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Review

Chemokine receptors and virus entry in the central nervous system

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Several members of the chemokine receptor family are used as coreceptors together with CD4 for HIV and SIV entry in the central nervous system (CNS). CCR5 is the major coreceptor for HIV-1 infection of macrophages and 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 receptors that can function as HIV-1 and SIV coreceptors for a subset of viruses are expressed in the brain (i.e. Apj, CX3CR1, STRL33/BONZO, and gpr1), but their role in CNS infection has not been defined. The expression of CXCR4, and possibly other chemokine receptors, on subpopulations of neurons and glial cells may contribute to mechanisms of CNS injury that are independent of viral infection. Understanding the role of chemokine receptors and their chemokine ligands in HIV-1 and SIV infection of the CNS will elucidate mechanisms of viral tropism and pathogenesis and advance the development of new therapeutic strategies.

Keywords: HIV-1; SIV; chemokine receptor; coreceptor; microglia; brain

Introduction

Human Immunodeficiency Virus type I (HIV-1) infects the brain and frequently causes dementia and other neurologic disorders in patients with AIDS (reviewed in Lipton and Gendelman, 1995; Price, 1996; Gabuzda *et al*, 1998). HIV-1 enters the brain early in the course of infection through the passage of infected monocytes and possibly CD4+ T lymphocytes, across the blood-brain barrier (reviewed in Gendelman *et al*, 1994; Gabuzda *et al*, 1998). Most of the HIV-1-infected cells in the brain are macrophages and microglia. Infected astrocytes and brain capillary endothelial cells are infrequently detected. HIV-1 entry into macrophages and microglia requires CD4, the primary receptor for HIV-1, whereas entry into astrocytes, endothelial cells, and some neurally-derived cell lines is CD4-independent (Kunsch *et al*, 1989; Harouse *et al*, 1989, 1991; Moses *et al*, 1993). Simian immunodeficiency virus (SIV) infection of macaques can cause neurological disease with clinical and pathological similarities to HIV-1 dementia.

Chemokine receptors are G protein-coupled seven-transmembrane domain receptors that mediate cellular responses involved in chemotaxis, cell migration and trafficking, and activation of leukocytes when activated by their chemokine ligands (reviewed in Baggiolini, 1997; Rollins, 1997; Luster, 1998). Several members of the chemokine receptor family are used together with CD4 for HIV and SIV entry (Table 1) (reviewed in Berger, 1997; Doms and Peiper, 1997; Littman, 1998; Rucker and Doms, 1998; Dimitrov *et al*, 1998; Berger *et al*, 1999; Edinger *et al*, 1999). CCR5 and CXCR4 are the major coreceptors for HIV-1 infection. The tropism of HIV-1 is determined by the specificity of the interaction of the HIV-1 gp120 envelope glycoprotein with a particular coreceptor. Macrophage-tropic (R5 or M-tropic) HIV-1 viruses use CCR5 as a coreceptor, (Alkhatib *et al*, 1996; Deng *et al*, 1996; Dragic *et al*, 1996; Doranz *et al*, 1996; Choe *et al*, 1996) whereas T cell line-tropic (X4 or T-tropic) HIV-1 viruses use CXCR4 (previously called fusin, HUMSTR, LESTER) (Feng *et al*, 1996). Dual-tropic viruses (R5X4)

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Table 1 Expression of HIV/SIV chemokine coreceptors in the CNS

