Over 55 million individuals, worldwide, have been infected by the human immunodeficiency virus, type 1 (HIV-1); greater than 90% of those infected will develop a neurological disease. The development of AIDS is defined by a decline in CD4 T cell levels below 200 cells/µl in blood, with or without an AIDS-defining illness. This fall in CD4 cells is accompanied by increasing viremia and opportunistic infections (Figure 1). Since the publication of our earlier review, the understanding and treatment of HIV/AIDS-related neurological disorders has advanced, particularly with the advent of highly active antiretroviral therapy (HAART). This review highlights new developments in HIV neuropathogenesis.

**Neurological Aspects**

Early in the HIV epidemic, two general categories of neurological diseases were recognized as HIV infection progressed to AIDS; the first group includes opportunistic infections within the nervous system and the role of viral expression and diversity are emphasized in relation to neurovirulence. Induction of innate immune responses within the central and peripheral nervous systems, largely mediated by cells of macrophage lineage, appear to be common to the development of primary HIV-related neurological disease. Activation of these cell types results in the release of a cascade of inflammatory molecules including cytokines, chemokines, matrix metalloproteinases, and arachidonic acid metabolites that influence neuronal survival. Individual viral proteins encoded by envelope and tat genes and discrete sequences within these genes influence the extent to which these pro-inflammatory molecules are induced. At the same time, systemic immune suppression may influence the occurrence and severity of HIV-related neurological diseases. Implementation of HAART and neuroprotective treatments improves neurological function although the evolution of drug-resistant viral strains limits the sustained benefits of HAART.
Figure 1: Disease course in HIV infection. Following primary infection there is a rapid transient rise in plasma viremia accompanied by a decline CD4+ lymphocyte count, which resolves over time. However, viral replication continues throughout infection and escalates with the development of AIDS with a concomitant drop in CD4+ cells below 200-cells/µl in blood. (After Johnson RT. Viral Infections of the Nervous System. Lippincott-Raven Publishers, 1998, with permission from Lippincott-Raven Publishers) ARC = AIDS related complex

Figure 2: Neurological diseases occurring during the course of HIV infection. All levels of the neural axis may be affected by HIV infection but the individual syndromes usually emerge depending on the level of immune suppression. HAD, MCMD and DSP present during advanced infection. (After Johnson RT. Viral Infections of the Nervous System. Lippincott-Raven Publishers, 1998, with permission from Lippincott-Raven Publishers)

GBS/CIDP = Guillain-Barré syndrome/chronic inflammatory demyelinating polyneuropathy; DSP = distal sensory polyneuropathy; HAD = HIV-related dementia; MCDC = minor cognitive and motor deficit
displays remarkable diversity in its clinical phenotype. The range from 5-20% among patients with AIDS, while MCMD Neurocognitive impairment advent of HAART including stroke-like events. Neurological syndromes have emerged, particularly with the previous reports. Despite the wide availability of HAART, HIV-diagnostic criteria for both conditions have been defined in (VM), and several peripheral neuropathies4 (Figure 2). Other cognitive and motor deficit (MCMD), vacuolar myelopathy syndromes include HIV-associated dementia (HAD), minor group are the primary HIV-induced syndromes. The latter results on neuropsychological testing). 81%) were receiving three or more antiretroviral drugs (NP+; abnormal patients (n=150) with HIV infection, followed at the Southern Alberta Clinic, Calgary AB. The level of immune suppression among patients with or without neurocognitive impairment did not differ significantly. Patients treated with HAART (HAD/MCMD-75%; ND (nondemented)-81%) were receiving three or more antiretroviral drugs (NP+: abnormal levels). Figure 3: Prevalence of neurocognitive impairment in a subset of patients (n=150) with HIV infection, followed at the Southern Alberta Clinic, Calgary AB. The level of immune suppression among patients with or without neurocognitive impairment did not differ significantly. Patients treated with HAART (HAD/MCMD-75%; ND (nondemented)-81%) were receiving three or more antiretroviral drugs (NP+: abnormal results on neuropsychological testing).

infecions of the central nervous system (CNS), while the second group are the primary HIV-induced syndromes. The latter syndromes include HIV-associated dementia (HAD), minor cognitive and motor deficit (MCMG), vacuolar myelopathy (VM), and several peripheral neuropathies4 (Figure 2). Other neurological syndromes have emerged, particularly with the advent of HAART including stroke-like events.

