Genetic Variation and HIV-Associated Neurologic Disease

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Abstract

HIV-associated neurologic disease continues to be a significant complication in the era of highly active antiretroviral therapy. A substantial subset of the HIV-infected population shows impaired neuropsychological performance as a result of HIV-mediated neuroinflammation and eventual central nervous system (CNS) injury. CNS compartmentalization of HIV, coupled with the evolution of genetically isolated populations in the CNS, is responsible for poor prognosis in patients with AIDS, warranting further investigation and possible additions to the current therapeutic strategy. This chapter reviews key advances in the field of neuropathogenesis and studies that have highlighted how molecular diversity within the HIV genome may impact HIV-associated neurologic disease. We also discuss the possible functional implications of genetic variation within the viral promoter and possibly other regions of the viral genome, especially in the cells of monocyte–macrophage lineage, which are arguably key cellular players in HIV-associated CNS disease.

1. INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) infects the central nervous system (CNS) initiating a cascade of neuroinflammation and eventually CNS injury. Despite the success of highly active antiretroviral therapy (HAART), neurocognitive impairment (NCI) continues to affect a significant proportion of infected patients. Although the incidence of HIV-1-associated dementia (HAD) has decreased, the overall prevalence of HIV-1-associated neurological disorders (HAND) has increased in the HAART era, primarily because the incidence of subtle forms of HIV-1-associated cognitive impairment has increased. In resource-limited settings, especially in the developing world, poor access to antiretroviral medication results in a much more severe prognosis for HIV-related CNS complications in late-stage HIV infection. HIV enters the nervous system within the first few weeks after initial systemic infection (Pilcher et al., 2001; Schacker, Collier, Hughes, Shea, & Corey, 1996), initiating a cascade of neuroinflammation and eventual CNS invasion and subsequent injury. CNS compartmentalization, including the cerebrospinal fluid (CSF), of HIV species may begin within the first year of infection. Thus, the CNS may be a potential independent

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site of HIV replication. Genetic variation within the HIV genome and associated selective pressures may lead to an increase in the prevalence of specialized variants that find a niche and begin evolving in the early stages of the disease. This review discusses the key features of HAND, the implications of the molecular and genetic diversity of the HIV-1 genome for HIV disease, and the importance of cells of the monocyte–macrophage lineage in the overall neuropathogenesis of HIV-1.

2. OVERVIEW OF HIV-1 CNS PATHOGENESIS

Entry of HIV-1 into the brain results in a chain of events leading to CNS disease and neurologic impairment. The virus must first circumvent the blood–brain barrier (BBB), a selectively permeable barrier separating the CNS from the peripheral circulation (Fig. 6.1). One route of entry into the CNS involves transit of HIV-1 across the BBB by means of infected cells trafficking from the periphery into the brain. This "Trojan horse" method of entry likely involves infected circulating monocytes carrying HIV-1 into the brain in the form of integrated provirus or infectious viral particles (Haase, 1986). Alternatively, HIV may also traffic into the CNS by lymphocytes that harbor viruses that replicate in macrophages, or as a cell-free virus entering through the endothelial cells or across cells of the choroid plexus (Collman et al., 1992; Spudich & Gonzalez-Scarano, 2012). Broad systemic infection and immune system activation may exacerbate this process, when infected (Hickey, 1999) and possibly uninfected cells within the CNS release chemotactic mediators into circulation, thereby drawing more activated cells harboring HIV-1 into the brain. This process may establish a positive feedback mechanism of viral entry and subsequent neuroinflammation (Fontaine, Poudrier, & Roger, 2011; Liu, Tang, McArthur, Scott, & Gartner, 2000; Yadav & Collman, 2009). HIV-1 infection of cells of the monocyte–macrophage lineage also induces increased expression of adhesion molecules on vascular endothelial cells, facilitating HIV-1 transit across the BBB (Blodget et al., 2012; Nottet et al., 1996; Rappaport et al., 1999). Infected macrophages induce greater expression of the adhesion molecules E-selectin and vascular cell adhesion molecule-1 (VCAM-1) on the surface of brain endothelial cells than do uninfected macrophages, suggesting that immune cell activation of monocytic cells following HIV-1 infection of the CNS likely plays a key role in facilitating transendothelial migration across the BBB (Miller et al., 2012; Nottet et al., 1996; Persidsky et al., 1997; Rappaport et al., 1999). Cells of the monocyte–macrophage lineage are the only cells in the CNS that routinely are shown to express HIV RNA or protein, although other cell types, such as astrocytes, have been shown to harbor HIV sequences but do not show a robust expression of HIV RNA or proteins (Spudich & Gonzalez-Scarano, 2012; Wiley, Schrier, Nelson, Lampert, & Oldstone, 1986). Among the different macrophage subtypes, perivascular macrophages are highly infected in the brains of HIV-1-infected individuals (Kim et al., 2006). Initially, it was thought that perivascular macrophages could not contribute to the long-term presence of HIV-1 in the brain owing to their rapid turnover rate, but reports have suggested that this cell population can harbor virus for long periods and can, therefore, serve as a reservoir for HIV-1, as previously reviewed (Spudich & Gonzalez-Scarano, 2012).

Neurologic disease rarely manifests prior to the onset of immune system dysfunction (McArthur et al., 1997), and patients who do not show early signs of neurologic impairment

typically progress through the asymptomatic stage of infection without experiencing a decline in neurologic status (Gannon, Khan, & Kolson, 2011; Selnes et al., 1990). The disconnect between initial infection of the CNS and the presentation of associated neurologic impairment may be explained by an initial immune system clearance of the virus followed by a reseeding of the CNS by HIV-1 at later time points in disease after the immune system has been functionally degraded. This is supported by evidence of increased levels of HIV-1-specific immunoglobulins within the CSF, intrathecally produced anti-HIV-1 antibodies, and increased numbers of HIV-1-specific CD8^{+} cytotoxic T lymphocytes (CTLs) (Krebs, Ross, McAllister, & Wigdahl, 2000). Recently, using the simian immunodeficiency virus model, it was shown that the intrathecal immune responses correlate inversely with the macrophage-tropic strains in the CNS (Selnes et al., 1990). An alternative explanation for the late onset of neurologic disease could be the selective infection of the CNS with a less neurovirulent strain of HIV-1, which results in a less cytotoxic but more chronic dissemination of the virus within the CNS (Krebs et al., 2000). Further explanation for the late-stage onset of neurological impairment comes from phylogenetic studies of HIV-1 gp160 sequences isolated from multiple tissue compartments as well as multiple compartments within the brain of HIV-1-infected patients (Liu et al., 2000). Recently, it was shown that HIV-1 R5 envelope (Env) sequences evolve with an increased positive charge and that this R5 subset evolves independently from highly macrophage-tropic variants with low-charge gp120s (Gonzalez-Perez et al., 2012). This study and others (Duncan & Sattentau, 2011; Peters, Duenas-Decamp, Sullivan, & Clapham, 2007) highlight the observations that HIV-1 R5 Envs evolve with very distinct properties at different sites in the body and are driven by powerful tissue-specific evolutionary pressures. Additional variables such as the effects of aging on brain, long-term CNS toxicity of HAART, and the impact of drugs of abuse need to be evaluated in detail to provide us with a conclusive model of these correlates of HIV disease.

Others have suggested that infected lymphocytes may be involved in the "Trojan horse" entry of HIV-1 into the CNS (Sloand et al., 1992; Weidenheim, Epshteyn, & Lyman, 1993). Similar to the case with cells of the monocyte–macrophage lineage, HIV-1 infection of CD4^{+} T cells results in upregulation of cellular adhesion molecules, including leukocyte function antigen-1 and very late antigen-4, which interact with vascular endothelial cell ligands intercellular adhesion molecule-1 (ICAM-1) and VCAM-1, thereby facilitating binding of lymphocytes to the endothelial cell surface (Sloand et al., 1992; Weidenheim et al., 1993). Lymphocytes may also secrete specific enzymes that degrade the basement membrane of endothelial cells, allowing the migration of HIV-1-infected T cells across the BBB (Sloand et al., 1992). However, owing to the relatively low frequency of lymphocytes infiltrating into the CNS, their contribution to HIV-1 trafficking to the brain and subsequent establishment and maintenance of infection remain a topic of debate.

3. CLINICAL DIAGNOSES OF HIV-1 CNS INFECTION

HIV-1 infection of the CNS can result in numerous motor and cognitive deficiencies (Cosenza, Zhao, Si, & Lee, 2002; Robertson, Liner, & Heaton, 2009; Wiley et al., 1986; Williams et al., 2001). Two distinctly recognizable conditions resulting from HIV-1 infection of the CNS are HAD and the less severe, subsyndromic condition called minor

cognitive motor disorder (MCMD) (Cherner et al., 2002; Gartner, 2000; McArthur et al., 2003; Minagar et al., 2008; Williams & Hickey, 2002). Patients with either of these conditions are classified collectively as having NCI. Prior to the introduction of HAART in the industrialized world, ~20–30% of HIV-1-infected individuals developed HAD (Childs et al., 1999). The onset of HAD corresponds with high plasma viral loads, and although a reduced incidence of HAD has been observed with patients on HAART, the longer life expectancy of HIV patients has increased the prevalence of the disease (Childs et al., 1999). With the widespread use of HAART, MCMD has become more common (Cherner et al., 2002; McArthur et al., 2003). In the HAART era, it is estimated that ~10% of HIV-infected adults develop HAD; however, MCMD may be several times more common, involving as many as 30% of the HIV-infected population (Cherner et al., 2002; Sacktor et al., 2002). Furthermore, the clinical presentation of MCMD has been associated with neuropathological changes characteristic of HIV encephalitis, and MCMD is associated with a worse overall prognostic outlook (Cherner et al., 2002; McArthur et al., 2003; Mothobi & Brew, 2012; Sacktor et al., 2002). One means of explaining the development of MCMD is that the low-level viral replication associated with successful HAART regimens may lead to slowly progressing neurodegeneration. This is consistent with the longer life spans of patients receiving HAART, and possibly with the inability of certain antiretroviral drugs to effectively penetrate into the brain (Letendre et al., 2004). A recent comparative study (Heaton et al., 2011) of HIV-associated NCI before and after the introduction of HAART concludes that although HAD (the most severe form of HAND) appears to be less common in the HAART era, the long-term benefits of therapy with respect to milder forms of HAND remain in question because their prevalence appears to be increasing (Heaton et al., 2011; McArthur & Brew, 2010). To achieve an optimal comparison of HAND in a longitudinal framework would require development of more consistent clinical definitions of neurologic disease, clinical predictors, and a better-defined characterization of comorbid conditions. Study and compilation of all these variables would be greatly facilitated by developing larger and more representative HIV/AIDS cohorts. Nevertheless, we can safely conclude that the beneficial effects of HAART on neurological complications associated with HIV have been less than complete and that continuous efforts are needed to improve prognosis. Additional variables that need more precise definition in relation to neurologic complications of HIV include drugs of abuse, age, viral strains, genetic variation within the HIV genome during the course of disease, and timing of HAART initiation.

HAD cannot be defined in terms of a single disease entity but must rather be characterized in terms of a broad collection of symptoms encompassing cognitive, motor, and behavioral deficiencies corresponding to the presence of actively replicating HIV-1 within the CNS. HAD is a subcortical dementia, which differentiates it from dementia induced by Alzheimer's disease, that presents clinically as a progressive decline in neurocognitive function (Kolson, Lavi, & Gonzalez-Scarano, 1998; Wendelken & Valcour, 2012). It may also include loss of memory, diminished ability to concentrate, psychomotor retardation, and frequent headache. Early in the course, HAD patients typically experience complications involving mental slowing, impaired motor control and lack of coordination, and behavioral alterations such as apathy, social withdrawal, and personality changes (Price, 1994). In late-stage disease, HAD patients exhibit severe, clinically recognizable cognitive, motor, and

behavior deficits. Severe cases of HAD, which have become increasingly rare with the advent of effective combination antiretroviral therapeutics, may manifest with almost absolute mutism, incontinence, and severe, debilitating dementia (del Palacio, Alvarez, & Munoz-Fernandez, 2012; Price, 1994).

