HIV treatment: mechanisms of neurotoxicity and implications for targeted therapy

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Abstract: The central nervous system is known to act as a unique compartment where the human immunodeficiency virus (HIV) can replicate independently from the plasma and as a sanctuary in which the virus is largely protected from the host immune system and combination antiretroviral therapy. Although combination antiretroviral therapy has dramatically decreased the rate of HIV-caused mortality and associated diseases, neurological complications are increasingly common. However, our knowledge of the complicated pathogenesis and clinical symptoms of HIV-associated neurocognitive dysfunction is limited by a lack of complete understanding of the biology of HIV and its interaction with host cells in the central nervous system. This review focuses on the mechanisms of HIV entry and replication in the central nervous system, neurotoxicity caused by viral proteins and cytokine/chemokines derived from affected host cells, their implications for targeted therapy, and advances in the development of animal models for novel therapeutics in the context of combination antiretroviral therapy regimens.

Keywords: HIV, HIV-associated neurocognitive dysfunction, neurotoxin, cytokines, chemokines, animal model

Introduction
Lentiviruses of human immunodeficiency virus type-1 (HIV-1) and HIV-2 are etiologic agents of the acquired immunodeficiency syndrome (AIDS).1 HIV infection has become pandemic and the World Health Organization reported that approximately 0.6% of the world’s population was affected by this virus in 2006.2 AIDS caused over 1.8 million human deaths in 2009 including 260,000 children, which dropped from a global peak of 2.1 million in 2004.3 Since its discovery in 2006, AIDS caused the loss of 25 million human lives.4 Although treatment with combination antiretroviral therapy (cART) has reduced the mortality and morbidity of HIV infection and public awareness and preventive measures has been significantly intensified, and despite the effects of the natural course of the epidemic, an estimated 2.6 million people were newly infected in 2009.5 HIV infects cells in the immune system such as T-helper cells, macrophages, and dendritic cells,4 and leads to low levels of cluster of differentiation-4+ (CD4+) T-cells. When the CD4+ T-cell numbers decline below a critical level of 200 cells/µL, cell-mediated immunity becomes lost, secondary infections with a variety of opportunistic microbes appear, and most untreated HIV-infected people eventually develop AIDS.5

HIV infection not only destroys cells in the immune system, chronic viral infection also leads to the development of a variety of neurological syndromes.6 HIV invades the central nervous system (CNS) shortly following primary infection, most likely soon...
after seroconversion, and subsequently induces progressive multiple systems of motor and cognitive dysfunction, psychiatric disturbances, and behavioral changes. Although the discovery of the neural expression of chemokine receptors as potential cellular binding sites for the virus and viral proteins – as well as progresses in neuroinflammation and neural stem cell biology – provides massive insights, mechanisms contributing to HIV-associated neuropathogenesis remain to be completely elucidated.

**HIV-associated neurocognitive dysfunction (HAND) associated with HIV infection**

HIV-positive patients develop HAND in the late phase of their infection. Pathological features such as neuronal loss, as well as cortical and subcortical atrophy, are revealed through examination of autopsied brains of HIV-positive individuals. Moreover, neurons in HIV-infected brains show dendritic and synaptic damage, and activation of apoptotic pathways ultimately leads to cell death.

HAND includes asymptomatic neurocognitive impairment, minor neurocognitive impairment, and HIV-associated dementia, and remains among the most frequently occurring disorders in HIV-infected people, even in an era that cART is widely deployed. A related study published in 2010 by the CHARTER (CNS HIV Antiretroviral Therapy Effects Research) group showed that 52% of HIV-infected adults exhibited signs of neuropsychological impairment. On the other hand, although cART has not dramatically changed the overall rate of this disease, it may alter presentation/severity of HAND from the fact that the asymptomatic neurocognitive impairment becomes the most common subdiagnosis in persons with HAND.

Before the introduction of cART, the majority of severe neurological symptoms took place in the late stage of HIV infection, with an estimation of the prevalence of HIV-associated dementia to be as high as 20%–30% in patients with low CD4+ T-cell counts. Following the era of cART, the life expectancy of HIV-infected people has been extended and the incidence of HIV-associated dementia has decreased. This is consistent with reports of more pronounced impairment of executive function and memory/learning in the cART era, compared to the pre-cART period. For instance, new cases of moderate or severe dementia fell from approximately 70% in 1989 to only 1% in 2000. This leads to the point that the effects on the brain caused by HIV infection should be considered along with systemic conditions, and that peripheral infection, induced immune response, and inflammation processes can affect all cell types in the CNS. However, due to the poor efficiency of drug penetration into the CNS, as well as other as yet unclear mechanisms, cART fails to provide protection to HIV-1-infected patients from the development of HAND, or to reverse the disease. To develop effective therapeutics, a better clarification and understanding of the pathogenic mechanisms of HIV-associated neurotoxicity is important. For instance, chemokine receptors that mediate the HIV infection, such as C-X-C chemokine receptor type-4 (CXCR4) and C-C chemokine receptor type-5 (CCR5), play particularly important roles in neurotoxicity. CXCR4 and CCR5 mediate the infection of immunocompetent cells through the viral envelope glycoprotein-120 (gp120). Even though neurons are not infected by the virus, due to the fact that they express high levels of chemokine receptors, they are subjected to massive apoptotic cell death in the presence of HIV proteins. This has led to a hypothesis that the chemokine receptor signaling itself is responsible for the neuronal apoptosis and atrophy observed in individuals with HAND.

**Other HIV-associated neurological diseases**

In addition to HAND, numerous neurological syndromes occur throughout the course of HIV infection that affects the CNS and peripheral nervous system. These HIV-associated neurological diseases either occur with opportunistic infections of the nervous system or are caused directly or indirectly by the virus itself. Some of these disorders are manifested early and some occur during the late stage of the infection, and the neuropathology includes inflammatory, demyelinating, and degenerative changes.

Early manifestations of these neurological diseases are relatively rare, and may occur following the initial infection, even before seroconversion. These early manifestations include aseptic meningitis or encephalitis, acute and chronic inflammatory demyelinating polyneuropathies, mononeuritis multiplex associated with peripheral nerve vasculitis, and HIV-associated polymyositis. Autoimmune pathogenesis is believed to be involved in these diseases, and patients with these diseases are typically responsive to immunosuppressive or immunomodulatory therapies in a similar manner to HIV-negative patients with the same diseases.

