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Chemokine receptors and mechanisms of cell death in HIV neuropathogenesis

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Several chemokine receptors are used as coreceptors for HIV-1 entry in the central nervous system (CNS). CCR5 is the major coreceptor together with CD4 for HIV-1 infection of microglia, the major target cells for HIV-1 infection in the CNS. CXCR4 and CCR3 are also expressed on microglia and can mediate infection by certain HIV-1 isolates but at lower efficiency than CCR5. Additional chemokine coreceptors are expressed in the brain, but their role in HIV-1 neuropathogenesis has not been defined. The expression of CXCR4, and possibly other chemokine receptors, on subpopulations of neurons and glial cells may render neurons vulnerable to mechanisms of CNS injury induced by the HIV-1 gp120 Env protein. HIV-1 viruses which use CXCR4 and emerge during the late stages of HIV-1 infection may impact disease progression in the CNS by inducing apoptosis of neurons and other cell types. The neurodegenerative mechanisms may involve infection of microglia by certain CXCR4 tropic viruses in addition to cellular dysfunction and apoptosis induced by HIV-1 gp120 binding to CXCR4. Understanding the role of CXCR4 and other chemokine receptors in HIV-1 neuropathogenesis will help to advance the development of new therapeutic strategies for the prevention and treatment of neurologic disorders associated with HIV-1 infection. *Journal of NeuroVirology* (2000) **6,** S24–S32.

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Introduction

Human immunodeficiency virus type I (HIV-1) infects the brain and frequently causes dementia and related neurologic disorders in children and adults with AIDS (reviewed in Lipton and Gendelman, 1995; Price, 1996). HIV-1 enters the brain via the passage of infected monocytes, and possibly CD4+ T lymphocytes, across the blood-brain barrier. Most of the HIV-1-infected cells in the brain are macrophages and microglia. A low level of infected astrocytes and brain capillary endothelial cells has also been detected. HIV-1 entry into macrophages and microglia requires CD4, the primary receptor for HIV-1, whereas entry into astrocytes and endothelial cells is CD4-independent. The mechanisms that lead to brain injury in HIV-1 infection are poorly understood. Neuropathological abnormalities in the brains of patients with HIV-1 encephalitis include brain atrophy, reactive astrocytosis, myelin pallor, microglial nodules, perivascular inflammation, multinucleated giant cells, abnormal blood-brain barrier permeability, and neuronal loss (Lipton and Gendelman, 1995; Price, 1996). Diffuse proliferation and immune activation of macrophages and microglia in the brain correlates with the severity of clinical dementia. The most commonly affected brain regions are the white matter and basal ganglia, followed by the cerebral cortex.

Apoptosis of neurons and possibly other cell types is a likely result of CNS injury in AIDS. Apoptosis of neurons and astrocytes is induced by infection with certain HIV-1 isolates *in vitro* (Shi *et al*, 1996; Öhagen *et al*, 1999; Power *et al*, 1998; Zheng *et al*, 1999a) and has been demonstrated in autopsy brain tissues from children and adults with AIDS (Adie-Biassette *et al*, 1995; Gelbard *et al*, 1995; Petito and Roberts, 1995; An *et al*, 1996; Shi *et*

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al, 1996; Vallat *et al*, 1998; Gray *et al*, 1999). Neurons are not directly infected by HIV-1, suggesting that neuronal apoptosis is likely to be induced by soluble factors. Several candidates for soluble proapoptotic factors that may lead to neuronal cell death in HIV-1 infection have been proposed based on *in vitro* and animal model studies (reviewed in Lipton and Gendelman, 1995). These include soluble forms of the HIV-1 gp120 and Tat proteins, and factors secreted by infected or activated macrophages and microglia, such as TNF-α, oxygen free radicals, nitric oxide, and excitatory amino acids, and other yet unknown factors. However, the *in vivo* role of these factors in contributing to apoptosis in the brains of AIDS patients has not been established.