Coreceptor	*Ligand(s)*	*Chemokine coreceptor expression in CNS*	*Viral usage*
Major coreceptors			
CCR5	MIP-1α, MIP-1β, RANTES	Macrophages, microglia	M-tropic HIV-1
CXCR4	SDF-1		HIV-2, SIV
		Macrophages, microglia, T cells, astrocytes, neuronal subpopulations, endothelial cells	T-tropic HIV-1 HIV-2
Minor coreceptors			
CCR2b	MCP-1, MCP-2, MCP-3	?	Some M-tropic HIV-1 Some HIV-2
CCR3	Eotaxin, RANTES, MCP-3, MCP-4	Microglia, Th2 cells	Some M- or T-tropic HIV-1 Some HIV-2
CCR8 (Chem R1/TER-1)	I-309	?	Some HIV-1, HIV-2, SIV
CX3CR1 (V28)	Fractalkine	Macrophages, microglia	HIV-2 (minor HIV-1)
APJ	?	Glial and neuronal subpopulations	Some HIV-1, HIV-2, SIV
STRL33/BONZO	?	? Glial subpopulations	HIV-2, SIV (minor HIV-1)
GPR1	?	? Glial subpopulations	SIV (minor HIV-1 and HIV-2)
GPR15 (BOB)	?	?	HIV-2 SIV (minor HIV-1)
Chem R23	?	?	Some HIV-2, SIV

use both coreceptors. A subset of viruses can also use CCR3 or other chemokine receptors such as CCR2b, CCR8 (ChemR1/TER-1), CX3CR1 (V28), or orphan receptors such as Apj, STRL33/BONZO, gpr1, gpr15/BOB, and ChemR23 (Choe *et al*, 1996, 1998; Doranz *et al*, 1996; Deng *et al*, 1997; Farzan *et al*, 1997; Liao *et al*, 1997; Reeves *et al*, 1997; Rucker *et al*, 1997; Edinger *et al*, 1998a,b; Horuk *et al*, 1998; Jinno *et al*, 1998; Samson *et al*, 1998; Shimizu *et al*, 1999), but the role of these alternative coreceptors *in vivo* is unknown. The US28 chemokine receptor homolog encoded by human cytomegalovirus can also be used by some HIV and SIV viruses (Pleskoff *et al*, 1997; Rucker *et al*, 1997). The use of these alternative coreceptors by most strains of HIV-1 is inefficient compared to the use of CCR5 or CXCR4. Infection by most M- and T-tropic strains of simian immunodeficiency virus (SIV) is mediated by CCR5 but not CXCR4 (Chen *et al*, 1997; Marcon *et al*, 1997; reviewed in Edinger *et al*, 1999). Alternative coreceptors such as CCR2b, gpr1, gpr15/BOB, and STRL33/BONZO can also serve as coreceptors for some SIV isolates (Deng *et al*, 1997; Farzan *et al*, 1997; Liao *et al*, 1997; Edinger *et al*, 1998a). In contrast to HIV-1, some HIV-2 or SIV isolates can use certain alternative coreceptors such as BONZO/STRL33 and gpr15/BOB as efficiently as CCR5. Feline immunodeficiency virus (FIV) infection can be mediated by CXCR4 (Willett *et al*, 1997), although CXCR4 does not appear to be the primary receptor. The usage of chemokine receptors as coreceptors by other lentiviruses such as visna virus and caprine arthritis encephalitis virus has not been determined.

Changes in coreceptor use frequently correlate with disease progression in HIV-1-infected individuals (Jansson *et al*, 1996; Simmons *et al*, 1996; Connor *et al*, 1997; Björndal *et al*, 1997). HIV-1 viruses that use only CCR5 and exhibit a non-syncytium-inducing M-tropic phenotype are usually involved in initial infections, are isolated in the early stages, and are usually present throughout the course of disease. In contrast, viruses isolated from patients who have progressed to AIDS exhibit a phenotypic switch in approximately 50% of infected adults to a syncytium-inducing X4 or R5X4 phenotype (Björndal *et al*, 1997; Connor *et al*, 1997). In some individuals, disease progression is associated with a general broadening of virus tropism with expansion of coreceptor usage to include CCR3 and other alternative coreceptors (Björndal *et al*, 1997; Connor *et al*, 1997; Doms and Peiper, 1997; Littman, 1998). However, not all patients who progress to AIDS harbor syncytium-inducing X4 or R5X4 viruses. Notably, the majority of children progress to AIDS in the absence of CXCR4-using viruses (Fitzgibbon *et al*, 1998). Thus, other characteristics of HIV-1 are important for its pathogenicity.

