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*Review*

# Human immunodeficiency virus type 1 genetic diversity in the nervous system: Evolutionary epiphenomenon or disease determinant?

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**Over the past decade there has been a revolution in the understanding and care of human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS)-associated disease. Much of this progress stems from a broader recognition of the importance of differences in viral types, including receptor preference(s), replication properties, and reservoirs, as contributing factors to immunosuppresion and disease progression. In contrast, there is limited conceptualizatin of viral diversity and turnover in the brain and circulation in relation to neurocognitive impairments. Herein, the authors review current concepts regarding viral molecular diversity and phenotypes together with features of HIV-1 neuroinvasion, neurotropism, neurovirulence, and neurosusceptiblity. Viral genetic and antigenic diversity is reduced within the brain compared to blood or other systemic organs within individuals. Conversely, viral molecular heterogeneity is greater in patients with HIV-associated dementia compared to nondemented patients, depending on the viral gene examined. Individual viral proteins exert multiple neuropathogenic effects, although the neurological consequences of different viral polymorphisms remain uncertain. Nonetheless, host genetic polymorphisms clearly influence neurological disease outcomes and likely dictate both acquired and innate immune responses, which in turn shape viral evolution within the host. Emerging issues include widespread antiretroviral therapy resistance and increasing awareness of viral superinfections together with viral recombination, all of which are likely to impact on both HIV genetic variation and neuropathogenesis. With the increasing prevalence of HIV-induced neurocognitive disabilities, despite marked improvements in managing immunosuppression, it remains imperative to fully define and understand the mechanisms by which viral dynamics and diversity contribute to neurological disease, permitting the development of new therapeutic strategies.** *Journal of NeuroVirology* (2005) **11,** 107–128.

**Keywords:** brain; HIV-1; molecular diversity; neuropathogenesis; selection pressures

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## Introduction

Retroviral infections frequently result in nervous system disease (Patrick *et al*, 2002; Sanders *et al*, 2001), but human immunodeficiency virus type 1 (HIV-1) infection exhibits the broadest range of associated neurological phenotypes. Autopsy studies show that over 90% of patients dying with acquired immunodeficiency syndrome (AIDS) manifest some type of neurological disease (Johnson, 1998). The broad

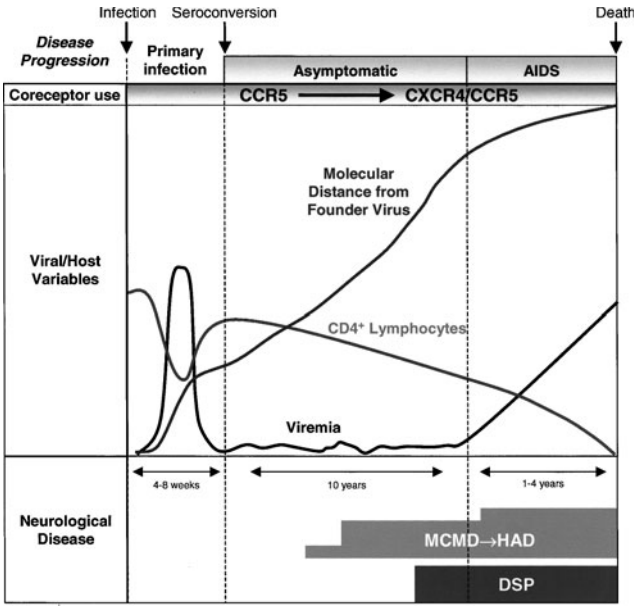


**Figure 1** The temporal interrelationships between HIV-1 systemic disease progression, immunosuppression, viral diversity and load, coreceptor preference, and primary neurological disease occurrence. As viral diversity in blood increases with concurrent immunosuppression, neurological disease develops (MCMD, minor cognitive motor disorder; HAD, HIV-associated dementia; DSP, distal sensory polyneuropathy), and X4 viruses emerge.

spectrum of primary HIV-induced neurological diseases affects both the peripheral (PNS) and central nervous (CNS) systems (Figure 1) (Power *et al*, 2002). Because 15% of untreated AIDS-defined patients will develop frank dementia and another 25% to 35% exhibit mild cognitive dysfunction, termed HIV-associated dementia (HAD) and minor cognitive and motor disorder (MCMD), respectively, the impact of HIV-induced neurocognitive impairment is substantial. Moreover, the onset of HAD heralds a significantly worsened survival prognosis (McArthur *et al*, 1993). Similarly, HIV-1– related peripheral neuropathies may affect greater than 50% of treated patients (Keswani *et al*, 2002). Advancing immunosuppression and increased HIV-1 molecular diversity within the host usually accompany the development of neurological disease. The understanding of HIV-1 dynamics and evolution in relation to systemic disease has advanced markedly over the past decade, with concomitant improved therapeutic strategies, such as the availability of highly active antiretroviral therapy (HAART). In contrast, similar progress in understanding nervous system infection by HIV-1 has not been forthcoming despite the significant burden of neurological disease. The limited understanding of HIV neuropathogenesis primarily stems from the complexity in diagnosing neurological disease, dynamic and codependent viral reservoirs within the CNS, i.e., brain and cerebrospinal fluid (CSF), and a dependence on autopsy studies with reliable clinical assessment before death. Overall, studies suggest that although incidence of HAD has dropped with the advent of HAART, its prevalence is rising (Brew and Dore, 2000; Dore *et al*, 1999; Neuenburg *et al*, 2002; Sacktor *et al*, 2002). An improved knowledge of viral dynamics in the CNS would enhance insights into HIV-induced neurological disease and perhaps lead to more effective therapeutics, as shown for systemic HIV-associated disease. It is important to appreciate that systemic immune suppression *per se* does not cause primary neurological disease despite its proclivity for increasing susceptibility to opportunistic infections. On the other hand, the development of HIV-related neurological disease is closely correlated with increasing immunosuppression and viral diversity (Figure 1). Bearing in mind that activation (and dysregulation) of innate immunity is increasingly recognized as an important determining factor of neurodegeneration (Holmes *et al*, 2003; Nguyen *et al*, 2004), immunosuppression with subsequent systemic intercurrent infections is also likely contributing to HIV-induced neurological disease. Virus presence in the brain is not sufficient for the development of CNS disease as many studies show detectable virus in the brains of patients without HAD who are profoundly immune suppressed. The extent to which viral molecular and antigenic diversity participate in HIV-1 neuropathogenesis is unclear. Herein, we review the current understanding of viral dynamics in the CNS together with the impact of viral diversity on HIV neuropathogenesis.

## Viral evolution and dynamics

Natural selection is usually assumed to improve the fitness of an infectious agent over time, reflected in maximized reproductive capacity (Nowak and Sigmund, 2004). Indeed, conventional evolutionary thinking implies that host factors determine the pathogen's adaptations but it is also worth remembering that those same adaptations may also influence the host's fate(s). Paradoxically, increased viral pathogenesis may actually reflect reduced viral fitness as it could diminish survival of the host, and thereby limiting the virus' ability to propagate itself (DeFillipis and Villarreal, 2001). The two principal processes by which the genetic composition of a viral population is regulated include selection and random genetic drift. Selection is defined as *positive* when fitter viral variants exhibit increased frequency in a population whereas *negative* selection reflects eradication of the less fit variants from the population. Conventional assays of viral fitness are usually predicated on comparing replication properties using *in vitro* assays, but this does not always reflect the *in vivo* circumstances. In many neurovirological infections, enhanced virus replication is associated with increased pathogenesis (DeFillipis and Villarreal, 2001; Johnson, 1998).

HIV-1 is one of the fastest evolving organisms, in large part due to (1) the high error rate of its reverse transcriptase (~0.2 errors per genome for cycle of replication); (2) extraordinary replication dynamics (~$10^{10}$ to $10^{12}$ viruses per day); (3) frequent recombination; and (4) intense selection (reviewed in Rambaut *et al*, 2004). The extent of HIV-1 genetic diversity or heterogeneity within a host is correlated with the duration of infection because of protracted immunological selection (Lukashov *et al*, 1995; Markham *et al*, 1998; Ross and Rodrigo, 2002; Shankarappa *et al*, 1999), as part of the ongoing battle between the virus and the immune system. Viral molecular diversity is often described as point mutations manifested as nonsynonymous (amino acid changing) or synonymous (non–amino acid changing) mutations, but also involves changes in nucleic acid sequence as a result of insertions, deletions, and recombination events within a viral population or quasispecies. Ultimately these changes lead to altered overall structure and function at the protein level, but also affect noncoding nucleic acid sequences that are important for viral replication, transcription, and translation. The term quasispecies describes an error-prone self-replicating, -organizing, and -adapting population of viral genomes, first described in studies of molecular evolution of primitive replicons (Eigen, 1971; Eigen and Biebricher, 1988). HIV-1 molecular and phenotypic diversity exists within host populations as well as within individuals, depending on the infected cell type or organ. Phylogenetic tools, by which the degree of relatedness and evolution among different viral sequences can be inferred, permit insights into the complex makeup of these viral populations or quasispecies. These inferences are also invaluable for epidemiological analyses (Gaschen *et al*, 2002; Korber *et al*, 2001).

