



HIV-1-Related Central Nervous System Disease: Current Issues in Pathogenesis, Diagnosis, and Treatment

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HIV-associated central nervous system (CNS) injury continues to be clinically significant in the modern era of HIV infection and therapy. A substantial proportion of patients with suppressed HIV infection on optimal antiretroviral therapy have impaired performance on neuropsychological testing, suggesting persistence of neurological abnormalities despite treatment and projected long-term survival. In the underresourced setting, limited accessibility to antiretroviral medications means that CNS complications of later-stage HIV infection continue to be a major concern. This article reviews key recent advances in our understanding of the neuropathogenesis of HIV, focusing on basic and clinical studies that reveal viral and host features associated with viral neuroinvasion, persistence, and immunopathogenesis in the CNS, as well as issues related to monitoring and treatment of HIV-associated CNS injury in the current era.

HIV-1 infects the nervous system in virtually all patients with systemic infection and frequently causes central nervous system (CNS) and peripheral nervous system (PNS) disorders. Until the introduction of combination antiretroviral therapy (cART) in the mid-1990s, HIV-1-associated dementia (HAD) and related cognitive and motor disorders affected 20%–30% of patients with advanced immunosuppression or AIDS. The incidence of overt HAD in countries where effective combination antiretroviral medications are widely available is now markedly diminished. However, in the setting of chronic, apparently systemically

suppressive treatment, there appears to be a continued prevalence of mild-moderate neurocognitive impairment in a significant proportion or even a majority of patients. This disquieting finding, combined with the staggering numbers of patients who continue to be newly infected with HIV worldwide, and the limited availability of optimal antiretroviral treatment in many of the persons affected with this condition, make understanding and effectively preventing HIV-1-related neurological injury a continued key area of investigation. To encompass this more complex range of disorders seen in patients treated with cART, most

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investigators now refer to HIV-associated neurocognitive disorders (HAND) rather than HAD as the principal primary CNS complication of HIV infection.

HISTORY

A dementing illness characterized by attention and memory deficits, motor impairment, and personality changes was recognized in a significant proportion of patients with advanced AIDS within the first years of the HIV epidemic (Navia et al. 1986b). Further investigation of this disorder revealed that these complications were a direct result of HIV-1 infection and attendant inflammation in the CNS. The neuropathology was characterized by diffuse brain atrophy with large ventricles, widespread low-grade inflammation with microglial nodules, perivascular lymphocyte cuffing, multinucleated cells expressing HIV p24 and other antigens, and patchy demyelination and white matter gliosis (Gabuzda et al. 1986; Navia et al. 1986a). Although inexorably progressive to severe disability and death in the absence of disease-modifying HIV therapy, the course of this clinical disorder has been altered considerably by treatment with antiretroviral therapy and especially cART. Originally defined as the AIDS-dementia complex (ADC) based on motor, cognitive, and behavioral symptoms and signs, current research nosology defines a broader spectrum now called “HIV-associated neurocognitive disorder,” with graded classifications based on abnormal performance on neuropsychological testing, and the presence or absence of a patient’s perception of functional limitation related to cognitive impairment (Antinori et al. 2007). Changes in the severity of neurological disease in the current era may also be accompanied by alterations in the underlying etiology of neurological morbidity in the setting of long-term survival with HIV, including the consequences of possible ongoing low-grade viral replication and inflammation within the CNS, cumulative exposure to antiretroviral and other medications, chronic systemic inflammation leading to accelerated vascular disease, and the effects

of comorbidities and neurodegeneration that occur with aging. Additionally, because cART appears to be beneficial in the amelioration and prevention of the most severe forms of HAND, newfound attention has been focused on the possible long-term cognitive benefits of initiation of cART in early stages of HIV infection.

KEY ADVANCES IN THE AREA

Viral Entry and Maintenance of Infection in the Nervous System

As with some other viruses that circulate in the bloodstream, HIV entry into the CNS is largely mediated through blood lymphocytes and monocytes that enter the perivascular spaces either in the course of their natural surveillance, or because they are attracted by chemokines to sites of inflammation. Viral strains isolated from the brain are more commonly CCR5-tropic and replicate effectively in cultured macrophages, suggesting that monocytes may predominate as “Trojan horses” in the process of CNS entry, as described years ago for classically described lentiviruses such as visna virus of sheep (Haase 1986). Alternatively, HIV may be brought into the CNS by lymphocytes, which can harbor viruses that replicate in macrophages (Collman et al. 1992), or conceivably as free virions, where the means of entry would be through endothelial cells. Regardless of the mechanism of entry, cells of the macrophage lineage are the only cells in the brain that are routinely found to harbor HIV antigens or RNA by conventional methods such as immunohistochemistry or in situ hybridization for viral RNA, although other cell types such as astrocytes may harbor HIV sequences without robust expression of RNA (or proteins) (Wiley et al. 1986). Detection of such infection requires other methodology such as in situ polymerase chain reaction (PCR) amplification or laser capture microdissection followed by PCR (Churchill et al. 2009). Recent studies have also concentrated on determining whether a subset of monocytes is particularly important in either delivering virus to the CNS or in

amplifying a local inflammatory process, whether such a subset is increased or enhanced by systemic inflammation mediated by circulating bacterial products, and which cytokines and chemokines increase the recruitment of monocytes and lymphocytes into the CNS and by extension are more likely to deliver virus into the brain (Fig. 1).