Genetic studies have provided important insights into the *in vivo* roles of coreceptors and their ligands (reviewed in Berger *et al*, 1999). Individuals homozygous for defective CCR5 alleles with the Δ32 mutation (approximately 1% of Caucasians) exhibit resistance to HIV-1 infection (Huang *et al*, 1996; Liu *et al*, 1996; Samson *et al*, 1996). Heterozygosity for the CCR5 Δ32 mutation (approximately 10–15% of Caucasians) is associated with slower HIV-1 disease progression (Dean *et al*, 1996; Michael *et al*, 1997). In addition, genetic polymorphisms in the CCR5 promoter may also be associated with effects on disease progression (Mummidi *et al*, 1998; Kostrikis *et al*, 1998). Furthermore, in individuals with normal CCR5 alleles, the level of CCR5 expression on PBMC is heterogeneous, and correlates with susceptibility to HIV-1 infection *in vitro* (Wu *et al*,

1997). These studies indicate that CCR5 plays a critical *in vivo* role in HIV-1 replication. Genetic polymorphisms in two other genes, SDF-1 (nucleotide 801 G to A) and CCR2 (amino acid 645 V to I), were associated with a slower rate of disease progression in some studies (Smith *et al*, 1997; Winkler *et al*, 1998). However, studies of other cohorts found minor effects or no association between these mutations and a slower rate of disease progression (Michael *et al*, 1997; Mummidi *et al*, 1998; Eugen-Olsen *et al*, 1998; van Rij *et al*, 1998). Thus, there is still some controversy regarding the effects of polymorphisms in the SDF-1 and CCR2 genes on HIV-1 disease progression. Furthermore, a linkage disequilibrium between the CCR2 645 V/I genetic polymorphism and a CCR5 promoter polymorphism may explain the effect of the CCR2 polymorphism on disease progression (Kostrikis *et al*, 1998). Together, these results indicate that individual variation in the level or pattern of expression of CCR5, and possibly other chemokine receptors or ligands, can determine host susceptibility to HIV-1 infection or affect the rate of disease progression.

The chemokine receptors that mediate HIV-1 entry vary in their cellular expression and tissue distribution (reviewed in Luster, 1998; Berger *et al*, 1999). CCR5 is expressed predominantly on activated memory CD45RO+ T cells, monocyte/macrophages, dendritic cells, and granulocyte precursors (Alkhatib *et al*, 1996; Deng *et al*, 1996; Bleul *et al*, 1997; Wu *et al*, 1997; Di Marzo *et al*, 1998; Rubbert *et al*, 1998). CXCR4 is expressed in a broader range of tissues and cell types, including naive CD45RA+ T cells, CD8+ T cells, monocyte/macrophages, and other immune cell types (Feng *et al*, 1996; Bleul *et al*, 1997; Di Marzio *et al*, 1998; Rubbert *et al*, 1998; Verani *et al*, 1998). CCR3 is expressed on eosinophils, basophils, Th2 helper cells, and dendritic cells (Sallusto *et al*, 1997; Luster, 1998; Rubbert *et al*, 1998). The expression of CCR5, CXCR4, and other chemokine receptors on T cells, monocyte/macrophages, and other cell types is regulated by cytokines and other stimuli. For example, CCR5 expression on T cells is upregulated by PHA/IL-2, and downregulated by CD3/CD28 costimulation (Bleul *et al*, 1997; Carroll *et al*, 1997). CCR5 expression on monocytes is upregulated by IL-10 (Sozzani *et al*, 1998). CXCR4 expression on T cells is upregulated by IL-4 and downregulated by IFN-γ. CCR5 and CXCR4 expression can also be regulated by other cytokines and stimuli (Bleul *et al*, 1997; Carroll *et al*, 1997; Di Marzio *et al*, 1998). Thus, one mechanism by which cytokines and other inflammatory stimuli can regulate HIV-1 replication is by regulating the expression of HIV-1 coreceptors.