Neurocognitive impairment

Estimates of HAD prevalence rates in the pre-HAART era range from 5-20% among patients with AIDS, while MCMG may affect as many as 30% of HIV/AIDS patients. The diagnostic criteria for both conditions have been defined in previous reports. Despite the wide availability of HAART, HIV-related neurocognitive impairment is a common problem observed in HIV clinics (Figure 3). Risk factors for HAD include low CD4 levels, high viral loads in CSF or plasma, anemia and extremes of age. HIV-associated dementia is characterized by progressive motor, cognitive and behavioral abnormalities and displays remarkable diversity in its clinical phenotype. The course of the dementia is variable with an abrupt decline in function over weeks among some individuals, while others display a protracted course over several years. Prior to the availability of HAART in 1996, the mean survival with HAD was three to six months. Since 1996, the incidence of HAD has diminished, but the prevalence may be rising due to longer survival times. Minor cognitive and motor deficit exhibits many clinical aspects of HAD although the signs and symptoms are less severe. The relationship between HAD and MCMG remains uncertain but a subset of patients progress from MCMG to HAD. Radiological features accompanying HAD include cerebral and basal ganglia atrophy and white matter hyperintensities on MRI T2 weighted images. Recent magnetic resonance spectroscopy studies show diminished N-acetyl aspartate levels in brain, implying neuronal injury or loss.

The neuropathological hallmarks of HIV in the adult include multinucleated giant cells, diffuse white matter pallor, perivascular cuffs comprised of monocytes and lymphocytes, microglial nodules or the presence of HIV-1 antigens. HIV encephalitis is defined by the presence of multinucleated giant cells and/or the presence of viral antigens. Among HIV-infected adults with dementia, approximately 20-80% of individuals will display multinucleated giant cells. Diffuse white matter pallor shows sparing of the U fibers and preserved myelin proteins; deposition of serum proteins in white matter suggests altered permeability of the blood brain barrier rather than demyelination. These findings are complemented by studies showing apoptotic cell death in cerebral endothelia in brains of HIV-infected patients. A limited correlation exists between HAD (a clinical entity) and HIV encephalitis (a pathological entity). Over half of adult AIDS patients with dementia do not exhibit diffuse myelin pallor or multinucleated giant cells at autopsy while microglial nodules may be present in 90% of autopsied AIDS patients including those without dementia. A correlation between HIV antigen abundance and HAD has been proposed. Other studies have shown that macrophage and microglia activation, particularly in the basal ganglia, is a stronger predictive marker for HIV dementia. Neuronal injury and death, in the frontal cortex and deep gray matter occurs in the brains of patients with AIDS. These neuronal abnormalities are likely responsible for the phenotypic expression of HAD.

Myelopathy

HIV-associated VM affects 5-10% of AIDS-defined patients, usually manifesting as subacute progressive gait ataxia, leg weakness, spasticity, incontinence and may occur independently of neurocognitive impairment. The incidence of VM has also dropped with HAART to a point that it is infrequently seen in HIV clinics except in severely immunosuppressed patients. The diagnosis is one of exclusion of other conditions causing chronic myelopathy, which requires cerebrospinal fluid (CSF) analysis and imaging studies. Autopsy studies prior to HAART showed that 20-50% of AIDS patients exhibited axonal injury, macrophage infiltration and activation including multinucleated giant cells, and a vacular appearance that was primarily localized in the lateral and dorsal columns of thoracic spinal cord. The vacular appearance may reflect intramyelnic edema. Approximately 25% of patients with pathologically confirmed VM presented with antemortem symptoms or signs suggestive of a myelopathy. Although HAART appears to reduce the incidence of VM, limited reversal of the signs or symptoms is observed after therapy is implemented.

Neuropathies

Despite a decline in incidence of CNS manifestations associated with advanced HIV infection, the incidence and
prevalence of peripheral neuropathies remains high. At present, there are two major groups of neuropathy observed among patients with HIV infection, which may overlap to some extent in clinical features and occurrence. Distal sensory polyneuropathy (DSP), acute and chronic demyelinating neuropathies, and mononeuropathies multiplex. Distal sensory polyneuropathy is the most frequently encountered, affecting 35-45% of AIDS-defined patients, and is associated with advanced HIV infection and is usually manifested by chronic or subacute complaints of burning foot pain, paresthesiae, dysesthesiae, distal sensory loss with diminished or absent distal deep tendon reflexes. This neuropathy may improve with HAART although several antiretrovirals are clearly toxic to peripheral nerves. The second group of neuropathies, frequently encountered among treated HIV/AIDS patients, includes the toxic neuropathies (TN), arising due to the use of antiretrovirals including didanosine (DDI), zalcitabine (DDC), stavudine (D4T) and to a lesser extent, lamivudine (3TC). The symptoms and signs of toxic neuropathies are similar to DSP and the two entities are frequently indistinguishable except by history of recent onset neuropathy with initiation of a neurotoxic drug within several months. The pathological features of DSP and TN include distal “Wallerian” axonal degeneration of long fibers with small diameter sensory fibers being chiefly affected. Macrophage infiltration and activation is also frequently observed with detection of viral protein or nucleic acid in macrophages of 30-50% of biopsies. Similarly, inflammatory cells, including macrophages and lymphocytes, have been reported in the dorsal root ganglia (DRG) of AIDS patients with neuropathy, which may be accompanied by a decrease in the number of DRG neurons. Recent skin biopsy studies indicate a loss or degeneration of small diameter C and Aδ fibers in epidermis among HIV/AIDS patients with and without concurrent HAART, and fiber degeneration may precede symptoms and signs of neuropathy.

