia cells and promotes their proliferation; however, it has also been localized in various neuronal populations *in vivo*, including cortical and striatal neurons, cerebellar Purkinje cells, and hippocampal pyramidal neurons (Westmoreland et al., 2002; Avdoshina et al., 2011).

CXCR4 and CCR5 ligands, CXCL12 and CCL5, respectively, can be produced by nonneuronal as well as neuronal cells. It has been known for a while that microglia, or astrocytes, can produce and release chemokines in response to brain inflammation [reviewed in (Cartier et al., 2005)]. This is consistent with their role as chemoattractants for non-neuronal cells to the site of inflammation. However, much is still unknown about their role in neuronal cells and their co-localization with primary neurotransmitters. For instance, dopamine neurons of the *substantia nigra* express both CXCL12 (Banisadr et al., 2003;

Shimoji et al., 2009) and CCL5 (Fig. 1). Such co-localization of chemokines with a biogenic amine neurotransmitter is reminiscent of a scenario described for neuroactive peptides acting as neuromodulators. By definition, a neuromodulator must be synthesized in nerve cells, stored in synaptic vesicles and released in a Ca^{2+} dependent manner by neuronal activity. Evidence that chemokines are stored in large core dense presynaptic vesicles was recently obtained for at least two chemokines, CCL21 (de Jong et al., 2008) and CXCL12 (Callewaere et al., 2008). Moreover, depolarization or activation of N-methyl-D-aspartate (NMDA) receptors increases the accumulation of CCL5 in the medium of primary cultured cortical neurons (Fig. 2), suggesting an activity-dependent regulation of its release not dissimilar to that seen for neurotransmitters. Once released, a neurotransmitter regulates synaptic function in an autocrine or paracrine fashion, producing synaptic potentials that can last hundreds of milliseconds. An autocrine function may include neurotransmitter release whereas a paracrine function may include perception of pain, or complex responses to stress. For instance, the CXCR4/CXCL12 system has been shown to modulate the release of GABA (Guyon et al., 2005) and dopamine (Skrzydelski et al., 2007), and consequently to induce motor activity. Thus, it has been suggested that these and other chemokines play a role in neuronal communication and plasticity (Adler and Rogers, 2005; Rostene et al., 2007). Based on these considerations, we put forth the idea that chemokines within the brain have assumed an additional role and serve not only as immunomodulators but possibly also as neuropeptide neurotransmitters to modulate neuronal-glia and glia-glia interactions.

The ability of CXCL12 and CCL5 to release neurotransmitters could be relevant for the neuropathological changes seen in HAD. In fact, both chemokines modulate glutamate release and transmission (Guyon et al., 2005; Musante et al., 2008). Glutamate has been suggested to cause neuronal loss in HAD and other neurological diseases through the activation of NMDA receptors (Kaul et al., 2001). Paradoxically, when glutamate is released at physiological (subtoxic) concentrations, it protects neurons against excitotoxic levels of glutamate or NMDA (Marini et al., 1998). This phenomenon is also known as preconditioning, a way whereby neurons develop tolerance to lethal insults induced by exposing them to a prior sublethal stimulus (Marini et al., 2007). Mechanisms of preconditioning could be multiple. For instance, glutamate could release neurotrophic factors that, in turn, modulate the opening of NMDA channel by down-regulating one or more receptor subunits (Brandoli et al., 1998). CXCL12 has been shown to induce the release of glutamate (Guyon et al., 2005) whereas CCL5 exhibits a dual modulation on the release of this amino acid; it facilitates its basal release and inhibits glutamate exocytosis evoked by depolarization (Musante et al., 2008). The CXCL12 or CCL5-mediated release of glutamate may play a role in neuroprotection conferred by glutamate preconditioning. Indeed, both CXCL12 (Nicolai et al., 2010) and CCL5 (Bruno et al., 2000) are neuroprotective against NMDA-mediated cell death. Thus, it is possible that, at physiological concentration, these chemokines play a direct as well as an indirect neuroprotective role.

