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Pathogenesis of human immunodeficiency virus–induced neurological disease

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Infection of the central nervous system by the type 1 human immunodeficiency virus (HIV-1) commonly results in a number of neurological impairments known, in their most severe form, as HIV-associated dementia (HAD). The persistence of HIV encephalitis (HIVE), the pathological correlate of HAD, in spite of highly active antiretroviral therapy (HAART) underscores the importance of continued research focused on the neurobiology of HIV. To elucidate direct and indirect mechanisms of HIV neuropathogenesis, current investigation is focused on neuroinvasion, HIV-1–mediated mechanisms of neuronal damage and apoptosis, and compartmentalized evolution of virus in the brain. The aim of this review is to provide a selective overview of the most recent research on the neurobiology of HIV, adding only a brief introduction regarding established principles. *Journal of NeuroVirology* (2003) **9,** 222–227.

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Introduction

In a significant subset of infected individuals, human immunodeficiency virus type 1 (HIV-1) infection is associated with a variety of neurological problems that are independent of any opportunistic infection. These include motor disturbances, cognitive impairments, and behavioral changes in various degrees of severity. The term HIV-associated dementia (HAD) is now used to describe this collection of neurological symptoms, particularly in its most severe form; minor cognitive motor disorder (MCMD) refers to milder versions of this problem, now more common since the advent of highly active antiretroviral therapy (HAART). In some patients, HAD is the initial manifestation of acquired immunodeficiency syndrome (AIDS); overall, it has been estimated to occur in 20% to 30% of *untreated* adults and in approximately half of pediatric cases (McArthur *et al*, 1993). HAART has reduced the incidence of HAD by approximately 50% (Maschke *et al*, 2000; Sacktor *et al*, 2002), but the frequency of HIV encephalitis (HIVE), its pathological manifestation usually characterized only in postmortem tissue, has remained constant (Masliah *et al*, 2000), suggesting that HAART does not eliminate HIV infection in the central nervous system (CNS), although HAART improves HIV infection symptoms quite dramatically. To better understand the pathological mechanisms of HAD and HIVE, current research is focused on HIV-1 entry into the (CNS), on the pathological and neurotoxic events that occur in response to CNS infection, and on the process of viral evolution within the CNS.

HIV entry into the CNS

HIV-1 enters the CNS early in the course of infection (Powderly, 2000), and the CNS is believed to remain a viral reservoir throughout the course of the infection, perhaps because of its relative immunological sequestration (Sinclair *et al*, 1994). The significant differences observed in nucleotide sequences and the biological properties of HIV-1 isolates from the brain and peripheral blood of the same patients suggest that the brain may harbor virus for years (Wong *et al*, 1997), as discussed in the last section of this review.

In order for HIV to enter a cell, it must bind to CD4, typically found on T lymphocytes, blood monocytes, macrophages, and some dendritic cells,

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and subsequently to one of a family of μ or μ chemokine coreceptors. CCR5, the chemokine receptor for macrophage inflammatory protein (MIP)-1μ and MIP-1μ, is the primary coreceptor used by most HIV isolates recovered from the CNS, whereas CXCR4 is used by isolates in the periphery, particularly towards the later stages of AIDS. Viruses using CCR5 are referred to as R5 tropic, and those that use CXCR4 as X4 tropic (some strains are dual-tropic, and can use either receptor). Within the CNS, HIV-1 infects mainly microglia, and monocyte-derived macrophages (Wiley *et al*, 1986). Because cells of the macrophage lineage are the only cells in the CNS that express both CD4 and CCR5, they are also the only productively infected cells within the brain (Bagasra *et al*, 1996). HIV-1 glycoprotein–mediated syncytia formation, which results from fusion of microglia or brain macrophages, occurs in many HIV-infected cell types *in vitro* and may be observed as multinucleated giant cells (MNGCs) *in situ*. MNGCs are the defining feature of HIVE. In addition to microglia, other cells within the brain, including a subset of astrocytes expressing CXCR4, can be infected by HIV and harbor viral sequences. Although infection of blood-brain barrier (BBB) endothelial cells has been reported in some instances, there is no convincing evidence that endothelial cells are significant targets of HIV infection in humans (Edinger *et al*, 1997). However, because human brain microvascular endothelial cells express several of the cell surface glycoproteins involved in HIV-1 entry and infection (namely CXCR4, CCR5, and DC-SIGN and L-SIGN), a potential role for this cell type in HIV entry has not been ruled out (Mukhtar *et al*, 2002).