VIROLOGICAL ASPECTS

HIV-1 belongs to the lentivirus genus of retroviruses, which are defined by a relatively slow disease course in their natural hosts. Other lentiviruses include visna-maedi virus, simian (SIV), feline (FIV), and bovine (BIV) immunodeficiency viruses, which are also neurotropic. HIV-2, which is chiefly found in West Africa and is genetically closely related to SIV, exhibits less systemic virulence but is of uncertain neurovirulence. Like all retroviruses, the HIV-1 genome is defined by gag, pol, and env genes, but HIV also contains several nonstructural genes that influence splicing and transcriptional events, for a total of 10 open reading frames within approximately 10 kilobase pairs. Extensive variation within different HIV-1 strains is manifested both genotypically as well as phenotypically, this arises from poor fidelity during reverse transcription and recombination in the virion between the diploid RNA molecules. Viral phenotypes have been defined in terms of cell tropism: most HIV strains are either macrophage- or T cell-tropic but dual tropic viruses have also been described. HIV-1 cell tropism is chiefly defined by which chemokine receptor the virus uses as a co-receptor; CXCR4 on T cells and CCR5 receptor on macrophages function together with the CD4, for infection; although viruses using CCR5 can be isolated from T-lymphocytes (Figure 5). In addition, different HIV strains have also been defined in vitro as syncytia (SI)- or nonsyncytia-inducing (NSI).
Neurovirological infections are defined by the following attributes: neuroinvasiveness or ability of virus to enter the nervous system; neurotropism or ability of virus to infect brain cells (including the selective infection of neurons, termed neuronotropism) and neurovirulence or ability of virus to cause nervous system disease. HIV-1 fulfills each of the above criteria. Several properties that contribute to HIV’s complex neurobiology include: (i) its predilection to genomic mutation, (ii) its induction of both innate and adaptive immune responses within the nervous system and (iii) its ability to cause disease simultaneously outside the nervous system.

Neuroinvasiveness

Neuroinvasion by HIV-1 occurs early after initial (primary) infection and has occurred in most patients by the time of death. HIV antigens and/or genome have been detected in the brains of HIV-infected patients at all stages of infection. Studies of patients who have died soon after infection of other causes exhibit viral antigen and neuropathological findings indicative of HIV infection. The mechanism by which HIV enters the CNS has been assumed to be infection of macrophages or lymphocytes, which cross the blood-brain barrier and infect cells of macrophage/microglia lineage (microglia and perivascular macrophages) in the brain. This has been termed the “Trojan Horse” hypothesis (Figure 5). An alternative pathway for entry into the CNS includes infiltration of the choroid plexus with subsequent seeding of the brain by infected macrophages or CD4 lymphocytes harbouring CCR5-using viral strains.

The viruses recovered from the brains of AIDS and pre-AIDS patients are macrophage-tropic viruses in terms of viral gene sequence analyses and in vitro cell tropism. However, monocyte/macrophage traffic through the CNS is limited, unless some injury or infection has occurred within the brain. Several
chemokines, including macrophage inflammatory protein (MIP)-1α and MCP-1, show enhanced expression in the brains of HIV infected patients suggesting that monocyte/macrophages (HIV-infected or uninfected) may be recruited into the CNS through a chemotactic mechanism. Additionally, upregulation of adhesion molecules such as intercellular adhesion molecule on the luminal aspect of brain endothelial cells has been demonstrated in vivo, which may facilitate monocyte/macrophage adherence and subsequent CNS entry. Free virus may directly enter the CNS during initial plasma viremia; this route of entry has appeal because of the high levels of macrophage-tropic virus early in infection in the plasma and CSF. The relationship, if any, of CSF viral subtype and quantity to infection of the brain also remains unclear. As discussed below, viral load in CSF may be a predictor of severity of HIV dementia. How HIV enters the CNS remains a pivotal issue, because strategies to prevent CNS entry by HIV will be of value in preventing the development of neurological disease.

**Neurotropism**

HIV antigens and genome (DNA and RNA) in the brain are found primarily in microglia and perivascular macrophages and in astrocytes, albeit less frequently. Infected cells are principally localized in the central white matter and the deep gray matter structures including the basal ganglia. In situ hybridization, with and without PCR, confirms the immunocytochemical findings. To date, endothelia and oligodendrocytes have not been shown consistently to be infected in vivo although in vitro studies indicate these cell types are permissive to some strains of HIV-1. The question of in vivo infection of neurons remains controversial: Nuovo et al have reported detection of viral genome in neurons by in situ PCR and this finding has been confirmed by at least one other group. However, the preponderance of studies has failed to show viral nucleic acids in neurons. In vivo productive infection of neurons has not been shown to date, despite studies showing in vitro productive infection of neuronal cell lines.