The relative rate of nonsynonymous ($d_N$) to synonymous ($d_S$) mutations reflects different selection pressures with a ratio ($d_N/d_S$) of $<1$ indicating negative (purifying) selection whereas a ratio of $>1$ suggests positive selection pressure and ratios approximating $\approx 1$ point to random genetic drift (Overbaugh and Bangham, 2001). Multiple factors including host immune response, intrinsic properties of the virus, and environmental factors influence selection pressures (Domingo and Holland, 1999; Overbaugh and Bangham, 2001). The consequences of increasing viral molecular diversity in HIV-1 consist of an enhanced ability to evade the immune system with ensuing immunological exhaustion and a gain or loss of select functions such as receptor binding or replication competence (Coffin, 1995). HIV-1 dynamics and phenotypes outside of the CNS are characterized by high levels of replication, depending on the individual virus strain, infected cell types and organs, coreceptor preference, susceptibility to immune inactivation and selective activation of both innate and acquired immune mechanisms with accompanying cytopathogenicity. Aside from obvious viral qualities that distinguish individual viral strains, including coreceptor(s) utilization, cell and organ tropism, and pathogenesis (Khanna *et al*, 2000; Kreisberg *et al*, 2001; Schramm *et al*, 2000; Voulgaropoulou *et al*, 1999), it is also evident that at the level of whole human populations, different HIV-1 subtypes or clades may vary in their ability to cause disease (Kanki *et al*, 1999). However, the prime example of HIV genetic diversity influencing disease outcome lies in the emergence of drug resistant viruses. Distinct mutations within the protease and reverse transcriptase sequences lead to drug failure and resulting disease progression (Richman, 2001). As HIV-1 infection progresses over time, viral molecular diversity expands with immunosuppression. In addition, increased viral diversity at the onset of infection may predict a higher viral load set point and accelerated disease progression (Lavreys *et al*, 2002; Neilson *et al*, 1999). Thus, the collision of a highly diverse and dynamic viral population with a large and genetically outbred species such as humans lends itself to the emergence of new and potentially more virulent viral variants.

## Neuroinvasion, neurotropism, and neurovirulence

HIV-1 infects cells of the brain during primary infection (Bell *et al*, 1993; Davis *et al*, 1992), termed *neuroinvasion*. Although HIV-1 is inherently *neurotropic*, i.e., able to infect and replicate in cells of the nervous system, not all HIV/AIDS patient will develop neurological disease, indicating that HIV-1 is not always *neurovirulent*, i.e., able to cause neurological disease. This raises the intriguing question of what factors influence the heterogeneity of clinical presentation and underlying neuropathogenesis of HIV-1–associated neurological disease. The obvious determinants include the virus' intrinsic pathogenic properties and the *neurosusceptibility* of the infected individual, i.e., genetic background and age (Corder *et al*, 1998; Gonzalez *et al*, 2002; Janssen *et al*, 1992; Quasney *et al*, 2001; van Rij *et al*, 1999). The level of immunosuppression caused by HIV-1 is also an integral determinant of the development of CNS disease (Figure 1). HIV-1 belongs to the genus of lentiviruses, all of which cause neurological disease (Patrick *et al*, 2002). Simian (SIV), feline (FIV), and bovine (BIV) immunodeficiency viruses, but also the non–immune-suppressing animal lentiviruses, such as caprine arthritis encephalitis virus (CAEV), maedi-visna virus (MVV), and equine infectious anemia virus (EIAV) cause CNS disease (reviewed in Patrick *et al*, 2002). In addition to HIV-1, HIV type 2 (HIV-2) has been shown to infect the brain with subsequent development of neurological disorders, but its neuropathogenic mechanisms remain largely unknown (Lucas *et al*, 1993; Sankale *et al*, 1996). The disease pattern characterizing lentiviral infections

generally consists of acute primary infection, which elicits an intense immune response, followed by a long period of asymptomatic infection and in the final stages of disease defined by immune suppression or immune activation, depending on the virus (Clements and Zink, 1996). HIV-1–, FIV-, and SIV-associated neurological diseases predominantly occur during advanced systemic immunosuppression but also hasten the host's demise, irrespective of the level of immune suppression (McArthur *et al*, 1993; Narayan *et al*, 1995; Patrick *et al*, 2002). Like other immunodeficiency lentiviruses, including SIV and FIV, HIV-1 appears in the nervous system early after infection (Davis *et al*, 1992; Poli *et al*, 1999; Sasseville and Lackner, 1997). The mechanism by which HIV-1 crosses the blood-brain barrier (BBB) is unclear. Several potential routes have been proposed and include direct infection of endothelial cells and subsequent release of virus into the brain, transcytosis of virions across brain endothelial cells, trafficking of infected cells (monocytes as well as lymphocytes) from the periphery into the nervous system, or disruption of the BBB or blood–cerebrospinal fluid barrier (B-CSF-B) at the level of the choroid plexus (reviewed in Strelow *et al*, 2001). Viral sequence diversity is lower in the brain compared to blood or other organs, as measured by the relative number of nonsynonymous mutations, whereas CSF displays intermediate levels of diversity (Wong *et al*, 1997). The $d_N/d_S$ ratio in brain is also lower, likely due to fewer immunological constraints on viral replication in the brain because of (1) its immune privileged nature with absent lymphatics or lymphoid tissue to support viral replication, together with (2) lower permissiveness of brain cells to HIV-1 infection, perhaps due to low CD4 expression. In fact, these conditions suggest that a bottleneck for viral replication occurs in the brain and only specific viral strains cross the BBB and subsequently infect the CNS.

*Neurotropism*, the ability to infect the nervous system, is determined in part by the individual cell types' permissiveness to viral binding, entry, and replication together with the specific strain of infecting virus. Cells infected by HIV-1 in the brain are primarily microglia, perivascular macrophages, and to a lower degree astrocytes (Bagasra *et al*, 1996; Nuovo *et al*, 1994; Torres-Munoz *et al*, 2001; Trillo-Pazos *et al*, 2002). The invading/perivascular macrophage and to a lesser extent the resident microglia are considered the principal sites for active lentivirus replication in the brain (reviewed in Clements and Zink, 1996; Lipton and Gendelman, 1995). This is complemented by the formation of multinucleated giant cells, which express abundant viral antigen and are the neuropathological hallmark of HIV-1 infection, representing HIV encephalitis (HIVE) (Wiley, 1995). Astrocytes are permissive to infection but limited to early virus gene expression with minimal viral replication and release (Gorry *et al*, 1999; Messam and Major, 2000; Neumann *et al*, 1995; Tornatore *et al*, 1991, 1994). To what extent direct infection of neurons (*neuronotropism*) plays a role in HIV-1 neuropathogenesis is unknown and remains controversial (Bagasra *et al*, 1996; Nuovo *et al*, 1994; Torres-Munoz *et al*, 2001; Trillo-Pazos *et al*, 2002).

In phylogenetic analyses of HIV-1, viral sequences cluster together by organ and vary with duration of disease (Ball *et al*, 1994), whereas in the CNS, brain parenchyma and CSF constitute overlapping reservoirs (Bratanich *et al*, 1998; Chang *et al*, 1998; Shapshak *et al*, 1999; Wong *et al*, 1997). In addition, the brain may be further compartmentalized as viral envelope sequences cluster by individual anatomical region (Chang *et al*, 1998; Liu *et al*, 2000; Shapshak *et al*, 1999). In patients with encephalitis, evidence of compartmentalization may be obscured in phylogenetic analyses (Gatanaga *et al*, 1999; Hughes *et al*, 1997; Wang *et al*, 2001). Some groups have also proposed that distinct brain-specific "signature" sequences can be defined (Korber *et al*, 1994) with a predominance of nonsyncytia-inducing viruses (van't Wout *et al*, 1998). Comparisons of brain- and spleen-derived envelope sequences from different HIV-1 clades show that the rate of nonsynonymous mutations ($d_N$) varied among individual clades, which was also dependent on the individual envelope domain (Zhang *et al*, 2001). However, purifying (or negative) selection was significantly greater in the brain-derived compared to spleen-derived sequences, reflecting the constraints on viral replication in the brain, as mentioned earlier.