HIV is believed to enter the CNS either as free virus or via infected immune cells, crossing one of the physical barriers that protect and encase the CNS: the BBB and/or the cerebrospinal fluid (CSF)-brain barrier. A likely source of the initial neuroinvasion is infected CD4+ lymphocytes, although it is also possible that cell-free virus penetrates the brain (Massari *et al*, 1990). Recent evidence suggests that cell-free HIV-1 particles may penetrate brain microvascular endothelial cells (BMVECs) through a mitogen-activated protein kinase (MAPK)-dependent macropinocytosis (Liu *et al*, 2002). BMVECs exposed to HIV-1 up-regulate expression of the intercellular adhesion molecule ICAM-1, which in turn may facilitate leukocyte migration across the BBB and increase the access of both cell-free HIV and infected monocytes/macrophages to the CNS (Liu *et al*, 2002). In addition, tumor necrosis factor (TNF)-μ, a proinflammatory cytokine secreted by infected macrophages (Mayne *et al*, 2000), increases BBB permeability by activation of guanylate cyclase and tyrosine kinase (Mayhan, 2002). Regardless of the potential role of cell-free virus and BMVECs, the most accepted model for entry of HIV into the CNS, also known as the "Trojan horse" hypothesis (Liu *et al*, 2000), suggests that HIV-1 enters the brain through the infiltration of infected monocytes that later differentiate into macrophages. Trojan horse transport of virus into the brain by infected monocytes and macrophages has also been described for visna virus, feline immunodeficiency viruses (FIV), simian immunodeficiency virus (SIV), and human T-cell leukemia virus type I (HTLV-I) (Georgsson *et al*, 1994; Romero *et al*, 2000), and it may therefore be a common mechanism for retroviral and lentiviral penetration of the brain. After crossing the BBB into the CNS, macrophages produce viral particles and spread productive HIV infection to neighboring microglia. The turnover rate of these perivascular microglia is relatively high compared to parenchymal microglia, which are long-lived (Kennedy and Abkowitz, 1997). A recent report differentiating between perivascular macrophages and parenchymal microglia based on the expression of myeloid markers suggests that the primary macrophage lineage cell type infected by SIV is the perivascular macrophage (Williams and Hickey, 2002). Similar results have been found for HIV infection. Regardless of the macrophage subtype infected in the CNS, all infected macrophages have the potential for secretion of neurotoxic factors.

The potential role of the CSF and the choroid plexus, the vascularized structure that makes up the boundary between the circulating blood and CSF, as a means for virus entry into the brain is a current area of research. The CSF functions as a partially separate compartment, and HIV strains derived from the choroid plexus are related to strains isolated from both the brain parenchyma and the periphery (Chen *et al*, 2002). Moreover, macrophages infected with some CSF isolates are able to mediate neuronal damage, possibly via apoptosis, as discussed later in this review (Chen *et al*, 2002). A recent study using the FIV model demonstrated productive infection of the choroid plexus and of macrophage-enriched choroid plexus cultures (Bragg *et al*, 2002), suggesting that the choroid plexus may be an important site for the trafficking of lentivirus into the CNS (Bragg *et al*, 2002). Other studies documented the infectability of choroids plexus cells, *in vitro* and *in vivo* (Petito *et al*, 1999; Harouse *et al*, 1989). Furthermore, if the CSF is seeded with more virulent X4 virus, direct neurotoxicity may occur if the CSF-brain barrier allows extracellular HIV particles to cross into the brain (Kaul and Lipton, 1999).