HIV infection is mediated by CD4 as the principal receptor on lymphocytes and macrophages. Several co-receptors have been shown to exist on blood-derived cells such as CCR5 on macrophages and CXCR4 on lymphocytes. Other co-receptors have been reported, although their roles are less well-defined. In the brain, CCR5 and CCR3 have been postulated as potential co-receptors on microglia. Thus, HIV-1 strains isolated from brain appear to be principally macrophage-tropic and have also been shown to infect microglia in vitro. The hypervariable V3 of HIV gp120 (Figure 4) derived from brain has been shown to be critical for HIV infection of macrophages and microglia. Recently, it has been demonstrated that brain-derived HIV-1 V3 sequences mediate the use of CCR5 and CCR3, but not CXCR4, as co-receptors. Repeated passages of HIV in vitro can result in mutations in the envelope that resemble mutations identified in brain-derived sequences, suggesting that the virus may adapt to the brain. How the different regions of gp120 interact with plasma membrane receptors is uncertain but a conformationally dependent interaction between multiple regions of the envelope (V2, V3 and C3 regions) and the different receptors, has been proposed. CXCR4 has also been demonstrated on microglia, in addition to cells such as neurons. The failure of T-cell tropic (CXCR4-using) HIV-1 strains to establish productive infection of microglia, despite the presence of CXCR4, is enigmatic and may reflect a requirement for interaction between multiple co-receptors for infection or importance of replicative steps subsequent to attachment and penetration. Nonetheless, the role of this latter co-receptor in HIV neuropathogenesis remains intriguing because it may have important implications in mediating neuronal injury and death. Other HIV receptors in the brain have been reported, including galactosyl ceramide and a 260Kd-astrocyte cell membrane protein. Hence, the mechanisms of viral fusion and entry into brain cells remain uncertain and may differ from blood cells.

Cell tropism or infectivity of a retrovirus is determined by multiple viral genes that influence events during infection, including viral entry, reverse transcription, integration, transport of viral proteins and genome to the cell surface and budding of virions. Several genes found within HIV are likely to influence its tropism. HIV entry appears to be modulated by different regions of gp120 that is encoded by the env gene. Studies of other viral genes and their ability to influence neurotropism indicate that distinct gag and tat sequences may influence neurotropism but functional confirmatory studies are pending. Other groups using transgenic mice have shown that the HIV-1 LTR may be important for expression in the brain.

**Neurovirulence**

(i) **Viral strains**

The role of distinct lentivirus strains that do or do not cause neurological disease has remained controversial, largely because the immense molecular diversity within lentiviruses obscures discrete or specific viral sequences associated with disease. Nonetheless, evidence for virulent HIV-1 strains comes from several sources including (1) studies showing that specific HIV-1 subtypes (clades) are associated with an accelerated disease course compared to socio-economically and geographically-matched patients infected with other HIV-1 clades; (2) the occurrence of drug resistant viral strains that are defined by specific mutations in the HIV-1 pol gene; (3) the use of CXCR4 by more pathogenic blood-derived viral species instead of CCR5 as disease progresses; (4) organ-specific compartmentalization of HIV-1 quasispecies. Evidence that individual viral strains play a direct or causative role in lentivirus-induced brain disease is derived from studies showing that distinct SIV and FIV strains are responsible for disease development in animal models. Additionally, specific sequences within the env gene have been shown to influence the development of animal lentivirus neurological disease.

In studies of a well-characterized, prospective cohort of AIDS patients with and without HAD, specific mutations within the V3 and C1 domains of brain-derived HIV-1 envelope sequences differed between AIDS patients with and without dementia. These findings are supported by subsequent studies. These same domains also determined the ability of
Table 1: Potentially neurotoxic molecules implicated in HIV neuropathogenesis

<table>
<thead>
<tr>
<th>Source</th>
<th>Molecules</th>
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<tbody>
<tr>
<td>Viral proteins</td>
<td>Tat, Nef, gp41, gp120, Vpr</td>
</tr>
<tr>
<td>Macrophage factors</td>
<td>Low molecular weight toxic factors: Ntox, Quinolinic acid, glutamate</td>
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<tr>
<td></td>
<td>Arachidonic acid metabolites (prostaglandins E₂, F₂α, thromboxane B₂)</td>
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<tr>
<td></td>
<td>platelet activating factor</td>
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<tr>
<td></td>
<td>Matrix metalloproteinases (MMP-2, MMP-7, MMP-9, TACE) and their substrates</td>
</tr>
<tr>
<td></td>
<td>NO, super anion, peroxynitrite</td>
</tr>
<tr>
<td></td>
<td>Cytokines: TNF-α, IL-1β, IL-6, IFN-α</td>
</tr>
<tr>
<td></td>
<td>Chemokines: MCP-1, RANTES, MIP-1α, SDF-1α</td>
</tr>
<tr>
<td></td>
<td>IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; MCP-1, monocyte chemotaxtractant protein-1; RANTES, regulated upon activation normal T cell suppressed and secreted; MIP-1α, macrophage inflammatory protein-1α; SDF, stromal derived factor</td>
</tr>
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</table>