Besides the early manifestations, neurological morbidity and mortality mainly occur following the onset of immuno-deficiency with significantly lowered CD4+ T-cell count. Demyelinating leukoencephalopathy, which occurs in AIDS patients failing cART, is caused by massive infiltration of HIV-infected monocytes/macrophages into the brain with...
extensive white matter destruction. This may be attributable to the interaction of antiretrovirals with cerebrovascular endothelium, astroglial cells, and white matter, leading to cerebral ischemia, increased blood–brain barrier (BBB) permeability, and demyelination.43,44 Acute encephalopathy associated with the immune reconstitution inflammatory syndrome occurs in patients with advanced HIV disease with low CD4+ T-cell counts and high viral loads, caused by an acute infiltration of CD8+ T-cells into the CNS when treated with cART. It is suggested that the rapid immune reconstitution induced by cART leads to a redistribution of lymphocytes into the peripheral blood, followed by the recruitment of CD8+ lymphocytes into the brain, which results in diffuse infiltration.45–47 Vacuolar myelopathy is the most common chronic myelopathy associated with HIV infection, and often occurs in conjunction with AIDS dementia complex, peripheral neuropathies, and opportunistic infections or malignancies of the CNS or peripheral nervous system, eg, cytomegalovirus, progressive multifocal leukoencephalopathy, and lymphoma.48–50 Vacuolar leukoencephalopathy occurs during immune recovery following cART treatment,51 and the histopathology is similar to that of vacuolar myelopathy.52 In fulminant HIV dementia, symptoms progress over days and result in death within 2 months from the onset of neurological symptoms.53 In this disease, the basal ganglia is a common site of involvement for unclear reasons.54 and functional imaging and pathologic studies demonstrate the presence of basal ganglia abnormalities in HIV-infected individuals.55,56

Involvement of the peripheral nervous system is also part of AIDS. Like the CNS, the peripheral nervous system is a target of both the virus and other infectious agents. Although the development of neuropathic symptoms does not occur until the patient starts to show symptoms of early AIDS, electrophysiological evidence of peripheral nerve involvement is found in many patients with normal or near-normal CD4+ T-cell counts. Although pathological involvement of peripheral nerves is present virtually in all patients dying of AIDS, not all patients with HIV-1 infection develop clinical neuropathy, and the peripheral neuropathic symptoms may remain unrecognized or unappreciated in the presence of additional lesions in the spinal cord or brain in end-stage AIDS.57 However, HIV-associated sensory neuropathy (HIV-SN) represents major neurological disorder in persons with HIV/AIDS, affecting more than 30% of HIV-infected individuals globally.9 Painful peripheral neuropathy may actually be the most common and main neurological complication, and is also the most frequent neurological complaint observed among HIV-infected individuals in the industrialized world.57,58 Significant pain from HIV-SN affects up to 40% of HIV-infected individuals treated with cART. Prevalence of HIV-SN has increased despite increased access to and more widespread use of cART by infected individuals, and painful HIV-SN remains a major and expanding health problem worldwide.59 HIV-SN includes neuropathy directly related to HIV infection, eg, distal symmetrical polyneuropathy, and neuropathy associated with use of nucleoside analogs. Neuropathy associated with HIV infection alone should be distinguished from cART-induced toxic neuropathy, in that the latter is likely due to interferences with DNA integrity and mitochondrial dysfunction introduced by cART.60,61 However, cART-associated neuropathy may be caused by the synergism between the neurotoxicity of HIV infection and the neuropathy associated with cART. Furthermore, in all HIV-infected individuals, risk of infections, both opportunistic and nonopportunistic, increases as absolute CD4+ T-cell count falls, especially when fewer than 200 cells/µL, and nonopportunistic infections tend to be more common and more severe among HIV-infected individuals. HIV proteins may interact with the proteins introduced by these infections, particularly viral infections, and induce synergistic deleterious effects, which may play critical roles in the pathology of diseases, eg, cryptosporidiosis, microsporidiosis, and progressive multifocal leukoencephalopathy.62 For example, HIV transactivator of transcription (Tat) protein can transactivate the John Cunningham virus promoter in John Cunningham virus-infected oligodendrocytes, and may contribute to the neuropathogenesis of progressive multifocal leukoencephalopathy in patients with AIDS.63–67

Another burgeoning field that has attracted increasing interest is the interaction between intravenous drug abuse, HIV-1 infection, and neurological impairment. Abuse of intravenous drugs contributes substantially to the global HIV-1 epidemic, with 20% of intravenous drug users estimated to be infected with HIV-1. It causes about 30% of HIV-1 infections worldwide, except for sub-Saharan Africa, and is the third most frequently reported risk factor for HIV-1 infection in the United States.68,69 Risk behaviors associated with intravenous drug abuse is primarily the sharing of syringes. However, aside from providing a route of viral transmission, drug abuse also intrinsically affects HIV-1 pathogenesis, particularly in the CNS. Abuse of opiate drugs, such as heroin and morphine, promotes HIV-1 infection and the progression to AIDS through the suppression of cell-mediated immunity of the host, thus contributing to increased susceptibility to HIV infection and the eventual
development of AIDS. In addition, opiate abuse appears to increase the frequency and severity of HAND through the activation of microglia, breakdown of the BBB, and direct neurotoxicity. Furthermore, recent studies have indicated that opiates can exacerbate the neurodegenerative effect of HIV-1 Tat protein and hasten the progression of HIV-associated dementia, possibly through eliciting high levels of nicotinamide adenine dinucleotide phosphate-dependent reactive oxygen species and significant alterations in mitochondrial membrane homeostasis in neurons. Opiates can enhance the vulnerability of macroglia and macroglial precursors to the HIV-1 Tat protein through direct actions on glial precursors and/or astroglia. With sustained exposure to morphine, HIV-1 Tat protein induces preferential death of glial precursors and some astrocytes through processes that are mediated by μ-opioid receptors and accompanied by the activation of caspase-3. In addition, opiates enhance HIV-1 Tat protein-induced inflammatory effectors that are released by glia, and consequently elevate reactive oxygen species, increase 3-nitrotyrosine production by microglia, and reduce the ability of glia to buffer glutamate. In addition to opiates, another common drug of abuse in HIV-1-infected individuals is methamphetamine, an extremely addictive sympathomimetic stimulant that is chemically similar to the CNS stimulant amphetamine, and abuse of either drug facilitates HIV transmission, promotes HIV infection of target cells, and causes more serious neurological impairment. Furthermore, although both HIV infection and methamphetamine abuse are known to be associated with neurological dysfunction in an independent manner, the combined effects of HIV infection and chronic methamphetamine use are consistent with a synergistic model and can lead to additional neuronal injury and glial activation due to the comorbid conditions.

Although neurological complications of HIV infection are numerous, most current studies have been directed to understanding HAND, which is the main focus of this review, including HIV invasion and replication in the CNS, mechanisms of neurotoxicity, their implications for targeted therapy, and advances in development of animal models.

**HIV entry into the CNS**

Probably due to its immunological sequestration, CNS is believed to remain a viral reservoir throughout the course of viral infection. Due to a significant difference in nucleotide sequences and biological properties that are observed on HIV-1 isolates from the brain and peripheral blood of the same patients, it is suggested that the brain may harbor the virus for many years. HIV-1 invades lymphoid and nervous systems through infecting target cells that contain major receptors, either CD4 or CD8, as well as various chemokine receptors as coreceptors that are typically found on T-cells, blood monocytes, and some dendritic cells. These receptors facilitate viral entry into host cells, and infected CD4+ T-cells and monocytes that circulate in the blood are a potential source of CNS infection.

Of the chemokine receptors that are utilized by HIV-1, CXCR4 is found on lymphocytes, and CCR5 is found on monocytes, macrophages, and microglia. According to their use of coreceptors, HIV-1 is designated as R5 or X4 tropic, respectively, with some strains being dual tropic due to their use of both receptors. Within the CNS, HIV-1 infects mainly microglia and monocyte-derived macrophages. These cell types are the only cells that express both CD4 and CCR5 and they are the only cells that allow productive HIV-1 infection within the brain. In addition to viral receptors, the intracellular environment plays an important role in HIV-1 replication. HIV-1-infected cells can be either highly active producers of progeny viruses or low/nonproducers, defined as “productive” or “restricted” infection. Productively infected cells support production of progeny virus, are engaged in transmission of infection as well as in the evolution of the viral genome, and die ultimately due to expression of toxic viral proteins. On the other hand, restrictedly infected cells are permissive to HIV-1 infection but are refractory to efficient viral gene expression, and thus do not support virus replication but serve as a virus reservoir with replication-competent viral genome. Although these cells do not produce viral structural proteins, they can express accessory proteins such as regulator of virion expression and negative regulatory factor. The restricted infection can be caused by blockade at various stages of the HIV-1 life cycle such as nucleocyttoplasmic transportation, translation of viral ribonucleic acids (RNAs), assembly, or maturation of progeny virion. Restricted infection, however, can be reactivated by changes in the intracellular environment, such as increased expression of cytokines like tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-1β.