Clinical assessment of HIV-1-associated CNS disease requires surrogate biomarkers because brain and spinal cord are relatively inaccessible. HIV-1 RNA measurement in the CSF is one of the most practical means of examining CNS viral load (Marra, Maxwell, Collier, Robertson, & Imrie, 2007; Spudich et al., 2005). CSF markers of immune activation and inflammation have also been used as indicators of disease activity, including CCL2/ monocyte chemotactic protein-1 (MCP1) and CXCL10/IP10 (chemokines that facilitate ingress of macrophages and lymphocytes across the BBB) (Chang, Ernst, St Hillaire, & Conant, 2004; Conant et al., 1998), β_2-microglobulin and neopterin (Brew et al., 1992; Brew, Dunbar, Pemberton, & Kaldor, 1996; Enting et al., 2000), quinolic acid (Heyes et al., 2001), arachidonic acid metabolites (Genis et al., 1992), and oxidative stress markers (Schifitto et al., 2009) (Fig. 6.2). Reduced levels of *N*-acetylaspartate, which indicate decreased neuronal function, and elevated levels of choline, which indicate inflammation and membrane turnover, can also be utilized for overt HAD assessment using magnetic resonance spectroscopy (Chang, 1995; Meyerhoff et al., 1994).

Several pathologies are associated with HAD (Rosenblum, 1990), including white matter pallor, multinucleated-cell encephalitis (associated with the multinucleated giant cells, or MNGCs, or syncytia), and vacuolar myelopathy (Price, 1994). MNGCs are observed in only about half of all HAD patients on postmortem examination (Kato, Hirano, Llena, & Dembitzer, 1987; Wiley & Achim, 1994), and they are comprised of resident CNS mononuclear phagocytes often concentrated around blood vessels (Price, 1994). The presence of MNGCs in infected patients indicates active HIV-1 replication within the CNS (Budka, 1991; Budka et al., 1987). MNGC encephalitis occurs in the subcortical regions of the brain and is often associated with the presence of gliosis and white matter pallor (Chrysikopoulos, Press, Grafe, Hesselink, & Wiley, 1990; Epstein et al., 1984). In addition to these diagnostic approaches, functional magnetic resonance imaging and diffusion tensor imaging have been utilized to assess the changes in brain hemodynamics including alterations in cerebral blood flow, blood oxygen level dependence, and white matter morphometric changes (Ances et al., 2010; Spudich & Gonzalez-Scarano, 2012).

The onset and progression of HIV-1-associated neurodegeneration and subsequent decline in cognitive ability are likely dependent on multiple host and viral factors, some yet to be characterized (Fig. 6.3). This chapter reviews the relationship between genetic variation within the HIV-1 genome and host and the onset and progression of HAND.

4. THE EVOLUTION AND ADAPTATION OF HIV-1 AND THE ESTABLISHMENT OF MOLECULAR DIVERSITY

HIV-1 molecular (and consequent phenotypic) diversity is present at the population level as well as among infected individuals. The rapidly evolving nature of HIV-1 results from several factors, including the error-prone nature of reverse transcriptase, selective pressures

from the host and from antiretroviral therapy, replication dynamics, and genomic recombination. HIV-1 molecular heterogeneity is most often characterized within the context of specific point mutations, which present as synonymous (nonamino acid altering) and nonsynonymous (amino acid altering) variations (Rambaut, Posada, Crandall, & Holmes, 2004). However, this genetic diversity may also manifest as polymorphic nucleotides throughout the viral genome resulting from insertion, deletion, and recombinatorial events occurring within a viral population (Rambaut et al., 2004). The end consequence is altered viral protein structure and function, as well as changes within noncoding nucleic acid sequences, such as the viral promoter, the long terminal repeat (LTR), which are critical viral components that may dramatically alter the course of viral gene expression.

The clinical trajectory of HIV disease is generally well characterized. Substantial variability occurs with respect to the rate of disease progression among infected individuals; however, the role host factors play in HIV disease progression cannot be discounted (Lackner, Lederman, & Rodriguez, 2012; Lemey et al., 2007). The asymptomatic phase of infection is highly variable and can range from several months to more than 20 years (Lemey et al., 2007). This differential rate of disease progression likely results from the dynamic relationship between virus and host and from specific selective pressures placed upon the virus within a given host (Lackner et al., 2012; Lemey et al., 2007). External factors such as other exogenously acquired infectious diseases may also affect disease progression. Following HIV-1 infection, both a humoral and a cell-mediated immune response are mounted by the host to combat the virus, but without avail, both are eventually defeated as HIV evolves and adapts, enabling it to efficiently replicate (Frost et al., 2005; Richman, Wrin, Little, & Petropoulos, 2003). This process ultimately leads to the destruction of the host's immune system and the establishment of multiple opportunistic diseases, which define the clinical progression to AIDS (Frost et al., 2005; Richman et al., 2003). The humoral immune response involves the production of neutralizing antibodies, which exert strong selective pressure on the HIV envelope gene *(env)* but do not effectively control viral replication (Cecilia, Kleeberger, Munoz, Giorgi, & Zolla-Pazner, 1999). Recent structural studies of the HIV-1 Env proteins have fueled interest in rational antibody design using candidate-induced antibodies to Env, as previously reviewed (Bonsignori et al., 2012; Walker & Burton, 2010). The CD8^{+} T-cell response likely serves in a protective capacity during HIV-1 infection, and evidence suggests that at least partial control of virus replication *in vivo* can be associated with the appearance of CTLs (Koup et al., 1994) and that the rate of disease progression is critically dependent on HLA class I alleles (Carrington, Dean, Martin, & O'Brien, 1999; Trachtenberg et al., 2003). It has been suggested that although the majority of the killing of HIV-1-infected cells results from the CTL response, small differences in CTL killing efficiency may be clinically relevant and may correspond with altered disease course (Asquith, Edwards, Lipsitch, & McLean, 2006).

HIV-1 possesses an enormous potential for evolutionary change, a consequence which is loss of the host's immune response to effectively control viral replication (Lemey et al., 2007). A genetically diverse viral population exhibiting a high rate of mutation and recombination, in concert with rapid replication dynamics, facilitates the propagation of

infection to a large population of HIV-1-susceptible cells and enables the virus to readily adapt to constantly changing physiological conditions within each individual host (Lemey et al., 2007). Multiple amino acid alterations have been shown to occur within the hypervariable region of HIV-1 Env, changes that allow the virus to evade the host's humoral immune response but that do not negatively impact viral entry into target cells (Frost et al., 2005). HIV-1-specific CD8^{+} T cells more efficiently target other regions of the virus such as *gag* and *nef* (Addo et al., 2003; Cao, McNevin, Malhotra, & McElrath, 2003; Lichterfeld et al., 2004), especially early in infection. Although viral evolution facilitates the establishment of escape variants capable of evading the CTL response, studies have suggested that evolution of these escape (or partial escape) mutants during chronic HIV-1 infection may occur with, however, an associated loss in replicative fitness (Ganusov et al., 2011; Leslie et al., 2004; Lewis, Dagarag, Khan, Ali, & Yang, 2012; Martinez-Picado et al., 2006).

The HIV-1-specific immune response is becoming increasingly understood. The effect of viral evolution and establishment of genetic diversity on the course and outcome of HIV-1-associated immune and nervous system disease, however, remain unresolved. One issue that is generally accepted is the critical nature of viral evolution with respect to our understanding of the dynamic relationship between virus and host during chronic or persistent infection. Consistent patterns of HIV-1 evolution have been observed throughout the course of infection (Shankarappa et al., 1999); however, investigations of viral diversity and mean divergence from the founder strain of HIV-1 within patients with differing rates of disease progression often conflict with one another (Ganeshan, Dickover, Korber, Bryson, & Wolinsky, 1997). Studies aimed at distinguishing between adaptive and selective neutral mutations have led some to believe that delayed disease progression may be associated with increased positive selection of sites within and accelerated adaptation rates of HIV-1 Env (Ross & Rodrigo, 2002; Williamson, 2003). It remains a topic of debate, however, whether viral adaptation results from, or is the consequence of, differential rates of HIV-1-associated disease progression (Lemey et al., 2007). Moreover, the dynamics of antiretroviral drugs also determine the path of HIV evolution and play a role in therapy outcome. Mathematical simulation studies have established that simulation of clinical trials with new and untested HIV treatment protocols could be used as a potent tool in selecting novel antiretroviral combinations (Hill, Rosenbloom, & Nowak, 2012; Rosenbloom, Hill, Rabi, Siliciano, & Nowak, 2012). Although this simulation approach may have biases toward success and may be limited with respect to the incorporation of all possible sequence variants that would render the compound combination inferior in clinical trials, the simulation can still be used as a preliminary step to strengthen a proposed antiretroviral combination prior to testing in preliminary efficacy analyses.

Studies analyzing the ratio of synonymous (nonamino acid changing) to nonsynonymous (amino acid changing) mutations within HIV-1 have provided insight into the mechanisms governing viral adaptation and the establishment of genetic diversity (Seo, Thorne, Hasegawa, & Kishino, 2002). Fluctuations in the rate of synonymous substitutions have been suggested to reflect changes in mutation rates, whereas nonsynonymous substitution rates may also be affected by changes in selective pressure and effective population size (Lemey et al., 2007). Studies of HIV-1 evolution have been based on the assumption that the

rate of synonymous changes is relatively constant among HIV-1-infected patients, an assumption that has been questioned (Ganeshan et al., 1997; Lemey et al., 2007). The rate of synonymous change depends on multiple factors including viral generation time, which may vary considerably among individuals (Lemey et al., 2007). In addition, it does not take into account the potential impact of synonymous changes on *cis*-acting effects between interactions of the genome with virion components that may alter the overall virion maturation and infectivity. Viral replication rates may depend on the activation state of the host's immune system (Silvestri & Feinberg, 2003), the physiological environment (Martinez-Picado et al., 2006), and the environmental conditions existing within latently infected cell populations (Kelly, 1996; Kelly & Morrow, 2003). An investigation utilizing a new computational technique to estimate absolute rates of synonymous and nonsynonymous mutations and characterize how these rates change over time has suggested that the trajectory of HIV-1-associated disease progression among infected individuals may be predicted by the rate of synonymous mutations and that nonsynonymous mutations evolve as a consequence of differential antibody selective pressure. This approach builds on previous relaxed-clock methodology (Drummond et al., 2006), and by comparing evolutionary rates for specific branches within HIV-1 phylogenies, potentially biasing effects of deleterious polymorphisms are corrected for (Lemey et al., 2007). Using this method, a previously unidentified association between the rate of silent HIV-1 evolution and the rate of disease progression has been discovered, demonstrating that host immune mechanisms associated with HIV-1 pathogenesis may also play a role in modulating viral replication and ultimately place restrictions on HIV-1 evolution (Lemey et al., 2007). Most investigations that have compared the rate of nonsynonymous to synonymous changes have concluded that evolution of *pol, env*, or *nef* in brain isolates is adaptive in nature (Gray et al., 2011; Huang, Alter, & Wooley, 2002; Spudich & Gonzalez-Scarano, 2012). The challenge now is to understand the selective pressures driving these adaptive changes. To this end, understanding the specific features of the immune response within the CNS during this adaptation period will be important.