Infectious recombinant clones to infect and spread in macrophage and mixed glial cultures; but these recombinants do not replicate in T cell analogue (HeLa/CD4) cultures. In addition, conditioned media from macrophages infected with recombinant viruses from HAD patients caused greater neuronal death when applied to human neural cultures. However, other studies have shown that T cell-tropic strains of HIV-1 induced the highest levels of neuronal injury when tested in an in vitro model of neuronal death, possibly through activation of CXCR4 expressed on neurons. Recent studies confirm the findings that brain-derived viruses use chiefly CCR5 and differ between patients with and without HAD, although the region of brain from which the virus was isolated may influence its replicative properties.

(ii) Viral proteins

There is extensive literature implicating several different virus-encoded proteins in HIV-1 neuropathogenesis. HIV-1 env-encoded gp120 has been shown to be directly and indirectly neurotoxic in vitro and in vivo. Specific domains within gp120 have been implicated as especially neuropathogenic, including the CD4 binding and the V3 regions. One proposed mechanism is the accumulation of intracellular calcium in neurons following activation of glutamate receptors and voltage-operated calcium channels. For example, indirect activation of the N-methyl D-aspartate (NMDA) receptor may result in neuronal death, through binding to the adjacent glycine receptor or increased free zinc concentrations. This binding can be blocked with several different NMDA receptor antagonists such as memantine and AP5. Other neurotransmitters may be affected by gp120-induced activation of NMDA receptors, such as impaired dopamine transport shown in dopaminergic neurons cultured from rat midbrain. Dawson et al have also shown that nitric oxide may modulate gp120 neurotoxicity. Transgenic mice expressing HIV-1 gp120 in astrocytes display neuropathological findings including astrogliosis, neuronal loss, and dendritic vacuolizations, resembling HIV encephalitis. Studies of gp120 action on glial cells suggest that the protein may alter glial function through perturbation of the Na+/H+ ion transporter(s) in astrocytes; this in turn might contribute to neuronal dysfunction and death. It has also been shown that gp120 affects intracellular signaling that controls the expression of different cell adhesion molecules, cytokines and perhaps nitric oxide through the JAK-STAT pathway. Recent studies suggest that mutations in gp120 may influence the induction of matrix metalloproteinases (MMP) by mechanism involving STAT-1 in macrophages. The above in vitro and in vivo findings support the hypothesis that gp120 is directly involved in the pathogenesis of HIV-induced neurological injury.

Other HIV proteins including Tat, gp41, and Nef have been shown to be neurotoxic in vitro. The transactivating protein, Tat, has attracted extensive attention in neuropathogenesis studies because early studies showed it was neurotoxic, released from infected lymphocytes, was taken up by cells and could, in turn, transactivate host genes such as TNF-α and IL-1. As with gp120, several domains within tat have been found to be especially neurotoxic including the basic region and the RGD amino acid residues in the second exon, but other groups have shown that the entire first exon is necessary for neurotoxicity. Conant and colleagues have shown that Tat induces the expression of MCP-1 in astrocytes, which may influence macrophage trafficking in the CNS. Our group has recently shown that brain-derived tat sequences induce the expression of MMP-2, which is neurotoxic in vitro and in vivo.

(iii) Viral load

Many studies indicate that viral replication in blood is extremely high in persons with HIV-1 infection and the balance established between viral replication and clearance shown in the viral load set point is predictive of the course of systemic disease. However, the role of viral load in brain in relation to the development of neurological disease is less clear. Viral load in CSF is usually several orders of magnitude less than plasma and is correlated to some extent with the presence of HAD. In addition, the source of virus in CSF may be derived from both blood and brain. Differing results depending on the method of viral or proviral quantitation have been reported from various groups examining the relationship between viral load in the CNS and the development of HIV dementia. QC-PCR studies of brain-derived viral mRNA and proviral DNA in brain indicate no significant difference in levels between AIDS patients with and without HIV dementia. In contrast, viral protein and RNA levels detected in CSF and viral antigen load, as detected by immunocytochemistry in brain, show elevated levels among patients with HAD compared to nondenminated AIDS controls. Other studies suggest that CSF viral load is correlated with neuropsychological abnormalities but is one hundred-fold less than that of plasma viral load. Viral load in the brain measured by immunostaining or quantitative molecular methods is closely associated with the extent of pathological change accompanying HIV encephalitis.