Env-coreceptor interactions that mediate virus entry

The proposed model for the HIV-1 Env-coreceptor interaction is shown in Figure 1. A high affinity binding of the HIV-1 gp120 envelope glycoprotein to CD4 induces a conformational change in gp120 that 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). For the HIV-1 gp120-CCR5 interaction, the chemokine receptor binding site in gp120 consists primarily of residues within the V3 loop (Cocchi *et al*, 1996; Trkola *et al*, 1996; Wu *et al*, 1996), in addition to a structure adjacent to the V3 loop derived from residues in the V1/2 stem and C4 region, which is formed or exposed upon CD4 binding (Rizzuto *et al*, 1998). This leads to a subsequent trimolecular interaction between the Env-CD4 complex and coreceptor, which triggers fusion by exposing the fusion domain of gp41.

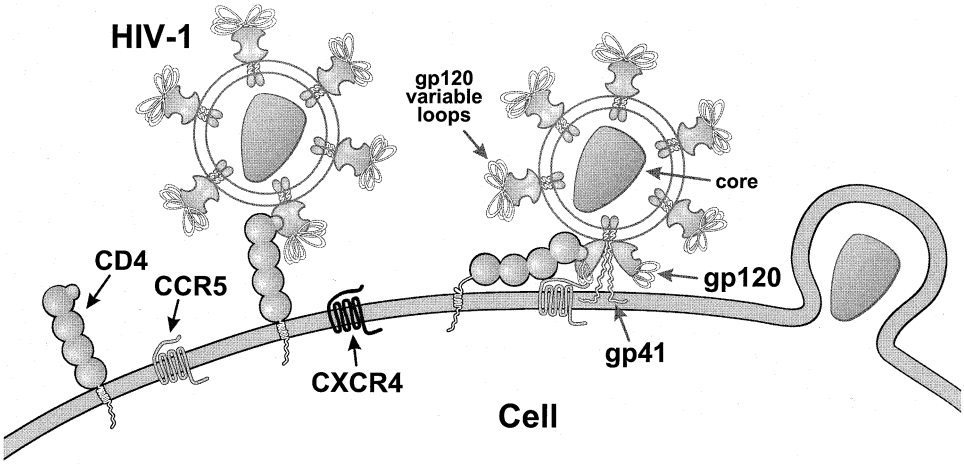

Figure 1 Model for HIV entry mediated by coreceptors together with CD4. The binding of gp120 to CD4 induces a conformational change in gp120 that exposes the V3 loop and thereby increases the affinity of gp120 for a given coreceptor. This leads to a subsequent interaction between the Env-CD4 complex and CCR5 or CXCR4, which triggers fusion by exposing the fusion domain of gp41. Graphic courtesy of Amy Emmert, Dana-Farber Cancer Insititute.

Factors that may influence the efficiency of virus entry in a cell-dependent manner include post-translational modifications of HIV and SIV coreceptors, such as glycosylation or tyrosine sulfation (Farzan *et al*, 1999; Berson and Doms, 1998) or the efficiency of chemokine receptor-CD4 interactions at the cell surface (Lapham *et al*, 1999; Dimitrov *et al*, 1999), and other cell type specific structural modifications (Hill *et al*, 1998; Lapham *et al*, 1999). Other cell surface factors such as glycosaminoglycans or adhesion molecules, which vary in a cell-dependent manner, may influence the efficiency of virus entry via non-specific interactions (Roderiquez *et al*, 1995; Mondor *et al*, 1998). 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 virus-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).

The natural ligands for chemokine receptors inhibit entry of HIV and SIV viruses that use those receptors (Table 1). Cocchi *et al* (1995) published the first study which demonstrated that the chemokines MIP-1α, MIP-1β, and RANTES are soluble factors produced by CD8 suppressor T lymphocytes that inhibit HIV-1 infection. M-tropic viruses that use CCR5 are inhibited by CCR5 ligands such as MIP-1α, MIP-1β, and RANTES (Cocchi *et al*, 1995; Alkhatib *et al*, 1996; Deng *et al*, 1996; Dragic *et al*, 1996; Choe *et al*, 1996). Similarly, SDF-1 inhibits entry by T-tropic or dual-tropic HIV-1 viruses that use CXCR4 (Bleul *et al*, 1996; Oberlin *et al*, 1996). Infection by the subset of M-tropic viruses that use CCR3 as a coreceptor is inhibited by eotaxin, a CCR3 ligand (Choe *et al*, 1996).