5. MOLECULAR DIVERSITY OF HIV-1 ENV AND NEUROLOGIC DISEASE

HIV-1 Env exists as a trimer in the virion and includes the surface glycoprotein transmembrane subunits gp120 and gp41. Initial viral attachment and subsequent entry into host cells are catalyzed by a high-affinity interaction between gp120 and the host cellular surface antigen CD4. This interaction results in gp120 undergoing a conformational change, exposing the coreceptor-binding site (Doms, 2000). The dynamic interaction between CD4-bound gp120 and the coreceptor then results in additional conformational changes culminating in the structural rearrangement of gp41 and facilitation of virus fusion and entry (Doms, 2000). The chemokine receptor CCR5 is the primary HIV-1 coreceptor utilized by the virus for infection of monocytic phagocytes, and viruses containing CCR5-utilizing envelopes (R5) are the predominant HIV-1 variants isolated from infected brain (Albright et al., 1999; Gorry et al., 2002; Smit et al., 2001). Brain microglia and tissue macrophages are known to express lower levels of CD4 and CCR5 on their surfaces than do peripheral blood CD4^{+} T cells (Lee, Sharron, Montaner, Weissman, & Doms, 1999; Lewin et al., 1996; Wang et al., 2002). CD4 and CCR5 by HIV-1 are interdependent on one another, with one

becoming more critical as the other becomes limiting (Doms & Moore, 2000). HIV Env proteins exhibiting increased tropism for cells of the monocyte–macrophage lineage also possess the ability to utilize low levels of both CD4 and CCR5 for fusion and entry, suggesting that decreased dependence on these surface molecules may represent an adaptation preferentially favoring viral replication within the CNS (Gorry et al., 2002; Gray et al., 2005; Martín, LaBranche, & González-Scarano, 2001; Peters et al., 2004; Thomas et al., 2007). However, the mechanistic details concerning how HIV-1 acquires this capacity to enter cells with limiting receptor and coreceptor levels are unclear. However, many of these studies focused on studying isolates derived from postmortem tissues and, therefore, would reflect an end-stage disease phenotype. Moreover, the CNS environment is that of an "immunological privileged" site with a low penetration of antibodies, thus allowing propagation of sequence configurations that would promote neutralization. In fact, isolates from brain are often sensitive to neutralizing antisera (Dunfee et al., 2007; Martin-Garcia, Cocklin, Chaiken, & Gonzalez-Scarano, 2005; Spudich & Gonzalez-Scarano, 2012).

The amino acid sequence of HIV Env determines its affinity for CD4 binding, and sequence alterations within the envelope protein may affect cellular tropism of HIV-1 by changing coreceptor utilization (Hoffman, Stephens, Narayan, & Doms, 1998; Wang et al., 1998). Efficient infection of the brain by HIV-1 is predicated on macrophage tropism, with the majority of viruses isolated from the brain of HIV-1-infected patients representing viruses that preferentially utilize CCR5 for attachment and entry into host cells (Albright et al., 1999; Gorry et al., 2001; Reddy et al., 1996). Studies have suggested that viruses isolated from the brain of HIV-1-infected patients may have higher affinity for CCR5 than do CCR5-utilizing viruses isolated from other tissue compartments, indicating a decreased dependence on CD4 expression by target cells (Gorry et al., 2002; Martín et al., 2001; Shieh, Martin, Baltuch, Malim, & Gonzalez-Scarano, 2000). The chemokine receptor CCR3 has also been implicated in the infection of microglia, and it has been shown that targeting either CCR5 or CCR3 protected both microglia and monocyte-derived macrophages (MDMs) from many strains of HIV-1 (Agrawal et al., 2009; He et al., 1997; Martin-Garcia, Cao, Varela-Rohena, Plassmeyer, & Gonzalez-Scarano, 2006). Additionally, CXCR4 (X4) utilizing viruses and dual-tropic (X4R5) viruses are rarely found in the brain, despite reports suggesting their ability to induce neuronal damage (Chan et al., 1999; Gorry et al., 2001; Reddy et al., 1996).

Studies have demonstrated that brain-derived HIV-1 sequences from HAD patients differ from those isolated from non-HAD patients, suggesting that specific HIV envelope sequences may be associated with clinical onset of dementia (Power et al., 1994; van Marle & Power, 2005). Furthermore, comparative molecular and biological analyses of HIV-1 isolates derived from both HAD and non-HAD patients have suggested that differences in viral tropism can discriminate between patients with and without dementia, indicating that neurotropic HIV-1 variants evolve independently within the brain and contribute to neuropathogenesis (Smit et al., 2001). Several studies have demonstrated that HIV-1 strains derived from patients with HAD differ from those derived from patients without dementia, primarily within the V1 and V3 region of gp120. Variation within the V1 region, in addition to the V2 region, has been associated with altered replication efficiency in macrophages (Toohey, Wehrly, Nishio, Perryman, & Chesebro, 1995), and the V3 region of Env has been implicated as a primary determinant of macrophage tropism and subsequent cytopathicity

(Chesebro, Wehrly, Nishio, & Perryman, 1992; Hwang, Boyle, Lyerly, & Cullen, 1991; Korber, MacInnes, Smith, & Myers, 1994; Rossi et al., 2008). Single amino acid alterations within this region have been shown to change viral tropism and entry (McKnight et al., 1995). In addition, association has been made between specific V3 sequences and HAD (Chang et al., 1998; Korber et al., 1994; Power et al., 1994). HAD patients exhibit impaired serological responses against CCR5-dependent HIV-1 strains and increased molecular diversity within the V3 region of Env, suggesting the emergence of viral mutants that may preferentially infect the brain and mediate neurodegeneration (Van Marle et al., 2002). One specific Env variant present in the CD4-binding site of gp120, N283, has been identified at high frequency in brain-derived viruses of HAD patients (Dunfee et al., 2006). N283 has been shown to increase the affinity of gp120 for CD4 by decreasing the dissociation rate between these two molecules, thus enabling HIV-1 to utilize lower levels of CD4 for binding and entry and subsequently enhancing viral replication within macrophages and microglia (Dunfee et al., 2006). The N283 HIV-1 Env variant is found significantly more often in brain-derived Envs from HAD patients (41%) compared with non-HAD patients (8%), suggesting that this macrophage-tropic HIV-1 variant may be specifically associated with neurologic disease (Dunfee et al., 2006). Studies have shown that brain-derived HIV-1 isolates differ from those typically found in systemic circulation (Epstein et al., 1991; Korber, Kunstman, et al., 1994; Wong et al., 1997), and comparisons between peripheral blood-derived viruses and those derived from multiple brain compartments in the same patient have suggested segregated evolution of viral strains present within differing brain regions (Chang et al., 1998). Several studies have shown that HIV-1 proteins may be directly neurotoxic (Dahiya, Nonnemacher, & Wigdahl, 2012; Li et al., 2012; Liu et al., 2000; Meucci et al., 1998; Muller, Schroder, Ushijima, Dapper, & Bormann, 1992) (also see sections below). Individual HIV-1 isolates have been shown to exhibit differential induction of neuronal apoptosis, and this induction is independent of viral replication capacity (Ohagen et al., 1999). Recently, HIV-1 infection was shown to upregulate cathepsin B in macrophages and to reduce cystatin–cathepsin interactions, eventually leading to neuronal apoptosis (Rodriguez-Franco et al., 2012). Also, it was shown that gp120 induced caspase-3-dependent neuronal apoptosis by enhancing A-type transient outward K^+ currents via CXCR4-protein kinase C signaling (Xu et al., 2011). The V3 region of the HIV-1 Env, in addition to conferring increased tropism and subsequent infection of macrophages and microglial cells (Strizki et al., 1996), has been shown to impact the release of neurotoxic molecules following infection of macrophages (Cunningham et al., 1997; Kaul, Garden, & Lipton, 2001; Khanna et al., 2000; Power et al., 1998) and may, itself, be neurotoxic (Pattarini, Pittaluga, & Raiteri, 1998).

6. GENETIC DIVERSITY WITHIN HIV-1 Tat, Vpr, AND THE LTR, AND ITS CONTRIBUTION TO THE ONSET AND SEVERITY OF HIV-1-ASSOCIATED NEUROLOGICAL SYSTEM DISEASE

6.1. HIV-1 transactivator protein Tat

HIV-1 transcription involves an early, Tat-independent and a late, Tat-dependent phase, and transactivation of the viral genome is a critical step in the viral replication cycle, as

previously reviewed (Dahiya et al., 2012; Li et al., 2012). The presence of Tat has been shown to increase LTR-mediated transcriptional activity by several hundredfold, and in the absence of Tat, viral replication falls to nearly undetectable levels (Doppler, Schalasta, Amtmann, & Sauer, 1992; Green, Ishino, & Loewenstein, 1989; Rice & Mathews, 1988). Tat is a unique transcription factor in that it binds to the "UCU" bulge of the transactivation response element (TAR), a *cis*-acting RNA enhancer element contained within the 5′ end of all viral transcripts (Brady & Kashanchi, 2005; Rappaport et al., 1999). The interaction of HIV-1 Tat with TAR RNA increases viral transcription and elongation (Raha, Cheng, & Green, 2005; Selby, Bain, Luciw, & Peterlin, 1989). Specifically, HIV-1 Tat is known to promote the binding of P-TEFb (cyclin T1 and cdk9) to the TAR region located within the viral promoter, immediately downstream of the transcriptional initiation site, and the interaction of Tat with P-TEFb and the TAR element results in hyperphosphorylation of the C-terminal domain and subsequent increased processivity of RNA polymerase II (pol II) (Raha et al., 2005; Zhou et al., 2000). The Tat-PTEFb crystal structure has shown that Tat forms extensive contacts with both the CycT1 and Cdk9 subunits in P-TEFb, resulting in a conformational change and constitutive activation of the enzyme (Tahirov et al., 2010). HIV-1 Tat may also be involved with the formation of the transcriptional preinitiation complex (Dahiya et al., 2012; Raha et al., 2005).

In addition to the HIV-1 LTR, Tat is known to upregulate several other viral as well as cellular genes. Within the CNS, Tat has been shown to stimulate HIV-1 LTR-mediated viral gene expression in the absence of TAR (Taylor & Khalili, 1994), an activity that may result from its ability to enhance the activity of cytokines such as tumor necrosis factor-α (TNF-α) (Sawaya et al., 1998). TNF-α also has the ability to activate the HIV-1 LTR via activation of cytoplasmic nuclear factor kappa B (NF-κB) (Nabel, Rice, Knipe, & Baltimore, 1988; Sawaya et al., 1998), and this positive feedback mechanism may lead to constitutive TNF-α and HIV-1 Tat synthesis by infected glial and microglial cell populations within the brain, ultimately resulting in paracrine dysregulation and damage to neighboring neurons and astrocytes. Similarly, evidence generated from stable expression studies has indicated that HIV-1 Tat may inhibit TNF-α-induced repression of TNF receptor p55, thereby resulting in the amplification of TNF-α activity (Chiao et al., 2001).