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(iv) Neuroinflammation

Excess production of host-encoded inflammatory molecules by microglia and perhaps astrocytes has been proposed as a chief cause of damage within the brain in a number of diseases including Alzheimer’s disease, stroke, multiple sclerosis and HAD. This hypothesis is predicated on data derived from cell culture experiments, animal models and studies of autopsy tissues. Among studies of HIV neuropathogenesis, this hypothesis has gained wide popularity because microglia and astrocytes are the principal cells infected or activated by HIV-1, resulting in the release of inflammatory and neurotoxic molecules (Table 1, Figure 4).

In 1990, Giulian and colleagues showed that HIV-infected monocytoid cell lines secreted diffusible molecules that killed several different neuronal cell types by a presumed excitotoxic mechanism, mediated by NMDA receptors. Pulliam et al also reported that HIV-infected macrophages produced a neurotoxic compound(s) causing cytopathic effects in cultured cell aggregates from human fetal brain tissue. In contrast, other groups have shown that only following direct contact with neurons in vitro, could HIV-infected monocytes induce neurotoxicity although a soluble neurotoxin could not be demonstrated. Despite the controversy surrounding this area, several potential neurotoxins have been identified and characterized in vitro and in vivo.

Multiple cytokines have been shown to be elevated in the brains and CSF of patients with HIV dementia, which include tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6) and tissue growth factor-beta (TGF-β). Although most cells in the CNS can produce cytokines, the chief sources of these small-secreted proteins are activated glial cells that include macrophages, microglia and astrocytes. TNF-α is an inflammatory cytokine that has received extensive attention for its potential neurotoxic effects in HIV infection and ability to influence the release of other cytokines. TNF-α is released by microglia and astrocytes in HIV infection and can prevent uptake of glutamate and may be directly toxic to neurons. Several studies have shown that TNF-α mRNA and protein levels are increased in the brains and CSF of patients with HIV infection. Notably, Wesselingh et al showed that TNF-α mRNA levels were increased in brains of patients with HIV dementia compared to AIDS patients without dementia or non-infected controls; furthermore, the level of mRNA was correlated with the severity of dementia. IL-6 has also been reported to be increased in the brains of patients with HIV infection and may mediate neurotoxicity indirectly. TGF-β and nerve growth factor (NGF) have been reported to be overexpressed in HIV-infected brain. The latter molecules have neurotrophic properties and, hence, their increased production may reflect a host defense response to the neurotoxic actions of HIV.

Cells of macrophage lineage also produce arachidonic acid and its metabolites. Griffin et al reported that prostaglandin E₂ and F₂α, and thromboxane B₂ were elevated in CSF from patients with HAD, compared to patients without dementia. Other groups have shown elevated levels of arachidonic acid metabolites including platelet-activating factor (PAF) in HIV-infected macrophages although most of the products were produced through the lipoxygenase pathway. It may be that arachidonic acid metabolites influence neurotoxicity indirectly by regulating the expression of glutamate uptake by astrocytes, as has been shown in vitro. Quinolinic acid (QA), a metabolite of tryptophan metabolism, is produced by macrophages following different types of stimulation. By binding to NMDA receptors, QA has been shown to have neurotoxic properties following acute or chronic exposure. QA levels in CSF appear to correlate with the severity of dementia. Likewise, increased levels of inducible nitric oxide synthase have been reported in the brains of patients with severe HAD, implicating nitric oxide as a potential neurotoxin. The inducible nitric oxide synthase levels also correlated with the levels of HIV gp41 expression in brain. An intriguing report described a novel neurotoxin, Ntox, which is released by activated microglial cells, although full characterization of the molecule is pending.

With the increased understanding in the role of chemokine receptors as co-receptors for HIV infection, a concomitant expanding interest has developed in the actions of chemokines in the nervous system in the context of HIV infection and other neurological diseases. Several chemokines are increased in the CSF and brains of patients with HIV infection, including MIP-1α and MIP-1β, regulated upon activation normal T cell suppressed and secreted (RANTES) and inflammatory protein-10. Other groups have shown that monocyte chemoattractant protein-1 (MCP-1) levels are increased in the CSF and brains of patients with HIV dementia while in vitro studies suggest that stromal derived factor-1 is neurotoxic. The exact role of these chemokines remains uncertain because several groups have shown that MIP-1α and RANTES are able to block gp120-induced neuronal death. Complementary studies show that different chemokines affect calcium signaling in neurons and demonstrate that chemokine receptors, which have shown to be expressed on neurons, may directly influence neuronal survival.