Entry and infection of the nervous system are not the sole determinants of neurological disease or *neurovirulence*. The inflammatory responses elicited by the infected cells as well as the activation and dysregulation of bystander microglia and astrocytes are considered key factors in HIV-1 neurological disease development (Kaul *et al*, 2001; Mollace *et al*, 2001; Wesselingh and Thompson, 2001). HIV-1, SIV, and FIV neuropathogenesis is characterized by direct and indirect activation of innate immune responses in the CNS with ensuing neuronal degeneration and death (Kaul *et al*, 2001). In HIV-induced neurological disease, the activation of innate immune responses in the CNS manifests itself as upregulation of cytokines, chemokines, and matrix metalloproteases (MMPs). The increase in proinflammatory molecules following infection may recruit additional inflammatory macrophage cells into the nervous system (Klein *et al*, 1999; Lane *et al*, 1996; Sanders *et al*, 2001; Sasseville *et al*, 1996), whereas both cytokines and chemokines through interactions with their cognate receptors present on astrocytes and neurons also have toxic effects on these cell types or result in the release of molecules with a neurotoxic action (Gabuzda and Wang, 2000; Klein *et al*, 1999; Zheng *et al*, 1999). Moreover, infection by HIV-1 or exposure to its gene products results in the release of other neurotoxic molecules by macrophages, microglia, and astrocytes (Kaul *et al*, 2001; Mollace *et al*, 2001; Wesselingh

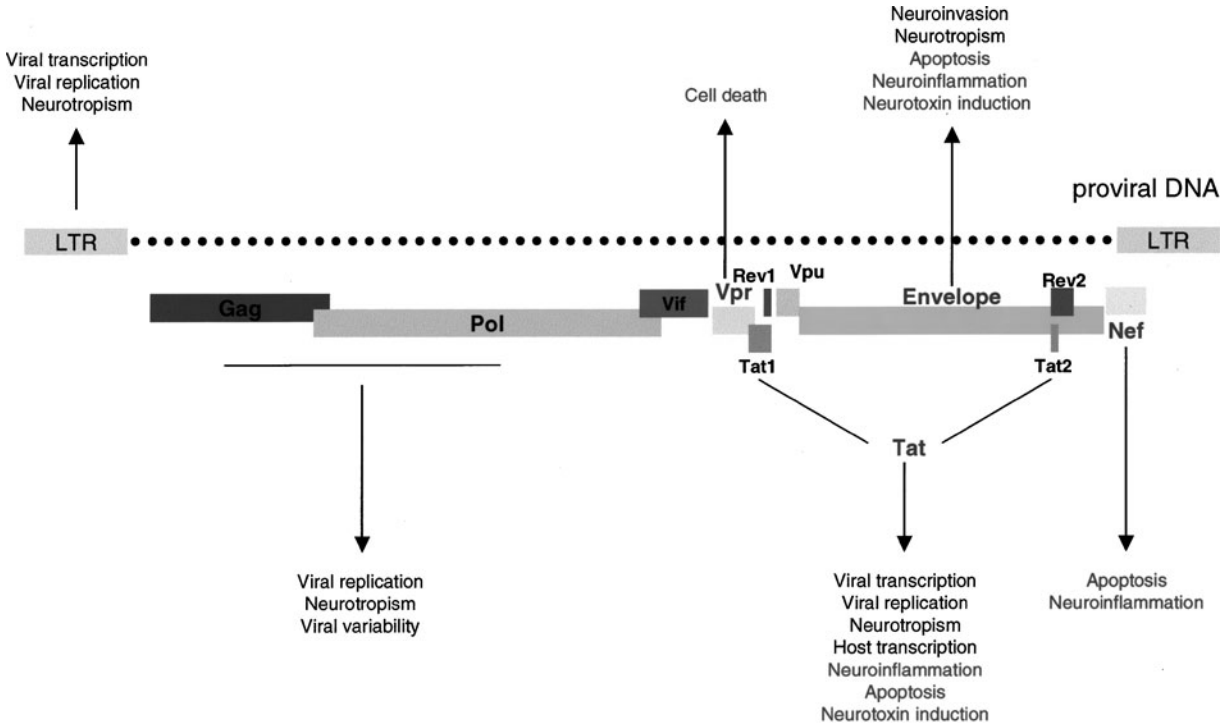


**Figure 2** Schematic representation of the impact of the different HIV-1 genes and regions identified in relation to HIV-1 neurovirulence. The HIV-1 genome is indicated as proviral DNA. The 2 exons making up the spliced *Tat* and *Rev* genes have been indicated as Tat1-Tat2 and Rev1-Rev2, respectively. The pathogenic effects for each gene expressed in the brain are highlighted.

and Thompson, 2001; Zhang *et al*, 2003b). The different neuropathogenic mechanisms appear to depend on individual viral genes (Figure 2). For example, HIV-1 Tat induces p53-mediated neuronal death (Silva *et al*, 2003). Conversely, the HIV-1 envelope protein triggers a highly novel pathogenic cascade, in which an MMP cleaves the chemokine, stromal cell–derived factor-1 (SDF-1), yielding a highly neurotoxic molecule that causes neuronal apoptosis (Zhang *et al*, 2003a). Other molecular pathways also impact on lentiviral neurovirulence, including elevated nitric oxide metabolites (i.e., peroxynitrite), altered tryptophan metabolism, and activation of arachidonic acid metabolism (Garden, 2002), whereas up-regulation of neuronal cell cycle regulators and deregulation of differentiation factors may also impair neuronal survival and function (Jordan-Sciutto *et al*, 2000; Peruzzi *et al*, 2002). Ultimately, each of the above mechanisms is driven by the presence of virus in the brain and stochastic events dictated by the interaction(s) between a particular viral protein and a host cellular pathway.

The individual host's *neurosusceptibility* is also an important disease determinant in lentivirus infections, similar to other infectious diseases in which age and genetic polymorphisms confer vulnerability to neurological disease (Clements and Zink, 1996; Dean *et al*, 2002; O'Brien and Moore, 2000; Patrick *et al*, 2002). For example, SIV infection of nonhuman African primates occurs naturally and is nonpathogenic (reviewed in Clements and Zink, 1996; Johnson, 1998; Sanders *et al*, 2001) despite high levels of virus in the brain. During cross-species transmission to Asian macaques, not normally infected with SIV, SIV induces simian AIDS and encephalitis. Host genetic studies in humans have identified polymorphisms in genes that are associated with the onset of AIDS or its progression (Berger *et al*, 1999; Dean *et al*, 2002), including the development of HIV-1 neurologic disease. The host neurosusceptibility genes with polymorphisms identified in relation to HIV-associated dementia include the chemokine receptor CCR5 and potentially pathogenic molecules such as apolipoprotein E (APOE), tumor necrosis factor-$\alpha$ (TNF-$\alpha$), SDF-1, and monocyte chemoattractant protein-1 (MCP-1) (Corder *et al*, 1998; Gonzalez *et al*, 2002; Quasney *et al*, 2001; Sei *et al*, 2001; van Rij *et al*, 1999). It is likely that more polymorphisms in host immune genes will be identified as risk factors for HIV-induced neurological diseases in the future. Such genetic variations in the host may also modify responses to individual HIV-1 genes, which have been reviewed elsewhere (Carrington *et al*, 1999a; Dean *et al*, 2002; O'Brien and Moore, 2000).

*HIV-1 gene products and neuropathogenesis*

Several viral gene products have been implicated in HIV-1 neurovirulence (Figure 2). The most prominently studied both with regard to pathogenic mechanism and sequence variability, are the HIV-1 envelope

and Tat (transactivator of transcription) proteins and will be discussed in more detail below. The Nef (Negative factor) protein, and more recently, the auxiliary viral protein R (Vpr) have been demonstrated to contribute to neuropathogenesis (Brack-Werner *et al*, 1992; Patel *et al*, 2000, 2002; Ranki *et al*, 1995; Saito *et al*, 1994; Speth *et al*, 2002), although in contrast to HIV-1 Tat and envelope, molecular diversity in Nef did not differ in blood from HIV/AIDS patients with and without HAD (van Marle *et al*, 2004). Likewise the *Gag/Pol* region and the noncoding long terminal repeat (LTR) sequences have also been implicated in development of neurological disease (Ait-Khaled *et al*, 1995; Corboy *et al*, 1992; Corboy and Garl, 1997; Huang *et al*, 2002; Ross *et al*, 2001). However, to date little is known about the neuropathogenic effects resulting from molecular diversity in these viral gene products. Although the direct contribution of individual viral proteins to pathogenesis of HIV-1 associated neurological disease remains uncertain, it is increasingly appreciated that each contributes to cell tropism and neurotropism, which ultimately influences neurovirulence, as discussed below.