The BBB, or cerebrospinal fluid (CSF)–brain barrier, also plays a crucial interactive role during HIV-1 infection of the CNS. The BBB is selectively permeable and constitutes a barrier for the exchange of cells and substances between the brain parenchyma and the bloodstream. In order to get entry into the brain, HIV has to cross the BBB through a mechanism (or mechanisms) that is not yet completely understood. Current studies support the entry of HIV-1 into the CNS either as a free virus, via release...
of the virus into the brain by infected brain microvascular endothelial cells (BMVECs), or via infected immune cells. 108 Although several studies identified HIV-1 structural proteins in BMVECs by immunohistochemistry, 109-111 the study by Bagasra et al primarily identifies HIV-1 DNA and messenger RNAs for regulatory factors, but not for structural proteins, 112 leading to the postulation that BMVECs are restrictedly infected. On the other hand, although it is possible that cell free viruses are able to cross the BBB, infected CD4+ T-cells and macrophages are more likely sources of initial neuroinvasion, 112,113 which is verified by histological studies from simian immunodeficiency virus (SIV)-infected rhesus macaques and HIV-1-infected humans demonstrating that lymphocytes and monocytes are capable of infiltrating into the brain. 114,115 Whilst pathophysiological significance of the CNS-infiltrating lymphocytes in HAND is subject to further delineation, 115,116 current evidence suggests that cell-free HIV-1 particles may penetrate BMVECs through a mitogen-activated protein kinase-dependent macropinocytosis, 112,117 and this process can be enhanced by lipopolysaccharide treatment. 118 BMVECs exposed to HIV-1 express an elevated amount of intercellular adhesive molecules such as intercellular adhesion molecule-1, which in turn may facilitate leukocyte migration across the BBB and offer access for both cell-free viral particles and infected monocytes/macrophages into the CNS. 113,119-122 Furthermore, proinflammatory cytokines, eg, TNF-α, and oxidative stress can increase BBB permeability by activation of guanylate cyclase and tyrosine kinase. 123,124

Regardless of the potential roles of infected lymphocytes, cell-free viruses, and BMVECs, the generally accepted model of HIV entry into the CNS, with the most compelling evidence, is that HIV enters the brain through infiltration of infected T-cells and monocytes, with the latter later differentiating into macrophages. This mechanism is known as the “Trojan horse” hypothesis. 125,126 According to this hypothesis, HIV and other lentiviruses cross the BBB and migrate into the CNS by hiding in infected cells trafficking to the brain. Besides HIV, other viruses, eg, visna virus, feline immunodeficiency viruses (FIV), SIV, and human T-cell leukemia virus type-1, have also been reported for being transported into the brain by infected monocytes and macrophages. 127-129 Therefore, Trojan horse might represent a common route for retroviral and lentiviral penetration into the brain.

HIV-associated neurotoxins
Pathogenic mechanisms behind HIV infection-associated CNS dysfunction have not reached a common agreement. Whilst it is generally accepted that neurons are not infected by HIV, the primary cause/mechanism of neuronal damage remains unclear. There are discrepancies between distribution and number of HIV-infected cells, severity of the clinical course, and brain tissue pathology. This may suggest other mechanisms than direct viral cytotoxicity as causes of CNS damage. 130 However, current evidence supports that neuronal damage can be caused by various HIV proteins, such as Tat, R protein (Vpr), negative regulatory factor, gp120, and gp41. 11,12 This has led to two different theories on how dysfunction in the CNS is caused, both centering on the productive infection of brain macrophages/microglia. These theories are named “direct injury” and “indirect injury,” with the latter also called “bystander effect,” 12 and they are not mutually exclusive. Although the indirect form of neurotoxicity seems to predominate, 11,131,132 current evidence supports a role for both. The direct injury hypothesis suggests that HIV proteins can directly cause neuronal injury without effects from intermediary functions of nonneuronal cells such as microglia and/or astrocytes, and the indirect hypothesis suggests neuronal dysfunction caused through immunoactivation of nonneuronal cells by HIV infection or shed viral proteins.

Virotoxins from HIV
HIV viral proteins are potentially neurotoxic through either direct or indirect mechanisms, or both, 36,133-136 with more information summarized in Table 1. Neurotoxicity caused directly by viral proteins is supported by evidence that gp120, which is soluble and can be shed from HIV-infected cells, has been demonstrated to be toxic to cultured neurons at low concentrations, 36,137 and can directly injure neurons in serum-free primary neuronal cultures and in neuroblastoma cell lines. 35,138,139 Evidence that blocking chemokine receptor signaling can prevent gp120-induced neuronal apoptosis suggests that gp120 is capable of interacting with several chemokine receptors in the absence of CD4, and HIV-induced neuronal injury may be directly mediated by distinct chemokine receptor signaling pathways. 39,140-142 Other evidence that gp120 interacts with the N-methyl-D-aspartate-type glutamate receptor (NMDAR) suggests another mechanism by which gp120 may directly cause neuron injury. 143,144 The other cleavage product of gp160, gp41, is neurotoxic through induction of nitric oxide. 133,145 Besides the envelope proteins, Tat is secreted by infected cells and may also directly induce neuronal apoptosis via a pathway involving disruption of the metabolic balance of lipoprotein receptor-related protein ligands and direct activation of neuronal genes, 146 or via an increase in intracellular calcium,
Table 1 Virotoxins from HIV proteins

<table>
<thead>
<tr>
<th>HIV proteins</th>
<th>Localization</th>
<th>Mode of action</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>gp120</td>
<td>Membrane of infected cell; secreted into extracellular milieu</td>
<td>Direct and indirect injury</td>
<td>Interferes with N-methyl-D-aspartate receptor; create oxidative stress; immunoactivation of monocyte/macrophage, microglia, and astrocytes; stimulate release of cytokines</td>
</tr>
<tr>
<td>gp41</td>
<td>Membrane of infected cell; ectodomain aggregates in the brain</td>
<td>Direct and indirect injury</td>
<td>Induction of neurotokines; production of nitric oxide through an inducible nitric oxide synthase</td>
</tr>
<tr>
<td>Tat</td>
<td>Secreted into extracellular milieu</td>
<td>Direct and indirect injury</td>
<td>Apoptosis via disruption of the balance of LRP ligands and activation of host genes; increases intracellular calcium; production of reactive oxygen intermediates; activation of caspase; activation of cells of immune system; stimulate release of cytokines</td>
</tr>
<tr>
<td>Rev</td>
<td>Unknown</td>
<td>Direct injury</td>
<td>Cause nucleolar ballooning and deformity with aberrant accumulation of ribosomal ribonucleic acids</td>
</tr>
<tr>
<td>Vpr</td>
<td>Detected in CSF of people with HAND</td>
<td>Direct injury</td>
<td>Form cation-selective ion channels across cell membrane, causing a large inward cation current and depolarization of the plasmalemma</td>
</tr>
<tr>
<td>Nef</td>
<td>Detected on the surface of infected cells; secreted into extracellular milieu</td>
<td>Direct injury</td>
<td>Share sequence and structural features with scorpion peptides; reversibly increase the total potassium current</td>
</tr>
<tr>
<td>Vpu</td>
<td>Unknown</td>
<td>Direct injury</td>
<td>Increase ion channel activity; interact with Fas</td>
</tr>
<tr>
<td>Vif</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Gag</td>
<td>Intracellular expression; assembly into virion-like particles</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Abbreviations: CSF, cerebral spinal fluid; Fas, tumor necrosis factor receptor superfamily, member 6; Gag, group-specific antigen; gp, glycoprotein; HAND, human immunodeficiency virus-associated neurocognitive dysfunction; HIV, human immunodeficiency virus; LRP, lipoprotein receptor-related protein; Nef, negative regulatory factor; Rev, regulator of virion expression; Tat, transactivator of transcription; Vif, viral infectivity factor; Vpr, viral protein R; Vpu, viral protein U.