Although there is general agreement that the predominant infected cells are macrophage-like, there is controversy regarding which of the several subtypes of CNS macrophages are harboring HIV (macrophage subtypes are reviewed by Perry et al. 2010). Most investigators agree that perivascular macrophages, which

are mostly derived from the circulating monocytes, are highly infected in the brains of HIV-infected persons or in macaques (rhesus or pig-tailed) that are infected experimentally with SIV (Kim et al. 2006); these may be labeled by CD163, a marker for this subtype that also appears to be increased in circulating monocytes in macaques with CNS infection (Borda et al. 2008). The life span of these perivascular macrophages was previously thought to be days or weeks; recent studies in rhesus macaques have indicated that they are probably longer-lived (Soulas et al. 2009). Multinucleated giant and other CNS lesions containing SIV in an experimental model or HIV in

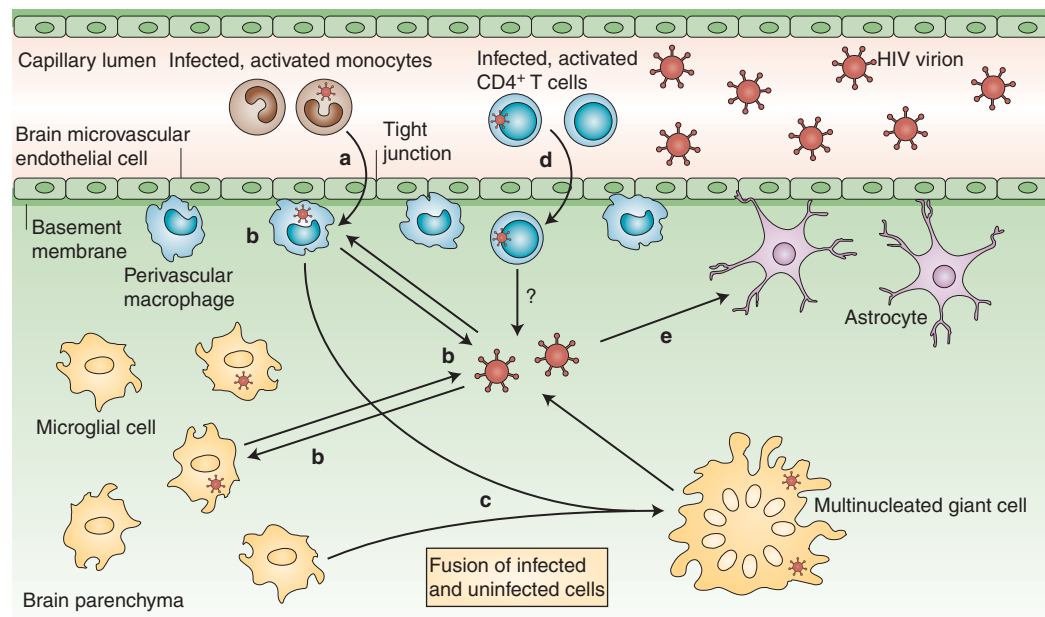


Figure 1. Potential models for HIV neuroinvasion and infection of the central nervous system (CNS). (a) HIV-infected monocytes with an activated phenotype may transport HIV into the nervous system via migration across the blood–brain barrier. (b) Infected monocytes likely differentiate into perivascular tissue macrophages and proceed to produce HIV within the CNS. This macrophage infection and replication allows for release of free virions and may facilitate infection of microglial cells. (c) Cell-to-cell fusion involving cells that express CD4 and HIV coreceptors results in formation of multinucleated giant cells within the brain, a hallmark of HIV-related brain pathology. (d) Infected CD4⁺ T lymphocytes may also serve as a mechanism of entry of HIV into the brain. There is varied evidence regarding the relative contribution of CD4⁺ T lymphocytes versus cells of the monocyte/macrophage in initiating and sustaining HIV infection within the CNS. (e) Although astrocytes might harbor HIV and also contribute to HIV-related brain disease through mechanisms of astrogliosis induced by local chemokines and cytokines, astrocytes infection is not thought to support ongoing replication within the CNS. (Adapted from Gonzalez-Scarano et al. 2005; with permission, from Macmillan Publishers Ltd. © 2005.)



autopsy samples are composed of cells expressing different macrophage markers, including CD163, CD68, and CD387; some cells appear productively infected; others do not express viral antigens (Soulas et al. 2011). Nevertheless, although it has previously been suggested that because of their rapid turnover perivascular macrophages could not contribute to the long-term presence of HIV/SIV within the CNS, which could then serve as a reservoir when systemic infection was cured, these more recent discoveries would propose that indeed this perivascular population could harbor virus for long periods of time. Parenthetically, the astrocytes found to be infected using sensitive methodologies are often close to the perivascular spaces.

Microglia, which are parenchymal, or deeper within the brain, are known to be long-lived and are replaced only infrequently during an individual's lifetime. Recent studies in mice have indicated that they may represent a separate ontogeny within bone marrow cells (Ginhoux et al. 2010), although hematopoietic circulating cells could potentially also give rise to morphologically appearing microglial cells, albeit a small minority. However, there is some controversy as to whether true microglia are commonly infected by HIV, and some investigators believe that they are never infected. Multinucleated giant cells, the pathological hallmark of HIV in the CNS, arise from macrophage-type cells, and although their frequent perivascular location would suggest they are from that perivascular macrophage population, there are no markers that can reliably confirm this. Furthermore, the life span of multinucleated giant cells is completely unknown, although one can speculate based on their presence in pathological specimens that they last at least days.