HIV-1 Tat can also be secreted from infected cells, including infected macrophages, microglia, and astrocytes and may consequently be taken up by neighboring, uninfected cells (Ensoli et al., 1993; Verhoef, Klein, & Berkhout, 1996). Tat protein has been detected within the brain of infected individuals, and the uptake of Tat by CNS cells has toxic consequences, resulting in large part from neuronal apoptosis (Hudson et al., 2000; Nath et al., 1996). Extracellular Tat can enter neurons via endocytosis through interaction with the low-density lipoprotein receptor-related protein present on the neuronal surface (Vendeville et al., 2004). Recently, it was shown that Tat could bind to the promoters of the phosphatase and the tensin homologue and protein phosphatase 2A (PP2A), eventually resulting in apoptosis of HIV-1-infected CD4^{+} T cells (Kim, Kukkonen, Gupta, & Aldovini, 2010). Furthermore, Tat has been shown to be transported along anatomical pathways within the brain, indicating that the neurotoxic effects of HIV-1 Tat may occur in regions far removed from the site of active infection (Bruce-Keller et al., 2003). Interestingly, secreted or

extracellular Tat has been shown to function as a specific CXCR4 antagonist, selecting against X4-utilizing viruses, and thereby greatly influencing the development and progression of HIV-1 disease (Xiao et al., 2000), specifically within the CNS where R5 viruses are thought to play the predominant role in pathogenesis.

The neuropathologic properties associated with HIV-1 Tat stem from its ability to either directly or indirectly induce apoptosis, upregulate cytokines and chemokines, and interact with matrix metalloproteinases (MMPs). The ability of HIV-1 Tat to upregulate TNF-α and interleukin-1β (IL-1β) has been associated with increased expression of cell adhesion molecules on endothelial cells. Likewise, Tat-induced upregulation of MCP-1 has been shown to exacerbate neuroinvasion, facilitating the loss of BBB integrity, a pathological hallmark of late-stage HAND (Avison et al., 2004; Mayne et al., 1998; Nath, Conant, Chen, Scott, & Major, 1999). Although HIV-1 Tat and various MMPs, including MMP-1, -2, and -9, are known to be independently cytotoxic to cells within the CNS, studies have suggested that the dynamic interaction between Tat and MMPs may be neuroprotective (Johnston et al., 2001; Zhang et al., 2003). Specifically, MMP-1 has been shown to selectively cleave HIV-1 Tat and thereby neutralize its neurotoxic potential (Rumbaugh et al., 2006).

The molecular diversity of HIV-1 Tat protein isolated from brains of patients infected with different HIV-1 clades has been examined, as previously reviewed (Li et al., 2012). Studies examining Tat proteins representative of HIV-1 subtypes B, C, and BF recombinants have demonstrated important structural and functional differences (Siddappa et al., 2006; Turk et al., 2006). BF recombinant HIV-1 isolates, from Argentina, appear to have a replicative advantage over subtype B isolates, possibly owing to the differential ability of Tat to interact with the LTR, and subtype C Tat protein has been shown to be more highly ordered than subtype B Tat. In addition, subtype C Tat protein has been demonstrated to be consistently inferior to subtype B Tat in biological assays with respect to its ability to promote viral proliferation, induce TNF-α and IL-6 expression, and upregulate chemokine coreceptor expression (Siddappa et al., 2006). However, studies have also shown that HIV-1 subtype C Tat exhibits greater transcriptional activity in the Jurkat CD4^{+} T-cell line when compared with subtypes B and E and that this higher level of transactivation is not LTR sequence dependent but results from variations in the C Tat sequence at amino acid residues 57 (Arg in B and E and Ser in C) and 63 (Glu in B, E, and C), which are within and close to the basic domain, respectively (Kurosu et al., 2002). Phylogenetic analyses of Tat sequences from patients with and without HAD have shown clustering of sequences with respect to clinical diagnosis of neurological impairment as well as tissue of origin (Bratanich et al., 1998; Mayne et al., 1998). Nonsynonymous versus synonymous mutation rates among brain-derived Tat sequences isolated from patients with NCI were shown to be significantly greater than those isolated from patients without clinical evidence of neurologic disease (Bratanich et al., 1998). Collectively, these studies suggest that differing selective pressures act on individual HIV-1 genes within the CNS and that these differing pressures may influence both the development and subsequent severity of NCI. Comparisons of matched brain- and spleen-derived Tat sequences have suggested that greater sequence homology exists among brain-derived Tat clones than what is observed between brain- and spleen-derived clones (Mayne et al., 1998). Additionally, significant sequence heterogeneity exists within brain-derived Tat in domains associated with viral replication and intracellular

transport (Mayne et al., 1998). Importantly, HIV-1 Tat derived from HAD patients has been associated with greater neuronal death both *in vitro* and *in vivo* compared with Tat from non-HAD patients, and this characteristic has been attributed, in part, to enhanced MMP-2 expression induced by brain-derived HIV-1 Tat (Johnston et al., 2001). Interestingly, however, these same brain-derived Tat isolates also appear to be limited in their ability to enhance viral gene expression despite their increased activation of host transcriptional machinery (Silva et al., 2003). However, one must remember that these viral gene activation studies were performed with a viral regulatory region that was derived from a non-CNS tissue source and may, therefore, not be naturally compatible with respect to optimal LTR activation by a Tat protein selected for CNS replication. This is particularly important because previous studies (Burdo, Gartner, Mauger, & Wigdahl, 2004; Hogan, Nonnemacher, Krebs, Henderson, & Wigdahl, 2003; Hogan, Stauff, et al., 2003) demonstrated that LTRs derived from the CNS are likely to be structurally and functionally different from LTRs derived from other tissue sources and that colinear Tat and LTR combinations may result in more efficient LTR activation (Li et al., 2011). Nonetheless, taken together, these reports suggest that genetic diversity of HIV-1 Tat very likely contributes to the establishment and severity of HAND.

6.2. HIV-1 Vpr

Viral protein r (Vpr) is a 96-amino acid accessory protein that is packaged into the HIV-1 virion via its association with the p6 domain of HIV-1 Gag (Emerman & Malim, 1998). Vpr is a multifunctional protein affecting both early and late stages of the HIV-1 viral life cycle and is associated with the nuclear localization and import of the HIV-1 preintegration complex (Lu, Spearman, & Ratner, 1993; Mahalingam, Collman, Patel, Monken, & Srinivasan, 1995; Mahalingam, Khan, Jabbar, et al., 1995; Mahalingam, Khan, Murali, et al., 1995). Lacking a true nuclear localization sequence, Vpr is known to localize to the nucleus when expressed *in vitro* (Lu et al., 1993; Mahalingam, Khan, Jabbar, et al., 1995; Mahalingam, Khan, Murali, et al., 1995). Therefore, Vpr likely facilitates nuclear localization via its interaction with cellular proteins involved in nuclear import, possibly karyopherin α and β (Lu et al., 1993; Mahalingam, Collman, et al., 1995; Mahalingam, Khan, Jabbar, et al., 1995; Mahalingam, Khan, Murali, et al., 1995). Vpr has been suggested by several studies to induce cell cycle arrest in HIV-1-infected cells, with Vpr-expressing cells accumulating in the G_2 phase (Ayyavoo et al., 1997; Jowett et al., 1995). The efficacy of Vpr-induced transactivation of the LTR has been shown to correlate with the induction of G_2 arrest in host cells (Ardon et al., 2006; DeHart et al., 2007; Zimmerman et al., 2004). Interestingly, cell cycle arrest of infected cells has been shown to increase HIV-1 LTR transcriptional activity, independent of Vpr (Cohen, Dehni, Sodroski, & Haseltine, 1990). Vpr has also been shown by several studies to transactivate the HIV-1 LTR by a variety of mechanisms (Cohen et al., 1990; Goh et al., 1998; McAllister et al., 2000; Sawaya et al., 1999). Vpr has been shown to activate the LTR via interaction with HIV-1 Tat and to indirectly increase LTR activity through its interaction with the transcription factor p300 (Felzien et al., 1998; Sawaya et al., 1999). Studies have suggested that Vpr may be involved in ternary complexes with Sp and the LTR, and investigations have indicated that Vpr is able to directly interact with the HIV-1 LTR via its binding to C/EBP-binding sites I and II (Burdo, Gartner, et al., 2004; Hogan, Nonnemacher, et al., 2003).

Studies of Vpr binding to HIV-1 LTR sequences encompassing the ATF/CREB, C/EBP site I, and the promoter-distal NF-κB site have suggested that the Vpr preferentially interacts with sequences spanning C/EBP site I and the adjacent NF-κB-binding site (Burdo, Nonnemacher, et al., 2004). This result in addition to the established proximity of the these two binding sites indicates that Vpr and NF-κB binding may be mutually exclusive; however, the downstream NF-κB element would still be available for binding independent of Vpr interactions upstream (Burdo, Gartner, et al., 2004). Importantly, it has been demonstrated that HIV-1 Vpr induces IL-8 production in monocytes through activation of both NF-κB and NF-IL-6 (C/EBP), and elevated levels of IL-8 are thought to be responsible for certain clinical manifestations observed among AIDS patients throughout the course of disease (Roux, Alfieri, Hrimech, Cohen, & Tanner, 2000). This finding suggests that HIV-1 LTR activity is likely influenced by a complex and dynamic balance between Vpr and members of the C/EBP transcription factor family, as well as NF-κB isoforms (Burdo, Nonnemacher, et al., 2004). Additionally, both Vpr and C/EBP factors are known to be required for efficient HIV-1 replication within cells of myeloid lineage. Studies have suggested that Vpr-regulated promoter activation may be enhanced as a result of increased binding of NF-κB and C/EBP factors to their respective binding sites (Roux et al., 2000), and other studies have suggested that Vpr may also mediate promoter activity via direct binding to C/EBP sites and other adjacent binding sequences (Burdo, Nonnemacher, et al., 2004; Hogan, Nonnemacher, et al., 2003). Evidence supports the concept that sequence-dependent interactions between Vpr and C/EBP site I may occur in the context of neurologic disease. Electrophoretic mobility shift (EMS) analyses have revealed a direct association between Vpr and HIV-1 LTR sequences, which include C/EBP site I, the promoter-distal NF-κB site, and the upstream ATF–CREB-binding site (Burdo, Nonnemacher, et al., 2004; Hogan, Nonnemacher, et al., 2003). This relationship was shown to be sequence-specific with respect to C/EBP site I (Burdo, Nonnemacher, et al., 2004). The 3T C/EBP-binding site variant, described earlier, which binds C/EBP factors with low relative affinity, has also been shown to be the C/EBP site I variant that binds Vpr with the highest relative affinity (Hogan, Nonnemacher, et al., 2003). Importantly, the affinity of C/EBP-binding sites for Vpr is associated with HAD, with high-affinity sites being more prevalent in HAD patients (Burdo, Nonnemacher, et al., 2004).

Cell types within the CNS that are capable of supporting productive HIV-1 infection are limited to macrophages and microglia; however, neuropathological abnormalities associated with cognitive impairment (MCMD and HAD) are thought to result, in large part, from neuronal dropout and apoptosis of neurons (Gelbard et al., 1995; Ohagen et al., 1999; Petito, 1995). Astrocytes do not support a high-level productive HIV-1 replication, potentially involving a defect in Rev function (Gorry et al., 1999; Messam & Major, 2000; Neumann et al., 1995; Tornatore, Chandra, Berger, & Major, 1994). Therefore, an indirect mechanism leading to apoptosis of neurons may exist, and HIV-1 Vpr is one of the viral gene products implicated in this process. Recently, it was shown that the effect of Vpr on neuronal death is in part via released proinflammatory factors. In this study, supernatants from Vpr-deleted HIV-1 mutant-infected MDMs contained lower concentrations of IL-1β, IL-8, and TNF-α and showed reduced neurotoxicity compared with wild-type HIV-1-infected MDM supernatants (Guha et al., 2012).