The role of strain variability in the pathogenesis of HIV-1 dementia is unknown. The genetic evolution of HIV-1 viruses within the brain is distinct from that in lymphoid tissues and other organs (Korber *et al*, 1994; Donaldson *et al*, 1994; Power *et al*, 1994; Hughes *et al*, 1997; Wong *et al*, 1997; van't Wout *et al*, 1998). Specific sequences in the Env, particularly the V3 region, are associated with brain infection (Korber *et al*, 1994; Power *et al*, 1994 and 1998; Hughes *et al*, 1997; Wong *et al*, 1997; van't Wout *et al*, 1998). However, specific determinants of HIV-1 neurotropism or neurovirulence have not been identified (Simmonds, 1996). Infection of the CNS by M-tropic strains of HIV-1 or SIV is not sufficient to cause dementia or encephalitis (Korber *et al*, 1994; Power *et al*, 1994; Mankowski *et al*, 1994; Joag *et al*, 1995), suggesting that neurovirulence is likely to be determined by genetic or biological characteristics that are distinct from macrophage-tropism.

Chemokine receptors mediate HIV-1 entry

Several chemokine receptors are used together with CD4 as coreceptors for HIV-1 entry (reviewed in Berger, 1997; Berger *et al*, 1999; Doms and Peiper, 1997; Littman, 1998; Rucker and Doms, 1998; Dimitrov *et al*, 1998). The cellular tropism of HIV-1 is determined by the interaction of the HIV-1 gp120 envelope glycoprotein with a particular chemokine coreceptor. Macrophage-tropic (R5 or M-tropic) HIV-1 viruses use CCR5 as a coreceptor, whereas T cell line-tropic (X4 or T-tropic) HIV-1 viruses use CXCR4. Dual-tropic viruses (R5X4) use both coreceptors. A subset of HIV-1, HIV-2, or simian immunodeficiency (SIV) viruses can also use CCR3 or one or more of several alternative chemokine coreceptors such as CCR2b, CCR8 (ChemR1/TER-1), CX3CR1 (V28), Apj, STRL33/BONZO, gpr1, gpr15/BOB, ChemR23, and US28 (reviewed in Berger *et al*, 1999; Doms and Peiper, 1997; Littman, 1998; Rucker and Doms, 1998; Dimitrov *et al*, 1998), but the role of these alternative coreceptors *in vivo* is unknown. R5 viruses are usually involved in virus transmission and can be isolated throughout the course of disease. X4 or R5X4 viruses arise in the later stages of disease in 40–50% of infected adults and are frequently associated with rapid disease progression (Björndal *et al*, 1997; Connor *et al*, 1997). It is late in the course of disease progression, when X4 and R5X4 isolates emerge, that neurological symptoms such as dementia typically arise. In some individuals, disease progression is associated with a general broadening of virus tropism with expansion of coreceptor usage (Björndal *et al*, 1997; Connor *et al*, 1997). CCR5 is expressed on activated memory T cells and monocyte/macrophages. CXCR4 is expressed in a broader range of cell types which include naive T cells and monocyte/macrophages. The regulation of CCR5 and CXCR4 expression on the cell surface can affect the efficiency of HIV-1 replication (Bleul *et al*, 1997; Carroll *et al*, 1997; Di Marzio *et al*, 1998).

Genetic polymorphisms in CCR5, CCR2, and SDF-1 alleles, particularly the CCR5Δ32 mutation, have been associated with effects on susceptibility to HIV-1 infection or disease progression (reviewed in Berger *et al*, 1999). Regarding the possible role of these genetic polymorphisms in determining susceptibility to CNS disease, one study suggested that heterozygosity for the CCR5Δ32 allele does not reduce the risk of developing HIV-1 dementia (Barroga *et al*, 1997). However, this issue has not been fully explored and further studies are required to determine whether individuals who harbor chemokine or chemokine receptor polymorphisms show altered susceptibility for HIV-1 encephalopathy.