Studies performed well over a decade ago first proposed that a subset of monocytes, characterized by expression of the markers CD14 and CD69, were particularly prominent in patients with HIV neurological complications and specifically HAD (Pulliam et al. 1997). More recently, investigators from the same group related these original findings to a less impressive but still measurable increase

in CD14/CD69 positive cells in patients on cART with dementia in comparison to those without (Kusdra et al. 2002). Similarly, in the rhesus macaque model of SIV encephalitis (SIVE) increased monocyte turnover and expression of the CD163 marker are associated with brain penetration and encephalitic changes (Burdo et al. 2010).

While these observations were developing, independent evidence that systemic inflammation driven by depletion in the gut immunological system and consequent microbial translocation had a role in HIV pathogenesis arose from several areas, including animal models and human observations (see Lackner et al. 2011 for details). This led to a series of discoveries suggesting that microbial translocation and concomitant immunological activation are associated with the presence of neurological complications in HIV infections (Ancuta et al. 2008). Furthermore, this finding may explain why there has been a strong correlation between inflammatory activity, as characterized by the presence of macrophages expressing activation markers in the CNS, and the development of HAND or HAD. This correlation may be as strong as that of the presence of viral proteins and other evidence of HIV replication.

A model that incorporates current concepts of systemic pathogenesis and the CNS-specific observations would then propose that infection of the CNS is driven by systemic activation of monocytes—at least partly owing to microbial translocation from a depleted gut immune system—which then are more likely to invade the brain perivascular space. As some of these cells are infected, they bring in virus that spreads locally and sets up a nidus of replication independent from the systemic circulation (see next section).

Concomitantly, the perivascular inflammation results in the secretion of cytokines and chemokines that in turn amplify the reaction, attracting in addition other circulating monocytes and infected CD4⁺ T lymphocytes that can also add to the CNS viral burden (Xing et al. 2009). Chief among the chemokines associated with HIV infection of the CNS is MCP1 (CCL2), which is present in easily measurable

concentrations in the cerebrospinal fluid (CSF) and is associated with dementia, but also IP10 and others (see next sections).

CNS Compartmentalization

Studies in acute HIV infection have shown that virus is present in the CSF at early points during HIV infection, including in some patients with primary infection (Schnell et al. 2010); whether such an early seeding forms the basis for independent replication in the brain, or whether it is cleared and virus penetrates at other points in the course of the infection, has been the subject of many excellent studies without a clear consensus (Caragounis et al. 2008; Harrington et al. 2009). It is likely that different scenarios take place depending on the host, the route of infection, and possibly the individual isolates. Nevertheless, although the details may vary, long-term HIV infection leads to genetically isolated populations in the CNS, as evidenced by studies using *pol*, *env*, *nef*, and other genes (Thomas et al. 2007; Brown et al. 2011; Cowley et al. 2011; Gray et al. 2011). The most recent studies (Brown et al. 2011) have used single genome analysis (SGA) to overcome potential PCR artifacts and confirmed the conclusions derived with bulk amplification before the development of SGA. Additionally, studies in experimental infection of rhesus macaques with a cloned SIV isolate suggested that virus is not only compartmentalized in the CNS, but that different regions have potentially different *env* genotypes, setting the stage for independent entry events, some potentially early in the course of infection, some perhaps much later (Chen et al. 2006; Reeve et al. 2010).

Important yet still unresolved questions are whether the genetic compartmentalization observed in the CNS is the result of a founder effect with concomitant independent divergence, or whether there are specific selective pressures that promote selection, and what those selective pressures might be. Most likely the end result observed in cross-sectional analyses such as the ones cited previously are attributable to a combination of factors. There have been comparatively few studies designed to

differentiate between genetic divergence owing to a founder effect and adaptive evolution; even fewer have estimated the timing of divergence in the CNS. Most investigators who have compared the rate of nonsynonymous to synonymous changes have concluded that evolution in *pol*, *env*, or *nef* in brain isolates is adaptive (Huang et al. 2002; Thomas et al. 2007b; Gray et al. 2011). What is less clear is which specific pressures are driving the adaptation. Theoretically, enhanced replication in macrophages—elaborated below—response to antiretrovirals, or the peculiarities of the immune response within the CNS could be playing a role.

Chief among the potential selective pressures are the requirement for robust replication in macrophages. Macrophages express CD4 at lower levels than CD4⁺ lymphocytes (Lee et al. 1999). Accordingly, HIV isolates from the CNS tend to have an increased capacity to use reduced levels of CD4 for entry and infection (Peters et al. 2004; Martín-García et al. 2006; Thomas et al. 2007a); however, many of these isolates have been obtained postmortem, and could reflect an end-stage phenotype. Similarly, the CNS is an environment with a relatively low penetration of antibodies, and configurations that might promote neutralization are better “tolerated” under these circumstances of “immunological privilege.” In fact, isolates from the brain have been shown to be sensitive to neutralizing sera, and particularly to a monoclonal antibody (b12) that overlaps the CD4 binding site (van Marle et al. 2002; Martín-García et al. 2005; Dunfee et al. 2007).

Antiretroviral use and its penetration into the CNS is another potential selective mechanism for HIV strains in this regard, and a few studies have shown discordance between the resistance phenotypes in the blood and CSF-derived strains (Haas 2004; Smit et al. 2004). However, the turnover of virus-infected cells can be different between the plasma and CSF compartments (Schnell et al. 2009) consistent with the predominantly infected cell type in each compartment—lymphocytes in the circulation, longer-lived macrophages in the CNS—and discordance in the sensitivity to antiretrovirals may be attributable to viral

genetic information being obtained as a “snapshot” rather than representing a true biological phenomenon (Haas 2004). For example, resistance genotypes detected in the circulation at one point may potentially not appear in the CSF until later because of slower replication cycles.

