The neuropathogenesis of HIV-1 infection

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Abstract

HIV encephalitis is the common pathologic correlate of HIV-dementia (HAD). HIV-infected brain mononuclear phagocytes (MP) (macrophages and microglia) are reservoirs for persistent viral infection. When activated, MP contribute to neuronal damage. Such activated and virus-infected macrophages secrete cellular and viral factors, triggering neural destructive immune responses. Our Center's laboratories have begun to decipher the molecular and biochemical pathways for MP-mediated neuronal damage in HAD. This review will discuss the salient clinical and pathological features of HAD and highlight the recent advances made, by our scientists and elsewhere, in unraveling disease mechanisms, including the role of chemokines and their receptors in the neuropathogenesis of HIV-1 encephalitis. ß 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Early in the course of HIV-1 disease, at or around the acute seroconversion reaction, HIV-1 invades the central nervous system (CNS) [1,2]. Virus may be carried into the brain through infected T-lymphocytes and/or monocytes. However, terminally differentiated brain mononuclear phagocytes (MP) (brain macrophages and microglia) harbor the majority of integrated virus in brain tissue. Commonly, in late stage HIV-1 disease, when peripheral CD4+ T cell counts decline, HIV-infected MP become immunologically active and initiate an inflammatory cascade within the brain parenchyma culminating in neuronal destruction. Laboratory investigations suggest cytokines, chemokines and their receptors expressed in the CNS may predispose a subset of HIV patients to virus- and/or MP-mediated neuronal damage. In this subset of individuals, viral and/or host immune factors secreted by MP may disrupt neuronal homeostasis resulting in HIV-associated dementia (HAD).

Recent advances in anti-retroviral regimens have retarded the progression of HIV disease in affected individuals and decreased the incidence of HAD. Moreover, because infection and activation of MP are necessary for macrophage-mediated neuronal damage, adjunctive anti-inflammatory and/or neuroprotective therapeutic strategies have received intense attention recently. Treatments aimed at disabling...
macrophage activation and/or its neurotoxic secretions may positively affect the progression of HIV-1 neuropathogenesis.

2. HIV-1 associated neurological disorders: clinical studies

Soon after seroconversion, 15–20% of infected patients display symptoms of aseptic meningitis. Additional neurologic symptoms present in late stage HIV-1 disease. Progressive viral infection with associated immunosuppression can cause cognitive and behavioral dysfunction. Neurologic deficits often have an insidious onset, are mild initially, and frequently are detected only by the patient. Early cognitive deficits include distraction, confusion (when faced with multiple tasks) and general forgetfulness. Early behavioral deficits include unexplained apathy and withdrawal, inappropriate disinterest and emotional lability.

Progression is highly variable and may depend on the genetic background of the infected host, systemic and/or brain HIV burden, blood-brain-barrier (BBB) integrity, CD4\(^+\) decline and the somewhat stochastic evolution of viral strains. HAD patients usually exhibit a progressive loss of cognitive function. Confusion and impoverishment of thought render patients unable to work or engage in basic social behavior. A subset of patients suffer impaired fine motor function, Parkinsonian tremor, ataxia, and rarely, limb weakness. Often, individuals with HAD do not seek neurologic evaluation. Unlike other AIDS defining illnesses, HAD is not widely recognized as a direct cause of death.

Clinical findings alone are not sufficient for a diagnosis of HAD. Indeed other AIDS defining illnesses that affect the brain (for example, lymphoma, CMV encephalitis, toxoplasmosis and others) can produce a similar constellation of neurologic symptoms. HAD is a diagnosis of exclusion and as such relies on neuroimaging techniques and cerebrospinal fluid (CSF) analysis to exclude other dementia-causing CNS disease. Radiographic evaluation demonstrates general atrophy, premature narrowing of gyri and widening of sulci, and mild edema of subcortical and deep structures. This is strikingly similar to the normal aged brain. There are no anatomical lesions associated with HAD. Interestingly, reduced N-acetylaspartate levels have been demonstrated by proton magnetic resonance spectroscopy (MRS), showing neuronal dropout in association with progressive disease [3].