In addition to being a critical point for HIV entry into the CNS, the BBB and the CSF-brain barrier present physical impediments to the delivery of antiviral drugs. The therapeutic efficacy of HAART in the CNS is dependent at least in part upon its ability to achieve inhibitory concentrations. Protease inhibitors, which are eliminated from the CNS by the efflux transporters, P-glycoprotein and multidrug resistance–associated protein 1, are less likely to achieve effective dose concentrations than other anti-HIV pharmacologic compounds (Thomas *et al*, 2001). Additionally suboptimal concentrations of HAART

in the CNS could lead to the development of drug-resistant HIV strains. A recent report by Thomas and colleagues (2001) investigated the ability of abacavir, a nucleoside analog reverse transcriptase inhibitor, to cross these barriers and enter the brain in a guinea pig model. The authors found that abacavir can enter the brain at physiologically relevant concentrations, and is not inhibited by nucleoside transport inhibitors (Thomas *et al*, 2001). Conversely, it is reported that 98% of all drugs do not cross the BBB (Miller, 2002). Therefore, finding new means of allowing drugs to cross the BBB and gain access to the CNS is critical for treatment of HAD and elimination of the brain as a potential viral reservoir (Miller, 2002).

Mechanisms of HIV-1–induced neurological disease

There is no consensus on the underlying mechanisms of HIV-mediated neuropathogenesis, and therefore, it remains the subject of active research. Two predominant models have been proposed to explain the development of CNS abnormalities, both centering on the productive infection of brain macrophages and microglia. As mentioned previously, microglia and brain macrophages are the most frequently infected cell type of the CNS during all stages of infection and are the only productively infected cell type in the brain (Bagasra *et al*, 1996; Wiley *et al*, 1986; Koenig *et al*, 1986). They are believed to mediate neurological disease, either by producing neurotoxic viruses or viral proteins (Kaul and Lipton, 1999; Adamson *et al*, 1999; Johnston *et al*, 2001; Smith *et al*, 2001) or alternatively releasing endogenous compounds as part of the host response to virus infection (Kelder *et al*, 1998; McManus *et al*, 2000).

Viral proteins, including the HIV-1 envelope glycoprotein 160 (gp160), which is cleaved into two noncovalently associated products (gp120 and gp41), and the HIV transactivator protein, Tat, have the potential for neurotoxicity (Kaul and Lipton, 1999; Adamson *et al*, 1999; Johnston *et al*, 2001). Cleaved gp120 is soluble and can be shed from HIV-infected cells. At low concentrations, gp120 has been demonstrated to damage cultured neurons (Kaul and Lipton, 1999; Kanmogne *et al*, 2002). Although it was long suggested that gp120-mediated neuronal damage was achieved by a direct effect of gp120 on neurons, recent *in vitro* evidence suggests that gp120 neurotoxicity occurs indirectly and relies on the presence of toxic intermediates and activated chemokine receptors on macrophages/microglia. These toxic intermediates are thought to be inflammatory cytokines and arachidonic acid metabolites that are produced when macrophages or microglia are exposed to gp120 (Kaul and Lipton, 1999). An interesting model proposed that astrocytes, which express CXCR4, may also be involved in mediating such toxicity (Bezzi *et al*, 2001). This follows experiments that had indicated that nitric oxide synthase is induced in astrocytes exposed to gp120 (Nath, 1999). Nitric oxide production may impair the ability of astrocytes to protect neurons from damage. *In vitro*, neuronal toxicity and apoptosis has been demonstrated through the use of both X4 and dual-tropic envelopes, which is consistent with the finding that CXCR4 is present in a number of neural cell types (Kaul and Lipton, 1999; Gorry *et al*, 2002).