*HIV-1 envelope protein*

The HIV-1 envelope glycoprotein is responsible for viral binding and entry into the cell. Apart from using CD4 as primary receptor, HIV-1 also requires chemokine receptors as coreceptors (reviewed in Berger *et al*, 1999). HIV-1 predominantly uses the CXCR4 or CCR5 receptors as coreceptor, but the use of several other chemokine receptors has also been reported (Choe *et al*, 1996; Doranz *et al*, 1996; Hoffman *et al*, 1998). Viruses that exclusively use CCR5 (R5) are largely macrophage tropic strains and those that use CXCR4 exclusively (X4) are largely lymphotropic strains and emerge late in the course of disease. Viral strains that are able to use both receptors have been isolated and termed as X4R5 or dual tropic strains (Berger *et al*, 1999; Collman *et al*, 1992; Doranz *et al*, 1996). In the case of each coreceptors, the HIV-1 envelope protein's sequence dictates the affinity with which it binds to the receptor (Berger *et al*, 1999). Amino acid residue changes in the envelope protein can change the tropism of HIV-1 by changing coreceptor use (Cho *et al*, 1998; Hoffman *et al*, 1998; Speck *et al*, 1997; Wang *et al*, 1999).

In the brain, macrophage tropism and use of CCR5 as a coreceptor for viral entry appear to be pivotal prerequisites for infection (Albright *et al*, 1999; Chan *et al*, 1999; Gorry *et al*, 2001; Reddy *et al*, 1996). The majority of the viruses found in the brain use CCR5 for viral entry. In some cases, CCR3 participates in the infection of microglia (He *et al*, 1997). Cells of macrophage/microglial lineage express CCR5, CXCR4 and CCR3 (Albright *et al*, 1999; He *et al*, 1997). Although astrocytes do not express CD4, they express both CXCR4 and CCR5 (Flynn *et al*, 2003). The infection of astrocytes is not efficient and, perhaps due to a block in Rev function, results in the expression of early viral gene products such as Tat and Nef (Gorry *et al*, 1999; Messam and Major, 2000; Neumann *et al*, 1995; Tornatore *et al*, 1994). X4-dependent viruses and dual tropic (X4R5) viruses are infrequently found in the brain (Chan *et al*, 1999; Gorry *et al*, 2001; Reddy *et al*, 1996), despite reports of their ability to cause neuronal injury (Buch *et al*, 2000; Ohagen *et al*, 1999; Yi *et al*, 2003). It has been reported that certain brain-derived viruses appear to have a higher affinity for CCR5, making them less dependent on high levels of CD4 for infection (Gorry *et al*, 2002; Martin *et al*, 2001; Shieh *et al*, 2000). Brain-derived HIV-1 envelope sequences from patients with HAD exhibit higher sequence diversity (Figure 3A), reflected in a trend towards random genetic drift, compared to brain-derived viral sequences

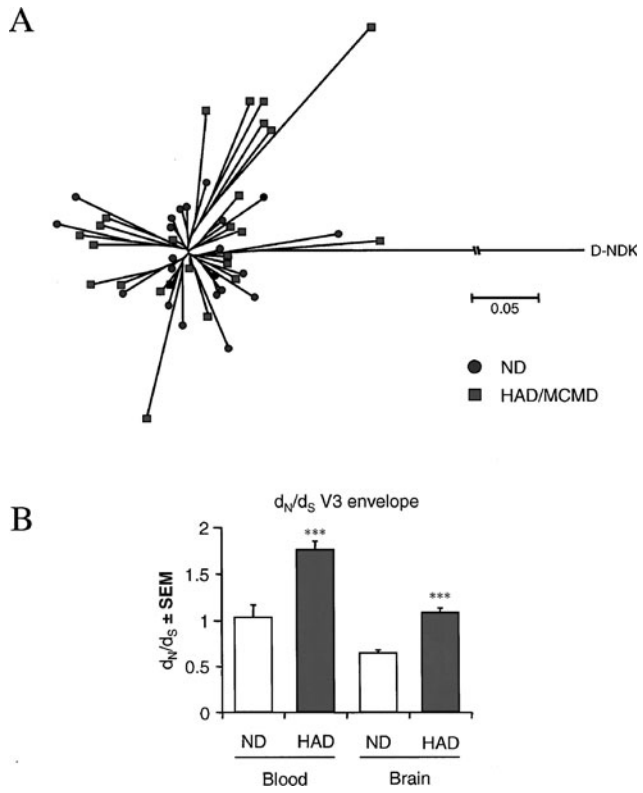


**Figure 3** (**A**) Total DNA distance displayed by phylogenetic neighbor-joining tree (Jukes-Cantor correction), based on the blood-derived consensus envelope (V3 region) sequences obtained from AIDS patients with neurocognitive impairment (HAD or MCMD) or nondemented (ND) patients, rooted to the V3 envelope sequence of the HIV-1 D clade virus strain NDK. The more extended branches for the HAD/MCMD patients compared to ND patients indicate higher viral diversity among sequences obtained from HAD/MCMD patients, reflecting differences in selection pressures acting on this region for each patient group. (**B**) The differences in selection pressure is reflected in a higher ratio of nonsynonymous (i.e., amino acid changing) over synonymous (i.e., non–amino acid changing) nucleotide substitutions ($d_N/d_S$), which were the most evident for the blood-derived sequences from HAD patients. A $d_N/d_S$ larger than 1 indicates a replicating nucleic acid sequence is under positive selection pressure. $^{*}P < .05$; $^{***}P < .001$. Adapted from van Marle *et al* 2002.

from AIDS patients without dementia, which exhibited purifying selection ($d_N/d_S < 1$) (van Marle *et al*, 2002) (Figure 3B). Blood-derived envelope sequences exhibited different profiles among clinical groups, with HAD patients having a higher $d_N/d_S$ values ($>1$) compared to nondemented (ND) patients ($d_N/d_S \approx 1$), despite matched levels and durations of HIV infection and systemic immunosuppression (van Marle *et al*, 2002). Of particular interest was the finding that the $d_N$ value for the envelope protein V3 loop derived from blood was highly correlated with presence or absence of HAD (van Marle and Power, unpublished results) (Figure 4A). These latter observations may point to immunological failure to curtail viral replication in blood in late stages of disease among patients with HAD, but also suggest the relative selection pressures that influence virus evolution in brain and blood differ.

Apart from influencing viral entry and spread, lentivirus envelope proteins influence neuropathogenesis by other mechanisms. The interaction of the HIV-1 and FIV envelope protein with chemokine receptors has been shown to initiate signaling events, such as the signal transducer and activator of transcription (STAT)/Janus kinase (JAK) pathway (Shrikant *et al*, 1996), in an envelope sequence–dependent manner (Johnston *et al*, 2000). Activation of this pathway in monocytoid cells induces neuronal death directly and indirectly by eliciting the release of molecules with neurotoxic actions in both infected and uninfected cells (Johnston *et al*, 2000; Martin-Garcia *et al*, 2002). Indeed, envelope molecular diversity appears to drive activation of MMP-2, which in turn cleaves SDF-1 to a neurotoxic form (Zhang *et al*, 2003a). The full-length envelope protein gp160 and the processed forms gp120 or surface unit (SU) and gp41, containing the transmembrane region, have been reported to induce neuronal death directly and indirectly by inducing the release of neurotoxic molecules (Adamson *et al*, 1996, 1999; Barks *et al*, 1997; Berrada *et al*, 1995; Dreyer *et al*, 1990; Gemma *et al*, 2000; Kaiser *et al*, 1990). Recombinant SIV gp120 induces a calcium flux in cultured macaque neurons that could be blocked by treatment with the CCR5 chemokine RANTES, suggesting that interactions of the envelope protein with CCR5 may initiate an intracellular cascade that results in neuronal death (Klein *et al*, 1999). The FIV envelope protein also causes neuronal injury through an excitotoxic mechanism as exposure of neuronal cultures to neurovirulent FIV particles or purified FIV envelope proteins results in increased calcium signaling that is dependent on glutamate (Gruol *et al*, 1998). Sequence differences in the viral envelope distinguish neurovirulent and non-neurovirulent FIV isolates by modulating the activity of intracellular signaling pathways, thereby altering the expression of host molecules including MMPs, which mediate neuronal injury (Johnston *et al*, 2002b, 2001; Yong *et al*, 1998). The precise nature of the interaction(s) between the recombinant envelope proteins with cell surfaces remains uncertain since these proteins exist as monomers while infection is dependent on trimer formation (Berger *et al*, 1999).