The mechanisms of chemokine inhibition of viral infection are complex. Chemokines can inhibit HIV-1 infection by one or more of several 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 affect 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; Rucker and Doms, 1998; Dimitrov *et al*, 1998). Furthermore, different HIV-1 isolates vary in their sensitivity to chemokine inhibition (Jansson *et al*, 1996; Dragic *et al*, 1996; Cocchi *et al*, 1996). The complexity of these interactions is further highlighted by the finding that 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, possibly by activating cellular signaling pathways via interaction with cell surface glycosaminoglycans (Gordon *et al*, 1999). This finding may help to explain discrepancies between different studies variably reporting either RANTES inhibition or enhancement of HIV-1 infection in monocyte/macrophages and other cell types (Berger, 1997; Kelly *et al*, 1998; Gordon *et al*, 1999). The finding that vMIP-II, a chemokine homolog encoded by Kaposi's sarcoma associated herpes virus (KSHV), inhibits HIV-1 infection via CCR3, CCR5 and other coreceptors together with the observation that HIV-1 infected individuals with Kaposi's sarcoma have a lower incidence of HIV-1 dementia (Baldewg *et al*, 1998; Liestoel *et al*, 1998) has led to speculation that increased expression of vMIP-II in these individuals may reduce CNS complications by inhibiting virus entry in macrophages and microglia. Together, these observations demonstrate that chemokines can inhibit virus entry, but these effects can vary depending on the particular cell type, viral isolate, and cell culture conditions.

Chemokine receptors are coreceptors for HIV and SIV entry in the CNS

Many chemokine receptors are expressed on neuronal and non-neuronal cells in the CNS (Table 1) (reviewed in Lavi *et al*, 1998; Glabinski and Ransohoff, 1999; Hesselgesser and Horuk, 1999). CCR5, CCR3, CXCR4, and CX3CR1 in addition to several chemokine receptors that do not function as coreceptors are expressed on microglia (He *et al*, 1997; Lavi *et al*, 1997; Pan *et al*, 1997; Ghorpade *et al*, 1998a; Vallat *et al*, 1998; Harrison *et al*, 1998; Sanders *et al*, 1998; Westmoreland *et al*, 1998; Albright *et al*, 1999; Sørensen *et al*, 1999; Shieh *et al*, 1998; Zhang *et al*, 1998; Rottman *et al*, 1997). CXCR4 is widely expressed on subpopulations of neurons 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). CXCR4 is also expressed on subpopulations of astrocytes (Lavi *et al*, 1997; Tanabe *et al*, 1997; Westmoreland *et al*, 1997; Sanders, 1998) and endothelial cells (Gupta *et al*, 1998; Lavi *et al*, 1997; Tachibana *et al*, 1998). Some studies have reported expression of CCR5 and CCR3 on subpopulations of neurons (Rottman *et al*, 1997; Lavi *et al*, 1998; Westmoreland *et al*, 1998; Zhang *et al*, 1998; Sanders *et al*, 1998), but this finding has not been confirmed by other groups (Vallat *et al*, 1998; Sørensen *et al*, 1999) and requires further study. Apj, STRL33/BONZO, and gpr1 are also expressed in the brain or neurally-derived cell lines (Table 1) (Farzan *et al*, 1997; Deng *et al*, 1997; Liao *et al*, 1997; Edinger *et al*, 1998b). Several other