thereby stimulating production of reactive oxygen intermediates and caspase activation. More recent evidence suggests that Tat toxicity is dependent upon a polyamine-sensitive site on NMDAR. In addition, Tat and negative regulatory factor have been reported to increase production of neurotoxic quinolinic acid, a glutamate receptor agonist. Vpr is capable of causing apoptosis in human neurons (via cytochrome c extravasation), protein 53 induction, activation of caspase-9, and exerting a depressive effect on whole-cell currents in neurons. Another study using cultured hippocampal neurons revealed that Vpr causes a large inward cation current and depolarization of the plasmalemma, eventually resulting in cell death.

Although several HIV proteins have been reported to cause neurotoxicity, further studies are necessary to determine whether or not the concentrations of these proteins required for neurotoxicity in vitro are within the range that is present in vivo in an infected brain, and findings from these in vitro experiments should be interpreted under context of the specific conditions of the experimental paradigm, in that most of the results are obtained in the absence of nonneuronal cells and therefore predominantly the indirect effect is not involved. Moreover, concentrations of the HIV proteins employed in these studies are frequently significantly higher than the picomolar or lower range that is thought to be present in the brain or CSF of patients with HIV-associated dementia.

Besides neuronal injury caused directly by HIV proteins, other in vitro evidence suggests that gp120 neurotoxicity may occur indirectly and rely on the presence of toxic intermediates and activated chemokine receptors on macrophages/microglia. These toxic intermediates include proinflammatory cytokines and arachidonic acid metabolites that are produced when macrophages/microglia cells are exposed to gp120. Tat is also capable of causing neurotoxicity indirectly via stimulation of macrophages to produce matrix metalloproteinases that are capable of inducing neuronal apoptosis. Another model proposes that astrocytes may be involved in mediating such toxicity, supported by experimental findings that nitric oxide synthase is induced in astrocytes exposed to gp120 and the nitric oxide production may impair the ability of astrocytes to protect neurons from damage. In vitro, neuronal toxicity and apoptosis has been demonstrated through the use of X4 and R5 dual tropic envelopes, which is consistent with the finding that CXCR4 is present in a number of neural cell types.

Toxins from host cells affected by HIV

Various cell types in the brain can be infected by HIV and/or dysregulated by HIV proteins as summarized in Table 2. Severity of HIV encephalitis, a pathological correlate of the most severe form of HAND, correlates better with glial activation rather than viral load. It is often characterized by multinu-
Table 2  Influence on host cells by viretoxins

<table>
<thead>
<tr>
<th>Cells of origin</th>
<th>Source of influence</th>
<th>Upregulation</th>
<th>Downregulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocytes/macrophages, microglia</td>
<td>gp120, gp41, Tat, Nef, Vpu</td>
<td>TNF-α, IL-1β, IL-6, PGE2, protein 53, NTox, TGFβ1, endothelin-1, CCR5, platelet activating factor, MMP-9</td>
<td>cAMP by Tat</td>
</tr>
<tr>
<td>Astrocytes</td>
<td>gp120, gp41, Tat, Nef, Vpu</td>
<td>iNOS, tyrosine kinase, ICAM-1, CXCL10, endothelin-1, CD23, complement factor-3, IL-6, IL-8, IL-10, MMP-1, MMP-2, MCP-1, IκB, NF-κB, MAPK, JNK</td>
<td>β-adrenergic function, glutamate influx, GFAP, glutamate transporter EAAT2, Claudin-1, claudin-5, ZO-2</td>
</tr>
<tr>
<td>BMVECs</td>
<td>gp120, gp41, Tat, Nef, Vpu</td>
<td>ICAM-1, ζ-oidi receptor, PKC, MCP-1, IL-6, IL-8, E-selectin, NF-κB, IL-1, FAK, iNOS</td>
<td>BDNF, neuron specific enolase, glutathione, nephrilin, LRIP ligands</td>
</tr>
<tr>
<td>Neurons</td>
<td>gp120, gp41, Tat, Nef, Vpu</td>
<td>Calcium uptake, CXCR4, oxidative stress, sphingomyelinase, protein 53, PKC, NMDAR, JNK, ERK, CXCL10, calcium release from IP-3 pool, GSK-3β, MAPK, quinolinic acid</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AP-1, activator protein-1; BDNF, brain-derived neurotrophic factor; BMVECs, brain microvascular endothelial cells; CAMP, cyclic adenosine monophosphate; CCR5, C-C chemokine receptor type-5; CD23, cluster of differentiation-23; CXCL10, C-X-C chemokine ligand-10; CXCR4, C-X-C chemokine receptor type-4; EAAT2, excitatory amino acid transporter-2; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; GFAP, glial fibrillary acidic protein; gp, glycoprotein; GSK-3β, glycogen synthase kinase-3β; ICAM-1, intercellular adhesion molecule-1; IκB, inhibitor of DNA binding-1; IL, interleukin; iNOS, inducible nitric oxide synthase; IP3, inositol triphosphate; JNK, c-Jun N-terminal kinase; LRP, lipoprotein receptor-related protein; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemotactic protein-1; MMP, matrix metalloproteinase; Nef, negative regulatory factor; NF-κB, nuclear factor-κB; NTox, neurotoxin; NMDAR, N-methyl-D-aspartate-type glutamate receptor; PGE2, prostaglandin E2; PKC, protein kinase C; Tat, transactivator of transcription; TGFβ1, transforming growth factor-β1; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1; Vpr, viral protein R, Vpu, viral protein U; ZO-2, zona occludens-2.

cleaved giant cells, widespread reactive astrocytosis, activated resident microglia, microglial nodules, cytokine/chemokine dysregulation, and infiltration by monocytoid cells including mainly blood-derived mono- and multinucleated macrophages, as well as neuronal loss. In addition, a variable degree of white matter and deep gray matter pathology with evidence of a broad range of myelin damage, and the presence of HIV-1 in the CSF, have been reported. Degree of neurocognitive impairment, however, does not correlate well with the presence and amount of HIV-infected cells in the brain, the presence of multinucleated giant cells and microglial nodules, nor the concentration of viral antigens in CNS tissue. In contrast, pathologic features that are most firmly associated with the clinical signs of neurodegeneration include increased numbers of microglia, elevated TNF-α messenger RNA in microglia and astrocytes, evidence of excitotoxins, decreased synaptic and dendritic density, selective neuronal loss, and signs of neuronal apoptosis. Furthermore, severity of neuronal apoptosis is topographically correlated with structural atrophy, signs of microglial activation, and axonal damage, especially within the subcortical deep gray structures.