Mechanisms of CNS Injury

The pathophysiology of HAD and HAND must eventually involve neurons, the principal effector cells of the nervous system. To understand the apparent paradox of cognitive and motor symptoms in what is principally a macrophage and microglial infection, investigators have developed a number of in vitro and in vivo models, many using combinations of infected monocyte-derived macrophages with mammalian (often rat, but also human) neurons. A somewhat simplistic summary of the vast literature on this subject divides the putative neurotoxic molecules into those that are the direct result of virus in the extracellular fluid, and those that propose that neurotoxicity is the end result of macrophage and microglial reaction to a chronic infection with HIV.

Among the viral proteins that have been implicated in neurotoxicity are gp120, Tat, Vpu, and Vpr. Of these, gp120 and Tat have received the widest attention, and unquestionably they can cause neurotoxicity in vitro. More complicated is the question of whether the concentrations of proteins that can be achieved in the in vivo extracellular fluid ever approach the concentrations required to affect neurons. For gp120, this may be the case, but extracellular concentrations of Tat in the nanogram range are difficult to visualize.

A second pathway of neurotoxicity involves the production of potentially neurotoxic factors in association with macrophage infection. Among the factors implicated in neurotoxicity are quinolinic acid, tumor necrosis factor, platelet activating factor, and arachidonic acid metabolites. Some of these have been detected in the CSF, and related to neurocognitive functioning, whereas others have been primarily tested in vitro. Many investigators have

proposed that a common end pathway of toxicity is through excitation of *N*-methyl-D-aspartate (NMDA)-subtype glutamate receptors, which has the potential for mediating apoptosis. Evidence supporting this model includes experiments that show that decreasing glutamate secretion by infected macrophages is partially protective in an in vitro model of macrophage-mediated neurotoxicity (O'Donnell et al. 2006), that toxicity associated with viral proteins is dependent on expression of these receptors, and that the areas that are most affected are associated with a concentration of NMDA receptors.

An intriguing recent finding, seemingly unrelated to HIV, comes from the work of Shau-Kwaun Chen and colleagues. They described a mouse mutant in the *Hoxb8* gene that has a phenotype of excessive grooming (Chen et al. 2010). However, in the relevant CNS regions, this gene is normally expressed in microglia only, and the phenotype could be rescued by bone marrow transplantation. This article raises the possibility that microglia can affect complex behaviors even in the absence of neurodegeneration, although it is still quite possible that the effects are attributable to aberrant secretion of cytokines. As such, it opens the door for a mechanism of HIV effect, potentially reversible, that does not depend on the classic findings of neuronal dropout, and could occur without such histopathological changes.

Strain-Specific Neuropathogenesis

A number of studies have related specific HIV sequences, primarily in gp120, to the development of HAND. Among these, those that associated either tropism in macrophages/microglia with specific genotypes are the most worthy of note. For example, position 306 influenced M tropism and CCR5 binding in a subset of brain-derived isolates (Dunfee et al. 2007). Similarly, a variant at position 283 (N283) was associated with brain infection and also had enhanced tropism for macrophages. These collective findings suggest that changes that enhance M tropism are associated with brain infection, but also that specific mutations are often

context-dependent and that a universal brain signature is unlikely.

Similarly, in view of the considerable burden of HIV infection in Africa as well as other developing countries, many investigators have examined the prevalence of neurological disorders in individuals who are infected with HIV clades other than the clade B that is predominant in the developed world. Those studies are difficult, because neurological and psychiatric care is suboptimal in developing countries, and because most if not all of the more sophisticated instruments that are used to determine CNS involvement depend on cultural context. Nevertheless, it is clear that HIV strains from clades other than B are associated with HAND. For example, Mahadevan et al. (2007) studied the brains of patients with clade C infection and found evidence of p24 antigen expression in macrophage-like cells in patients who had opportunistic infections such as toxoplasmosis. The pattern was similar to that of clade B CNS infiltration, but there were no multinucleated giant cells. In addition, Sacktor and coworkers in Uganda (Sacktor et al. 2009) showed that dementia occurs in patients with clade D infection, possibly in greater proportion than in patients with clade A infection in the same region.

Biomarkers of CNS Disease

As the brain and spinal cord are relatively inaccessible for assessment, surrogate biomarkers of CNS disease may provide some insight into ongoing processes relevant to HIV infection. However, a major problem has been identifying markers that are specific enough and also measurable with assays routinely available.

Measurement of HIV RNA in the CSF is the most practical means of assessing CNS “viral load.” CSF HIV RNA is ubiquitous during chronic untreated HIV infection, with levels that trend as the levels in blood but typically are 10-fold lower in absolute terms (Spudich et al. 2005; Marra et al. 2007). In untreated patients, HIV RNA levels may be higher in patients with active neurological disease as compared with asymptomatic individuals (Brew et al.

1997; Robertson et al. 1998). However, the CSF HIV RNA may arise from sources other than the CNS, and in addition to the brain, may also reflect virus in the systemic circulation that has been transported or “leaked” to the nervous system. Importantly, genetic compartmentalization of virus and detection of divergent viral quasiespecies between these tissues indicates that CSF HIV is not entirely a spillover from that present in blood (see previous sections for references).