The neurotoxicity of HIV isolates is closely associated with the ability to induce fusion in monocyte-derived macrophages, which may result from the increased affinity for CCR5 exhibited by some HIV isolates of the CNS (Gorry *et al*, 2002). *In vitro*, this is indeed the case, as an isolate that was cultured for multiple rounds in microglia evolved into a highly fusogenic and pathogenic phenotype (Strizki *et al*, 1996). Further studies with that same passaged isolate indicated that specific amino acid changes correlate with increased fusion and decreased dependence on CD4 (Martín *et al*, 2001). Recently, CD4 binding of gp120 was demonstrated to induce a conformational change in the gp120 V1/V2 variable loops, which exposes the high affinity binding site for CCR5 (Kolchinsky *et al*, 2001; Zhang *et al*, 2001; Kwong *et al*, 2002). The loss of an *N*-linked glycosylation site in the V1/V2 stem appears to prevent occlusion of the CCR5-binding site and in some instances is sufficient for CD4-independent binding of gp120 to CCR5 (Kolchinsky, 2001). Therefore, the increased affinity for CCR5 exhibited by some neurotropic strains of HIV and SIV may result from changes in the V1/V2 stem. In addition, the CCR5-binding site of gp120 overlaps with an immunodominant epitope for neutralizing antibodies. Therefore, it has been postulated that in circumstances where the CCR5-binding site is exposed, the virus has an increased susceptibility to neutralization. One could then extrapolate that persistence of HIV/SIV strains with an increased affinity for CCR5 may result from the paucity of selective pressure exerted by neutralizing antibodies in the CNS, although this hypothesis is only now being addressed (Puffer *et al*, 2002).

The other cleavage product of gp160, gp41, was also shown to be neurotoxic in a study that demonstrated gp41 induction of nitric oxide production (Adamson *et al*, 1999). Tat is also secreted by infected cells and may induce neuronal death through apoptosis directly, via increases in intracellular calcium thereby stimulating the production of reactive oxygen intermediates and caspase activation; or indirectly, by stimulating macrophages to produce matrix metalloproteinases that induce neuronal apoptosis and whose expression is up-regulated in the brains of patients with HAD (Johnston *et al*, 2001). Recent evidence suggests that Tat toxicity is dependent upon a polyamine sensitive site on the *N*-methyl-D-aspartate receptor (Prendergast *et al*, 2002). In addition, it has been reported that both Tat and Nef increase production of neurotoxic quinolinic acid, a glutamate

receptor agonist (Smith *et al*, 2001), whereas Vpr may cause apoptosis in human neurons (Patel *et al*, 2002). Although several HIV proteins have been reported to cause neurotoxicity, future studies should consider whether or not the concentrations of these viral proteins required for neurotoxicity *in vitro* are within the range present *in vivo* in infected brain.

Activated microglia, whether HIV infected or not, are important mediators of the innate immune system and are capable of producing a long list of soluble molecules and potential neurotoxins, including nitric oxide, superoxide anions, μ-chemokines, matrix metalloproteinases, glutamate receptor agonists, proinflammatory cytokines, growth factors, and superoxide anions (Bagasra *et al*, 1996, Gendelman *et al*, 1994). The neurotoxins produced by activated microglia have been implicated in several chronic progressive neurologic disorders, including HAD, Alzheimer's disease, Parkinson's disease, and multiple sclerosis. Levels of cytokine production, in particular interleukin (IL)-1, interferon (IFN)-μ, TNF-μ, tissue growth factor (TGF)-μ, and IL-6, correlate roughly with the presence of HAD and may be secreted by macrophages, astrocytes, neurons, and endothelial cells when infected by HIV-1 or stimulated by activated macrophages (Kelder *et al*, 1998; Nath, 1999). Cytokines secreted by activated macrophages may result in bystander damage of both neurons and glia.