The number of activated macrophages in the white matter correlates well with the pathology of HIV dementia. Multinucleated giant cells that are derived from HIV infection-induced fusion of macrophages, microglial nodules, and perivascular mononuclear inflammation are hallmarks of HIV dementia pathology. A clear association between the amount of circulating activated monocytes and the development of HIV dementia has been established, which is likely caused by increased inflammatory mediators from these monocytes and/or infected monocytes that are increasingly trafficking into the brain.

Although brain macrophages and microglia from HIV-infected individuals may have neuroprotective properties, especially in the early phase of the infection, their predominant roles in the pathogenesis of HIV dementia are neuroinflammatory and neurotoxic – through the increased expression of cytokines such as TNF-α, IL-1, interferon-α, and inducible nitric oxide synthase. Furthermore, a number of neurotrophins are normally secreted by macrophages, eg, brain-derived neurotrophic factor, insulin-like growth factor, β-nerve growth factor, transforming growth factor-β, neurotrophin-3, neurophinin-4/5, and glial-derived neurotrophic factor. Expression of these neurotrophins is affected by cytokines and growth factors. For instance, acidic fibroblast growth factor induces higher IL-4, IL-10, and IL-13 levels, as well as expression of nerve growth factor and brain-derived neurotrophic factor in transected rat spinal cords. Cytokine mixtures derived from monocytes/macrophages induce a unique pattern of changes in genes for neurotrophins, growth and maturation factors, and related receptors through the downregulation of an alternatively spliced form of neurotrophin-3 growth factor receptor (trkC). Consequently, a dysregulation of macrophage-derived neurotrophic factors by viral infection or immunoactivation occurs during the course of HIV infection, which may elicit neuronal damage to considerable extent via the production of neurotoxins. Cytokines and chemokines, particularly those expressed by activated macrophages, appear to play a prominent role in the pathogenesis of HAND and other neurological com-
Implications of HIV. Although the relationship is not definitively demonstrated, current evidence shows that elevated levels of CSF and serum TNF-α correlate with presence of dementia. Although TNF-α may mediate neuroprotection under certain circumstances, it is neurotoxic through multiple regulatory roles, eg, promoting formation of reactive oxygen species, inhibiting glutamate uptake by glial cells, increasing the activation of NF-κB, expression of inducible nitric oxide synthase, inducing other chemokines and cytokines such as IL-6, IL-8, and CXCL10, upregulating expression of endothelial intercellular cell adhesion molecules, increasing the permeability of the BBB and subsequently facilitating HIV-infected monocytes entry into the brain, and promoting HIV replication.

In addition to macrophages/microglia, reactive astrocytes are also commonly observed in the brains of HIV-infected individuals. Astrocytes do not possess CD4 receptor, but they express CXCR4 and possibly other coreceptors including CCR5. In the brain, whilst macrophages and microglia are productively infected, astrocytes are involved in restricted infection through a CD4-independent pathway and viral regulatory proteins are expressed. Since HIV invasion of the CNS occurs early following initial infection, substantial infection of astrocytes is therefore likely to take place before cART is initiated, and the infected astrocytes can thus serve as a significant source of viral proteins that are not affected by cART. These toxic viral proteins in turn are capable of stimulating the release of other neurotoxic substances from affected glial cells and macrophages, or otherwise altering glial cell function and leading to the loss of support for neurons. Furthermore, activated astrocytes themselves – induced by HIV Tat and gp120 – are also capable of secreting inflammatory mediators such as TNF-α.

Besides glia, macrophages, and astrocytes, BMVECs may play a role in the pathogenesis of HIV in the CNS. Although direct infection of endothelial cells with HIV remains uncertain, cells exposed to HIV or viral proteins may undergo apoptosis or increase expression of adhesion molecules, consequently enhancing monocyte adhesion. The mechanisms involved implicate the activation of vascular endothelial growth factor receptor and phosphatidylinositol 3-kinase, as well as the induction of oxidative stress. Dysfunction of BMVECs may also play an important role in regulating the penetration of antiretroviral drugs across the BBB.

Implications for targeted therapy

Although the introduction of cART has significantly reduced the morbidity and mortality rate of HIV infection, it cannot provide complete protection for HIV-infected individuals from HAND, largely due to inefficient drug delivery across the BBB. HAND, as well as sensory neuropathies, remain common in the cART era, with up to half of HIV-1 patients affected. Although cART regimens may be able to play more significant roles in the prevention/treatment of HAND with the development of improved delivery systems, the challenging complexity of the treatment of AIDS patients impedes previous approaches to coping with HAND. Approaches tested so far include various antiretroviral compounds, applied either alone or in combination, including abacavir, amprenavir, atazanavir, darunavir, didanosine, efavirenz, emtricitabine, enfuvirtide, etravirine, fosamprenavir, indinavir, lamivudine, lopinavir, maraviroc, nelfinavir, nevirapine, raltegravir, ritonavir, saquinavir, stavudine, tenofovir disoproxil, zalcitabine, and zidovudine. However, due to poor penetration across the BBB, effects of most of these drugs are limited. Drugs that belong to the nucleoside reverse transcriptase (RT) inhibitor group have the advantage of good penetration because of low molecular weight and the lowest rates of protein binding; among which, zidovudine has the best partition coefficient in the brain and cerebral tissue. However, it is not long lasting in spite of the beneficial effect it has on crossing the BBB.

In the current anti-HIV armamentarium, nevirapine, indinavir, lopinavir, maraviroc, nevirapine, nelfinavir, saquinavir, stavudine, emtricitabine, darunavir, and raltegravir are the only ones found to have CSF levels that are sufficient to inhibit HIV replication. This dilemma calls for further development of alternative strategies other than antiretroviral drugs for the treatment of HIV in the CNS. Among these, agents such as NMDAR antagonists, cytokines, chemokines, chemokine and cytokine receptor antagonists, protein 38 mitogen-activated protein kinase inhibitors, caspase inhibitors, and antioxidants can be considered.

Studies have shown that neuronal damages caused either indirectly through HIV-infected macrophages or directly by the HIV proteins, both in vitro and in vivo, can be attenuated through NMDAR antagonists. One such agent, memantine – which inhibits excessive NMDAR activity while maintaining physiological function, is capable of blocking the neurotoxicity of HIV-1 gp120 and Tat in vitro, as well as improving synaptic transmission in hippocampal brain slices in a mouse model of HIV encephalitis and other studies. In addition, memantine has proven safe and effective in a number of clinical trials for amyotrophic lateral sclerosis, Alzheimer’s disease, and vascular dementia. Furthermore, it provides clinical benefits in the functional abilities of patients with moderate to severe Alzheimer’s disease.
However, it does not induce significant improvement in the neuropsychological tests of patients with moderate-to-severe neurocognitive impairment during a 16-week treatment despite magnetic resonance spectroscopy demonstrating potential neuroprotective effects, as reflected by an improvement of the neuronal metabolism in the frontal white matter and parietal cortex of treated patients. In a subsequent open-label trial, long-term use of memantine for up to 60-weeks did not provide clear evidence of cognitive benefit. Besides NMDAR antagonists, calcium channel blockers, CCR5 antagonists, TNF antagonists, and platelet-activating factor antagonists used in small clinical trials suggest some therapeutic benefit but fail to show clear cognitive improvement.