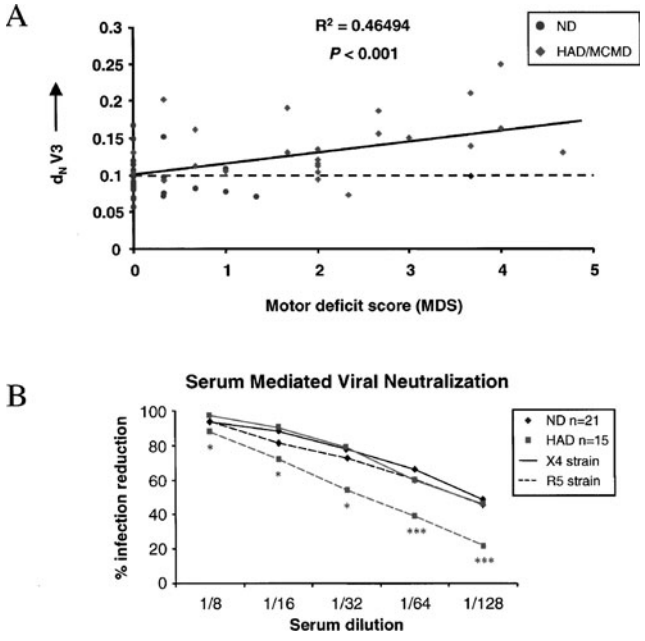


**Figure 4** (**A**) A higher number of nonsynonymous, i.e., amino acid changing nucleotide substitutions ($d_N$) observed for HAD/MCMD compared to nondemented (ND) HIV-infected patients for blood-derived sequences, which was correlated with the severity of neurological impairment, represented by the mean deficit score (MDS). Moreover, a $d_N$ value for the V3 region $> 0.1$ (indicated by the dashed line) was predictive of HAD whereas a $d_N < 0.1$ was predictive for a nondemented neurological status (sensitivity = 88%, specificity = 81%; $P < .001$, Fisher's Exact test). (**B**) Viral neutralization by sera obtained from demented (HAD $n = 15$) and nondemented (ND $n = 21$) AIDS patients. R5 strains were consistently neutralized less efficiently by sera from HAD patients, whereas no difference in neutralization between the two patients groups was observed for X4 strains. Adapted from van Marle *et al* (2002) and unpublished data.

With regard to the impact of HIV-1 envelope sequence variability on neuronal survival, brain-derived envelope protein sequences derived from patients with HAD, which differed at several amino acid positions from sequences derived from the brains of ND patients, also caused significantly more neuronal death when expressed in infectious recombinant viruses for neurotoxicity assays (Power *et al*, 1998b). These sequences differed between patient groups chiefly within and near the V3 region, which is also an important determinant of calcium-mediated neurodegeneration (Pattarini *et al*, 1998). Of interest, several investigators have also reported differences in the envelope sequences and biological properties of viruses isolated from brains of patients with and without HAD (Smit *et al*, 2001; Smith *et al*, 2000) Other studies have reported differences in the ability to induce TNF-$\alpha$ synthesis by different HIV-1 strains that mapped to the V3 envelope region and may

account for the differences in systemic disease course observed among patients (Khanna *et al*, 2000). Viral isolates from brain also selectively activate a neurotoxic pathway involving tryptophan metabolism (Burudi *et al*, 2002; Grant *et al*, 2000). In addition, envelope proteins from different HIV-1 strains have been shown to differentially induce cell signaling pathways through chemokine receptors such as CCR5, which not only influences viral replication but may also impact on systemic HIV-1 pathogenesis by altering cellular function (reviewed in Kinter *et al*, 2000). Furthermore, recombinant HIV-1 gp120s from different viral strains differed in their ability to activate calcium signaling in CD4-negative epithelial cells, underscoring the importance of sequence diversity in modulating cell function (Clayton *et al*, 2001). Indeed, recent studies from our laboratory indicate that both replication-competent and -incompetent viruses encoding brain-derived C2V3 sequences from multiple HIV-1 clades differed in their ability to induce neuronal death directly or indirectly through activation of macrophages (Zhang *et al*, 2003b). These latter observations were associated with cytokine induction that was mediated by a non–CCR5-driven signaling cascade that varied depending on the virus envelope sequence. These observations emphasize the importance of HIV-1 envelope variability in pathogenic outcomes.

It has been consistently demonstrated for murine leukemia retrovirus (MuLV)-associated infections that mutations within the envelope protein contribute to neurovirulence (Hasenkrug *et al*, 1996; Peterson *et al*, 2001; Poulsen *et al*, 1998; Robertson *et al*, 1997). However, these mutations did not influence pathogenesis by changing cell tropism or levels of infection (Hasenkrug *et al*, 1996; Poulsen *et al*, 1998, 1999, Robertson *et al*, 1997) but rather by altering the induced inflammatory responses (Peterson *et al*, 2001). Although the interaction of the envelope protein with its cognate receptors and subsequent activation of cell signaling pathways with ensuing release of neurotoxins may be a major mechanism by which the envelope contributes to pathology in the brain (reviewed in Martin-Garcia *et al*, 2002), alternative envelope-mediated mechanisms may also participate in neuropathogenesis. Differences in MuLV neurovirulence have been associated with abnormal intercellular expression of the envelope protein in microglia due to specific mutations (Kamps *et al*, 1991; Lynch *et al*, 1995; Lynch and Sharpe, 2000). The potential underlying cellular mechanisms include misfolding or an altered expression pattern of the envelope protein within the endoplasmic reticulum as a consequence of individual mutations, leading to a stress response that results in the release of neurotoxins, as suggested for studies of misfolded proteins in other neurodegenerative diseases (Soto, 2003). The release of cytokines, chemokines, arachidonic acid metabolites, and reactive oxygen species observed in HIV infection by microglia and astrocytes are considered stress responses (Wesselingh and Thompson, 2001). Similar mechanisms may play a role in HIV envelope–associated neuropathogenesis, as astrocytes expressing, but apparently not releasing, detectable amounts of the HIV-1 envelope protein are a potent source of neurotoxins (van Marle *et al*, 2003).

*HIV-1 Tat protein*

The HIV-1 regulatory protein Tat is chiefly recognized for its regulation of viral transcription (Freed and Martin, 2001). In addition, Tat has a potent immunosuppressive action, inhibits T-cell proliferation in response to antigen stimulation and may block CXCR4 (Ghezzi *et al*, 2000; Viscidi *et al*, 1989; Xiao *et al*, 2000; Zagury *et al*, 1998). Many studies of Tat's effects on host neuroimmune activation and associated signaling pathways support a role for Tat in HIV-1 neuropathogenesis (Rappaport *et al*, 1999). Following infection, Tat is produced by macrophages and microglia, in addition to astrocytes (Nath, 2002). Tat has also been shown to be taken up by neurons and translocated to the nucleus rapidly *in vitro* and *in vivo* (Fawell *et al*, 1994; Kolson *et al*, 1994). Intracerebral injection of the complete Tat protein results in inflammation, microglial and astrocyte activation, macrophage invasion, and neuronal degeneration (Jones *et al*, 1998; Philippon *et al*, 1994). These pathogenic features have been attributed to the ability of Tat to directly or indirectly induce apoptosis, cytokines, chemokines, and MMPs, disturbing the glutamate and calcium homeostasis (Cheng *et al*, 1998; Johnston *et al*, 2001; Nath *et al*, 1996; Philippon *et al*, 1994; Rappaport *et al*, 1999). The up-regulation of cytokines, including TNF-$\alpha$ and interleukin (IL)-1$\beta$, are linked to an increase of cell adhesion molecule expression on endothelial cells (Lafrenie *et al*, 1996; Nottet *et al*, 1996; Rappaport *et al*, 1999). Up-regulation of the chemokine MCP-1 by HIV-1 Tat (Conant *et al*, 1998) may facilitate inflammatory cell invasion of the brain, and contribute to the loss of BBB integrity observed late in disease (Berger *et al*, 2000; Power *et al*, 1993; Shi *et al*, 1996). The effects of Tat in many studies have been associated with high levels of exogenously supplied Tat. In the human brain, Tat transcripts are readily detectable, but extracellular Tat protein has not been detected to date (Hudson *et al*, 2000). High turnover rates for the Tat protein as well as other HIV-1 proteins may account for this discrepancy (Nath *et al*, 1999).