With the recognition that markers of cellular activation and inflammation were useful indicators of disease activity in systemic HIV infection, attention turned to the utility of following such measures in the CNS compartment. Soluble CSF markers of macrophage activation (neopterin), chemokines stimulating ingress of macrophages and lymphocytes across the blood–brain barrier (CCL2/MCP1 and CXCL10/IP10), and molecules involved at various stages in the pathways for cell turnover and activation within the nervous system compartment are used to monitor processes that are thought to serve as the substrate for neuropathology in HAND (for review, see Cinque et al. 2007). In one small study, moderately elevated CSF neopterin predicted subsequent progression to HAD (Brew et al. 1996). However, although such markers have been correlated to disease activity, they have not been clinically used for diagnosis or monitoring of Neuro-AIDS owing to lack of specificity for active neurological disease in the setting of the immune activation characterizing HIV infection (Gisslén et al. 2009). Recently, attention has turned to plasma markers related to immunopathogenesis of systemic HIV, including soluble CD14 and lipopolysaccharide (Ancuta et al. 2008; Sun et al. 2010). Direct markers of neurological injury assayed in CSF, including neurofilament light chain protein (NFL), tau protein, and precursors and products of amyloid protein (amyloid precursor proteins and A β 1-42) may be more valuable as measures of active neurodegeneration or injury (Hagberg et al. 2000; Gisslén et al. 2007, 2009; Clifford et al. 2009).

Imaging of the brain has been extensively investigated and used; overt HAD may be



characterized by cerebral atrophy with or without periventricular white matter hyperintensities, which are diffuse, largely symmetric, and not characterized by edema or mass effect (Price et al. 1991). However, these findings are neither specific for nor ubiquitous in HAD, especially in its earlier stages, and, conversely, brain atrophy is noted in many neuroasymptomatic HIV-infected patients. Magnetic resonance spectroscopy (MRS), which detects cellular and biochemical processes based on diffusion of molecules through cerebral tissues, has yielded more specific insight into the inflammatory and neuronal processes occurring in the nervous system throughout the course of HIV infection. Overt HAD is associated with reduced relative levels of *N*-acetylaspartate, indicating decreased neuronal function, and elevated levels of choline, associated with brain inflammation and membrane turnover (Meyerhoff et al. 1994; Chang 1995). Similar, although less severe, patterns are seen in asymptomatic, untreated HIV infection, indicating that MRS may be a valuable preclinical marker of active CNS disease (Meyerhoff et al. 1999). Although changes in cerebral metabolites may indicate regional inflammation and neuronal injury, more subtle and potentially more neuropathologically relevant information may be obtained by systematic evaluation of white matter tracts or white matter morphometry in the brain by diffusion tensor imaging (DTI). Some early work in this area suggests that in neuroasymptomatic HIV, there are reductions in major white matter tracts in a number of brain regions (Pomara et al. 2001; Thurnher et al. 2005; Chang et al. 2008). More global sophisticated brain morphometry measurement may be used to detect focal atrophy of gray or white matter structures (Wang et al. 2009). Functional magnetic resonance imaging (fMRI), which takes advantage of the fact that hemodynamics in the brain are closely linked to neural activity, uses techniques that measure cerebral blood flow and blood oxygen level dependence (BOLD) signals. Early studies in this area show reduced baseline cerebral blood flow and increased functional demand in the brain parenchyma in HIV-infected patients

(Ances et al. 2008, 2010). Detection of abnormalities in neuroasymptomatic patients underscores the potential utility of functional MRI as assessment before development of overt neurological disease. Similar pathology of impaired blood flow may be detected by simpler cerebral perfusion imaging, which may additionally have a role in the assessment and monitoring of HIV-related CNS disease (Ances et al. 2009).

Beneficial Effects of Antiretroviral Therapy in HIV-Associated CNS Disease

Combination antiretroviral therapy has had a dramatic beneficial impact on the incidence and prevalence of severe forms of HAND or HAD. The Euro-SIDA cohort study clearly showed a decline in incidence of severe dementia (then termed AIDS-dementia complex, or ADC), related to introduction of protease inhibitors and use of cART (d'Arminio Monforte et al. 2004). More recent evidence from the CHARTER study indicates a greatly reduced prevalence (2% overall) of severe HAD in a cohort of HIV-infected individuals in the current era (recruited between 2003 and 2007) (Heaton et al. 2010). This improvement in the prevalence and incidence of severe HAND with cART reflects the generally beneficial effect of initiation of cART on neurocognitive performance witnessed in studies of initiation of antiretroviral therapy (Marra et al. 2003; Robertson et al. 2004; Cysique et al. 2006).

What are the biological underpinnings of this improvement? Blood and CSF viral burden are clearly reduced by cART (Marra et al. 2003; Spudich et al. 2005, 2006), and the initiation of cART is associated with sequential reduction in HIV RNA levels in both compartments over time (Ellis et al. 2000). CNS inflammation, the putative substrate of ongoing CNS injury in the setting of HIV, is also partly ameliorated by cART. Treatment is associated with reduced levels of markers of intrathecal inflammation, such as cellular markers of T-cell activation, CSF white blood cell (WBC), CSF neopterin, and β -2 microglobulin (Yilmaz et al. 2004; Spudich et al. 2006; Sinclair et al. 2008). Finally, markers of active neural injury in the CSF,

including CSF NFL and tau protein are reduced in the setting of cART and have been observed to decay over time in subjects initiating therapy (Mellgren et al. 2007).