Importantly, free Vpr has been identified in the serum of HIV-1-infected patients and in the CSF of HIV/AIDS patients with neurological disease (Levy, Refaeli, & Weiner, 1995). Studies have linked Vpr to the induction of apoptosis of T cells, and although the HIV-1 envelope glycoprotein gp120 and Tat regulatory protein have been most commonly associated with cellular death during HIV-1 infection, virion-encapsulated Vpr may also be involved in CNS cell death *in vivo*. Experiments using extracellular Vpr have demonstrated that Vpr is able to bind promonocytic and lymphoid cells and increase permissiveness to HIV-1 replication (Levy et al., 1995). Extracellular Vpr has been shown to associate directly with the plasmalemma of cultured rat hippocampal neurons and causes a large inward sodium current, depolarization, and cell death (Levy et al., 1995; Piller, Jans, Gage, & Jans, 1998). In addition, HIV-1 Vpr has been shown to potently induce apoptosis both in the undifferentiated neuronal precursor cell line NT-2 and in mature human neurons (Patel, Mukhtar, & Pomerantz, 2000). Thus, based on the cytotoxic and neurotoxic effects of extracellular Vpr, one may postulate that cell-free Vpr likely contributes to cellular depletion within lymphoid, peripheral blood, and CNS tissue compartments. Furthermore, the fact that extracellular Vpr is present in the serum and CSF of AIDS patients with neurologic disease suggests that extracellular Vpr may play a significant role in AIDS pathology and HIV-1-associated neurologic complications (Levy et al., 1995; Piller et al., 1998). Moreover, Vpr has been implicated in modulating the host glucocorticoid receptor to affect transcription from the LTR as well as other host genes (Refaeli, Levy, & Weiner, 1995). Vpr has also been shown to transactivate promoters containing glucocorticoid-responsive elements. This modulation is most likely via direct interaction with the glucocorticoid receptor, with Vpr acting as a coactivator of glucocorticoid receptor (Kino et al., 1999). Vpr has also been shown to induce oxidative stress in microglial cells via the hypoxia-inducible factor pathway (Deshmane et al., 2009) and has been shown to interact with ANT, PP2A, and HAX-1, which have been shown to play important roles in pathways that culminate in neuronal degeneration (Na et al., 2011; Zhao, Li, & Bukrinsky, 2011). Nonetheless, extensive studies related to the role Vpr plays in HIV pathogenesis have established it as a crucial accessory protein with a multitude of functions spread across different stages of the viral life cycle.

6.3. HIV-1 LTR activity within cells of the monocyte–macrophage lineage

The HIV-1 LTR is approximately 640 bp long and consists of the U3, R, and U5 segmented regions. The U3 region is further divided into the modulatory, enhancer, and core regions, which facilitate the interaction of both viral and cellular proteins involved with regulating viral gene expression (Cullen, 1991; Pereira, Bentley, Peeters, Churchill, & Deacon, 2000). With HIV-1 subtype B, the core region contains the TATAA box and a GC-rich sequence, which facilitates binding of members of the Sp family of transcription factors. The TATAA box binds TBP (TATAA-binding protein), in addition to other cellular proteins involved with the pol II transcriptional complex (Jones & Peterlin, 1994). The enhancer element is located immediately upstream of the core region and is associated primarily with the presence of two 10-bp NF-κB-binding sites (Nabel & Baltimore, 1987). The modulatory region, which consists of sequences located upstream of the NF-κB-binding sites, contains numerous transcription factor-binding sites specific for factors including C/EBP, ATF/CREB, LEF-1, NF-AT, and many others (Krebs et al., 2000). Studies of the modulatory region have revealed that this region is rich in *cis*-acting-binding elements, which serve to

both repress and activate the HIV-1 LTR (Pereira et al., 2000). Furthermore, the interaction of viral proteins, specifically Vpr and Tat, with the LTR provides an additional element of complexity to the regulation of viral gene expression.

One of the primary regulators of HIV-1 LTR activity in all susceptible host cell populations, including cells of the monocyte–macrophage lineage, is NF-κB (Asin, Bren, Carmona, Solan, & Paya, 2001). Several studies have been aimed at determining the dependence of NF-κB family members with respect to transcriptional activation of the HIV-1 LTR in T cells and its subsequent effect on reactivation of HIV-1 from latency (Chen, Feinberg, & Baltimore, 1997; Folks et al., 1986; Ross, Buckler-White, Rabson, Englund, & Martin, 1991). Depending on the type of T cell examined, and differences in the experimental approaches employed, results from these studies have been conflicting. Generally, HIV-1 LTR NF-κB-binding sites are indispensable with respect to viral replication in CD4$^+$ T-cell lines (Alcami et al., 1995; Chen et al., 1997). Studies involving human monocytic cells and transformed human monocyte and macrophage cell lines have centered, in large part, on determining how monocytic differentiation affects HIV-1 expression and how HIV-1 infection results in NF-κB activation (Griffin, Leung, Folks, Kunkel, & Nabel, 1989; Raziuddin et al., 1991; Schuitemaker et al., 1992). Interestingly, differentiated macrophages already contain a constitutive nuclear pool of NF-κB, and what role this preexisting pool of NF-κB plays with respect to modulating HIV-1 gene expression remains unclear (Asin et al., 2001). However, studies have suggested that preexisting NF-κB heterodimers within the nuclei of these cells play a role in transcriptional initiation following infection by HIV-1 (Asin et al., 2001). Overall, studies have concluded that NF-κB *cis*-acting elements within the HIV-1 LTR are critical for efficient LTR activity and subsequent viral gene expression both within CD4$^+$ T cells and in cells of monocyte–macrophage lineage.

The importance of NF-κB can also be observed in the difference in LTR activity of different HIV-1 subtypes. HIV-1 subtype B is the predominant subtype in North America and in Europe, whereas subtypes C and E are most prevalent in other parts of the world, including east Asia, Thailand, India, and southern Africa (Janssens, Buve, & Nkengasong, 1997; Novitsky et al., 1999; Ping et al., 1999). One of the most striking differences between HIV-1 subtype C and other HIV-1 subtypes, such as B and E, resides in the LTR. Subtype C viruses have been shown to contain three NF-κB-binding sites within the enhancer element of the LTR, whereas subtypes B and E have only two and one, respectively (Gao et al., 1996; Kurosu et al., 2001; Montano et al., 2000). The functional consequence of this difference was revealed by transient transfection assay in HeLa cells, which showed that subtype C LTRs have higher promoter/enhancer activity compared with subtypes B and E. Subtype C does appear to be transmitted more efficiently than other subtypes (Essex, 1999), and specific genetic biological differences such as increased NF-κB-binding sites may play a role in this increased transmission efficiency.

Investigations of the transcriptional regulation of the HIV-1 LTR in cells of the monocyte– macrophage lineage have also focused on the role of C/EBP and Sp transcription factors with respect to regulation of viral gene expression within the CNS. C/EBP factors have been shown to be critically involved in the regulation of monocyte-specific gene expression (Matsusaka et al., 1993; Pope et al., 1994; Tanaka et al., 1995). C/EBPβ has been shown to

bind at least two sites within the HIV-1 LTR and has been demonstrated to activate viral transcription in transient expression analyses (Ross et al., 2001; Tesmer, Rajadhyaksha, Babin, & Bina, 1993). Studies have revealed that at least one intact C/EBP site is required for HIV-1 replication in monocytic cells; however, this is not required for replication in T cells (Henderson & Calame, 1997; Henderson, Connor, & Calame, 1996; Henderson, Zou, & Calame, 1995).

7. SEQUENCE VARIATION OF SPECIFIC TRANSCRIPTION FACTOR-BINDING SITES WITHIN THE HIV-1 LTR AND ITS CORRELATION WITH NERVOUS SYSTEM DISEASE

The relationship between LTR genetic diversity and HIV-1 disease is complex. Several reports have suggested that LTR sequence variation may alter promoter activity in varying cell types (Henderson & Calame, 1997; Henderson et al., 1996, 1995; Krebs, Mehrens, Pomeroy, Goodenow, & Wigdahl, 1998; McAllister et al., 2000). Numerous investigations have reported sequence variation within LTRs isolated from infected patients (Hogan, Nonnemacher, et al., 2003; Hogan, Stauff, et al., 2003; Michael, D'Arcy, Ehrenberg, & Redfield, 1994; Nonnemacher, Irish, Liu, Mauger, & Wigdahl, 2004; Ross et al., 2001). Comparative analysis of LTRs isolated from both peripheral blood mononuclear cells and brain across a population of individuals has also revealed compartmentalization of the specific LTR variants and showed that LTRs isolated from the CNS are more closely related to previously characterized brain-derived LTRs than to LTRs isolated from other physiological compartments (Hogan, Nonnemacher, et al., 2003; Hogan, Stauff, et al., 2003; Michael et al., 1994; Nonnemacher et al., 2004; Ross et al., 2001). These naturally occurring sequence alterations within the LTR, which appear to arise as a result of tissue-specific selective pressures, may have a profound impact on the ability of the LTR to support HIV-1 infection by differentially modulating the ability of critical transcription factors to bind the LTR (Hogan, Stauff, et al., 2003). Although several studies have suggested that there is no correlation between LTR sequence variation and altered viral tropism and replication (Pomerantz, Feinberg, Andino, & Baltimore, 1991; Schuitemaker et al., 1993; Velpandi, Nagashunmugam, Otsuka, Cartas, & Srinivasan, 1992), studies involving the analysis of two different LTR variants have demonstrated that increased LTR activity based on transient expression studies corresponds to increased viral replication (Golub, Li, & Volsky, 1991; McAllister et al., 2000). Studies utilizing an HIV-1 LAI infectious molecular clone revealed that when the native high-affinity NF-κB-proximal Sp-binding site III was replaced with a low-affinity site, replication within Jurkat CD4$^+$ T cells was markedly decreased, whereas little effect on replication was observed within U-937 monocytic cells (McAllister et al., 2000). These results were consistent with previously published results (Zeichner, Hirka, Andrews, & Alwine, 1992; Zeichner, Kim, & Alwine, 1991a, 1991b), demonstrating that mutations introduced into the HIV-1 LTR that resulted in altered transient expression activity also resulted in similar alteration in viral replication potential when the same mutations were placed into the context of a replication-competent virus. Transient transfection analyses of HIV-1 LAI LTR-luciferase constructs in the Jurkat T-cell line have suggested that the large reduction in viral replication within these cells, caused by low-affinity Sp-binding site III variants, may be the result of reduced basal, Vpr, and Tat-

mediated LTR activity (McAllister et al., 2000). When examined within the context of an HIV-1 YU-2 LTR-luciferase construct, the naturally low-affinity Sp-binding site III was replaced with a high-affinity site, which resulted in increased basal YU-2 LTR activity in Jurkat T cells and reduced LTR activity in U-937 monocytic cells (McAllister et al., 2000). In addition, LTRs derived from HIV-1-infected patients have been shown to differentially regulate transient expression in a cell type-specific manner, a finding that is reinforced by studies involving cell type-specific reporter gene expression directed by a brain-derived LTR in transgenic mouse CNS tissue (Corboy, Buzy, Zink, & Clements, 1992; McAllister et al., 2000; Michael et al., 1994).

Several investigations have compared LTR sequences from HIV-1-infected long-term nonprogressors (LTNPs) and rapid progressors, and in each of these studies, no direct relationship could be established between LTR sequence variation and disease progression (Zhang et al., 1997). Furthermore, transient expression analyses performed in both cell lines and primary monocytes demonstrated no simple correlation between promoter length and rapidity of disease course (Zhang et al., 1997). However, two LTNPs were shown to harbor virus that exhibited what could be defined as a defective LTR. G to A hypermutations were observed throughout the promoter region of LTRs derived from one LTNP (Zhang et al., 1997), and another LTNP was shown to harbor virus with multiple insertions and deletions across the LTR (Rousseau, Abrams, Lee, Urbano, & King, 1997), both indicating defects within the 5′ LTR structure and suggesting that impaired functionality of the HIV-1 LTR may correspond to long-term nonprogression in a subset of HIV-1-infected patients. Other reports have postulated that LTRs with increased activity may correlate with greater viral infectivity and propagation throughout high-risk populations. HIV-1 subtype C LTRs, which have been show to contain three NF-κB-binding sites, exhibit enhanced LTR activation when compared with LTRs containing only one or two of these sites. Additionally, studies have shown that subtype C viruses also produce increased levels of p24, thus indicating greater replication rates than viruses representative of other HIV-1 subtypes (Naghavi, Schwartz, Sonnerborg, & Vahlne, 1999).