HIV-1 Env-coreceptor interactions

The proposed model for chemokine coreceptor function is that high affinity binding of the HIV-1 gp120 envelope glycoprotein to CD4 induces a conformational change in gp120 which increases the affinity of gp120 for CCR5 or CXCR4 by exposing the chemokine receptor binding site (reviewed in Berson and Doms, 1998; Dimitrov *et al*, 1998; Berger *et al*, 1999). This leads to a subsequent trimolecular interaction between the Env-CD4 complex and coreceptor, which triggers fusion by exposing the fusion domain of gp41. Factors that may influence the efficiency of virus entry in a cell-dependent manner include post-translational modifications of HIV-1 coreceptors (Berson and Doms, 1998), the efficiency of coreceptor-CD4 interactions at the cell surface (Lapham *et al*, 1999; Dimitrov *et al*, 1999), and other cell surface factors such as glycosaminoglycans or adhesion molecules. Env-coreceptor interactions can occur in the absence of CD4 (Hesselgesser *et al*, 1997; Berson and Doms, 1998), but these CD4-independent interactions do not necessarily allow virus entry. Virus entry mediated by chemokine receptors does not appear to require signaling, since signaling-defective mutants of

CCR5 or CXCR4 can still support HIV-1 infection (Littman, 1998). However, binding of soluble or virion-associated gp120 to CCR5 or CXCR4 can activate cellular kinases and signaling pathways (Davis *et al*, 1997; Weissman *et al*, 1997; Hesselgesser *et al*, 1997), raising the possibility that Env-coreceptor interactions may lead to alterations in cellular signaling pathways that may contribute to mechanisms of pathogenesis in infected cells as well as uninfected bystander cells (Hesselgesser *et al*, 1998; Herbein *et al*, 1998).

Chemokine coreceptors in HIV-1 infection of the CNS

Several chemokine receptors that mediate HIV-1 entry are expressed in the CNS (reviewed in Lavi *et al*, 1998; Glabinski and Ransohoff, 1999; Hesselgesser and Horuk, 1999). The role of chemokine receptors in HIV-1 entry and pathogenesis in the CNS is reviewed in another issue of this journal (Gabuzda *et al*, 1999). In the CNS, CCR5 is predominantly expressed on perivascular mononuclear cells, macrophages, and microglia (He *et al*, 1997; Lavi *et al*, 1997; Ghorpade *et al*, 1998(a); Vallat *et al*, 1998; Sanders *et al*, 1998; Westmoreland *et al*, 1998; Albright *et al*, 1999; Shieh *et al*, 1998; Zhang *et al*, 1998; Rottman *et al*, 1997). In view of this, it will be of interest to determine the relationship between CCR5-positive mononuclear cells trafficking into the brain of individuals with HIV-1 encephalitis and the activated $CD14^+/CD16^+/CD69^+$ monocyte subset that is increased in peripheral blood of AIDS patients with dementia (Pulliam *et al*, 1997; Gartner *et al*, 1999). CXCR4 is expressed on perivascular mononuclear cells, macrophages, microglia, endothelial cells, and in neuronal and astrocyte subpopulations in various regions of cerebral cortex and other brain regions (Lavi *et al*, 1997; Hesselgesser *et al*, 1997; Vallat *et al*, 1998; Sanders *et al*, 1998; Zhang *et al*, 1998; Klein *et al*, 1999). CCR3 is expressed predominantly on microglia (He *et al*, 1997; Ghorpade *et al*, 1998b; Sanders *et al*, 1998; Albright *et al*, 1999; Zhang *et al*, 1998). Additional orphan chemokine coreceptors such as Apj, STRL33/BONZO, and gpr1 are also expressed in the brain or neurally-derived cell lines, but their expression on specific cell types has not been defined. Recent studies suggest that chemokine receptors and their chemokine ligands play a role in brain development and other biological functions in the CNS, in addition to their involvement in inflammatory responses and regulating cell trafficking across the blood-brain barrier. For example, CXCR4 knock-out mice demonstrate a defect in cerebellar development (Ma *et al*, 1998; Zou *et al*, 1998), and several studies suggest that chemokine receptor can mediate cell-to-cell communication between different CNS cell populations, neuronal signaling, and neuronal migration (Hesselgesser *et al*, 1997; Bolin *et al*, 1998; Harrison *et al*, 1998; Meucci, 1998; Zheng *et al*, 1999a,b).