Persistent Evidence of HAND despite Antiretroviral Therapy

HAND is a clinical diagnosis, currently defined based on abnormal cognitive and motor performance on neuropsychological tests according to criteria that denote three levels of HIV-associated neurological disease: asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HAD (Antinori et al. 2007, see Table 1). A number of recent studies have documented persistence of neurocognitive abnormalities, predominantly along the milder spectrum of HAND, in the setting of cART, with a prevalence ranging between 18% and 52% in varied settings (Robertson et al. 2007; Heaton et al. 2010; Cysique and Brew 2011). Studies including subjects with comorbidities that potentially confound the diagnosis of HAND, including current or past substance abuse, mental health disorders, head trauma, low education level, and coinfection with hepatitis, find a higher prevalence (up to 83% HAND in the highest “comorbidity” group in CHARTER), whereas those excluding subjects with significant relevant comorbidities and with low CD4 nadirs had

lower, but not negligible, frequency of impairment (Table 1) (Heaton et al. 2010).

The dramatic change in the severity and perhaps phenotype of the clinical disorders associated with HIV suggests that the etiology of Neuro-AIDS may have altered during this time. A small number of studies have focused on brain pathology in the era since the introduction of cART. Examination of 589 brains obtained mainly from subjects on antiretroviral therapy available through the National Neuro-AIDS Tissue Consortium (NNTC) revealed a significant reduction in the proportion of subjects with typical HIV-related brain pathology (including encephalitis, microglial nodules, and leukoencephalopathy) compared with the pre-antiretroviral era (Everall et al. 2009; Heaton et al. 2010). However, 78% overall had neuropathological abnormalities including vascular pathologies, Alzheimer type II gliosis, and other infectious and noninfectious pathologies. Premorbid HAND diagnosis (in 82%) in this cohort did not correlate with HIV-related brain pathology.

Although classical HIV-related brain pathology may no longer be the only substrate for HAND, HIV-driven mechanisms may still be important CNS abnormalities in the setting of cART. Real-time PCR quantification of HIV RNA in autopsy specimens from the NNTC has revealed detectable HIV-1 RNA in brain in a majority of cART-treated subjects (Kumar



Table 1. Diagnostic research criteria for HAND

Diagnostic entity	Cognitive performance	Functional status
Normal	Normal	Normal
Asymptomatic neurocognitive impairment (ANI)	Acquired impairment in at least two cognitive domains (<1 SD)	No perceived impact on daily function
Mild neurocognitive disorder (MND)	Acquired impairment in at least two cognitive domains (<1 SD)	Perceived interference with daily function to at least a mild degree (work inefficiency, reduced mental acuity)
HIV-associated dementia (HAD)	Acquired impairment in at least two domains, typically in multiple domains with at least two domains with severe impairment (<2 SD)	Marked impact on daily function

Adapted from Antinori et al. 2007; with permission, from Wolters Kluwer Health © 2007.

et al. 2007), and abnormal levels of microglial activation in the CNS were found to persist in neuroasymptomatic subjects with successful plasma viral suppression on cART in another recent neuropathological study (Anthony 2005). These studies are corroborated by evidence that intrathecal inflammation as detected in CSF persists in the setting of long-term, systemically effective cART (Eden et al. 2007; Yilmaz et al. 2008). Although even in the setting of therapy that fails to successfully suppress plasma HIV RNA (Spudich et al. 2006), cART usually successfully suppresses CSF HIV RNA below detectable levels, viral “escape” in the CSF may occur in up to 10% of individuals on current regimens (Eden et al. 2010), and in rare cases dramatic CSF “escape” has been associated with clinically progressive neurological disease (Canestri et al. 2010).

CNS Penetration of cART

The CNS is separated from the systemic circulation by blood–brain and blood–CSF barriers, thus potentially allowing for a “sanctuary” of infection that is only partially reached by some antiretroviral medications which, owing to molecular size or hydrophilicity, do not

readily cross the blood–brain barrier. Based on structural composition and effect on CSF HIV RNA, antiretrovirals have been ranked according to their estimated CNS penetration effectiveness (CPE), with a combination of drugs in a regimen assigned a combined “CPE” score based on the sum of their individual rankings (Table 2) (Letendre et al. 2008). Given that treatment with cART benefits the nervous system in terms of both the detection and magnitude of HIV infection and attendant inflammation within the CNS, more potent activity of antiretroviral medications within the CNS might provide additional benefit in the setting of treatment of HAND. Despite predominant concurrence between numerous studies that regimens with higher CPE scores tend to lead to more successful suppression of HIV RNA levels in the CNS (Letendre et al. 2008; Marra et al. 2009), the evidence that enhanced CPE scores are related to improved neurocognitive outcomes in subjects with HIV infection is less definitive. Whereas some observational studies show a cognitive benefit of initiation of regimens with higher CPE scores in the setting of HIV-related CNS disease (Letendre et al. 2004; Tozzi et al. 2009), others show poorer neurocognitive performance in subjects treated with

Table 2. Antiretroviral central nervous system penetration effectiveness (CPE) scoring system used in recent clinical studies

Increasing CNS penetration →	0	0.5	1
Nucleoside reverse transcriptase inhibitors	Didanosine Tenofovir Zalcitabine Adefovir	Emtricitabine Lamivudine Stavudine	Abacavir Zidovudine
Nonnucleoside reverse transcriptase inhibitors		Efavirenz	Delavirdine Nevirapine
Protease inhibitors	Nelfinavir Ritonavir Saquinavir Saquinavir/r Tipranavir/r	Amprenavir Atazanavir Fosamprenavir Indinavir	Amprenavir/r Atazanavir/r Fosamprenavir/r Indinavir/r Lopinavir/r
Entry inhibitors	Enfuvirtide T-1249		Maraviroc Vicriviroc
Integrase inhibitors		Raltegravir Elvitegravir	

Adapted from Smurzynski et al. 2011; with permission, from Wolters Kluwer Health © 2011.