Ultimately, HIV-associated neurotoxic events, whether mediated by viral or cellular proteins, result in neuronal death, consistent with the symptomatology of HAD. Although there is little consistent evidence of neuronal infection in HIV infection, neuronal apoptosis can be detected in the brains of individuals with HAD, particularly in regions of the brain associated with neuropathological changes of MNGC formation. Similar changes have been noted in SIV encephalitis, the neuropathological outcome of SIV infection of rhesus macaques. Therefore, it has been postulated that neuronal apoptosis is the proximate cause of HIV-associated dementia. Two pathways of apoptosis have been proposed as potentially playing a role in HIV-mediated apoptosis: the extrinsic pathway is mediated by "death receptors" (TNF-μ or fibroblast associated [FAS]), whereas the intrinsic pathway is mediated by mitochondria. The extrinsic and intrinsic pathways have common downstream partners, including activation of effector caspases, and they may engage in "crosstalk." In the intrinsic pathway, apoptosis is typically inhibited by the antiapoptosis Bcl2 family proteins. Although the predominant pathway involved in the neuronal apoptosis observed in HAD is unknown, it was recently shown that NMDA receptor/*bcl-2*–regulated intrinsic apoptotic pathway contributes to neuronal apoptosis and that the Bcl-2 family of proteins protects neurons from the neurotoxic affects of HIV (Chen *et al*, 2002). Modulation of *bcl-2* gene expression may confer neuroprotection and circumvent the development of HAD (Chen *et al*, 2002). Future research is focused on determining whether one or both of these pathways is involved with HIVD with the goal of blocking apoptosis in the brains of HIV-infected individuals, particularly if MCMD becomes a significant problem even in the face of adequate systemic therapy.

Viral evolution

As indicated previously in this review, HIV is known to penetrate the brain soon after the initial systemic infection. However, there is little evidence that primary viral seeding results in a permanently productive infection within the CNS. In fact, although the sampling error may be quite large, as most studies are performed at postmortem examination (i.e., after severe immunodeficiency has set in), viral RNA and antigens are infrequently detected during asymptomatic HIV infection (Gray *et al*, 1992). This is parallel to the findings in SIV infection, where there is minimal if any viral production until late in the course of simian AIDS (Williams *et al*, 2001). On the other hand, during the period of severe immunodeficiency (i.e., AIDS or SAIDS), viral replication in the CNS is quite robust, and strains with decreased dependence on CD4 and an increased sensitivity to neutralization can be isolated (Martin *et al*, 2001; Gorry *et al*, 2002; Puffer *et al*, 2002). The origin of this population indigenous to the CNS is still unclear; it may emerge as the result of waves of neuroinvasion during the course of the infection, or could be the result of low levels of replication in a quasilatent reservoir in relatively long-lived microglia. Activation of quiescently infected cells may provide low levels of ongoing replication and support adaptive evolution in the brain throughout infection. In addition, quasispecies may arise in the brain as a result of genetic drift of an isolated population or from selection or adaptation for growth in CNS cell types.

Recent work by our group examined the genomic SIV sequences found in individual SIV-infected MNGCs and compared these to the majority species present in brain and spleen (Ryzhova *et al*, 2002). The majority of genomic species present in the MNGCs were similar to the prevailing brain SIV genotype, indicating that at least a component of the population in this cell type comes from cells actively replicating virus. However, there was evidence of expression of archival genomes identical to the original inoculum in the region examined, indicating that some of the cells were seeded early during the course of infection. The isolation of archival sequences in the later stages of infection suggests that viral genomes remained in a latent, nonreplicating state of infection in at least a subpopulation of cells (Ryzhova *et al*, 2002). Latently infected microglia may support a low level of expression from this latent viral pool and contribute to compartmentalized virus evolution in the brain (Ryzhova *et al*, 2002).

Conclusions

Although the advent of HAART has significantly prolonged and improved the lives of individuals living with HIV, the incidence of HIVE may be unchanged and systemic HIV infection is resistant to therapy in approximately 20% of patients participating in a HAART regimen. In addition, the vast majority of AIDS patients worldwide do not have access to HAART (Powderly, 2000). Therefore, continued research focused on the neurobiology of HIV is imperative. An increased understanding of HIV infection and the pathogenesis of HAD and HIVE will be achieved by further research focused on neuroinvasion, HIV-mediated mechanisms of neuronal damage and apoptosis, antivirals with improved access to the CNS, and compartmentalized evolution of virus within the brain.

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