Chemokine coreceptors play an important role in HIV-1 neurotropism. CCR5 is the major coreceptor for HIV-1 infection of macrophages and microglia (He *et al*, 1997; Ghorpade *et al*, 1998a; Shieh *et al*, 1998; Albright *et al*, 1999). CXCR4 or CCR3 can also mediate infection of microglia by some neurotropic isolates (He *et al*, 1997; Albright *et al*, 1999). Most laboratory-adapted X4 viruses do not replicate efficiently in macrophages and microglia. The inefficient replication of many X4 HIV-1 viruses in macrophages and microglia may be due to a coreceptor-dependent block in post-entry events, such as the inability of CXCR4 to activate a requisite signal transduction pathway (Schmidtmayerova *et al*, 1998), or cell-specific factors such as the relatively low level of CD4 expression (Kozak *et al*, 1997; Platt *et al*, 1997) or a reduced ability of CXCR4 to associate with CD4 compared to CCR5 (Lapham *et al*, 1999; Dimitrov *et al*, 1999). However, we have shown that a subset of primary X4 viruses (i.e. SG3 and ELI) replicate relatively efficiently in microglia (Öhagen *et al*, 1999), consistent with previous studies in primary macrophages (Simmons *et al*, 1998). Apj, CCR8, gpr 15, and STRL33/BONZO can be used as coreceptors by some brain-derived viruses albeit at lower efficiency than CCR5 (Albright *et al*, 1999), but the role of these and other unidentified coreceptors (Ghorpade *et al*, 1998a) in mediating infection of microglia remains to be determined.

CCR5 chemokines (MIP-1, RANTES) and in some cases CXCR4 or CCR3 chemokines (SDF-1 and eotaxin, respectively), as well as anti-CCR5 and in some cases anti-CXCR4 or anti-CCR3 monoclonal antibodies can inhibit HIV-1 infection of microglia by isolates that use those coreceptors (He *et al*, 1997; Shieh *et al*, 1998; Ghorpade *et al*, 1998a). Chemokine inhibition of HIV-1 infection can involve one or more of several different mechanisms: (1) direct competitive inhibition of the HIV-1 gp120-coreceptor interaction; (2) downregulation of the autologous chemokine receptor; (3) cross-regulation of expression or function of a heterologous chemokine receptor; (4) effects on cellular signaling pathways that influence HIV-1 replication via effects on virus entry, post-entry events, viral gene expression, or other steps in the viral life cycle (Schmidtmayerova *et al*, 1998; Gordon *et al*, 1999; reviewed in Berger, 1997; Berger *et al*, 1999; Rucker and Doms, 1998; Dimitrov, 1998). Which of these mechanisms is involved in inhibition of microglial cell infection remains to be determined. Chemokine inhibition of virus entry can give variable results depending on the particular cell type, viral isolate, and cell culture conditions. For example, RANTES can actually enhance rather than inhibit HIV-1 entry in some contexts through a mechanism(s) that

appears to be independent of the route of virus entry (Gordon *et al*, 1999), a finding which may help to explain discrepancies between different studies variably reporting either RANTES inhibition or enhancement of HIV-1 infection (Berger, 1997; Gordon *et al*, 1999). In the case of CCR3-mediated HIV-1 infection of microglia, some studies found inhibition of viral infection (He *et al*, 1997), while others found minimal or no inhibitory effect (Shieh *et al*, 1998; Ghorpade *et al*, 1998a; Albright *et al*, 1999). Our studies on HIV-1 coreceptors on microglia were performed in primary human fetal brain cultures which contain a mixture of astrocytes, neurons, and microglia (He *et al*, 1997), while other studies have used purified human fetal or adult microglia (Shieh *et al*, 1998; Ghorpade *et al*, 1998a; Albright *et al*, 1999). In fact, we have found that the expression of CCR3 and CXCR4 and to a lesser extent CCR5 on microglia is highly dependent upon cell culture conditions and the cytokine environment (D Gabuzda and J Wang, unpublished data). Together, these findings suggest that the variable level of CCR5, CCR3, and CXCR4 expression or chemokine inhibition reported in different studies of cultured human microglia may reflect the cytokine environment, cell culture conditions, possible differences between fetal and adult cells, and other variables discussed above.