Viral proteins and Chemokines

Altered neuronal-glia interaction appears to be a common denominator for a variety of neurodegenerative diseases, including HAD. In HAD patients, a proportion of the synapses of the hippocampus and the fronto-striatal network undergo degeneration and retraction leading to neuronal death (Ellis et al., 2007). Additionally, there is increased macrophage trafficking into the brain and accumulation of activated microglia (Fischer-Smith et al., 2004). Similar changes were observed in animal models of HIV. For example, mice over-expressing gp120 under a glial fibrillary acidic protein promoter exhibited synaptic simplification that included loss of dendritic processes and decrease in synaptic density in various brain areas (Toggas et al., 1994). A similar scenario has been observed in

“humanized” NOD-SCID mice infected with HIV (Dash et al., 2011). These mice were generated by the combination of immunodeficient CB17-scid (SCID) mice with a phenotype lacking mature B and T cells (Goldman et al., 1998; Mazurier et al., 1999) and transplanted intra-hepatically with human CD34+ cells from fetal liver tissue. Lastly, HIV transgenic rats generated with an integrated HIV genome (Reid et al., 2001) exhibit a deficit in the synaptic marker debrin when the rats reach 8 months of age (Rao et al., 2011). Thus, there are “rodent” models of HAD undergoing synaptic loss caused by HIV.

The selective vulnerability of synapses in HAD does not result from direct intrinsic viral infection given that neurons are not infected. This consideration has prompted study of indirect mechanisms for HIV mediated neurotoxicity and has led to the discovery of viral proteins as a primary cause of neuronal injury. HIV produces nine proteins that play a role in the viral lifecycle. At least six of these proteins can damage neurons; these include the envelop proteins gp120 and gp41, the transcription activator Tat, the accessory proteins Nef and Vpr, and the RNA binding protein Rev [reviewed in (Li et al., 2005)]. Some HIV proteins, especially gp120 and Tat, can be released from infected cells and act on uninfected neurons even at distal sites (Bruce-Keller et al., 2003; Bachis et al., 2006). Gp120 can also be shed by the cell during viral entry. Most evidence favors the theory that gp120 and Tat evoke neuronal injury by promoting the release of pro-inflammatory cytokines and chemokines (Conant et al., 1998; Nath et al., 1999; Bezzi et al., 2001; Kaul et al., 2001; Gonzalez-Scarano and Martin-Garcia, 2005) from microglia and astrocytes. These data support an indirect role of cytotoxins released from nonneuronal cells in HIV neurotoxicity.

Other HIV proteins, such as Nef and Vpr are not released (Tornatore et al., 1994b)). However, they can also cause neuronal apoptosis (Patel et al., 2000; Trillo-Pazos et al., 2000) by altering and disrupting the supportive and neurotrophic role of non-neuronal cells (Radja et al., 2003) and/or by interacting with potassium or calcium channels (Piller et al., 1996; Zegarra-Moran et al., 1999). Although these mechanisms are far from being fully demonstrated *in vivo* they would undoubtedly impair survival of neurons even without inflammation or microglia activation.

Other experimental data have favored the hypothesis of HIV neurotoxicity that occurs by a direct interaction of viral proteins with neuronal pathways/stimuli that promote cell death. For instance, Tat can also activate NMDA receptors (Eugenin et al., 2007) and induce Ca^{2+} -mediated apoptosis (Haughey et al., 2001). The direct theory of neurotoxicity is also supported by data showing that gp120 binding to neuronal chemokine receptors activates signaling pathways coupled to these G protein receptors, such as extracellular signal-regulated kinases, or Erk (Meucci et al., 1998) and p38 mitogen-activated protein kinase (Medders et al., 2010), which can mediate cell death. In fact, direct activation of CXCR4 (Bagetta et al., 1995; Hesselgesser et al., 1998; Meucci et al., 1998; Kaul and Lipton, 1999; Zheng et al., 1999; Bansal et al., 2000) or CCR5 (Kaul et al., 2007; Bachis et al., 2009) by X4 and R5 tropic gp120s, respectively, has been shown to evoke neuronal apoptosis. Similarly, activation of CXCR3 by its ligand CXCL10 promotes neuronal loss (Sui et al., 2006). The neurotoxic signal of both gp120s involves apoptosis through a traditional caspase-3 regulated cell death effector pathway (Kaul et al., 2001; Bachis et al., 2003; Singh et al., 2004) that has also been shown in the brain of HIV subjects (Garden et al., 2002). Nevertheless, caspase-3 is considered a marker for apoptosis of cell bodies, whereas axonal/dendritic injury/retraction is associated with activation of caspase-6 (Nikolaev et al., 2009). Intriguingly, gp120 has also been shown to reduce endogenous growth factors such as brain-derived neurotrophic factor (Nosheny et al., 2004). This neurotrophin supports axonal and dendritic integrity required for neuronal survival (Horch, 2004; Tanaka et al., 2008). Thus, it is plausible to suggest that HIV may promote synaptodendritic degeneration by multiple mechanisms which include a reduction of relevant neurotrophic factors.