Phylogenetic analysis of Tat sequences from patients with and without HAD revealed clustering of sequences by diagnosis and tissue of origin (Bratanich *et al*, 1998; Mayne *et al*, 1998). Similar to brain-derived envelope sequences for AIDS patients with and without dementia, the extent of molecular diversity in Tat was greater among patients with dementia with evidence of purifying selection again acting on Tat sequences for nondemented patients (Bratanich *et al*, 1998). Sequence variation within specific domains of the Tat protein

has been associated with higher viral replication levels and TNF-$\alpha$ production (Chiao *et al*, 2001; Mayne *et al*, 1998; Munoz-Fernandez *et al*, 1997; Westendorp *et al*, 1995); interestingly, brain-derived Tat sequences derived from patients with HAD were more diverse in these regions (Mayne *et al*, 1998). Moreover, brain-derived Tat sequences from HAD patients have been shown to induce significantly more neuronal death *in vitro* and *in vivo* compared to Tat from nondemented HIV/AIDS patients, which was in part mediated by enhanced MMP-2 expression (Johnston *et al*, 2001). However, these same Tat sequences displayed minimal activation of viral transcription despite their ability to activate host gene expression (Silva *et al*, 2003). Collectively, these observations support the notion that Tat sequence variability also contributes to HIV-1 neurovirulence through differential effects on cellular responses.

*HIV-1 Gag/Pol region*

The HIV-1 polymerase (*Pol*) region of the *Gag/Pol* encodes for the integrase (IN), reverse transcriptase (RT), and viral protease (PRO), which are incorporated in the viral capsid (Freed and Martin, 2001). RT and IN catalyze the viral reverse transcription and integration steps whereas PRO is essential for Gag/Pol polyprotein proteolytic processing and viral capsid maturation. A recent study indicates that the RT sequences found in the CNS appear to be under a positive selection pressure compared to other tissues, which was influenced to some extent by HAART (Huang *et al*, 2002). Structural analysis indicated that the mutations found in the RT sequences were located within regions important for protein and structure and function. The distinct nature of the RT sequences might suggest a specialized RT for the brain, which is complemented by the observations that drug resistance mutations found in CNS viral sequences differ from those found in matched blood-derived viral sequences (Wong *et al*, 1997). Indeed, clustering of *Gag/Pol* sequences by tissue compartment, and the higher number of amino acid changing substitutions observed in brain- and CSF-derived sequences, when compared to blood-derived sequences (Huang *et al*, 2002; Lanier *et al*, 2001; Morris *et al*, 1999; Venturi *et al*, 2000; Wong *et al*, 1997) implies increased "neuro-adaptation," which could influence neurotropism and ultimately neurovirulence. Conversely, RT sequences from HAD and nondemented HIV/AIDS patients did not show differences in sequence diversity or phylogenetic clustering by clinical group (Bratanich *et al*, 1998). The relationship between drug resistance mutations in reverse transcriptase and protease and the subsequent development of neurological disease remains ill-defined, but patients in whom systemic antiretroviral resistance occurs are at greater risk of primary (and secondary opportunistic) neurological disease (Power and Johnson, 2001).

*HIV-1 LTR*

The HIV-1 LTR is a repeat region of approximately 600 bp in length located at the 5′ and 3′ ends of the integrated proviral DNA (Freed and Martin, 2001). Within the proviral DNA, the LTRs act as promoters and polyadenylation signal for viral transcription by the host cell RNA polymerase (Freed and Martin, 2001). Variations in the LTR sequence can determine retroviral gene expression and the production of progeny virus. The impact of LTR sequence diversity on pathogenesis is evidenced by the differences in replication and transcriptional activity of LTRs from different HIV-1 clades that may correlate with the differences observed in pathogenicity among these clades (Jeeninga *et al*, 2000; Kanki *et al*, 1999). The lower transcriptional activation of the HIV-2 LTR by TNF-$\alpha$ compared to HIV-1, has been suggested to be one of the reasons HIV-2 is less pathogenic than HIV-1 (Hannibal *et al*, 1993). Several LTR polymorphisms have been observed, but their influence on systemic pathogenesis is controversial (Chen *et al*, 2000; Estable *et al*, 1996, 1998; Hiebenthal-Millow and Kirchhoff, 2002; Koken *et al*, 1992).

The distinct differences in HIV-1 LTR sequences between different tissue compartments, including the brain, suggest an important role for the LTR in HIV-1 tissue tropism and adaptation (Ait-Khaled *et al*, 1995; Corboy and Garl, 1997). This concept is highlighted by a study with transgenic mice containing a $\beta$-galactosidase gene driven by blood- or brain-derived LTRs (Corboy *et al*, 1992). Expression of the transgene in the brain was observed only with the brain-derived LTRs. Moreover, there also appeared to be subtle differences in locations of gene expression mediated by different brain LTRs. Differences in the LTR of MVV have also been shown to influence neural cytotropism (Agnarsdottir *et al*, 2000; Andresdottir *et al*, 1998). Evidence for functional differences of brain-derived HIV-1 LTRs comes from a study by Ross *et al* (2001) examining the effects of mutations in two CAAT/enhancer binding protein (C/EBP) sites in the HIV-1 LTR. These two transcription factor binding sites are considered important for viral replication and transcription in cells of macrophage/microglial lineage (Henderson *et al*, 1995, 1996; Schwartz *et al*, 2000), which is the principal cell type infected in the brain. Within brain-derived LTRs, there was a tendency for particular C/EBP binding sites to preferentially bind more C/EBP factors. These differences in LTR sequences were also correlated with differences in Tat-dependent and -independent transcription activation (Hogan *et al*, 2002; Ross *et al*, 2001). The latter observation suggests a coevolution of Tat and the LTR. A direct link between this transcription factor binding site and HAD has not been established, but recent observations emphasize that LTR differences not only contribute to HIV-1 neurotropism but also appear to be associated with neurological disease development (Hogan *et al*, 2003).

## Immune selection and viral diversity in the CNS

Molecular heterogeneity among viral isolates consisting of overall viral diversity and specific mutations within individual genes can dramatically influence viral pathogenesis (Domingo and Holland, 1999). Given that host immune competence is a key determinant of HIV-1 molecular diversity and systemic pathogenesis, differences in humoral and cellular immune responses select for or against viruses that infect the brain and cause neurological damage. Because the viruses present in the brain are likely generated in the blood and then cross the BBB, differences in neutralization ability of antibodies in the serum could influence sequence variability of the HIV-1 in blood and determine which viruses infect the brain. Studies from several groups have established a link between lower efficiency in HIV-1 neutralization and non-neurological disease progression (Carotenuto *et al*, 1998; Cecilia *et al*, 1999; Liu *et al*, 1997; Loomis-Price *et al*, 1998; Pilgrim *et al*, 1997). Differences in immune responses to and neutralization sensitivity of CCR5- and CXCR4-using HIV-1 strains have also been postulated (Trkola *et al*, 1998; Wodarz and Nowak, 1999). A humoral immune response directed primarily against X4 viruses with less impact on R5 viruses would result in a predominance of the R5 phenotype with greater potential to infect the brain (Wodarz and Nowak, 1999). This phenomenon might arise due to increased immune tolerance to R5-dependent viruses, because they have been present since the onset of infection in latent reservoirs in the body (Pierson *et al*, 2000).

Differences in viral neutralization between sera from HIV/AIDS patients with and without neurological disease have also been reported (Beilke *et al*, 1991). Indeed, neutralization ability of sera differs in patients with and without HAD that is dependent on virus coreceptor preference and perhaps molecular or antigenic diversity (van Marle *et al*, 2002) (Figures 3 and 4). R5-dependent but not X4-dependent viruses were less efficiently neutralized by sera from patients with HAD compared to matched nondemented HIV/AIDS patients, which was dependent on the C2V3 envelope region of the HIV-1 envelope (Figure 4B). Of interest, the C2V3 sequences amplified from the same blood samples exhibited greater sequence diversity among patients with HAD versus nondemented patients, with greater diversity among the HAD patients. Furthermore, the observations that neurotropic (and neurovirulent) HIV-1 strains have increased affinity for CCR5 and are more sensitive to neutralization by antibodies underscores our observations (Gorry *et al*, 2002; Martin *et al*, 2001; Song *et al*, 2004). Less efficient neutralization of R5-dependent viruses in HIV-1 patients would give potentially neurovirulent R5 strains a better chance of escaping into the immune privileged milieu of the brain than in patients with an efficient virus neutralization antibody response.