To combat the effects of excitotoxicity through minimizing the impact of free radicals, antioxidants are tried in an attempt to reduce oxidative stress-induced neuronal injury due to the toxic interactions between HIV-infected macrophages and neurons. One study utilizing OPC-14117, a lipophilic antioxidant, shows only a trend toward cognitive improvement. Two studies using seleagine and transdermal seleagine show significant efficacy on HAND, but in larger trials show neither cognitive benefit nor changes in brain metabolism. Studies using antiapoptotic drugs are also conducted to prevent or delay neural injury, with no clear benefit observed in neuropsychological measures. However, in one of these studies, neuroimaging reveals a decrease in the glutamate/glutamine ratio peak in the frontal grey matter, an increase in fractional anisotropy, a decrease in mean diffusivity in several brain areas, and changes in brain activation patterns, thus suggesting improvement of the HIV-associated CNS injury.

Other chemicals, such as minocycline and valproic acid, have also been tested. Sodium valproate (VPA) functions as a mood stabilizer that moderates signal transduction. Because mood changes to the level of disorders are one of the problems associated with HIV-1 disease, VPA might be beneficial when incorporated into the therapeutic armamentarium for HAND. Studies have shown that VPA inhibits the apoptosis-inducing glycogen synthase kinase-3β, a multifaceted kinase involved in numerous cell processes and known to be present in platelets. Glycogen synthase kinase-3β is activated by the platelet-activating factor, an inflammatory instrument that is upregulated during HIV-1 infection, and is thought to play a role in cytoskeletal rearrangement and lamellipodia formation in some cell types. When used at the therapeutic concentration (0.6 mM), VPA significantly increases β-catenin protein levels, decreases the level of protein α-kinase C and epsilon isoymes, and downregulates the myristoylated alanine-rich C-kinase substrate through inositol-independent mechanisms. However, it is suggested that further studies of VPA in advanced HIV infection should cautiously include high doses over prolonged periods of at least 18 months so that it can be more accurately determined whether the posited neuroprotective benefit of VPA still occurs or whether it is replaced by toxicity.

Several lines of evidence have shown that some cytokines, eg, IL-4, IL-5, IL-6, IL-10, IL-11, and interferon-α, may be capable of protecting neurons through antiinflammatory effects. These antiinflammatory cytokines may confer protection by antagonizing proinflammatory substances released from activated macrophages/microglia, or by inhibiting production of the inflammatory cytokines and activation of macrophages/microglia. In addition, erythropoietin, a cytokine that controls erythropoiesis, acts synergistically with another cytokine, insulin-like growth factor-1, as neuroprotectants by activating the phosphatidylinositol 3-kinase/protein kinase B pathway. Besides the application of an antiinflammatory cytokine, chemokine/cytokine receptors may represent potential therapeutic targets against HAND or AIDS. For instance, antagonists of CXCR4 and CCR5 may be capable of inhibiting HIV entry into cells, and expression of a soluble TNF-α receptor-Fc fusion protein confers protection of neuron cells from TNF-α, HIV-1 Tat-, and gp120-mediated neurotoxicity. Furthermore, expression of these molecules in genetically modified cells, such as monocytes and macrophages that possess a natural capability of crossing the BBB, and use of the genetically modified cells as delivery vehicles into the CNS, may constitute a novel gene therapy strategy against the neurological complications of AIDS.

Certain chemokines appear to be capable of protecting HIV-associated neurotoxicity, despite the fact that neurons are not infected by HIV. For example, fractalkine and β-chemokines are capable of preventing gp120-induced neuronal apoptosis in vitro, and some β-chemokines can ameliorate NMDAR-mediated neurotoxicity similarly. Neurotrophic factors, eg, brain-derived neurotrophic factor, glial-derived neurotrophic factor, and neurotrophin-3, are constitutively expressed in the CNS. Dysregulation of neurotrophic factors is known to be associated with neurotoxicity and affect pathogenesis of HAND, with downregulation of neurotrophic factors contributing to neuronal injury and death. Through binding of their cognate receptors, neurotrophins act to limit neurotoxin- and
lesion-induced neuropathologic damage and affect individual neuronal populations, dendritic length, spine density, synaptic transmission, antiapoptotic signaling, and signaling to limit oxidative stress.\textsuperscript{310,318–324} Moreover, neurotrophins confer neuronal protection by preventing apoptosis.\textsuperscript{325–328}

Since caspases are essential in the regulation of the apoptotic program, their inhibitors may be capable of forestalling apoptosis and thwarting neuronal loss.\textsuperscript{329–331} Similarly, protein 38 mitogen-activated protein kinase inhibitors have been shown to protect neurons from apoptosis that is caused by excitotoxicity, HIV/gp120 exposure, or a chemokine (stromal cell-derived factor-1)-mediated toxicity.\textsuperscript{324,332–334}

The future development of drugs for the treatment of HAND probably needs to focus on solving the challenges currently confronting the detection and treatment of HAND. First, the optimal cART regimen in terms of preventing and curing HAND, whether cART has neurotoxic effects, and the causative compounds if cART shows neurotoxicity need to be determined. Second, new therapies with minimal or no neuronal damage need to be developed. Given that the results of clinical trials so far with antinflammatory and/or neuroprotective agents were largely disappointing, newer compounds, administered either alone or in combination, in large and long-term studies are necessary. Third, biomarkers to differentiate patients developing HAND and those developing other neurodegenerative diseases and to detect changes in clinical settings need to be identified. Overcoming these challenges would be of great significance for the development of new therapeutics against HAND.

### Application of animal models

Finding an appropriate animal model for HIV has proven difficult. HIV naturally infects human beings and can only infect a small number of nonhuman primate species.\textsuperscript{335} This has severely limited the development of animal models for the study of HIV infection and HAND. An ideal animal model would be one that maintains the virological, immunological, and pathological aspects of human HIV infection and disease. However, such an animal model remains to be developed. It is important to acknowledge that each of the currently available animal models has its limitations. Accordingly, ongoing comparison of results obtained in animal models with those observed in human studies is necessary for the validation of the various models and for further improvement.\textsuperscript{336}

Currently, although infection with several lentiviruses of certain farm animals results in slowly progressive degenerative diseases, these viruses infect only macrophages/monocytes and not CD4\(^+\) T-helper cells. Consequently, they do not cause immunosuppression in the infected hosts, thus their relevance as a model for HIV immunopathogenesis is limited,\textsuperscript{337} and models that have been used for HIV study are mainly based on mouse/rats, felines, and nonhuman primates. Table 3 provides a summary of the advantages and disadvantages of the animal models used for HIV study.