CSF studies show pleocytosis in one-third of HAD patients, oligoclonal bands in one-third and slightly elevated protein levels in two thirds [4,5]. CSF HIV RNA levels correlate better with clinical HAD than do plasma HIV RNA,[6] but still have a high false-negative rate. CSF HIV RNA levels generally are about 10 times lower than those seen in plasma. Elevated CSF levels of neopterin, quinolate, \(\beta_2\)-microglobulin, TNF-\(\alpha\), IL-1 and IL-6 in HAD have been reported [5,7–11] but are not used for establishing a diagnosis as they all lack disease specificity. Early radiographic or CSF are not predictive for HAD progression.

The multicenter AIDS cohort study (MACS) estimated that roughly 20% of AIDS patients suffered HAD from 1985–1995, before introduction of highly active anti-retroviral therapy (HAART) [12]. It has been estimated that an additional 30–40% of AIDS patient suffered mild or subclinical cognitive decline [13]. The MACS determined no detectable clinical difference in cognitive function between otherwise asymptomatic HIV seropositive patients and HIV seronegative controls [14,15]. Multiple studies confirm this finding [16–18]. In 0.8–10% of previously undiagnosed HIV-positive patients, neurologic impairment is the presenting complaint [19]. The introduction of zidovudine (ZDV, AZT), a highly CNS-penetrant nucleoside analogue reduced HAD incidence. CSF zidovudine levels can reach peaks of 0.5 \(\mu\)g ml\(^{-1}\) [20]. Highly active anti-retroviral therapies (HAART) have significantly diminished the disease even further. In the years 1995–1997, the prevalence of HAD has fallen to less than 7% (McArthur, J.C., unpublished observation).

3. Pathology and pathogenesis of HIV encephalitis

During the later stages of HIV infection, a subset of patients experience nervous system disease. Peripheral macrophage infiltration into brain is a widely observed component of HIV encephalitis [21,22]. Clinical-pathologic investigation has shown
that proliferation and immune activation of infiltrated and resident brain MP correlates better with HAD progression than does the CNS viral load [23,24]. Perivascular and parenchymal brain macrophages fuse with one another and with resident microglia to form multinucleated giant cells [22]. Pathological signs of a giant cell encephalitis can be found in cortex but preferentially affects subcortical white matter, deep white tracts and basal ganglia. Other histologic findings include microglial nodules, neuronal dropout, diffuse myelin pallor and reactive astrogliosis. Myelin pallor of HIV encephalitis represents disruption of the neuron-oligodendrocyte interaction. The HIV encephalitic brain may appear atrophic with ventricular dilatation but is otherwise unremarkable. Interestingly, the pathological features of HIV-encephalitis do not always correlate with clinical neurologic deficits.

MP are the target cells for HIV infection in the CNS. Three distinct MP are found in brain parenchyma and represent targets for the virus. They include ramified ‘resting’ microglia, activated ‘amoeboïd’ microglia, and perivascular macrophages. Between weeks 6–8 of embryogenesis, mesodermal cells migrate into the CNS and give rise to microglia [25,26]. Brains from humans infected in utero display increased proportions of infected microglia compared to brains from humans infected horizontally. Resting microglia display an elliptical, biramified morphology and appear to have reduced phagocytic and secretory potential compared to peripheral macrophages. Microglia represent up to 10% of the parenchymal brain cell population in some regions. Neighboring elliptical microglia contact each other in series and in parallel, forming a network that extends through gray and white matter. Microglia arrange in fence-like patterns along parenchymal boundaries and may contact migratory cells of vascular or perivascular origin. Unlike microglia, parenchymal and perivascular macrophages usually appear amoeboïd. In morphology and function, these cells more closely resemble tissue macrophages in other organs. Brain macrophages have great migratory potential within parenchyma and may traffic in and out of the microvasculature.