In addition to differences in the humoral immune response, cellular immunity may also play an important role in the development of HAD. The cellular immune response in HIV-1 infection is directed against the envelope, Gag, Pol, Tat, Nef, Rev, Vif, and Vpr proteins, whereas Vpu is infrequently recognized by cytotoxic lymphocytes (CTLs) (Addo *et al*, 2002; Altfeld *et al*, 2001; Walker and Goulder, 2000). Many observations suggest a role for CTL escape by HIV-1 and SIV in systemic disease progression (Barouch *et al*, 2002; Carrington *et al*, 1999b; Evans *et al*, 1999; Goulder *et al*, 2001; Goulder and Walker, 1999; McMichael and Rowland-Jones, 2001). Although limited evidence exists for CTL participation in HIV-1 neuropathogenesis, there are data to suggest that CTLs are present in the CSF early in infection following SIV infection (von Herrath *et al*, 1995). Infiltration of the brain by CTLs in SIV-infected Rhesus macaques may also accelerate the onset of encephalitis (Marcondes *et al*, 2001). However, in a murine model of HIV-1 encephalitis, CTLs may directly participate in viral clearance from the brain (Poluektova *et al*, 2002), which is supported by the observation that depletion of CTLs accelerates the onset of systemic disease (and encephalitis) in SIV-infected macaques and in pediatric HIV-infected patients with progressive encephalopathy (Sanchez-Ramon *et al*, 2003). In addition, other studies suggest that HIV-1 diversity in the brain may reflect CTL escape mutants, which have entered the brain (Morris *et al*, 1999). Thus, the mechanisms by which CTLs regulate lentivirus neuropathogenesis by acting on specific viral sequences remain a critical area of research as it is for systemic pathogenesis.

## Viral replication and neurological disease

Although immune selection is a principal driving force of viral diversity, augmented viral replication rates also contribute to increased viral heterogeneity (Overbaugh and Bangham, 2001). Conversely, in nonpathogenic SIV infections limited viral sequence diversity at the protein level was observed despite high replication levels, whereas pathogenic infection showed substantial protein sequence diversity at similar replication levels (Rey-Cuille *et al*, 1998). In general, HIV-1 strains isolated from the CNS replicate at lower levels compared to blood-derived viruses, which in part may reflect brain-derived viruses' preference for CCR5 and macrophage tropism. In HIV-1 systemic disease, increased viral load in blood is a key parameter by which viral burden can be assessed (Mellors *et al*, 1997). In contrast, the association between plasma, brain, or CSF viral load and the development of neurological disease remains controversial. Several reports propose correlations between HIV-1 RNA, provirus, and antigen levels in the brain

and CSF with HAD presence and severity (Brew *et al*, 1995; De Luca *et al*, 2002; Demuth *et al*, 2000; Ellis *et al*, 1997; Zink *et al*, 1999). Viral load in the CSF is likely derived from both brain parenchyma and blood (Ellis *et al*, 2000) and rapid turnover of virus in these compartments (Eggers *et al*, 2000). Other studies have not shown associations between HIV-1 plasma or brain viral (RNA and provirus) load and the development and severity of HAD (Johnson *et al*, 1996; Lazarini *et al*, 1997; McArthur *et al*, 1997; McClernon *et al*, 2001). Similarly, brain viral loads in lentivirus animal models do not differ between neurovirulent and non-neurovirulent viral strains (Johnston *et al*, 2002b) and are not correlated with the severity of neurobehavioral abnormalities (Murray *et al*, 1992; Power *et al*, 1998a), unlike initial virus input titer in the brain, which seems to govern the subsequent severity of neurobehavioral dysfunction in some models (Johnston *et al*, 2002a). Nonetheless, viral load in select regions of the brain and in CSF are positively correlated with the presence of HIV encephalitis (Wiley *et al*, 1998). In autopsied brain tissue from AIDS patients, unintegrated circular proviral DNA (ccDNA) has been found in HIV-1–infected patients with clinical signs of HAD but not in nondemented patients (Pang *et al*, 1990; Teo *et al*, 1997). The circular forms are considered unstable dead-end products of the reverse transcription step but indicative of ongoing viral replication (Bukrinsky *et al*, 1992; Pauza *et al*, 1994; Sharkey *et al*, 2000). Two recent studies showed that among patients on HAART with prolonged low or undetectable viral loads, ccDNAs were easily detectable in their peripheral blood mononuclear cells (PBMCs) (Cara *et al*, 2002; Sharkey *et al*, 2000). This observation may indicate continuing replication in a viral reservoir not susceptible to antiretroviral drugs or alternatively ccDNA may be more stable than previously thought and can persist for months (Cara *et al*, 2002). If the latter is the case, the presence of ccDNA in the brain of patients with clinical disease may reflect viral replication that preceded the onset of neurological disease and explain the lack of correlation between parenchymal viral load and neurological disease development.

Additional mechanisms by which increased HIV-1 genetic diversity can arise include recombination with or without preceding superinfection by another HIV strain. Recombination would allow for the introduction of many genetic changes simultaneously, resembling the antigenic shifts observed with influenza virus genome segment reassortment (Malim and Emerman, 2001). Recombinant HIV-1 genomes are present in infected humans and their recent classification as separate clade illustrates the importance of this phenomenon in the HIV-1 pandemic. Brain-derived viral species have also been found to undergo recombination (Morris *et al*, 1999; Zhang *et al*, 2001), although rigorous phylogenetic analysis revealed that these represented a minor subset of viral sequences (Zhang *et al*, 2001). The impact of recombinant viral strains on neurological disease development has not been assessed to date. Moreover, technical problems such as recombination during polymerase chain reaction (PCR) amplification and phenomena such as transplicing may complicate the identification of these viral species (Fang *et al*, 1998). However, the power of viral recombination to generate novel viral varieties warrants further attention, especially with the growing number of reports of patients infected simultaneously and persistently with multiple HIV strains (Altfeld *et al*, 2002; Blackard *et al*, 2002; Jost *et al*, 2002). In SIV encephalitis, phylogenetic studies suggest that recurrent neuroinvasion by different viral mutants arising because rapid evolution within the host contributes to viral diversity in the brain (Ryzhova *et al*, 2002). To date there is limited evidence for HIV-1 superinfections contributing to viral diversity in the brain and/or neuropathogenesis but viral superinfection nonetheless remains one of the chief mechanisms by which an agent's virulence is augmented (Blackard *et al*, 2002; Gottlieb *et al*, 2004; Lipsitch *et al*, 1995). Together with the burgeoning emergence of drug resistant viruses, superinfection may become a more important factor in the future.

## Viral diversity and therapeutics

Many of the drugs used as antiretroviral therapies do not readily cross the BBB, leading to the realization that the CNS as an important viral reservoir, is not efficiently targeted by the current treatment regimens (reviewed in Richman, 2001). Drug resistant viral strains have been found in both the CSF and brain of patients receiving antiviral therapy including drugs considered to have efficient CNS penetration (Bratanich *et al*, 1998; Lanier *et al*, 2001; Venturi *et al*, 2000; Wong *et al*, 1997). In one study, drug-resistant viral sequences were found in the brain of a patient 14 months after termination of antiviral therapy, thereby illustrating the long-lived nature of viruses in the CNS reservoir (Gatanaga *et al*, 1999). The continuous replication of drug resistant HIV-1 during antiviral therapy not only can escalate clinical drug resistance (Richman, 2001), but also allows genes not targeted by antiviral drugs to evolve more rapidly (Brown and Cleland, 1996; Frost *et al*, 2001; Sheehy *et al*, 1996). To combat the ongoing viral replication in the brain, efforts have been increased in designing antiviral drugs, such as abacavir, which show high CNS penetration (Lanier *et al*, 2001). However, despite the ongoing development of therapeutics, viral drug resistance will remain a challenge, especially in patients previously treated with drugs that exhibit limited BBB penetration.

Although HAART has been remarkably successful in increasing life expectancy and reducing neurological disease incidence, it has resulted in an increase

of HIV-related neurological disease prevalence (Brew and Dore, 2000; Dore *et al*, 1999; Neuenburg *et al*, 2002; Sacktor *et al*, 2002), likely because survival times have concomitantly been extended. Nonetheless, among patients in whom HAART is successful in reducing the plasma viral load, the viral envelope sequence continues to evolve (Frost *et al*, 2001). Moreover, fluctuations in the viral load, with changes in HAART regimens or structured treatment interruptions (STIs) (Garcia *et al*, 2001; Ruiz *et al*, 2001), may increase viral molecular variability and perhaps indirectly influence neurological disease onset and or progression. Although no detectable viremia may be present, active replication occurs in other compartments in the body among patients receiving HAART (Natarajan *et al*, 1999; Schrager and D'Souza, 1998). These issues have yet to be critically assessed with respect to neurological disease emergence (Price *et al*, 2001).