chemokine receptors that do not mediate virus entry are expressed in the CNS (reviewed in Lavi *et al*, 1998; Glabinski and Ransohoff, 1999; Hesselgesser and Horuk, 1999). For example, CXCR2 (a receptor for IL-8) is expressed in primary human neurons, and by subsets of projection neurons in the brain and spinal cord (Horuk *et al*, 1997; Hesselgesser *et al*, 1997). Duffy antigen (which binds many different chemokines) is expressed exclusively by Purkinje cells in the cerebellum (Horuk *et al*, 1997). CCR1, CXCR3, and CXCR5 are also expressed in the brain (Sørensen *et al*, 1999; Glabinski and Ransohoff, 1999). These findings together with studies demonstrating that expression of particular chemokine receptors is increased in the brain of patients with HIV-1 encephalitis, multiple sclerosis, and Alzheimer's disease (Horuk *et al*, 1997; Vallat *et al*, 1998; Sanders *et al*, 1998; Sørensen *et al*, 1999; Glabinski and Ransohoff, 1999; Hesselgesser *et al*, 1997) suggest that chemokine receptors and their ligands are likely to contribute to disease pathogenesis in a variety of inflammatory and degenerative CNS diseases.

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. CXCR4 knock-out mouse models demonstrate a defect in cerebellar development associated with abnormal migration of the granule cells, in addition to B cell, myeloid, and cardiac abnormalities (Ma *et al*, 1998; Zou *et al*, 1998). Thus, CXCR4 and possibly other chemokine receptors play a role in brain development. Chemokines or chemokine-like molecules may also be involved in mediating cell-to-cell communication between different cell populations (e.g. glial cells and neurons). For example, a recent study provided evidence that neuronally derived fractalkine, a CX3CR1 ligand, may mediate interactions between neurons and CX3CR1-expressing microglia in certain pathological states associated with neuronal injury (Harrison *et al*, 1998). Other studies suggest that chemokine receptors can mediate neuronal signaling, or neuronal migration (Hesselgesser *et al*, 1997; Bolin *et al*, 1998; Meucci, 1998; Zheng *et al* 1999a,b. Chemokine receptors may also play a role in vascularization (Tachibana *et al*, 1998). Studies are in progress to define the biological roles of chemokines and chemokine receptors in the CNS during brain development and other normal processes such as neuronal migration, neuronal signaling, and cell-to-cell communication as well as their role in the pathogenesis of CNS diseases.

The ability of HIV-1 viruses in the brain to use CCR5 as a coreceptor is an important determinant of neurotropism. CCR5 is the major coreceptor for HIV-1 infection of macrophages and microglia in the CNS (He *et al*, 1997; Ghorpade *et al*, 1998a; Shieh *et al*, 1998; Albright *et al*, 1999). Furthermore, most HIV-1 viruses isolated from brain use CCR5 for virus entry. The HIV-1 YU2 and JRFL Env proteins, which were cloned directly from brain, use CCR3 in addition to CCR5 (Choe *et al*, 1996; He *et al*, 1997). Minor use of CCR3 or CXCR4 has been demonstrated for other brain-derived viruses (Shieh *et al*, 1998; Albright *et al*, 1999). In addition, alternative coreceptors such as Apj, CCR8, gpr15, and STRL33/BONZO can be used by some brain-derived viruses, albeit at lower efficiency than CCR5. These findings suggest that the pattern of coreceptor usage is an important determinant of HIV-1 neurotropism.

Natural ligands for CCR5 (MIP-1β, RANTES) and in some cases CXCR4 (SDF-1) or CCR3 (eotaxin), as well as an 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). It remains to be determined whether the mechanisms of inhibition involve direct competition with gp120 binding, non-competitive inhibition, coreceptor downmodulation, or other ligand-mediated mechanisms. Studies on chemokine receptor expression and the chemokine inhibitory effects on HIV-1 infection of primary cells can give variable results depending on the cell culture conditions and other variables as discussed above. In the case of CCR3-mediated HIV-1 infection of microglia, some studies found inhibition (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, 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). Thus, the variable level of expression of CCR5, CCR3, and CXCR4 and variable chemokine inhibition reported in different studies may reflect the cytokine environment and cell culture conditions as well as possible differences between fetal and adult cells.