Studies have demonstrated that specific HIV-1 LTR C/EBP-binding site sequence configurations may be preferentially compartmentalized in the brain of infected patients and that these LTR variants exhibit enhanced LTR-mediated transcriptional activity (Hogan, Stauff, et al., 2003; Ross et al., 2001). Transient expression studies have suggested that an NF-κB-proximal C/EBP site I that binds C/EBP factors with high relative affinity results in increased basal as well as IL-6-induced LTR activity (Ross et al., 2001). Investigations have revealed that specific HIV-1 LTR C/EBP configurations preferentially encountered in the brain exhibit enhanced LTR-specific activity (Ross et al., 2001). A high relative affinity 6G C/EBP site I (T to G change at nucleotide position 6) was commonly found in brain-derived LTRs but was infrequently encountered in peripheral blood-derived LTRs, as demonstrated by analyses of variations at each nucleotide position within C/EBP site I (Hogan, Stauff, et al., 2003). A differential level of conservation was also observed at C/EBP site II. Analyses of overall conservation of each site demonstrated that C/EBP site II was highly conserved in LTRs derived from brain and less conserved among those derived from the peripheral blood compartment. Overall, these studies demonstrated that brain-derived LTRs contain two high

relative affinity C/EBP-binding sites and suggest that these sites may play a particular role in LTR-directed transcription with respect to CNS disease.

Studies have also suggested a direct correlation between specific C/EBP sequence variants and HAD. Sequence analysis of C/EBP-binding sites I and II using peripheral blood-derived HIV-1 LTR sequences was reported in three studies (Estable et al., 1996; Kirchhoff, Greenough, Hamacher, Sullivan, & Desrosiers, 1997; Michael et al., 1994). Because these published reports used different classification systems to describe disease severity, LTRs were designated as belonging to one of three groups prior to analysis. HIV-1 LTR sequences from asymptomatic patients with nonprogressing or stage I disease were assigned to disease severity group 1 (DSG 1) (Hogan, Stauff, et al., 2003). DSG 2 was comprised of patients characterized as having slow-progressing, stage II or III disease (Hogan, Stauff, et al., 2003). DSG 3 consisted of patients who were originally classified as having progressing, stage IV HIV-1 disease (Hogan, Stauff, et al., 2003). At C/EBP-binding site I, a 3T (C to T change at nucleotide position 3) configuration was observed at low prevalence within LTRs isolated from the peripheral blood of HIV-1-infected patients early in disease, and at relatively high prevalence from patients with late-stage disease (Hogan, Stauff, et al., 2003). The prevalence of the 3T C/EBP site I variant was not identified among LTRs from DSG1 patients and increased from approximately 8% of all DSG 2 LTRs to nearly 50% of all DSG 3 LTRs (Hogan, Stauff, et al., 2003). Within C/EBP site II, the consensus B (conB) configuration increased significantly throughout disease progression, whereas the prevalence of the 6G and 4C (T to C change at nucleotide position 4) variants decreased (Hogan, Stauff, et al., 2003). The conB configuration at C/EBP site II increased in prevalence from approximately 24% of all DSG 1 LTRs to approximately 93% of all DSG 3 LTRs. Conversely, the 4C C/EBP site II sequence variant decreased from approximately 28% in DSG 1 to approximately 8% in DSG 2 and was completely absent among DSG 3 LTRs (Hogan, Stauff, et al., 2003). Likewise, the 6G C/EBP site sequence variant decreased in prevalence from nearly 35% in DSG 1 to approximately 1% in DSG 3 LTRs. Interestingly, in this as well as similar studies, described in more detail below, involving analysis of Sp transcription factor-binding sites I, II, and III, both NF-κB-binding sites I and II were shown to be highly conserved in the conB configuration throughout disease progression (Hogan, Stauff, et al., 2003; Nonnemacher et al., 2004).

With respect to the impact of the genetic variants in C/EBP sites I and II and CNS disease, the 3T C/EBP site I variant was also observed in 25% of all brain-derived LTRs from patients diagnosed with dementia but was absent in brain-derived LTRs from patients without dementia (Hogan, Stauff, et al., 2003). The 3T C/EBP site I sequence configuration has been shown to have low relative affinity for C/EBP factors (Burdo, Gartner, et al., 2004; Hogan, Stauff, et al., 2003). Taken together, these results suggest that the 3T C/EBP site I configuration may provide a valuable tool in evaluating the likelihood of HIV-1-infected patients developing HAD. Similar to the observations made concerning the 3T C/EBP site I variant, the 6G and 4C C/EBP site II variants were observed in approximately 10% and 7% of HAD patients, respectively, and neither the 6G nor the 4C variant was found in LTRs derived from patients without dementia (Hogan, Stauff, et al., 2003). One study examining the regional distribution of HIV-1 LTRs containing the 6G and 4C C/EBP site II sequence variants within the brains of patients with HAD revealed statistically significant differences,

with the high-affinity 6G C/EBP site II accumulating in the midfrontal gyrus and the low-affinity 4C C/EBP site II accumulating in the cerebellum (Burdo, Gartner, et al., 2004). These observations are consistent with reports of viral replication rates within these neuroanatomical regions, with the midfrontal gyrus representing a neuroanatomical region known to exhibit high-level HIV-1 replication (Glass, Fedor, Wesselingh, & McArthur, 1995), whereas viral gene expression within the cerebellum has been shown to occur at very low levels in patients with HAD (Burdo, Gartner, et al., 2004). This suggests that the presence of the 4C C/EBP site II variant within the cerebellum may represent a means by which HIV-1 maintains a silent genome and establishes a latent viral reservoir in the brain.

The three Sp-binding sites that comprise the remaining sequences within the core region of the HIV-1 LTR are also very important to HIV-1 basal and Tat-mediated transactivation (McAllister et al., 2000). Mutation of these binding sites diminishes both basal promoter activity and viral replication (although not in all cell types) (J.J. McAllister and B. Wigdahl, unpublished observation). Specific sequence variations in the NF-κB-proximal Sp site III present within the brain-derived HIV-1 variant, YU-2, result in a failure to interact efficiently with members of the Sp transcription factor family (McAllister et al., 2000) (unpublished observation). This result, combined with the observation that the ratio of Sp1:Sp3 factor binding to Sp site III is increased during monocytic differentiation, suggests that HIV-1 replication within cells of monocyte–macrophage lineage in the brain may be impacted by changes in Sp factor expression that accompany monocytic differentiation as well as alterations of the functional interactions between Sp factors and the NF-κB proximal, G/C-rich Sp-binding site (McAllister et al., 2000) (unpublished observation). Sequence variation within the Sp-binding sites and altered Sp factor recruitment may also impact the ability of HIV-1 Vpr to interact with and subsequently upregulate LTR activity (McAllister et al., 2000 and unpublished observations). Studies aimed at characterizing specific Sp-binding site sequence variants within 348 peripheral blood-derived HIV-1 LTRs isolated from patients with disease ranging in severity from DSG 1 to DSG 3 have demonstrated the presence of a low-affinity 5T (C to T change at nucleotide position 5) variant in Sp site III (Nonnemacher et al., 2004). The 5T Sp site III was shown to increase in prevalence throughout disease progression, with approximately 60% of all DSG 3 LTRs (Nonnemacher et al., 2004). Similar to the 3T C/EBP site I variant, the 5T sequence configuration results in substantially decreased binding affinity for Sp transcription factors, as demonstrated by both EMS (Nonnemacher et al., 2004) and surface plasmon resonance analyses (Nonnemacher and Wigdahl, unpublished observations). Interestingly, when the 3T C/EBP site I containing LTRs were examined for the presence of the 5T Sp site III sequence variant, an absolute correlation was observed with respect to DSG3. Of nine DSG2 LTRs that contained the 3T C/EBP site I sequence variant, six also contained the 5T Sp site III variant; and of 44 DSG3 LTRs that contained the 3T C/EBP site I sequence variant, all 44 also contained the 5T Sp site III variant (unpublished data). Importantly, the 5T Sp site III variant was also observed in 16% of all brain-derived LTRs from patients diagnosed with dementia but was absent in brain-derived LTRs from patients without dementia (unpublished observations). Taking into consideration that decreased binding of C/EBP and Sp factors corresponds with impaired HIV-1 LTR activity, the sequence variation observed

within this region is almost certainly relevant to viral pathogenesis and may also influence both the course and severity of immunologic as well neurologic HIV-1 disease progression.

8. HOST GENETIC DETERMINANTS OF HIV-1 INFECTION AND CNS DISEASE

The course of HIV-1 infection is determined by a complex and dynamic interplay between viral and host factors. The very existence of LTNPs, in addition to individuals who have been exposed to HIV but remain uninfected, strongly suggests the existence of predisposing factors that may represent major determinants of clinical disease outcome. Post-seroconversion, progression to AIDS may take as little as 2 years in some individuals, while others may remain symptom-free for more than a decade. To this end, several studies have determined that the marked heterogeneity among infected individuals is governed, at least in part, by host genetic variants that serve to modulate virus replication and antiviral immunity (Carrington & O'Brien, 2003; Fauci, 2003; O'Brien & Nelson, 2004). The most widely recognized host genetic variant to be associated with HIV-1 disease progression is the 32-bp deletion in the coding region of the CCR5 gene (CCR5- 32). CCR5- 32 is one of the most significant host polymorphism with respect to HIV infection, and it has been shown to effectively block HIV-1 infection in homozygous individuals and to significantly retard disease progression and development of AIDS in heterozygotes (Carrington et al., 1997; Dean et al., 1996; Liu et al., 1996; O'Brien & Nelson, 2004; Samson et al., 1996). Subsequent to the discovery of CCR5- 32, 13 additional host polymorphisms have been identified, and these genetic variants differ widely with respect to their influence on HIV-1-associated disease progression and development of AIDS-defining illnesses (O'Brien & Nelson, 2004). Here, we restrict our discussion to CCR5- 32 and CCR2-V64I, which have been linked to HIV-1 neuropathogenesis.

Chemokines and chemokine receptors play a critical role in the pathogenesis and transmission of HIV-1 (Michael, 1999; Paxton & Kang, 1998). The chemokine receptor CCR5 is utilized by R5 strains of HIV-1 to gain entry into host cells, particularly those of the monocyte–macrophage lineage, cells that have been shown to be intricately involved in HAND (Alkhatib et al., 1996; Berger et al., 1998; Choe et al., 1996; Roos et al., 1992). Several single-nucleotide polymorphisms identified within the regulatory region of CCR5 have been implicated in modulating the rate of HIV-1 disease progression (Kostrikis et al., 1999; Martin et al., 1998). The CCR5- 32 gene variant has been shown to result in the production of aberrant CCR5 protein and is known to provide considerable protection against HIV-1 infection in individuals homozygous for the mutation and to result in slowed disease progression in heterozygous patients infected with HIV-1 (Barroga et al., 2000; Liu et al., 1996; Singh et al., 2003). Chemokines and their cognate receptors are also expressed within the brain (Glabinski et al., 1995), and CCR5 has been shown to be the primary coreceptor utilized by brain-derived HIV-1 strains isolated from HAD patients (Albright et al., 1999; Boven, van der Bruggen, van Asbeck, Marx, & Nottet, 1999). This, in addition to resident brain microglia and macrophages being the primary cells supporting replication of R5 HIV-1 strains within the CNS, leads to the conclusion that any host genetic variant that may inhibit or at least decrease the ability of HIV-1 to infect these cell types could have a

profound effect on the neuropathogenesis of HIV-1 infection and subsequent neurocognitive abnormalities.