Activated microglia and HIV

As mentioned above, macrophage trafficking into the brain and proliferation of activated microglia have also been identified in HIV (Fischer-Smith et al., 2004). Upon infection, microglia cells become multinucleated and form clusters that are subsequently found in the late phase of infection (Sharer et al., 1985; Budka, 1986). These findings have led to the hypothesis that activated microglia play a role in the pathogenesis of HIV. In addition to a neurodestructive role, microglia exhibit properties that, contrary to being pro-inflammatory, may be neuroprotective. In their non-activated state, microglia have a characteristic 'ramified' morphology in the brain parenchyma (Graeber, 2010), a unique property that distinguishes them from resident macrophages of any other tissue. With the presence of damaged cells, microglia assume an amoeboid morphology at the site of injury to eliminate cellular debris (Graeber, 2010). Microglia of this activated phenotype express chemokine receptors and exhibit increased size of cytoplasm and retraction of processes (Graeber, 2010). Like macrophages, through their ability to recognize and phagocytize pathogens and dying cells, activated microglia produce chemokines that orchestrate the outcome of an inflammatory process by recruiting other inflammatory cells and secreting cytokines. Importantly, the physical interaction between phagocytotic microglia and dying neurons suppresses inflammatory responses and, as it has been proposed, upon successful clearance of debris, microglia alter their morphology and switch to a phenotype favoring tissue repair and regeneration (Lucas et al., 2006).

Experimental findings have demonstrated that phagocytic microglia, macrophages, and dendritic cells respond to an encounter with apoptotic cells by increasing production of anti-inflammatory cytokines (e.g. transforming growth factor and interleukin-10). This suggests that activated microglia and released chemokines could be beneficial for recruiting other cells in order to prevent any further damage to the infected brain (Cardona et al., 2006). Thus, the microglia theory alone, although supporting a role that these cells may play in neuroinflammation seen in HIV, does not explain neuronal simplification and loss seen in HIV-mediated dementia. Moreover, it does not consider the new notion that activated microglia could be a defense mechanism that is beneficial for the survival and regeneration of neurons (Batchelor et al., 2002; Yong and Rivest, 2009; Colton and Wilcock, 2010). For instance, remyelination is impaired in demyelinated mice that are devoid of T cells, macrophages, particular cytokines such as tumor necrosis factor alpha (TNF- α) and interleukin 1 beta (IL-1 β), or leukocyte-derived proteases such as the matrix metalloproteinases (Yong, 2005). Similarly, deficiency of CX3CR1, which is essential for microglia migration and response to systemic inflammation, limits neuronal survival (Cho et al., 2011). Microglia also internalize and degrade oligomer proteins that otherwise promote neuronal degeneration (Mandrekar et al., 2009; Stefanova et al., 2011). Thus, both beneficial and detrimental effects can be associated with microglia activation.

Activated microglia are present in HAD subjects, which also exhibit an impairment in the hippocampal function. Nevertheless, microglia proliferation/activation is not always seen in animal models of HIV. For instance, HIV transgenic (HIV tg) rats at 5 months of age, display spatial learning deficits; yet, the expression of pro-inflammatory cytokines in their hippocampus is similar to that of wild type littermates (Fig. 3). At 8 months of age, HIV tg rats exhibit neuronal loss and synapto-dendritic injury, but do not show significant difference in microglia morphology as compared to control rats (Rao et al., 2011), nor do the microglia have a prominent evidence of activation or of a phagocytic phenotype (Fig. 4). Such evidence poses the question as to whether microglia proliferation/activation leads to the neuronal dysfunction and cognitive decline in HAD.