Although macrophages and microglia express CXCR4, cells from most donors support productive infection by a subset of primary but not lab-adapted X4 HIV-1 viruses (Simmons *et al*, 1996, 1998; Strizki *et al*, 1996; Ghorpade *et al*, 1998b; Yi *et al*, 1998; Verani *et al*, 1998; Öhagen *et al*, 1999). The inefficient replication of many X4 HIV-1 viruses in macrophages 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). An alternative possibility is that other 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) may be responsible for the block in CXCR4 coreceptor function in macrophages. Further studies are needed to clarify the mechanisms that may account for the relatively inefficient use of CXCR4 by most X4 viruses in microglia. However, it is noteworthy that a subset of primary X4 viruses, including several that can use CCR3 in addition to CXCR4, can replicate relatively efficiently in microglia (Öhagen *et al*, 1999), consistent with previous studies in primary macrophages (Simmons *et al*, 1998). These findings together with the observation that R5X4 viruses can infect macrophages from donors homozygous for the CCR5 Δ32 allele (Yi *et al*, 1998) indicate that CXCR4 can mediate productive infection of macrophages and microglia by a subset of primary X4 HIV-1 isolates. The role of CXR4-mediated infection of macrophages and microglia by particular primary X4 or R5X4 HIV-1 viruses in disease pathogenesis merits further study.

HIV and SIV infection of astrocytes, brain capillary endothelial cells, and some brain-derived cell lines is CD4-independent (Kunsch *et al*, 1989; Harouse *et al*, 1989, 1991; Moses *et al*, 1993; Edinger *et al*, 1997; reviewed in Edinger *et al*, 1999). Infection of astrocytes and endothelial cells with HIV-1 in human adult brain is rare compared with macrophages and microglia. Naturally occurring HIV-1 variants that can use CXCR4 or other chemokine receptors for CD4-independent entry have not been identified. However, rare CD4-independent HIV-1 isolates have been generated *in vitro* (Dumonceaux *et al*, 1998). Some HIV-2 viruses use CXCR4 for CD4-independent entry (Endres *et al*, 1996; Reeves *et al*, 1997; Reeves and Schultz, 1997). A neurovirulent strain of SIV uses CCR5 for CD4-independent infection of brain capillary endothelial cells (Edinger *et al*, 1997). Together, these observations suggest that Env-coreceptor interactions can occur in the absence of CD4 (Berson and Doms, 1998). The receptors which mediate CD4-independent HIV-1 infection of astrocytes and capillary endothelial cells have not been identified. Previous studies have shown CXCR4 but not CCR5 expression on human astrocytes and endothelial cells (Lavi *et al*, 1997; Sanders *et al*, 1998; Gupta *et al*, 1998; Tachibana *et al*, 1998). Some studies have detected CCR5 or CCR3 on human or non-human primate astrocytes (Rottman *et al*, 1997; Westmoreland *et al*, 1998; Ghorpade *et al*, 1998a), but this finding was not observed in other studies (Vallat *et al*, 1998; Sanders *et al*, 1998; J Wang and Gabuzda, unpublished data). Further studies are needed to clarify the reasons for these discrepancies. Galactosylceramide, a glycolipid, has been implicated in mediating CD4-independent entry into some neurally-derived target cells (Harouse *et al*, 1989, 1991). However, galactosylceramide is not an efficient receptor for HIV-1 entry. Moreover, it is not expressed on astrocytes and endothelial cells. Studies are in progress to identify the receptor(s) that mediate CD4-independent HIV-1 infection of astrocytes and brain capillary endothelial cells.

Role of chemokine receptors in HIV and SIV neurotropism

The tropism of HIV and SIV viruses is determined by the envelope glycoproteins and their ability to interact with CCR5, CXCR4, or other chemokine receptors (reviewed in Berger, 1997; Doms and Peiper, 1997; Littman, 1998; Rucker and Doms, 1998). 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). Phylogenetic analysis of blood- and brain-derived Env sequences suggests that some trafficking of virus from blood into brain does occur in a subset of patients (Korber *et al*, 1994; Wong *et al*, 1997; van'T Wout *et al*, 1998; Chang *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, 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 (Simmons, 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. Consistent with this possibility, some primary HIV-1 isolates show preferential replication in microglia compared to blood-derived monocyte/macrophages (Strizki *et al*, 1996).