(v) Host susceptibility

Although multiple host molecules have been implicated in the inflammatory cascade of events causing HIV-associated neuronal and axonal injury, there have been few specific polymorphisms or mutations identified in genes associated with these molecules. However, the APOE E4 allele has been associated with an increased likelihood of developing HAD in one study and a recent study suggested that a polymorphism in TNF-α was associated with an increased risk of HAD. Two small studies have shown that a deletion in the CCR5 gene among heterozygotes is accompanied by a lower frequency of HAD occurrence. This latter finding complements other studies showing that CCR5 mediates both HIV infection and intracellular signaling pathways involved in inflammation. Clinical studies have reported slower progression of systemic disease among HIV-infection patients carrying the CCR5 deletion.

(v) Neuronal damage

There is increasing interest and understanding of the intracellular signaling pathways and mechanisms by which neurons are damaged and/or killed during ontogeny and disease. In the context of HIV infection, these questions are
just beginning to be addressed. Studies of autopsy tissues indicate several distinctive patterns of neuronal loss, including a reduction in neuronal cell body volume in the frontal cortices of patients. Diminished dendritic arborization and loss of presynaptic terminals have also been reported in the brains of HIV-infected individuals, which may reflect a retrograde phenomenon of white matter injury in some instances. Select neuronal sub-populations, including larger pyramidal cells within the cortex identified by stereological methods, are at greater risk of cell death and similarly neuronal populations defined by expression of certain neurotransmitters such as GABA or proteins, including parvalbumin and calbindin, are more likely to be diminished in HIV-infected brains. Synaptic density is diminished in patients with HIV-induced cognitive impairment. In contrast, other neuronal populations expressing neuropeptides such as somatostatin appear relatively resistant to HIV-induced injury. In some studies, the mechanism of cell death has been shown to be apoptotic, although this was not correlated with the occurrence of dementia. However, it is unclear at present if programmed cell death is the major mechanism of neuronal loss, nor is it apparent the extent to which glia die during the course of HIV infection of the brain, although a recent study indicated that HAD patients with rapid progression exhibited increased levels of astrocyte cell death. In vitro studies have implicated different intracellular signaling pathways in neurons as potential routes to cell death. For example, gp120 induces apoptosis in human fetal neurons through the activation of JNK and ERK pathways. Other studies suggest that gp120, derived from a T-cell tropic HIV strain, induced neuronal apoptosis that was mediated by p38 mitogen activated protein kinase. In vitro studies using HIV-1 tat indicate that the glycogen synthase kinase-3 beta and caspase 9 are also involved in neuronal death. Thus, these studies indicate that multiple pathways may determine the mechanism and frequency of HIV-related neuronal death.

THERAPEUTIC CONSIDERATIONS

The availability of HAART has revolutionized the care of patients with HIV infection in developed countries. Survival times have lengthened, and both general health and quality of life have been improved. HAART is usually comprised of three or more antiretroviral drugs including, most often, two nucleoside analogues and either a non-nucleoside reverse transcriptase inhibitor or an HIV viral protease inhibitor (Table 2). Signs and symptoms of HIV-related cognitive impairment, VM and DSP indicate several distinctive patterns of neuronal loss, including a reduction in neuronal cell body volume in the frontal cortices of patients. Diminished dendritic arborization and loss of presynaptic terminals have also been reported in the brains of HIV-infected individuals, which may reflect a retrograde phenomenon of white matter injury in some instances. Select neuronal sub-populations, including larger pyramidal cells within the cortex identified by stereological methods, are at greater risk of cell death and similarly neuronal populations defined by expression of certain neurotransmitters such as GABA or proteins, including parvalbumin and calbindin, are more likely to be diminished in HIV-infected brains. Synaptic density is diminished in patients with HIV-induced cognitive impairment. In contrast, other neuronal populations expressing neuropeptides such as somatostatin appear relatively resistant to HIV-induced injury. In some studies, the mechanism of cell death has been shown to be apoptotic, although this was not correlated with the occurrence of dementia. However, it is unclear at present if programmed cell death is the major mechanism of neuronal loss, nor is it apparent the extent to which glia die during the course of HIV infection of the brain, although a recent study indicated that HAD patients with rapid progression exhibited increased levels of astrocyte cell death. In vitro studies have implicated different intracellular signaling pathways in neurons as potential routes to cell death. For example, gp120 induces apoptosis in human fetal neurons through the activation of JNK and ERK pathways. Other studies suggest that gp120, derived from a T-cell tropic HIV strain, induced neuronal apoptosis that was mediated by p38 mitogen activated protein kinase. In vitro studies using HIV-1 tat indicate that the glycogen synthase kinase-3 beta and caspase 9 are also involved in neuronal death. Thus, these studies indicate that multiple pathways may determine the mechanism and frequency of HIV-related neuronal death.