Astrocytes and endothelial cells express low levels of CXCR4 (Lavi *et al*, 1997; Sanders *et al*, 1998; Klein *et al*, 1999). Astrocyte expression of CXCR4 can be upregulated by certain proinflammatory cytokines (TNF-α and IL-1β) (D Gabuzda and J Wang, unpublished data). However, the role of chemokine coreceptors in CD4-independent HIV-1 infection of astrocytes and capillary endothelial cells has not been determined. Previous studies have shown that Env-coreceptor interactions can occur in the absence of CD4. Some HIV-2 viruses use CXCR4 for CD4-independent entry (Endres *et al*, 1996). A neurovirulent strain of SIV uses CCR5 for CD4-independent infection of brain capillary endothelial cells (Edinger *et al*, 1997). In the case of HIV-1, CD4-independent HIV-1 isolates have been generated *in vitro* (Dumonceaux *et al*, 1998), but naturally occurring HIV-1 variants that can use CXCR4 or other chemokine receptors for CD4-independent entry have not been identified. Studies are in progress to determine the role of chemokine receptor(s) and other as yet unidentified receptors in mediating CD4-independent infection of astrocytes and endothelial cells.

Role of CXCR4 in neuronal apoptosis

Several lines of evidence suggest that the expression of CXCR4, and possibly other chemokine receptors, on subpopulations of neurons in cerebral cortex and other brain regions (Lavi *et al*, 1997; Hesselgesser *et al*, 1997; Vallat *et al*, 1998; Sanders *et al*, 1998; Zhang *et al*, 1998) may render neurons vulnerable to mechanisms of CNS injury induced by soluble forms of the HIV-1 gp 120 Env protein (Lipton and Gendelman, 1995) or by chemokines (Hesselgesser *et al*, 1998; Zheng *et al*, 1999a,b). Soluble HIV-1 gp 120 can bind to CXCR4 on neurons in the absence of CD4 (Hesselgesser *et al*, 1997) and thereby induce neuronal signaling and apoptosis (Hesselgesser *et al*, 1998; Meucci *et al*, 1998; Zheng *et al*, 1999a,b). In a recent study, we demonstrated that certain HIV-1 isolates which use CXCR4 can induce apoptosis of neurons and astrocytes *in vitro* (Öhagen *et al*, 1999). We examined the ability of diverse blood and brain HIV-1 isolates to replicate and induce neuronal and astrocyte apoptosis in primary human fetal brain cultures. HIV-1 isolates were shown to differ in the ability to induce neuronal apoptosis, and the ability to induce apoptosis was independent of replication capacity. Surprisingly, apoptosis was induced by infection with three X4 or X4R5 blood isolates (89.6, SG3, ELI) whereas five R5 brain isolates (YU2, JRFL, DS-br, KJ-br, and RC-br) replicated but did not induce apoptosis (Öhagen *et al*, 1999). These results suggest that X4 viruses are more cytopathic in the CNS. However, analysis of additional blood isolates showed that the R5 isolate ADA but not the X4 or X4R5 isolates DH123, NL4-3, and HXB2 induced neuronal apoptosis (Öhagen *et al*, 1999). Thus, CXCR4 usage is neither necessary nor sufficient to induce apoptosis. Replacement of the *env* gene in YU2 with the X4 Env of SG3 was sufficient to confer the apoptosis-inducing phenotype to an otherwise non-apoptosis inducing virus (Öhagen *et al*, 1999). Replacement of the Env V3 regions alone, which contain the major determinants of coreceptor usage, largely conferred the phenotype of the parental clones, but regions outside V3 also contributed to the cytopathic effects of Env. In a recent study, Zheng *et al* (1999a) showed that virions from X4 viruses (MN, IIIB, and Lai) induced the highest levels of neuronal apoptosis whereas virions from R5 viruses (ADA, JRFL, Bal, MS-CSF, and DJV) induced markedly less neuronal apoptosis. Together, these results provide evidence that the Env is a major determinant of neurodegenerative mechanisms associated with HIV-1 infection *in vitro* and raise the possibility that blood-derived viruses which use CXCR4 and emerge during the late stages of disease may impact disease progression in the CNS.