Microglia activation is also triggered by apoptotic cells through recognition and migratory cues (Peter et al., 2010). Microglia will then cluster at “injured sites” and help clear debris and reduce the potential for extended inflammatory events (Fiedorowicz et al., 2008). This is consistent with the notion that an encounter with apoptotic cells during an inflammatory response or tissue regeneration causes macrophages and microglia to adopt an alternative anti-inflammatory phenotype. Thus, activated microglia in HIVE might perform a beneficial function. This has been shown in animal models of HIV infection of the brain in which soluble gp120 was injected into the brain parenchyma at a concentration to elicit neuronal apoptosis. Gp120 promoted the accumulation of amoeboid CD11 positive microglia cells only around apoptotic neurons (Fig. 5). In addition, time-course experiments revealed that neuronal apoptosis occurs at least two days prior to the activation of amoeboid microglia (Ahmed et al., 2009). Therefore, at least for gp120-induced phagocytic microglia, this event is the result and not the cause of neuronal apoptosis. This type of response is consistent with the role that activated microglia play in the elimination of apoptotic cells (Fiedorowicz et al., 2008). Thus, the exact role of microglia in the neurotoxic effect of HIV remains to be identified.

CCL5 and neuronal protection

The pro-apoptotic and deleterious consequences of CXCR4 activation by gp120 do not appear to be limited to gp120, since CXCL12 also increases neuronal death through CXCR4 (Kaul and Lipton, 1999; Bachis et al., 2003). Paradoxically, CCL5 activation of CCR5 can be neuroprotective against the neurotoxic effect of T-tropic gp120 (Kaul *et al.*, 2007) or R5 gp120 (Bachis et al., 2009; Avdoshina et al., 2010). In addition, HIV-infected individuals with higher cerebrospinal fluid concentrations of CCL5 perform better on neuropsychological measures than those with low or undetectable levels (Sozzani *et al.*, 1997; Letendre *et al.*, 1999). The antiapoptotic activity of CCL5 against the neurotoxic effect of gp120 is not unique, as activation of the fractalkine receptor CX3CR1 inhibits apoptosis of hippocampal neurons (Meucci et al., 2000). Lastly, in animal models, R5 neurotoxicity is significantly weaker than that of X4 (Bachis et al., 2009; Bachis et al., 2010) despite the fact that R5 gp120 promotes microglia activation and proinflammatory cytokines such as IL-1 β (Bachis et al., 2010). Thus, much remains to be discovered about the role of this and other chemokine receptors in the CNS, considering that, as mentioned above, chemokines (and their receptors) coexist with other neurotransmitters, they are released in an activity-dependent manner and thus they may act as neuromodulators to promote/inhibit synaptic transmission. For example, CCL5 released from microglia or astrocytes, in addition to functioning as a chemoattractant for microglia, could bind to neuronal CCR5 and shift a neuroinflammatory signal to a neuroprotective one (Kaul et al., 2007; Avdoshina et al., 2010). Thus, CCL5 could be neuroprotective rather than neurotoxic. This finding might help explain the suggestion that M-tropic strains of HIV that infect the CNS are not sufficient to cause dementia or encephalitis (Power *et al.*, 1994).

Concluding remarks

When HIV meets chemokine co-receptors it initiates infection of immune cells which leads to AIDS and infection of microglia and macrophages. Consequently, HIV promotes profound neuronal loss and other neuropathological changes that culminate in HAD. Proliferation of activated microglia is a key feature observed in HAD. However, the role of microglia as the leading cause of the neuropathology of HIV remains speculative, as microglia can also be activated by distressed and dying neurons. Yet, neuroprotective therapies are needed. Chemokines and their receptors mediate HIV-mediated microglia proliferation and neuronal loss. Thus, it is urgent to develop antagonists for inhibition of viral infection as well as neuronal apoptosis caused by HIV. Any strategy to target

chemokine receptors must include the acknowledgement that individual chemokines may have a functional role in synaptic communication and CNS plasticity. Understanding how HIV envelope glycoproteins interact with chemokine receptors may facilitate the development of therapeutics to target neurological injury in AIDS patients.

Acknowledgments

This work was supported by grants from the National Institute of Drug Abuse 1R01DA026174 and 1F31DA032282, and National Institute of Neurological Disorders and Stroke 1R21NS074916 and the Intramural Research Program of the National Institute of Environmental Health Sciences. The views expressed in this article are those of the authors and they do not represent the views or policies of the National Toxicology Program or National Institute of Environmental Health Sciences.