The hypervariable V3 loop of the HIV-1 envelope protein gp120 has been shown to contain sequences necessary for binding CD4 and viral chemokine receptors [27–31]. gp120 interacts with CD4 and chemokine receptors to mediate viral entry. Brain MP express CD4 and chemokine receptors. Neurons do not express CD4 and can not support productive HIV infection. Similarly, astrocytes do not express CD4 and are not consistently infected. Ex vivo sequence analysis has shown that brain isolates are macrophage-tropic [32,33]. Although this finding may seem self-evident, late stage peripheral HIV disease is associated with a shift to lymphotropic strains [34]. Viral evolution may influence disease progression within the brain. Alternatively, the late-stage AIDS brain may experience an increase in vascular permeability associated with influx of serum isolates.

Early in HIV infection, virus penetrates the BBB. It has been shown that HIV-infected monocytes can traverse brain microvascular endothelium, the so-called ‘Trojan horse’ hypothesis [35,36]. Undifferentiated peripheral monocytes are infected at low frequency in circulation. Upon entry into tissue and differentiation into macrophage, however, these cells become easily infected. Neither immature monocytes nor mature macrophages divide. In contrast, because of their highly proliferative nature, infected CD4+ T-lymphocytes produce large levels of virus in the periphery. Thus monocytes, macrophages, and/or lymphocytes may carry the virus into the brain during disease. Free progeny virus may also cross the BBB before infecting monocyte lineage cells in the perivascular space. Recent data suggests that gp120 binds endothelial cell surface glycopolymers and mediates absorptive endothelial pinocytosis of infected cells or naked virus [37,38]. Any mechanism explaining CNS infection must account for a high degree of fidelity. Indeed, all post-mortem specimens, in demented and non-demented patients alike, implicate the brain as an important viral reservoir.

Chemokine receptors expressed on non-neuronal CNS cells may play an important role in progression of HAD. HIV-1 isolates that invade brain are macrophage-tropic (M-tropic). However evidence suggests M-tropic strains alone are not sufficient to cause clinical neurologic impairment. Because HIV-1 relies on chemokine receptors for entry into MP, chemokine receptor expression by brain macrophages and microglia may influence viral evolution within the brain reservoir leading to the emergence of neurovirulent progeny [39]. Diverse viral clones
may differ in their ability to infect MP and could prime them for activation.

Late in the course of systemic HIV disease, peripheral virus shifts from predominant macrophage-tropism to T-lymphocyte-tropism and peripheral CD4 cell counts decline. Concurrently, CNS MP may become activated. It has been proposed that infected CD4+ or uninfected CD8+ T-cells trafficking in and out of brain parenchyma activate MP through cell-to-cell contact. Monocytes express the TNF-family activation receptor CD40 and activated T-cells express CD40 ligand (CD40L). Binding of soluble CD40L activates infected and uninfected monocyte lineage cells in vitro (Cotter, R., Zheng J., Niemann, D., Gendelman, H.E., unpublished observation). Alternatively, healthy monocytes and T-cells may exist in antagonistic equilibrium in the CNS and other tissues. Decline in the CD4+ T cell population allows de-repressed macrophages to express a metabolically active, tissue-destructive phenotype. Alternatively, circulating pro-inflammatory cytokines trigger monocyte activation independent of lymphocytes. Indeed, late-stage HIV-1 patients consistently exhibit elevated serum TNF-α and TNF-α receptors. Whatever the mechanism, when activated, brain macrophages secrete a variety of neurotoxic immune and viral factors. Functionally, macrophage activity leads to gp120-mediated cell fusion, multinucleated giant cell formation, disruption of neuronal homeostasis and ultimately disease.