The possibility that blood-derived HIV-1 strains which use CXCR4 may contribute to disease progression in the CNS is consistent with several previous observations: (1) X4 viruses emerge in the later stages of disease, which is the time when HIV-1 dementia occurs; (2) X4 HIV-1 viruses are generally more cytopathic than R5 HIV-1 viruses for uninfected bystander cells in the immune system, including $CD4^+$ T cells, $CD8^+$ T cells, and B cells (Herbein *et al*, 1998 and references therein; Glush-

akova *et al*, 1998); (3) a neurovirulent SHIV (a chimeric simian-human immunodeficiency virus that contains the *tat, rev, vpu*, and *env* genes from the T-cell line-tropic laboratory-adapted HIV-1 IIIB isolate and adapted for growth in macrophages) that causes AIDS, neurological disease, and renal disease in rhesus macaques uses only CXCR4 (Liu *et al*, 1999). Furthermore, phylogenetic analysis of blood- and brain-derived Env sequences suggests that some trafficking of virus from blood into brain occurs in a subset of AIDS patients (Korber *et al*, 1994; Wong *et al*, 1997; van't Wout *et al*, 1998; Chang *et al*, 1998; Morris *et al*, 1999). Env V3 region sequences with characteristics of T-tropic of dual-tropic HIV-1 strains have been detected in the brain, albeit at low frequency (Korber *et al*, 1994; Chang *et al*, 1998). Blood-derived variants have also been detected in the choroid plexus (Morris *et al*, 1999). Disruption of the blood-brain barrier (Petito and Cash, 1992; Power *et al*, 1993) may increase CNS entry of blood-derived viruses in individuals with advanced disease.

CXCR4-mediated mechanisms of neuronal injury may not necessarily require virus replication. For example, gp120 binding to CXCR4 on the surface of macrophages, microglia, or astrocytes could activate production of a neurotoxin (Zheng *et al*, 1999a,b). SDF-1, the CXCR4 ligand, is expressed in the brain and can induce signaling and chemotaxis in human neurons *in vitro* (Hesselgesser *et al*, 1997; Zheng *et al*, 1999b). This effect can be inhibited by soluble HIV-1 gp120 (Hesselgesser *et al*, 1997), raising the possibility that gp120 neurotoxicity (reviewed in Lipton and Gendelman, 1995) may involve CD4-independent binding of gp120 to CXCR4 and competition for natural ligands (Madani *et al*, 1998; Meucci *et al*, 1998). However, effects of SDF-1 on neurons can be pro-apoptotic at least in some settings (Hesselgesser *et al*, 1998; Zheng *et al*, 1999b). This observation together with the finding that SDF-1 is expressed in the brain of patients with HIV-1 dementia and is upregulated in astrocytes exposed to HIV-1-infected or activated macrophage conditioned media (Zheng *et al*, 1999b) raises the possibility that increased SDF-1 production by astrocytes in the presence of X4 isolates is another potential mechanism that could lead to neuronal injury. Together, these findings suggest that the expression of CXCR4, and possibly other chemokine receptors, on neurons and other cell types in the CNS is likely to contribute to mechanisms of CNS injury associated with HIV-1 infection. Whether CXCR4-mediated mechanisms of neuronal apoptosis result from agonistic or antagonistic effects on signaling pathways that influence cell survival remains to be determined. A recent study examined the possible involvement of specific intracellular signaling pathways in chemokine receptor-mediated neuronal apoptosis induced by diverse HIV-1 isolates and found evidence for involvement of the PKA pathway and activation of inositol 1,4,5-triphosphate (Zheng, *et al*, 1999a). This study showed that HA1004, a drug which inhibits PKA, PKC, and calcium/calmodulin-dependent protein kinase II (but not drugs which selectively inhibit PKC or the p42/44 and p38 mitogen-activated protein kinases) blocked CXCR4-mediated neuronal apoptosis. However, the specific mechanisms which link intracellular signaling to neuronal apoptosis are unknown and require further study.