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Figure 1. Expression of CCL5 in adult rat brain

Sections (40 μ m) were obtained from the somatosensory cortex (A) and *substantia nigra* (B) of 3 month old male SD rats. **A.** Sections were stained with CCL5 (red, 1:100 dil) and class III β -tubulin (green, 1:1000 dil) antibodies. Cy3 TSA Biotin System (Perkin-Elmer) was used to detect CCL5 according to the manufacturer's instructions. White arrows indicate CCL5 positive neurons, black arrows indicate non-neuronal cells positive for CCL5. Mag 60X. **B.** Sections were stained with CCL5 (red) and tyrosine hydroxylase (TH, green, 1:1000 dil) antibodies. Mag 20X. Fluorescence was visualized with FV300 laser confocal scanning system attached to an Olympus IX-70 upright microscope. Orange denotes co-localization.

Figure 2. Activity-dependent release of CCL5

Cortical neurons were prepared from E17 rat embryos as described (Avdoshina et al., 2010). Neurons were exposed to KCl (25 M) or NMDA (15 μM) for 30 min, the medium was collected and CCL5 content determined by ELISA (R&D), according to the manufacturer's instructions. * $p < 0.001$ vs control (ANOVA and Scheffe's test).

Figure 3. mRNA levels for pro-inflammatory cytokines are not elevated in the hippocampus of HIV tg rats

The hippocampus was dissected from 5 month old Fischer 344 controls (white bars) and HIV tg (black bars) male rats [Hsd:HIV-1(F344); Harlan Laboratories]. Total RNA was isolated, and cDNA subjected to quantitative real time polymerase chain reaction for IL-1 β , IL-1 receptor antagonist (IL-1RA), the microglia marker Iba-1, and TNF- α . Data are represented as mean δ Ct $\pm$ SD (n=6).

Figure 4. Microglia morphology in the hippocampus of HIV tg rats

Representative images of Iba-1+ cells (red) within the CA1 pyramidal cell layer and molecular layer of the hippocampus of control (A) and HIV tg (B) rats at 8 months of age. The nuclear marker 4',6-diamidino-2-phenylindole or DAPI (blue) was used as a counterstain. Note that microglia in HIV tg rats maintain a normal appearance with fine ramified processes and has no prominent evidence of activation. Scale bar = 50 μ m.

Figure 5. Gp120 activates microglia

Rats received X4 gp120 into the superior colliculus (gp120IIIb, Immunodiagnostic Inc., 400 ng in 0.1% bovine serum albumin). Rats were euthanized 18 days after the injection and coronal sections through the visual cortex were prepared. Example shows a section of gp120-treated rats stained for caspase-3 (green), the microglia marker CD11b (red) and counterstained with DAPI (blue). Immunoreactivity was analyzed by confocal imaging. Note that CD11b positive cells have their processes encircling the outside of caspase-3 positive cells that were previously identified as neurons. Mag 40X. (Adapted from Ahmed et al., 2009).

Table 1

Chemokine ligands and their receptors in the CNS.

Ligands ¹	Receptors ²
CXC (α -chemokines)	
CXCL1, CXCL6, CXCL8	CXCR1
CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8	CXCR2
CXCL9, CXCL10, CXCL11	CXCR3
CXCL12	CXCR4 ³
CXCL13	CXCR5
CXCL16	CXCR6
CC (β -chemokines)	
CCL3, CCL5, CCL7, CCL14, CCL15, CCL16, CCL23	CCR1
CCL2, CCL7, CCL13, CCL16	CCR2
CCL4, CCL5, CCL8, CCL14, CCL3 ⁴ ,	CCR3
CCL17, CCL22	CCR4
CCL3, CCL4, CCL5, CCL8	CCR5
CCL20	CCR6
CCL19, CCL21	CCR7
CCL1	CCR8
CCL25	CCR9
CCL27, CCL28	CCR10
XC (γ -Chemokine) ⁵	
XCL1, XCL2	XCR1
CXC3C (δ -Chemokine or fractalkine)	
CX3CL1	CX3CR1

¹Including expression *in vitro*.²Chemokine receptors are G-protein coupled, seven-transmembrane receptors.³CXCL12 and extracellular ubiquitin activate CXCR4 through separate binding sites (Saini et al., 2011).⁴Expressed by brain endothelial cells.⁵Undetected in CNS cells.