Infected macrophages can cause neuronal damage and destruction through multiple mechanisms. First, the healthy CNS relies on microglia to produce neurotrophins. HIV hijacks constitutive protein synthesis machinery and prevents microglia-mediated neurotrophic activity. Second, macrophage secretory products disrupt neuron-astrocyte and/or neuron-oligodendrocyte interactions, creating functional deficit and ultimately neuronal death. Third, activated mononuclear cells kill neurons through secretion of inflammatory factors. Candidate neurotoxins include nitric oxide (NO), platelet activating factor (PAF), arachidonic acid metabolites, kynurenic metabolites, and the pro-inflammatory cytokines TNFα, interferon-alpha and -beta (IFNα, IFNβ) and IL-1β and IL-6. Fourth, the mononuclear cell compartment serves as a factory for the synthesis and export of viral products that are toxic to neurons. Tat and gp120 both have been shown to cause neuronal damage and destruction in vitro. It has been shown that gp120 can bind CXCR4 receptors expressed on neurons and effect intraneuronal events culminating in disruption of Ca²⁺ flux and ultimately, death. The last of these explanations suggests a potential therapeutic benefit for Ca²⁺ channel antagonists. Indeed, post-mortem evaluation has shown that virus is necessary, but not sufficient to induce neurologic dysfunction. In general, the pathogenesis of HIV encephalitis has not been limited to a single mechanism, but rather represents multiple genre neuronal insult.

4. Laboratory models for HAD

Human fetal tissue is a practical substrate for in vitro cellular models of HIV neurologic disease. Neursurgical specimens from temporal lobe resection can be cultured but specimens are rare and depend on surgical cooperation. Neurons from surgical preparations can not be cultured. 10^20% of microglia can survive but frequently have astrocyte contamination. In contrast, preparations of 12–16 week post-conception fetal tissue yields viable human neurons, astrocytes or microglia for subsequent analysis. Purification techniques can create 70% pure neuronal cultures, 99% pure microglia or astrocytes, as desired. Alternatively, rat, caprine, rabbit and simian sources can provide mammalian neurons, astrocytes and microglia for laboratory investigations. For HIV replication studies, peripheral monocytes and lymphocytes elutriated from uninfected human donors are a valuable cell viral targets.

Brain microvascular endothelial cells (BMVEC) from neursurgical specimens survive expansion and passage in culture. Because in vitro expansion of BMVEC is associated with phenotypic changes, experiments using BMVEC are conducted before the eighth passage. Our center has established an experimental BBB model in 24-well plates using BMVEC and human astrocytes to study monocyte migration [40]. Confluent BMVEC are cultured on one face of a porous membrane and confluent autologous human fetal astrocytes are cultured on the opposite face. Endothelial cells form tight junctions with each other and do not directly contact astro-
cytes. After adding monocytes to the endothelial chamber, endothelial cell morphology changes and monocyte migration occurs. Using this system, we have shown differential effects on monocyte migration under multiple experimental conditions.

5. Animal model systems for HAD

Because HIV exhibits species-specific tropism, construction of relevant animal model systems represents a tremendous scientific challenge. Our center, in conjunction with colleagues, has created a small animal model to recapitulate the pathology of HIV neurologic disease [41,42]. HIV-infected human peripheral monocytes are injected into the basal ganglia of SCID mice using a stereotactic injection apparatus. Human macrophages and multinucleated giant cells are observed in the needle tract in high density, but also at distant sites including the contralateral hemisphere. Spreading infection within the monocyte population is monitored using immunohistochemical analysis of p24 antigen. Reactive astrogliosis and neuronal dropout in the surrounding murine parenchyma is quantified using immunohistochemistry. Protein and RNA extraction also provide useful substrate for analysis of proinflammatory cytokines or other proteins of interest. This model of HIV encephalitis has been used to study the CNS activity of multiple systemic retroviral therapies and is currently used for studies of interaction between drugs of abuse and progression of HIV infection of the brain.