The *in vivo* role of CXCR4 and other chemokine receptors in HIV-1 dementia remains to be established. HIV-1 dementia or encephalopathy can occur in individuals who progress to AIDS in the absence of X4 or R5X4 viruses (Brew *et al*, 1996), particularly in children (Fitzgibbon *et al*, 1998). Furthermore, a neurovirulent strain of SIV uses CCR5, but not CXCR4 (Edinger *et al*, 1997). These findings together with the studies described above are consistent with a model in which viral determinants in addition to CXCR4 usage also contribute to viral cytopathicity in the CNS. Additional determinants for the apoptosis-inducing phenotype may also be outside the *env* gene. For example, the HIV-1 Nef and Tat proteins have been proposed to have neurotoxic activity. Studies are in progress to determine whether HIV-1 dementia and related neurological disorders is more frequent in patients who harbor syncytium-inducing X4 and X4R5 isolates. The apoptosis-inducing isolates identified in our studies were all blood-derived. However, our findings do not exclude the existence of brain-derived viruses that induce apoptosis or other cytopathic effects in neurons or other cell types, particularly since only a limited number of brain-derived viruses were analyzed. It will be important to analyze a larger series of blood-, CSF, and brain-derived viruses in future studies to elucidate the relationship between tissue-specific variants, viral phenotypes, coreceptor usage, and HIV-1 pathogenicity in the CNS.

Conclusions

Recent studies suggest that the Env is a major determinant of neurodegenerative mechanisms associated with HIV-1 infection and raise the possibility that blood-derived viruses which use CXCR4 and emerge during the late stages of disease may impact disease progression in the CNS by inducing cellular dysfunction and apoptosis of neurons and possibly other cell types. The neurodegenerative mechanisms may involve infection of microglia by certain X4 primary isolates in addition to effects induced by HIV-1 gp120 binding to CXCR4 on microglia, astrocytes, or neurons. Uninfected bystander cell death in the immune and central nervous systems may share common mechanisms, since X4 viruses have been shown to induce cellular dysfunction and apoptosis in

uninfected $CD8^+$ T cells and B cells (Herbein *et al*, 1998; Glushakova *et al*, 1998). Cytokines and other stimuli may impact CXCR4-mediated mechanisms of cell death by their effects on CXCR4 expression. HIV-1 chemokine coreceptors are promising targets for therapeutic intervention. Several compounds that selectively block virus entry without affecting normal physiological functions of these receptors or accelerating the selection of strains with broader tropism are being developed. HIV-1 infection mediated by CCR5 can be inhibited by drugs such as TAK-779 and RANTES derivatives such as AOP-RANTES. CXCR4-mediated infection can be inhibited by drugs such as AMD3100, ALX40-4C, and T22. Other therapeutic strategies targeted at inhibition of HIV-1 coreceptors are also being developed (reviewed in Cairns and D'Souza, 1998; Berson and Doms, 1998; Dimitrov, 1998; Berger *et al*, 1999; Michael and Moore, 1999). Understanding the role of CXCR4 and other chemokine receptors in HIV-1 neuropathogenesis will be important for advancing the development of new therapeutic strategies for the prevention and treatment of neurologic disorders associated with HIV-1 infection.

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