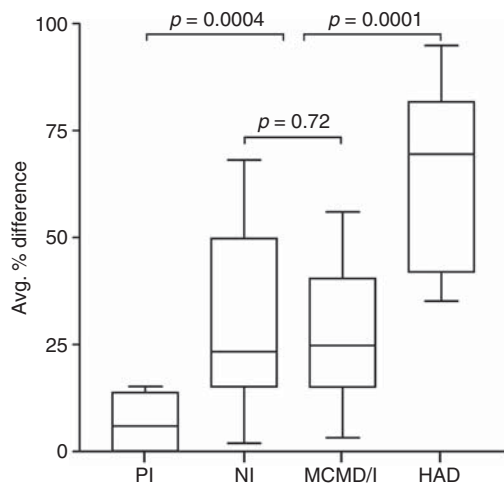


Figure 2. Heterduplex tracking assay (HTA) was used to compare V1/V2 and V4/V5 *env* populations in blood plasma (BP) and CSF from subjects with distinct stages of HIV infection and neurological status (PI, primary infection; NI, not impaired; MCMMD/I, minor cognitive and motor disorder; HAD, HIV-associated dementia). The % difference for BP/CSF HTA band patterns was determined for each patient and the data compiled. A higher percentage difference indicates more discordant BP/CSF viral genetic populations for the particular region of *env* analyzed. The mean of V1/V2 and V4/V5 percent difference results was determined for each patient to reflect global *env* compartmentalization between BP and CSF, and results were compiled for comparison between the different disease categories. *p* values shown were determined by Wilcoxon rank-sum test. (Adapted from Harrington et al. 2009; with permission, from Wolters Kluwer Health © 2009.)

regimens with higher CPE scores (Marra et al. 2009). A variety of issues may lead to the discrepant results of such studies. High CPE regimens may be especially important in conditions of robust brain and meningeal infection with HIV, which typically occur in later stages of chronic infection, when targeted therapy to CNS tissues may be key for control of compartmentalized viral replication (Fig. 2). Furthermore, retrospective, observational studies may be biased; for example, subjects with more profound neurological deficits may be started on regimens with higher CNS penetration. Such deficits may not be entirely reversible and thus

persist despite high CPE regimens. A recent study did find a modest neurocognitive benefit of higher CPE scores (although only between patients with more than three drugs in their regimens and higher or lower CPE scores) in the context of randomized assignment of antiretroviral regimens (Smurzynski et al. 2011); additional randomized studies are warranted to directly examine this issue.

New Research Areas

The Relationship among Aging, New Comorbidities, and HAND

In resource-rich regions where life expectancy after HIV diagnosis in a young adult is estimated to be approximately 12 years below that of a noninfected individual (Lohse et al. 2007), longer duration of survival with chronic HIV-1 infection may change the scope and etiology of disorders affecting the CNS. A great deal of recent attention has been paid to the question of whether aging and HIV-1 will have a synergistic effect on neurodegeneration within the brain, “accelerating” injury triggered earlier by neuropathological processes associated with HIV. Systemic immunologic changes noted in HIV infection are in part characterized by markers of immunosenescence, and chronic infection with HIV is associated with the earlier onset of cancers and vascular disorders seen in HIV-uninfected persons at more advanced age (Desai and Landay 2010). However, the evidence that aging and HIV have an enhanced combined deleterious effect in the brain is mixed. A large cohort study enrolling subjects with HIV and advanced age, the Hawaii aging cohort, found a higher prevalence of cognitive deficits in older HIV-infected patients, related to CD4 nadir, insulin resistance, and presence of Apo E alleles (Valcour et al. 2004), and a recent neuroimaging study found that HIV and aging caused independent reductions in cerebral blood flow (Ances et al. 2010). However, the Multicenter AIDS Cohort Study (MACS) found no difference between older HIV⁺ individuals and older HIV⁻ individuals in rate of change in performance on longitudinal testing,



suggesting the absence of an acceleration of neurological disease with aging (Becker et al. 2009). Two additional recent studies using well-matched HIV-uninfected comparison groups in analyses of age effects on neurocognitive performance showed no combined effects of HIV and age on cognitive function (Cysique et al. 2011; Valcour et al. 2011). Other conditions are emerging in the setting of long-term survival with chronic HIV infection that may influence the integrity of the CNS and alter the substrate for cognitive and neurological impairment in HIV. In particular, cardiovascular risk factors such as hyperlipidemia, hypertension, carotid intima-media thickness, and past history of cardiovascular disease have been identified recently as associated with reduced neurocognitive performance in HIV-infected subjects (Becker et al. 2009; Foley et al. 2010; Wright et al. 2010). It is unclear whether these risk factors are non-HIV-related cofactors that influence cognitive outcomes, HIV- or cART-related systemic effects that parallel processes of CNS injury, or conditions that are directly involved in the mechanisms of HAND in the current era. Further efforts to investigate the association between vascular changes and clinical, pathological, and imaging changes in the setting of HIV are warranted to expand possible treatment approaches to HAND.