Multiple transgenic mice have been used to study HIV-1 neurologic disease. Transgenic models are achieved by delivering a cistron of interest to the one- or two-cell stage embryo using an appropriate vector. After recombination, appropriate spatial expression is achieved by incorporating a tissue-specific promoter within the delivered cistron, though promoter leakiness precludes absolute tissue specificity. A gp120 transgenic mouse was created in which recombinant, truncated gp120 was introduced downstream of the glial fibrillary acid protein (GFAP) promoter [43,44]. Although cross reactivity between recombinant gp120 and a distinct astrocytic reaction marker prevented definitive detection of gp120 using immunohistochemistry and immunoprecipitation analyses, CNS gp120 expression was confirmed using a sophisticated method of peripheral T-cell invasion and antigen recognition [45]. The resulting model has been used to study gp120 effects on NMDA receptor dependent calcium flux and its effects on the developing nervous system [46–50].

A relevant model for the study of HIV disease, systemic, neurologic and otherwise, is macaque infection by simian immunodeficiency virus (SIV). The non-human primate model offers insight into CNS disease progression, in contrast to the end-stage view of CNS degeneration described in most human studies. HIV and SIV viruses share about 55% sequence homology, typical retroviral structure and many non-structural genes. Both depend on CD4 for entry and have similar tropisms. Variants of the lymphotropic isolate SIVmac239 have been shown to effectively invade the CNS [51], infect MPs [52] and yield pathology similar to HIV encephalitis. However neurologic impairment has not been demonstrated in adult animals. The SIV model holds exciting promise for discoveries pertaining to neuropathogenesis as the neurologic component is fashioned to more closely resemble clinical human disease.

6. Future outlook for investigations into HIV-1 neuropathogenesis: roles for chemokine receptors in neuroinflammation

Chemokine receptors in the brain can be involved in HAD pathogenesis in at least three ways. First, expression of chemokine by resident CNS cells and chemokine receptors by peripheral monocytes may predispose patient subsets to neurologic disease progression. Second, chemokine receptors on brain MPs may direct viral evolution, predisposing affected individuals to emergence of destructive viral progeny. Third, whole virus or soluble viral proteins may bind neuronal and/or astrocyte chemokine receptors. HIV-1 infects neither cell owing to low expresses of CD4 but virus-receptor interaction may cause functional damage through signal transduction.

In the simian model, α- and β-chemokines are overexpressed among the SIV-infected brains compared to controls [53]. In correlation, α- and β-chemokine receptors are over-expressed in the mono-
cytic infiltrate. In humans, HIV encephalitis patients display an upregulation of α- and β-chemokine receptors in the brain compared to uninfected controls [54–56]. Taken together, these data suggest that chemokine expression in the CNS is crucial for the recruitment of peripheral infiltrating monocytes into brain parenchyma. Monocytes expressing higher levels of chemokine receptors may then be predisposed to BBB trans-endothelial migration.

Once HIV-infected monocytes have entered the CNS, viral evolution is largely dependent on the ability of progeny virus to infect surrounding cells. Infection depends on interactions between the HIV-1 envelope protein gp120 and viral receptors, including CD4 and chemokine receptors [29–31]. Tropism is defined by the ability of an HIV isolate to infect primary human monocytes and immortalized lymphocytoid cells in vitro. Viral structural determinants of tropism have been mapped to the hypervariable V3 region. Determinants may also exist within p41 and other regions of gp120. M-tropic virus relies heavily on β-chemokine (CC) family receptors for entry. CCR5 is a major cofactor for viral entry into macrophages and microglia [57–59]. Entry and infection by M-tropic virus is blocked by β-chemokines [60–63]. T-tropic variants rely heavily on the α-chemokine receptor, CXCR-4 (fusin, LESTER, HUMSTR) for entry. Because T-cells also express CCR3 and CCR5, T-cells can be infected by T- and M-tropic virus. Brain macrophages and microglia express CCR5, the major cofactor for entry into monocyte lineage cells, and, in lower levels, CCR3 [62,63]. Microglia can express CXCR4, but infection levels using T-tropic isolates in vitro are limited [58]. Chemokine receptor regulation and constitutive expression in replication competent CNS MPs may influence emergence of new viral phenotypes over time. It is easy to imagine that, if neurovirulent clones emerge with increased affinity for MP chemokine receptors, tissue destruction would result.