### Mouse/rat models

A mouse model is attractive for HIV-1 research because it can be maintained in inbred strains, can be easily genetically engineered, and their immune system is extensively

### Table 3 Advantages and disadvantages of animal models used for human immunodeficiency virus/human immunodeficiency virus-associated neurocognitive dysfunction study

<table>
<thead>
<tr>
<th>Model</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>General mouse/rat</td>
<td>Availability of inbred and genetically engineered strains; well-known immune system; inexpensive for housing; fast reproduction</td>
<td>Species barrier for natural productive HIV infection</td>
</tr>
<tr>
<td>Transgenic mouse/rat</td>
<td>Presents good pathogenic manifestations of chronic HIV-1 diseases</td>
<td>No active HIV infection and replication; not all HIV genes included in a single animal</td>
</tr>
<tr>
<td>Mouse/rat with chimeric virus</td>
<td>Active virus infection and replication in vivo, with various hallmark features of HIV-1 infection and some brain pathology</td>
<td>gp160, a key neurotoxic HIV protein, is disabled; virus does not replicate as actively as natural HIV infection of human cells</td>
</tr>
<tr>
<td>SCID/human mouse model</td>
<td>HIV infection in human cells; different HIV strains can be used; genetically identical animal is possible with cells from the same donor; less overall cost of use and need less drugs</td>
<td>Lack of macrophages and robust anti-HIV immunity; technically challenging and time consuming; different physiology from humans and no real natural HIV infection; limited life span</td>
</tr>
<tr>
<td>FIV models</td>
<td>Natural infection in cats with an AIDS-like syndrome following a long incubation time; requires relatively low expense; present HIV/AIDS hallmark of CD4(^+) T lymphocyte depletion; many similarities of RTs of FIV and HIV</td>
<td>Most HIV nonnucleoside RT inhibitors and protease inhibitors are not active against FIV; FIV does not use the CD4 receptor, and infects not only CD4(^+) T lymphocytes but also CD8(^+) T lymphocytes and B lymphocytes</td>
</tr>
<tr>
<td>SIV models</td>
<td>Nonhuman primates are phylogenetically the closest to humans with similar immunology and physiology, viral pathogenesis, and antiviral immune response; long-term viral persistence</td>
<td>HIV cannot be directly used, requiring use of SIV or SHIV instead; expensive and very limited availability; large size requires more drugs; significant genetic differences between HIV and SIV</td>
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**Abbreviations:** AIDS, acquired immunodeficiency virus; CD, cluster of differentiation; Gp160, glycoprotein-160; FIV, feline immunodeficiency virus; HIV, human immunodeficiency virus; HIV-1, human immunodeficiency virus type-1; RT, reverse transcriptase; SCID, severe combined immunodeficiency; SHIV, simian human immunodeficiency virus; SIV, simian immunodeficiency virus.
known. Other advantages of using a mouse model include its relatively low cost and its ability to be housed in large numbers in a relatively small facility and reproduce quickly. Indeed, mouse AIDS models with distantly related oncoviruses (eg, murine leukemia virus) have been used in some early drug studies and have demonstrated efficacy of zidovudine and acyclic nucleoside phosphonates. However, mouse cells do not allow productive HIV infection, and HIV replication in mouse cells is generally disappointing. Blocks at viral entry and postentry are identified, and the HIV regulator of virion expression and Tat proteins are found to be less functional in mouse cells. Although engineered rats with human CD4, CCR5, and cyclin T1 support some HIV expression, additional obstacles for efficient viral replication and dissemination remain. For these reasons, transgenic rodents with specific HIV genes are produced to model select pathogenic manifestations of chronic HIV-1 diseases. Expression of the HIV gp120 protein in the CNS of a mouse model by astrocytes is the first model of its kind, and a genetic knockout mouse model of the two major HIV coreceptors, CXCR4 and CCR5, is created, with the latter model providing an in vivo system for further understanding of the CCR5-mediated pathway. A critical role of CCR5 is found for gp120-induced neuropathogenesis, which supports the indirect toxicity of HIV or viral proteins on neurons and corroborates other studies in linking neuropathogenic effects of HIV to chemokines and their receptors. Another transgenic mouse model with the HIV-1 Tat protein has been developed. Studies using this model have demonstrated that Tat expression in the absence of HIV-1 infection is sufficient to cause neuropathologies similar to most of those noted in the CNS of AIDS patients. It provides the first evidence in the context of a whole organism to support a critical role of the Tat protein in HIV-1 neuropathogenesis, and suggests that the doxycycline-inducible, brain-targeted Tat transgenic mice may offer an in vivo model for delineating the molecular mechanisms of Tat neurotoxicity and for developing novel therapeutic strategies for treating HAND. Another newer transgenic mouse model, in which the HIV Vpr protein is expressed in myeloid cells including those in the CNS and peripheral nervous system, has been developed. A transgenic mouse of this model manifests both structural and functional CNS abnormalities, as well as signs of peripheral neuropathy, and it links the peripheral neuropathy to effects on mitochondria. More importantly, studies using this model have examined the role of coinfection with hepatitis C virus – common in HIV-infected individuals including those with neurological complications of AIDS – through direct CNS injection of hepatitis C viral proteins, and revealed additional neuronal damage.

A transgenic rat model, with a group-specific antigen–polymerase-deleted HIV-1 genome expressed in many tissues including the CNS, is created and used to examine the interactions with drugs of abuse, specifically opiates. Study using this model have identified a wide range of interactions between the virus, host response, and the morphine receptor and its ligands in a preferred behavioral model for drug abuse. Besides transgenic models, direct injection of viral proteins into the brain has been studied. Except for the injection of the HIV Tat protein as the main model, effects of an additional injection of neuroprotective molecules, use of transgenic models and chemical inhibitors for mechanistic studies, and the monocyte migration into the brain under the influence of cocaine have been investigated.

To circumvent the species barrier to HIV in rodents, two rodent models using a chimeric virus are developed. The chimeric HIV virus is constructed through the use of a murine retrovirus envelope, which enables the infection of mouse cells including in vivo infection. This model is used to examine the antiviral immune response, by examining resistance to reinfection both peripherally and in the CNS, and shows the role of CD8+ T-cells in transferring immunity, indicating the potential utility of this model in studying host response and protective factors.

Moreover, nontransgenic models, eg, severe combined immunodeficiency (SCID)/human mouse, have been created. These models are produced through transplanting human transplants of thymus, fetal liver, or peripheral blood mononuclear cell into SCID mice. Although immune cells in the implanted human tissues allow reconstitution of the immune system of the SCID mice, most of these models have a limited repertoire of human cell types and limited distribution of the immune cells outside of the implant. Another similar model with humanized bone marrow/liver/thymus has better systemic reconstitution of all major human hematopoietic lineages, including T/B-lymphocytes, monocytes/macrophages, dendritic cells, and natural killer cells. Furthermore, a Trimera mouse model, by engrafting human peripheral blood lymphocytes in normal strains of mice, is used to study human immune responses and may be used for HIV infection as well. In this model, the normal hematopoietic system of the mouse is radiated by split-dose total body irradiation, with the immune system subsequently reconstituted by transplantation of murine SCID bone marrow, converting the normal hematopoietic system into a SCID-like system. The mice are subsequently converted
to Trimera by intraperitoneal injection of human peripheral blood lymphocytes.363,364 These models of a human immune system within an immunodeficient mouse host has enabled HIV infection and the study of many virus/host cell interactions.365 Furthermore, inclusion of myeloid cells in HIV neuropathogenesis studies has provided key findings in the effects of HIV on the brain and the science behind them.366,367 HIV-infected humanized mice brains are studied through noninvasive imaging studies to examine not only effects of HIV on the brain structure but also key metabolic effects that significantly impact neuronal and overall brain function.368 This makes longitudinal analyses for leukocyte migration and brain virus distribution possible,369 which are especially important in the current cART era of HIV infection.