The chemokine receptor CCR2 has been shown to function as a minor coreceptor for HIV-1, and its natural ligand is MCP-1, a β-chemokine, which has been linked to neuropsychological impairment (Conant et al., 1998; Weiss, Cuff, & Berman, 1999). A genetic variant within the promoter region of MCP-1 (−2518-G/A polymorphism) has been associated with increased HIV-1 disease progression and development of HAD (Singh et al., 2004), and homozygosity for an MCP-1 2578-G allele has been associated with a 50% decrease in the risk of becoming infected with HIV-1 (Gonzalez et al., 2002). A specific polymorphism (G to A change at nucleotide position 190) in the coding region of the CCR2 gene results in the expression of isoleucine in place of valine at amino acid position 64 (V64I), and this nonsynonymous mutation has been linked with impaired progression of HIV-1 disease in adults (Kostrikis et al., 1998; van Rij et al., 1998); however, this association has been contested by other studies (Michael et al., 1997; Mulherin et al., 2003). In a study investigating development of neurologic complications in 121 HIV-1 patients, the CCR2-V64I polymorphism correlated with more rapid progression to neuropsychological impairment (Singh et al., 2004). An observation that was further confirmed among individuals possessing the CCR5-wt/wt genotype suggested that heterozygosity for the CCR5- 32 genotype could not be used to explain the differential rate of progression to neurologic disease in this patient cohort (Singh et al., 2004). Interestingly, although the CCR2-V64I allele was associated with more rapid onset of neuropsychological impairment, it was shown not to have an impact on the overall rate of HIV-1-associated disease progression (Singh et al., 2004). Additionally, the CCR2-64 genotype did not correspond to differential HIV-1 plasma viral load or to $CD4^+$ T-cell count, suggesting that this genotype is not directly involved with viral entry or replication but with the host inflammatory response mediated by the binding of MCP-1 to the CCR2 receptor within the CNS (Singh et al., 2004). Moreover, no association was observed between the genotype of CCR2 and HIV disease progression or therapeutic response (Philpott et al., 2004), indicating that HAART therapy benefits may overshadow the advantages imparted by the V64I polymorphism.

More recently, to investigate the virus–host interactions and their role in HIV-1 pathogenesis, genome-wide association studies (GWAS) have been conducted (Table 6.1) that utilized unbiased searches at the genome-wide level to identify yet unidentified genetic factors and cellular pathways associated with HIV-1 infection and these studies have been previously reviewed (van Manen, van 't Wout, & Schuitemaker, 2012). These association studies were greatly facilitated by a number of clinical HIV-1 disease cohorts that have been established over the years. An alternative approach of sequencing the whole exome that selectively sequences the coding regions can prove to be more cost-effective, but the caveat is that many trait-related single-nucleotide polymorphisms fall in intergenic and noncoding regions (Hindorff et al., 2009; Manolio, 2010). Moreover, the gaps in our knowledge of the viral promoter, the LTR, can also be filled by application of comparative genomics and deep sequencing techniques to find key signatures within the LTR that associate with changes in viral pathogenesis and/or alterations in clinical measurements of disease severity such as viral load, $CD4^+$ T-cell count, HAND, and/or other pathologies associated with HIV disease.

Nevertheless, the efforts in these large-scale approaches (Fellay, Shianna, Telenti, & Goldstein, 2010) have enabled studies to examine HIV pathogenesis at a more global level, but the inter-individual variability that plays an important role in disease progression remains to be explored. The shift to whole genome sequencing will further help in accelerating the effort to find signatures that can be potentially used for therapeutic intervention regardless of whether they are of cellular or viral in origin.

9. CONCLUSION

The onset of HIV-1 CNS disease is largely dependent on the trafficking of infected monocytic cells from the periphery across the BBB, where virus is subsequently disseminated to susceptible cell populations within the brain including microglia and perivascular macrophages. Phylogenetic studies have suggested that cells of the monocyte–macrophage lineage may become infected within the bone marrow, and molecular genetic analyses of HIV-1 LTR, Tat, and Env sequences have revealed that evolutionary events occur both within the periphery and in the CNS (Liu et al., 2000). Current evidence indicates that HIV-1 within the brain may adapt specifically to selective pressures present within that physiological compartment, conferring upon the virus the capacity to utilize lower levels of CD4 and CCR5 for binding and entry (Doms, 2000; Doms & Moore, 2000). This observation, combined with results suggesting the presence of greater molecular diversity among HIV-1 quasispecies isolated from HAD versus non-HAD patients (Power et al., 1994), supports the idea that HIV-1 becomes specifically conditioned for efficient replication within the CNS. To date, no specific brain-adapted, neurovirulent virus has been identified, but lack of these observations may be due to limited experimental capabilities and incomplete patient information. If a brain-specific virus exists, one might postulate that it would result in greater neurologic disease and more severe NCI of its host. However, this also has not been observed, perhaps owing in part to the suppressive effects of combination antiretroviral therapy on reseeding of the virus in the brain owing to effective control of the viral replication in the periphery or more effective penetration of antiretroviral drug combinations into the CNS resulting in improved intra-CNS control of viral gene expression and replication.

The past two decades have witnessed an extraordinary experimental effort centered on elucidating both the causes and effects of HIV-1 infection of the CNS. Through these efforts, a number of viral adaptations and evolutionary events have been observed. Genetic mutations present throughout the HIV-1 genome have been identified and correlated both with peripheral immune and/or CNS disease progression and severity (Burdo, Gartner, et al., 2004; Dunfee et al., 2006; Hogan, Stauff, et al., 2003; Johnston et al., 2001). Multiple amino acid variants have been identified within the hypervariable regions of the HIV-1 Env that facilitate viral evasion of the host's adaptive immune response (Dunfee et al., 2006). Brain-derived HIV-1 Env sequences from HAD patients have been shown to differ from those of non-HAD patients, suggesting that specific HIV-1 Env sequences may correlate with neurotropism, neuroinvasion, CNS infection, and the subsequent onset of NCI (Van Marle et al., 2002). Investigations of the HIV-1 LTR within cells of the monocyte–macrophage lineage have focused heavily on the role of C/EBP and Sp transcription factor families. Specific C/EBP-binding site sequence variants contained within the LTR that are associated

with increased LTR-mediated transcriptional activity have been identified within the brains of HAD patients (Burdo, Gartner, et al., 2004; Hogan, Stauff, et al., 2003; Ross et al., 2001). Likewise, sequence variation within Sp transcription factor-binding sites, which results in altered Sp factor recruitment and thus diminished viral replication, may also impact the capacity of HIV-1 Vpr to modulate LTR activity (McAllister et al., 2000; Nonnemacher et al., 2004). Analyses of HIV-1 Tat derived from patients with and without clinical indications of neurological impairment have revealed evidence of phylogenetic clustering with respect to neuropathological status and tissue of origin (Bratanich et al., 1998; Mayne et al., 1998). HIV-1 Tat isolated from the brain of HAD patients has been linked with increased neuronal death (Johnston et al., 2001), and evidence suggests that brain-derived HIV-1 Tat may be limited in its ability to upregulate viral gene expression (Silva et al., 2003).

The knowledge obtained by studying these individual viral evolutionary events has greatly enhanced our understanding of how HIV-1 establishes infection within the CNS and ultimately causes disease; however, the overall value of this information is limited in that the analysis of these individual mutations alone does not allow for a complete appreciation of how the virus is adapting to global environmental changes within a given host or host population. In order to gain an accurate understanding of why HIV-1 selects for specific genetic variants, one must take a step back and determine whether multiple molecular alterations may be coselected for within the same viral genome. For example, the 3T C/EBP site I-binding site sequence variant would, at first glance, seem deleterious to the virus in that it prevents binding of a transcription factor that has been shown to be critical for viral replication with cells of the monocyte lineage. The same can be said for Tat variants isolated from the brains of infected patients, which appear to be more toxic to neuronal cells and less capable of transactivating the HIV-1 LTR. However, on further examination, these and other seemingly nonadvantageous viral structural and functional changes begin to make sense within the bigger picture of HIV-1 adaptation to changing host conditions. Although the 3T C/EBP site I variant binds C/EBP factors with low relative affinity, it has been shown to bind Vpr with high relative affinity, and studies have shown that only one functional C/EBP site is required for viral replication within cells of the monocyte–macrophage lineage (Burdo, Nonnemacher, et al., 2004; Hogan, Nonnemacher, et al., 2003). Interestingly, in all HIV-1 LTRs identified as containing the 3T C/EBP site I configuration, C/EBP-binding site II has been identified as containing a conB sequence configuration, a high-affinity C/EBP-binding site. Why, then, would the virus choose to sacrifice C/EBP binding in exchange for Vpr binding to the LTR? The answer to that question is not completely known, but one explanation may be found in the fact that Vpr binding to the HIV-1 LTR has been correlated with HAD (Burdo, Gartner, et al., 2004; Hogan, Stauff, et al., 2003). Therefore, within the brain, the virus contains a Tat protein that is inefficient with respect to driving viral gene expression, a deficiency that may be compensated for by Vpr binding to and subsequently transactivating the HIV-1 LTR, a process that can occur only after the virus has mutated in such a way as to ameliorate binding of C/EBP factors to C/EBP site I, while maintaining binding integrity at C/EBP site II. A similar story can be told regarding the 5T Sp site III sequence variant. Although this theory has not yet been demonstrated experimentally, perhaps by preventing binding of Sp factors to Sp-binding site III the virus may facilitate greater binding of NF-κB, a transcription factor that has been shown to be absolutely

required for viral replication within all HIV-1-susceptible host cell populations (McAllister et al., 2000; Nonnemacher et al., 2004).

Regional distribution analysis of HIV-1 LTR sequence variants has revealed a relationship between the accumulation patterns of specific C/EBP site II variants' viral replication rates (Burdo, Gartner, et al., 2004). The 6G C/EBP site II sequence configuration was shown to localize in the midfrontal gyrus, an area known to support relatively high levels of viral gene expression, whereas the 4C C/EBP site II variant was shown to accumulate in the cerebellum, a region exhibiting low-level viral replication (Burdo, Gartner, et al., 2004). These results, which provide evidence for the regional distribution of specific HIV-1 LTR genotypes within the brains of HAD and non-HAD patients, are consistent with investigations suggesting the neuroanatomical regionalization of HIV-1 Env sequences. It would be of great value to determine whether multiple HIV-1 gene products such as Env, LTR, and Tat evolve independently of one another, thus making the regional distribution of specific sequence variants merely coincidental, or whether HIV-1 evolves in a coordinated manner. Coordinated selection of multiple genetic variants within differing genomic regions could explain, at least in part, the occurrence of particular sequence alterations that have been identified throughout the HIV-1 genome and that correspond with neurologic disease. The idea that multiple HIV-1 genes may adapt and evolve together in response to changing physiological conditions within specific tissue compartments, as well as microenvironments within those compartments, raises the possibility that specific brain-adapted HIV-1 envelopes correspond to specific brain-adapted Tat proteins, which in turn correspond to specific brain-adapted LTR sequences. Further investigation is required to substantiate this concept.