The viral and host factors contributing to neurovirulence are not entirely understood. Our group has shown that HIV-infected human monocyte-derived macrophages (MDM) secrete neurotoxins that alter neuronal function and induce apoptosis [66]. To delineate the relative contributions of viral and cellular secretory products in disease, we have explored a myriad of potential receptor-mediated cytotoxic pathways in neurons and astrocytes. The most promising of these implicates the CXCR4-chemokine receptor in HIV-mediated toxicity. CXCR4 is expressed on neurons and microglia [67–69,71,75] and is a 46-kDa transmembrane Gi-protein coupled receptor. Stroma-derived factor 1α (SDF-1α) a natural ligand for CXCR4 [72,73] is a soluble protein secreted by bone marrow stroma, neuroglia and other cells.

Neurotoxicity observed in the presence of HIV-infected MDM secretory products is partially reversed by addition of 12G5, a monoclonal antibody directed against CXCR4. Human neurons treated with high concentrations of SDF-1α in vitro display a decrease in intracellular cAMP levels, activation of neuronal Caspase-3 and a 67% increase in apoptosis [74]. 12G5 and pertussis toxin abrogate second messenger alterations associated with SDF-1α treatment of human neurons. Taken together, these results suggest that a CXCR4 ligand is responsible, in part, for effecting neurotoxicity. A similar effect is observed in neurons treated with whole HIV, but not in neurons treated with soluble recombinant gp120.

In the bone marrow, SDF-1α exists in low concentration and plays a protective role for resident hematopoietic cells. SDF-1α may protect CNS neurons, as well, at lower concentrations than studied in vitro. RT-PCR analysis shows that astrocytes constitutively express SDF-1α and that SDF-1α message is upregulated by about 30% in response to secretory fluids from HIV-1 infected MDM [74]. The inflammatory response of HIV-encephalitis may trigger astrocyte secretion of neurotoxic levels of SDF-1α.

Neuronal and astrocyte chemokine receptor expression offers an attractive mechanism for direct virus-mediated toxicity. HIV can bind neuronal chemokine receptors and activate intracellular signaling cascades leading to dysfunction. Endogenous SDF-1α secreted in high concentrations in response to a CNS inflammatory response also may activate signal transduction in cells of neuroectodermal origin and activate damaging intracellular messenger cascades. Further investigation is aimed at reversing MP-mediated neurotoxicity and stimulating CNS neuroprotective pathways.
7. Conclusion

MPs are the centerpiece of HIV encephalitis and associated HAD. In the healthy patient, brain macrophages and microglia protect against infectious pathogens, remove cellular debris and provide support for the delicate neuronal network. When infected with HIV and activated, however, these cells assume a different role. MP migrate through brain parenchyma, destroying neurons and causing clinical functional deficits.

Chemokine receptors expressed on CNS cells hold great promise in future studies of HAD progression. Chemokine receptor expression on brain MP may influence the emergence of neurovirulent phenotypes. Chemokine receptors expressed on neurons and astrocytes may represent part of a final pathway for neuronal destruction, though other mechanisms for brain toxicity certainly exist.

Future therapeutics for HAD likely will include multiple approaches. First, the viral life cycle may be slowed within the CNS independent of host factors as central penetration of anti-retroviral medication continues to improve. Second, the macrophage and the toxins it secretes may be retarded with anti-inflammatory agents. Third, CXCR4 and other neuronal receptors may be blocked to alter neuronal evolution in the brain and/or affect terminal events in HIV mediated brain damage. Fourth, neuroprotective pathways may be stimulated within the brain, through regulation of neuronal calcium channels, chemokine receptors or other means. Strategies aimed at reducing the ability of the mononuclear cell to cause disease within the CNS offer multiple exciting avenues for further investigation in HAD as well as for other neurodegenerative disorders.

References