## Conclusions and future prospects

HIV-1 infection of the CNS is currently defined by the predominance of R5-dependent and nonsyncytia-inducing viruses, infection of chiefly macrophages and microglia with concurrent activation of these cells, together with viral evolution towards neurocompartmentalization (Figure 5). These latter findings are largely derived from molecular and phylogenetic analyses of HIV-1 LTR, Tat, Gag/Pol, and envelope sequences. The data collected to date indicate that viral diversity in both the brain and blood appears greater among patients with HAD compared to patients without neurological disease. Herein lies a major gap in the understanding of neurovirological dynamics, as most viral studies of the CNS are predicated on examining autopsied patients with end-stage disease, together with limited analysis of matched blood samples for which in many cases there

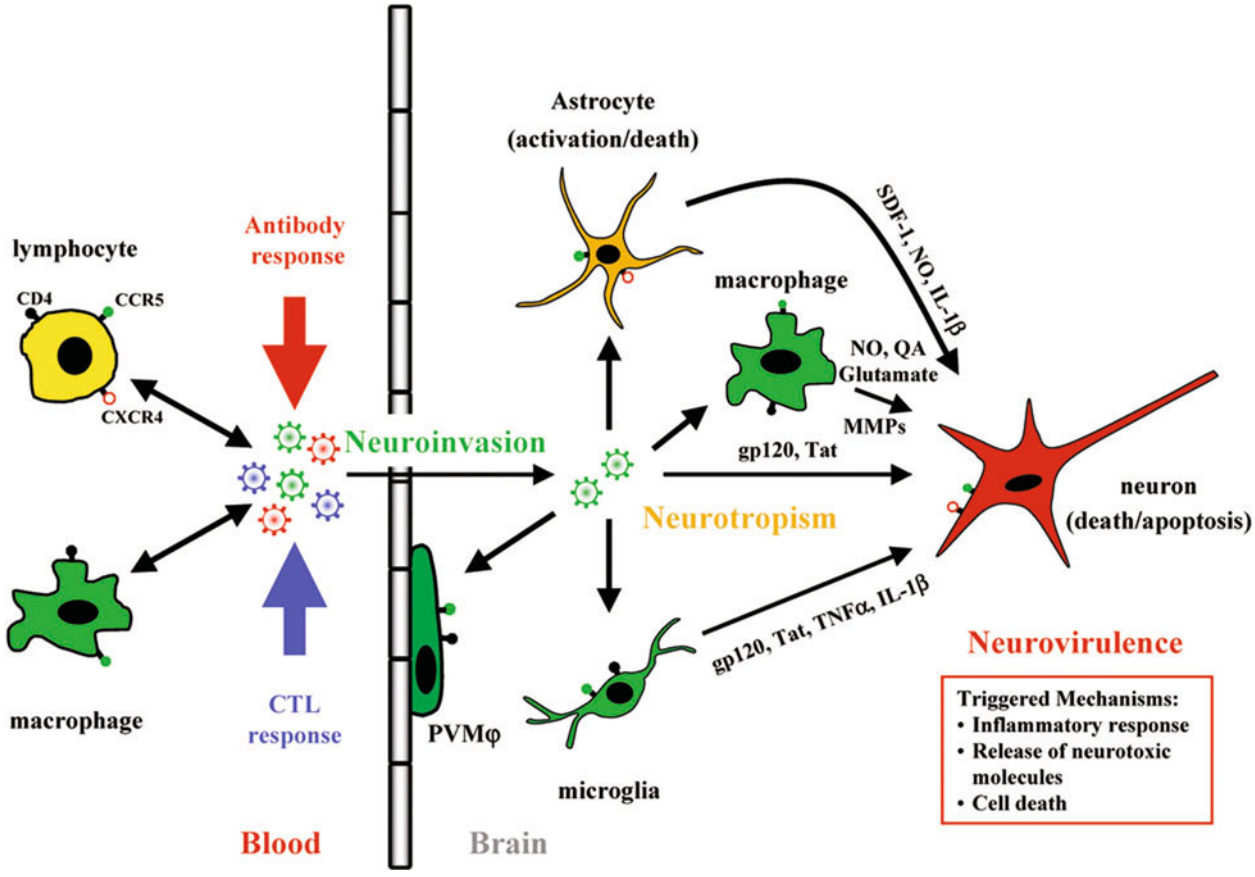


**Figure 5** The impact of differing selective pressures acting on HIV-1 during infection that influence viral diversity. In the periphery or blood, the immune response (either cellular or humoral) and the availability of cells for infection, such as lymphocytes and macrophages and cells in other viral reservoirs, determine viral evolution towards increased neuroinvasion (CNS entry), neurotropism (infection of microglia, macrophages, neurons, and astrocytes), and ultimately neurovirulence (neuronal damage or death). Viral diversity, represented by the color variation in the virions, results from differing selection pressures in the periphery. In turn after neuroinvasion, either through direct transport of virions or via trafficking of infected cells across the blood-brain barrier (BBB), the virus also drives neurovirulence following infection. Viral replication in the perivascular monocytoid cells (PVM$\varphi$) and microglia (neurotropism) together with the incomplete infection of astrocytes results in induction and release of neurotoxins (nitric oxide (NO), quinolinic acid (QA), glutamate, matrix metalloproteases (MMP) together with viral proteins (gp120, Tat), and in neuroinflammatory responses (TNF-$\alpha$, IL-1$\beta$, MMPs), culminating in cell death among neurons and astrocytes.

is limited clinical information. Although lentivirus animal models have provided insights into neuropathogenesis, animals infected with single (cloned) viruses may not represent the realities of patients infected with heterogenous quasipsecies. Indeed, viral heterogeneity may contribute to neuropathogenesis through multiple mechanisms, including differential activation of receptor-mediated pathways and stress responses. Distinct HIV-1 strains causing HAD have yet to be identified, although the select neuropathogenic effects of different HIV-1 Tat and envelope proteins raise the possibility of their existence. Viral load in the brain parenchyma is not consistently correlated with the occurrence of HAD, but CSF viral load is correlated with the severity of dementia and the presence of encephalitis. These observations coupled with the findings that brain-derived HIV-1 isolates selectively induce a myriad of pathogenic signaling pathways in neural cells underscore the importance of viral diversity in the CNS.

The precise mechanisms by which viral diversity is increased among HAD patients' blood and brain remains unclear. Greater viral diversity in blood among HAD patients may reflect fundamental immune dysregulation, enabling a broader quasispecies to emerge. By increasing viral diversity within the CNS, the potential to avoid host immune regulation is greater and subsequently the opportunities are increased for viruses to initiate a broader range of pathogenic pathways through interactions between virus-encoded proteins and host cells. This manifests as both direct effects of the virus on target cells such as neurons but also as indirect mechanisms through interactions with effector cells including macrophages, microglia and astrocytes (Figure 5). Although considerable progress has been made in our efforts to elucidate the underlying principles governing HIV-1 pathogenesis, the overwhelming viral diversity and the outbred nature of the human host make it difficult to pinpoint the key pathogenic determinants in both systemic and neurologic disease development. In the past, research into viral quasispecies was greatly hampered by the inability to sequence whole viral genomes efficiently. Current technologies have overcome these obstacles and the detailed analysis of whole viral genomes and their structure has become feasible, which will avoid biases in interpretation arising from focused sequence analysis of specific viral domains (DeFillipis and Villarreal, 2001).

Over the past 15 years research has focused mainly on HIV dementia because of its high prevalence, but the impact of HIV-induced peripheral neuropathies is now receiving increasing attention (Keswani *et al*, 2002), largely because of their growing prevalence. Little is known about the abundance of virus or its molecular diversity within the peripheral nervous system. There is also growing concern about the spread of drug resistant viruses (Little, 2000; Little *et al*, 2002; Richman, 2001; Salomon *et al*, 2000), but the impact of drug-resistant virus on neurovirulence remains uncertain. By the same token, the effects of HAART on HIV-1 neurovirulence will require further investigation together with evaluation of the influence of multiple viral passages through different individuals and thus differing selection pressures on HIV-1 neurovirulence. It has been posited that viral passage from patient to patient is not associated with an increase in systemic virulence (Malim and Emerman, 2001), but this is still uncertain and may not be true for neurovirulence. The consequences of CNS infection by HIV-1 on systemic immune regulation also remain unclear but potential injury to the hypothalamic-pituitary-adrenal axis may have profound effects on systemic immunity. Finally, given the plethora of evidence demonstrating interactions between HIV-1 proteins and neural cells that lead to neuronal injury and death, vaccine development based on attenuated HIV-1 strains or vectors expressing HIV-1 genes warrants greater attention to the potential for vaccine-mediated neurotropism and neurovirulence.

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