Importance of Acute/Early Infection

Although severe HAND clearly is a condition associated with long-term chronic HIV infection and immunosuppression, recent evidence that acute and early HIV are crucial for systemic disease pathogenesis has raised questions about whether early stages of HIV might also be important for neuropathogenesis. It has long been known that HIV may enter the nervous system within the first weeks after initial systemic infection (Schacker et al. 1996; Pilcher et al. 2001). It is now clear that acute infection also initiates a cascade of neuroinflammation, providing conditions for inflammation-mediated injury within the CNS. Analysis of CSF from 96 antiretroviral naïve subjects at a median

less than three months after HIV transmission revealed elevations in CSF WBC counts, neopterin, and CXCL10 equal to those in subjects with chronic HIV infection (Fig. 3) (Spudich et al. 2011).

Furthermore, neuroimaging studies in recently HIV-infected humans reveal lower *n*-acetylacetate in the frontal cortex during early infection, suggestive of neuronal dysfunction or injury during this early period (Lentz et al. 2009). Follow-up longitudinal studies in this group reveal dynamic patterns of cerebral metabolites over the first year of infection (Lentz et al. 2011). Given that early HIV infection is characterized by neuroinflammation and evolving metabolite changes within the CNS, it is possible that either antiretroviral or anti-inflammatory treatment initiated during early stages of infection may ameliorate injury sustained in the CNS during the early years of infection before immune systemic immunosuppression. Finally, the fact that CSF compartmentalization of HIV species may begin within the first year of HIV infection in some individuals (Schnell et al. 2010) suggests that treatment and eradication efforts may need to consider the CNS as a potential independent site of replication and mutation, beginning in the early stages of HIV.

CONCLUSIONS

There has been substantial progress in the recognition and treatment of the most severe forms of CNS HIV infection, and although the mechanisms leading to neurological dysfunction are still under investigation, the central role of macrophages and microglia is well established. Less well understood are the less severe forms of CNS disease now seen in the developed countries—where the use of cART is common—and the role of virus, inflammation, and CNS penetration of antiretrovirals are areas of potential new discoveries. Similarly, there has not been any successful “adjuvant” therapy: that is, one designed to treat the CNS specifically rather than the virus. Such adjuvant treatment may be important in preventing or ameliorating HAND in the setting of cART.

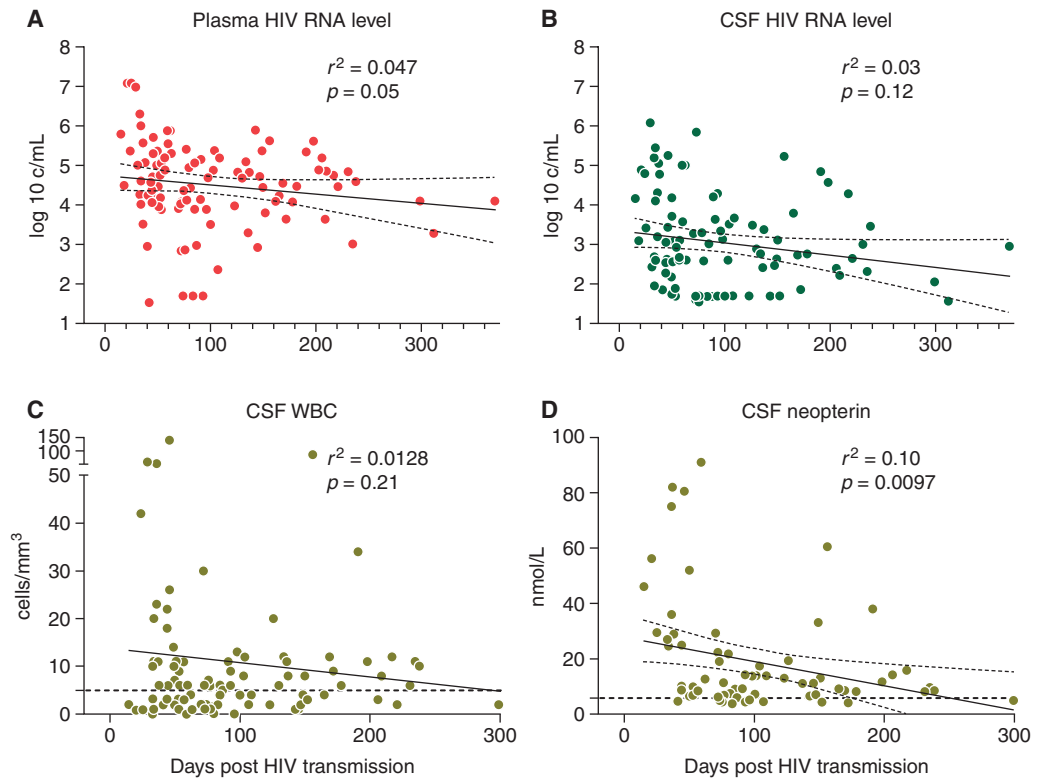


Figure 3. Baseline levels of markers of HIV infection and inflammation in a primary infection cohort are shown according to estimated days after transmission at blood and CSF sampling. Simple linear regression between the number of days post-estimated HIV transmission and (A) blood plasma HIV RNA levels, (B) CSF HIV RNA levels, (C) CSF WBC counts, and (D) CSF neopterin levels. Regression lines (solid) and 95% confidence intervals (dotted) are indicated; upper limit of normal values for CSF WBC and CSF neopterin are indicated on each graph by a dotted horizontal line. (Adapted from Spudich et al. 2011; with permission, from Oxford University Press © 2011.)

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