Whilst these rodent models are useful as initial in vivo screening methods for antiviral strategies, their disadvantages are that they remain technically challenging and time-consuming, they do not recapitulate the full spectrum of immunopathological events that occur during natural HIV infection, and their physiology remains quite different from that of humans.366

### FIV models

FIV is another lentivirus that infects domestic cats and develops an AIDS-like syndrome following a long incubation period.370 This has several advantages for modeling HIV-associated diseases including that it is a natural infection model, it requires relatively low expense, and cats experience a CD4+ T-cell depletion during the course of pathogenesis which is a hallmark of HIV/AIDS. Additionally, RTs of FIV and HIV have many similarities in sequence, function, and in vitro sensitivity to nucleoside RT inhibitors such as zidovudine, lamivudine, didanosine and the nucleotide RT inhibitor adefovir,371–376 except that most nonnucleoside RT inhibitors and protease inhibitors are not active against FIV.377 Following primary infection, the relatively long asymptomatic period before the onset of disease makes FIV an attractive model for the study of effects of chronic infection. Using a synthetic ligand of a neurotrophin receptor, neuroprotection is demonstrated in vitro in an FIV/feline neural culture system.314 However, despite the advantages of the FIV model as an early screening method, several factors have made it less popular than the nonhuman primate models. These factors include FIV not using the CD4 receptor and infecting not only CD4+ T-cells but also CD8+ T-cells and B-cells.378 In addition, the long incubation period is a disadvantage in screening the efficacy of drugs.

### SIV models

Whilst the rodent and feline models have their advantages for initial screening, further testing/confirmation is best done in nonhuman primate models that are closer to the HIV infection of humans, which allows a more reliable extrapolation of the results. Nonhuman primates are phylogenetically the closest to humans, and have similar immunology and physiology. However, direct use of HIV-1 is limited by various factors. Although chimpanzees are susceptible to infection with HIV-1, they are limited by the low availability, high price, ethical issues, and the observation that disease rarely develops following infection.379–381 Whilst HIV-1 infection can be induced in young pigtailed macaques, virus replication cannot last a long time and no disease was observed.382 Substitution of the viral infectivity factor protein with viral infectivity factor from pathogenic SIV enables replication of HIV-1 in pig-tailed macaque T-cells in vitro and results in acute viremia that approaches the levels observed in HIV-1-infected humans, significantly extending persistent infection for several months in vivo.383,384 However, additional adaptation of the virus may still be necessary to enhance viral replication.383 HIV-2 infection models have been developed with Hamadryas baboons and several macaque species. Depending on the HIV-2 isolates, the outcome varies from an AIDS-like disease with CD4+ T-cell decline to no disease.385–387

Other than HIV, many nonhuman primate species in Africa are naturally infected with SIV strains, such as African green monkeys and sooty mangabees. These viruses are closely related to HIV-2. However, probably because viral infections lead to little activation of the immune cells, these hosts rarely develop disease in spite of persistent high-level virus replication.388–390 In contrast, SIV infection of nonnatural hosts, such as Asian monkeys like rhesus and pigtailed macaques, tends to follow a similar disease course as human AIDS in many aspects.391,392 including cell tropism, generalized immune activation, CD4+ T-cell depletion, opportunistic infections, weight loss, and wasting.393,394 Furthermore, similar to the HIV-1 infection in humans, the same clinical and laboratory markers, e.g., viral RNA levels in plasma and CD4+ T-cell counts, can be used to monitor and predict disease progression.395–397

On the other hand, although SIV is closely related to HIV-1, significant genetic differences between them exist, resulting in distinct adaptive strengths, weaknesses, and peculiarities, as well as functional consequences. These differences make it difficult to study their effects on pathogenesis or their targeting by drugs.398 In order to overcome
these limitations, various SIV/HIV chimeric viruses (SHIVs) that more closely represent HIV-1 have been constructed and used in many macaque experiments. The first SHIV is an SIV<sub>Mac</sub> chimera containing the tat, rev, vpu, and env genes from HIV-1.<sup>399</sup> This virus replicates and causes disease in animals,<sup>400–402</sup> and many versions of this type of SHIV have been made thereafter. Although the most extensively studied SHIVs are made from X4-tropic HIV-1,<sup>401,403</sup> SHIVs with incorporation of the R5-tropic HIV-1 envelopes have also been developed.<sup>404–406</sup> Because most primary HIV-1 isolates are R5 tropic, these SHIVs are more valuable to studies of early HIV-1 infection and pathogenesis, as well as mucosal transmission.<sup>407–409</sup> Because many HIV-1 RT inhibitors do not inhibit SIV RT,<sup>410,411</sup> SIV RT is replaced with the HIV-1 RT and used for in vivo drug studies.<sup>410–412</sup> For similar reasons, another SHIV with HIV-1-derived protease is constructed,<sup>413</sup> making it a useful tool for in vivo efficacy tests of protease inhibitors. Animal models based on these SHIVs have greatly advanced HIV-1 research and are useful adjuncts to the SIV model.

Despite limitations of the SIV-macaque models, the similarities in virus, host, and disease pathogenesis have made them currently the premier animal model in HIV research, with many therapeutic agents and functions able to be assessed in monkeys using experimental protocols that cannot be done in humans. A rapid model of the neurological complications of AIDS in rhesus macaques is developed by depletion of CD8<sup>+</sup> T-cells via antibody treatment at the time of infection with SIV,<sup>414,415</sup> leading to altered neurological conditions with a high proportion of animals developing SIV encephalitis. This model is applicable for studying monocyte/macrophage imaging, trafficking, turnover, and linked biomarkers of disease, as well as understanding the key mechanisms of neuropathogenesis and its potential prevention and treatment through the application of experimental and therapeutic modalities. SIV-infected pigtailed macaques are used to study the effects of cocaine abuse on HIV pathogenesis,<sup>416</sup> but few differences attributable to cocaine administration have been found, despite extensive studies being performed on virological, neuroinflammatory, and behavioral parameters. This suggests that cocaine has no distinct effect on SIV in the brain. Furthermore, characterization of the brain transcriptome in SIV-infected rhesus monkeys can be studied through the use of bioinformatics, with altered pathways found at different stages of infection,<sup>417</sup> and a subset of altered messenger RNAs and microRNAs have been commonly identified in studies comparing SIV and HIV encephalitis.<sup>418</sup>

**Conclusion**

The HIV infection pandemic has proved a unique and difficult challenge. Infection by HIV causes neuronal dysfunction and loss of numerous interplaying mechanisms. It has become clear that HAND results from a complex interaction of effects caused by viral proteins and host inflammatory mediators. This article is a summary of current evidence that supports either the direct or indirect mechanisms by which neuronal death may occur during HIV infection. From the literature and ongoing studies, it is clear that a considerable amount of further investigations are still necessary to elucidate the mechanisms of HIV neurotoxicity and the pathogenesis of HAND. Understanding these mechanisms is tantamount to the development of therapeutics that would attenuate or prevent the neuronal degeneration associated with late-stage HIV infection.

Although cART has markedly reduced the morbidity and mortality of HIV-caused neurological diseases and the severe cognitive and motor dysfunctions of HAND, as well as the sensory neuropathies, HAND remains common in the cART era, with up to half of HIV-1 patients affected.<sup>22,248</sup> Part of the reason that current cART regimens are inadequate in providing complete protection from developing these neurological complications or eradication of HIV in the CNS is due to poor penetration of these drugs. With future development of improved systems for the delivery of drugs, or drugs with improved capability of penetrating BBB, cART regimens may play a more significant role in the treatment of HIV infection and its progression to neurological disease.

Since the discovery of HIV, despite many initial obstacles, the development of animal models for HIV infection has evolved dramatically. However, each of the available models has its intrinsic limitations and advantages. In spite of their limitations, these models have provided unique and valuable tools for studies addressing specific issues. Further development of novel models and refinement of the current ones to more accurately reflect the biological properties and physiological conditions of HIV infection of humans are required for the effective management of therapeutic strategies and the development of novel anti-HIV therapeutics.

**Disclosure**

The authors report no conflicts of interest in this work.

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