It may be useful to consider both the timing of HIV-1 entry into the CNS and the mechanisms by which it may distribute and accumulate within specific brain regions. As discussed previously, regionalization could very well be the result of brain-specific viral evolution whereby HIV-1 adapts to the particular physiological conditions present within varying CNS microenvironments. Another possibility is that, through the continual trafficking of HIV-1 to the CNS during the course of infection, only those viral species already genetically adapted for survival within particular brain regions remain viable, while all others die out. Another possible explanation of the region-specific distribution of HIV-1 within the brain is that trafficking of HIV-1 occurs primarily during late-stage disease and that viral evolutionary events occur prior to neuroinvasion within the peripheral blood and possibly the bone marrow. This latter possibility is, however, contradictory to evidence suggesting that the 6G and 4C C/EBP site II sequence configurations are found only in the peripheral blood in patients with early-stage disease but are present in the brain in HAD patients with late-stage disease. This finding would indicate that HIV-1 containing these LTR genotypes must invade the CNS during the early stages of disease.

One of the most profound advancements that might be realized by identifying and characterizing HIV-1 genetic mutations is their potential use as diagnostic markers of disease progression and as targets for preventive and therapeutic strategies. Several genetic mutations have been associated with varying degrees of disease severity, including the 3T C/EBP site I and 5T Sp site III variants. These markers of disease progression are currently

being evaluated for their potential prognostic value (Burdo, Gartner, et al., 2004; Burdo, Nonnemacher, et al., 2004; Hogan, Nonnemacher, et al., 2003; Hogan, Stauff, et al., 2003; Nonnemacher et al., 2004). Studies are ongoing to develop an HIV-1 sequence database that will allow for the identification of additional sequence variants that may also serve as molecular markers of HIV-1 immune and nervous system disease. Developing an understanding of viral as well as host genetic heterogeneity may also prove useful in the prevention and treatment of HIV-1 infection and disease. This factor is being utilized in microbicide development, where the knowledge of how HIV-1 selectively adapts to more readily and efficiently infect and replicate within specific cellular compartments may generate effective pharmacologic barriers to viral entry and infection. Of course, enhancing our appreciation of how HIV-1 adapts for replication within the CNS will greatly facilitate the development of the next generation of antiretroviral therapeutics that can more effectively target compartmentalized virus within the brain and other tissue reservoirs.

It is clear that individual genetic mutations cannot be fully understood in isolation and that specific genomic mutations may very well be coselected for within specific viruses and viral quasispecies in response to local and global physiological changes within the host's peripheral circulation as well as specific tissue compartments such as the brain. Based on the available data, there is little debate as to whether genetic diversity of HIV-1 contributes to the onset and severity of HIV-1-associated neurologic disease; however, further investigation is required to definitively determine the impact that the genomic heterogeneity of HIV-1, and that of the host, has on the development of neuropathology and the onset of NCI.

Acknowledgments

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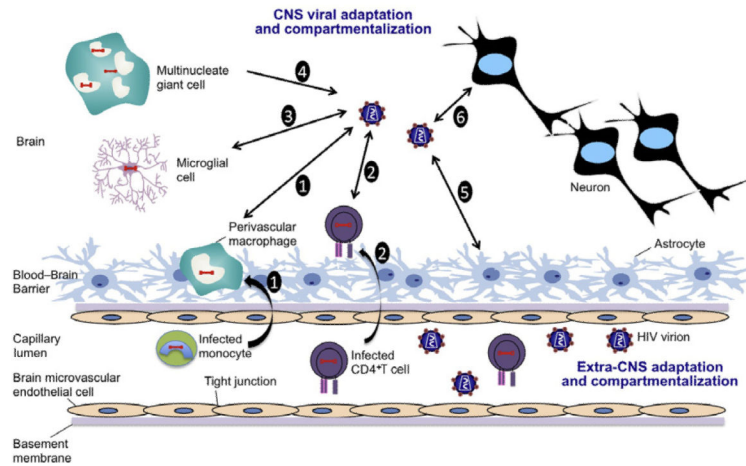
Figure 6.1.
Model of HIV trafficking across the BBB and its impact on the CNS. (1) Infected, activated monocytes transport HIV across the BBB through a "Trojan Horse" model, where they differentiate into perivascular macrophages. Infected perivascular macrophages then result in production of HIV within the CNS. (2) To a lesser extent, infected CD4^{+}T cells also serve to carry HIV across the BBB and may also contribute to HIV pools within the CNS. (3) HIV produced in the CNS can result in infection of resident microglial cells. (4) The presence of multinucleate giant cells is an important feature of HIV-related brain pathology; these are produced as a result of cell-to-cell fusion. (5) Astrocytes are known to harbor HIV, but it is well established that they do not result in a productive infection. (6) The viral evolution and adaptation within the CNS adversely affect the physiology of neurons via a variety of mediators including ROS, nitrous oxide (NO), MMPs, and viral proteins that exhibit neurotoxic properties resulting in compromised neurologic functions.
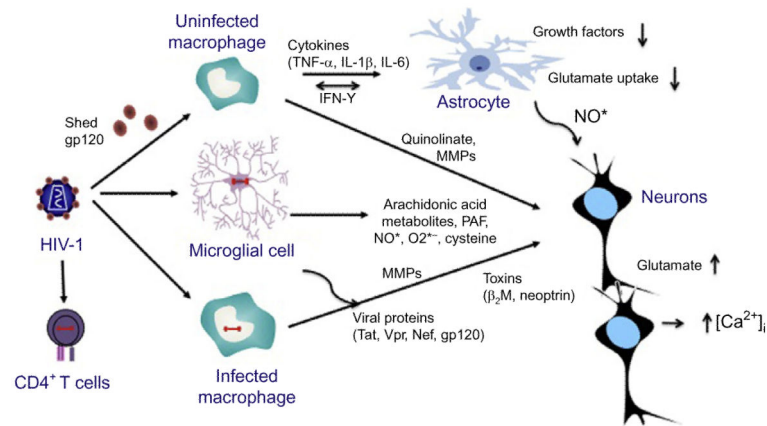
Figure 6.2.
Potential pathways and mediators of CNS damage. Subsequent to neuroinvasion, HIV-1-infected perivascular macrophages and brain microglial cells are likely to be the major producers of infectious virus and neurotoxic cellular and viral proteins such as gp120, Tat, and Nef. The extent of CNS dysfunction observed during HIV-1 infection is likely due to both host and viral factors. CNS damage may occur through increased viral replication within the CNS, production of viral neurotoxic proteins, and release of toxins including NO, TNF-α, and quinolinic acid, all of which target neurons, astrocytes, endothelial cells, and oligodendrocytes (not shown).
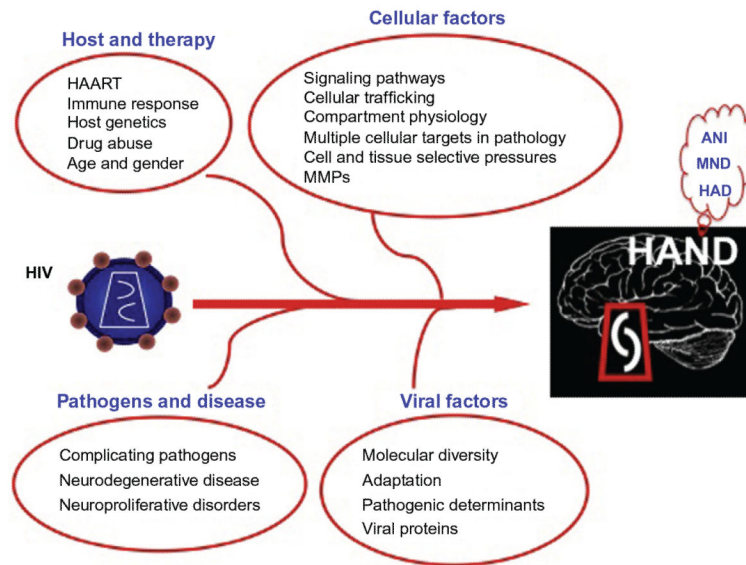
Figure 6.3.

The dynamic interplay of multiple viral and host factors contributes to both the onset and severity of HIV-1-associated neurocognitive impairment. An individual's HAART (highly active antiretroviral drug) and/or drugs of abuse status, as well as the presence of comorbidities resulting from opportunistic pathogens, greatly influence overall disease course and the establishment of central nervous system (CNS) pathology. One major complicating factor with respect to HIV-1-induced neurodegeneration is the ability of HIV-1 to adapt and evolve specific genetic variants in response to host immune pressures and subsequently to increase in its capacity to replicate within specific tissue compartments such as the CNS. This ability of HIV to evolve within the CNS eventually manifests as a variety of neurologic symptoms including asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HIV-associated dementia.

Table 6.1

Genetic factors and cellular pathways associated with HIV-1 infection identified through genome-wide association studies (GWAS) on HIV pathogenesis

	Significant and confirmed		
Phenotype	**Association**	**Validation technique**	**Reference(s)**
RNA VL set point	HCP5 (rs2395029)	Genotyping, Illumina's HumanHap550 BeadChip	Catano et al. (2008), Fellay et al. (2007), Stern et al. (2012), Telenti and Johnson (2012)
VL controllers	−35 HLAC (rs9264942)	Genotyping, genotype–phenotype associations	Catano et al. (2008), Fellay et al. (2007)
	HLA-B*5703	IFN-γ ELISPOT, tetramer staining, Illumina HumanHap BeadChip	Pereyra et al. (2010)
Long-term nonprogression	HCP5 (rs2395029)	Meta-analysis using Infinium II HumanHap300 BeadChips	Limou et al. (2009)
	CXCR6 (rs2234358)	Illumina Infinium II HumanHap300 BeadChips	Limou et al. (2010)
Progression to AIDS	PARD3B (rs11884476)	Regression analysis	Troyer et al. (2011)
	Significant		
VL controllers	MICA (rs4418214) >300 SNPs in MHC	Genotype association, regression modeling	Pereyra et al. (2010)
	Confirmed		
CD4 T-cell decline	ZNRD1 (rs9261174)	Genotyping, shRNA gene knockdown	Ballana et al. (2010), Fellay et al. (2007)
Progression to AIDS	PROX1 (rs17762192)	Meta-analysis; immunoblotting	Herbeck et al. (2010)
Nevirapine tolerance	CCHCR1 (rs1265112)	Illumina HumanHap550v3 Genotyping BeadChip	Chantarangsu et al. (2011), Lingappa et al. (2011)
	Putative		
Long-term nonprogression	C6orf48 (rs9368699)	Meta-analysis using Infinium II HumanHap300	Limou et al. (2009)

Significant and confirmed

Phenotype	Association	Validation technique	Reference(s)
Progression to AIDS	PRMT6 (rs4118325)	Illumina HumanHap300 BeadChips	Le Clerc et al. (2009)
	SOX5 (rs1522232)	Illumina HumanHap300 BeadChips	
	AGR3 (rs152363)	Illumina's Infinium HumanHap300 BeadChip	van Manen et al. (2011)
In vitro replication in macrophages	DYRK1A (rs12483205)	Illumina 610 Quad BeadChip, *in vitro* HIV-1 replication assays	Bol et al. (2011)
Motherto-child transmission	HS3ST3A1 (rs8069770)	Illumina's HumanHap650Y Genotyping BeadChip	Joubert et al. (2010)

Significant and confirmed: *p* value for association less than 5×10^{-8} and repeated independently to confirm the association with significance.

Significant: meets current standard for genome-wide significance in GWAS (has a *p* value below 5×10^{-8}); needs to be replicated independently. Confirmed: SNP tested to have association with a phenotype but failed to achieve a significant *p* value to have genome-wide significance (i.e., *p* value $>5 \times 10^{-8}$). Putative: *p* value $>5 \times 10^{-8}$ and yet to be confirmed by independent analysis.

Adapted from van Manen et al. (2012).