Table 2: Therapeutics for HIV-related neurological disorders

<table>
<thead>
<tr>
<th>Drug – type</th>
<th>Neurological syndrome</th>
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</thead>
<tbody>
<tr>
<td><strong>A) Antiretroviral therapies:</strong></td>
<td></td>
</tr>
<tr>
<td>Nucleoside analogues: (AZT, 3TC, dT4, abacavir, DDI, DDC)</td>
<td>HAD/MCMD, DSP</td>
</tr>
<tr>
<td>Protease inhibitors: indinavir, ritonavir, nelfinavir, lopinavir, amprenavir</td>
<td>HAD/MCMD, DSP</td>
</tr>
<tr>
<td>Non-nucleoside RT inhibitors: efavirenz, nevirapine</td>
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<tr>
<td><strong>B) Neuroprotective therapies:</strong></td>
<td></td>
</tr>
<tr>
<td>OPC 14117 - antioxidant</td>
<td>HAD/MCMD</td>
</tr>
<tr>
<td>nimodipine - calcium channel blocker</td>
<td>HAD</td>
</tr>
<tr>
<td>selegiline (deprenyl) - antioxidant</td>
<td>HAD/MCMD</td>
</tr>
<tr>
<td>lexipafant - anti-PAF</td>
<td>HAD/MCMD</td>
</tr>
<tr>
<td>memantine - NMDA receptor antagonist</td>
<td></td>
</tr>
<tr>
<td>CN1189 - anti-TNFα</td>
<td>HAD</td>
</tr>
<tr>
<td>NGF - neurotrophin</td>
<td>DSP/TN</td>
</tr>
<tr>
<td><strong>C) Symptomatic therapies:</strong></td>
<td></td>
</tr>
<tr>
<td>Gabapentin</td>
<td>DSP/TN</td>
</tr>
<tr>
<td>Tegretol</td>
<td>DSP/TN</td>
</tr>
<tr>
<td>Tricyclic antidepressants</td>
<td>DSP/TN</td>
</tr>
<tr>
<td>Amanitadine/L-DOPA</td>
<td>Parkinsonism/HAD</td>
</tr>
</tbody>
</table>

1 DDI and DDC are highly associated with the development of TN.
2 Trials in progress.
AZT = zidovudine; DDI = didanosine; DDC = zalcitabine; 3TC = lamivudine; D4T = stavudine; RT = reverse transcriptase; PAF = platelet activating factor

nucleoside analogue, abacavir, failed to confer any improvement in cognition, probably due to pre-existing drug resistance mutations. Antiretroviral resistance mutations in the reverse transcriptase and protease encoding genes have been identified in viruses from patients who show high viral loads in blood despite HAART. The extent to which these mutations are present in the viruses found in the brains of patients treated with antiretroviral drugs is unknown. Brain-derived viruses exhibit fewer mutations associated with drug-resistance than matched blood-derived HIV isolates. This may reflect poor CNS drug penetration and/or limited replication in the brain, which would diminish the potential for drug resistant mutations to emerge but may also simply reflect sampling artifact.

Neuroprotective strategies have also been employed for both neurocognitive impairment and peripheral neuropathy in HIV-1 infection. A recent randomized clinical trial (RCT) showed that NFG was beneficial in terms of reducing symptoms related to DSP and TN. The antioxidant, selegiline (deprenyl) has been shown to improve neuropsychological performance in patients with HIV-related neurocognitive impairment in a small RCT and this preliminary finding has led to the design of a larger trial. Lexipafant, an antagonist of the putative neurotoxin, PAF,
showed a trend towards improvement in HIV-related neurocognitive impairment in a small RCT. Likewise, an antioxidant, OPC-14117 and the calcium channel blocker, nimodipine showed beneficial trends in small RCT. Several trials are in progress testing compounds including a NMDA antagonist, memantine, and a TNF-α inhibitor, CN-1189.

**FUTURE ISSUES**

Although the AIDS epidemic continues to expand with ever increasing numbers of infected individuals, many important questions remain unanswered regarding the pathogenesis and optimal treatment of HIV-related neurological disorders. The potential role of the brain as a protected viral reservoir for drug resistant strains of virus or as a reservoir that can re-seed the systemic circulation after the virus has been eradicated in other sites is not resolved. Most HIV neuropathogenesis studies using human samples performed to date have focused on gay males infected with clade B viruses from North American and Europe. The extent to which this population sample reflects the entire spectrum of the neuropathogenesis of HIV-1 is uncertain. Finally, the exact function(s) and regulation of the plethora of inflammatory molecules released by macrophages infected by or exposed to HIV have yet to be defined. This latter question is of interest because it may provide valuable insights into the mechanisms of neuronal injury mediated by HIV infection. At the same time, understanding the role(s) of these inflammatory molecules will provide clues to the pathogenesis and rational therapy of other neurological diseases characterized by glial activation and neuronal damage.

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