HIV-1 viruses in brain are typically CCR5-tropic (Keys *et al*, 1993; Korber *et al*, 1994; Donaldson *et al*, 1994; Power *et al*, 1994; Di Stefano *et al*, 1996; Reddy *et al*, 1996; Simmons, 1996). CXCR4 or CCR3 can also mediate infection of microglia by some neurotropic isolates and may also be important for HIV-1 entry and pathogenesis in the CNS (He *et al*, 1997). The role of other coreceptors in mediating infection of microglia remains to be determined. Interestingly, some previous reports provide evidence that another unidentified coreceptor may also mediate microglial infection (Ghorpade *et al*, 1998a) The V3 loop of gp120 is an important determinant of coreceptor use, as well as sensitivity to chemokine inhibition (Cocchi *et al*, 1996; Choe *et al*, 1996; Wu *et al*, 1996; Trkola *et al*, 1996).

However, other regions of gp120 also influence the gp120-coreceptor interaction (Wu *et al*, 1996; Trkola *et al*, 1996). Multiple regions of the HIV-1 Env influence coreceptor usage in a manner that is both strain- and coreceptor-dependent (Bieniasz *et al*, 1997; Cho *et al*, 1998; Smyth *et al*, 1998; Ross and Cullen, 1998; Öhagen *et al*, 1999). Based on these observations, it is likely that the ability of the HIV-1 Env to use particular chemokine receptors for virus entry determines HIV-1 tropism for microglia and possibly other target cells in the brain. The selection for particular genetic variants may reflect biological selection among the variants due to factors in the CNS microenvironment that include constraints on virus entry. The pattern of chemokine and chemokine receptor expression in the brain may select for genetic variants with particular features of the HIV-1 Env. The finding that MIP-1α and MIP-1β are induced in the brain of AIDS patients (Schmidtmayerova *et al*, 1996; Sanders *et al*, 1998) raises the possibility that HIV-1 viruses continue to replicate in the CNS in the presence of these CCR5 ligands by using CCR3 or other alternative coreceptors for virus entry. Elucidating the relationship between coreceptor usage, the genetic evolution of HIV-1 viruses in the brain, and neurologic disease *in vivo* will provide important insights into understanding the role of chemokine receptors in HIV-1 replication and disease pathogenesis in the CNS.

Role of chemokine receptors in disease pathogenesis in the CNS

Several studies have examined expression of CCR5 and CXCR4 in the brain of children and adults with AIDS (Lavi *et al*, 1997; Vallat *et al*, 1998; Sanders *et al*, 1998). CCR5 is predominantly expressed on perivascular mononuclear cells and on macrophages and microglia in inflammatory lesions (Figure 2). The frequency and staining intensity of CCR5-positive perivascular mononuclear cells and macrophages is increased in the brain of pediatric and adult patients with HIV-1 encephalitis (Vallat *et al*, 1998). Most of these CCR5-positive cells appear to be uninfected. CXCR4 is expressed on perivascular mononuclear cells, microglia, and in some neuronal and astrocyte subpopulations (Lavi *et al*, 1997; Vallat *et al*, 1998; Sanders *et al*, 1998). CCR3 is expressed primarily on microglia associated with inflammatory lesions (Sanders *et al*, 1998; Zhang *et al*, 1998). Studies on expression of CCR5, CXCR4, and CCR3 in macaques with SIV encephalitis have shown similar results (Westmoreland *et al*, 1998). Together, these results suggest that increased entry of CCR5-positive mononuclear cells into the brain may contribute to disease progression in the CNS. Factors which may contribute to the increased trafficking of CCR5-positive mononuclear cells into the brain of AIDS patients include increased expression of monocyte chemoattractants such as MIP-1α, MIP-1β, and MCP-1 (Schmidtmayerova *et al*, 1996; Bernasconi *et al*, 1996; Conant *et al*, 1998; Cinque *et al*, 1998), increased expression of adhesion molecules involved in monocyte trafficking through the blood-brain barrier (Nottet *et al*, 1996), and abnormal blood-brain barrier permeability (Petito and Cash, 1992; Power *et al*, 1993). Furthermore, *in vitro* studies suggest that activated or HIV-1-infected monocytes are more likely to migrate through the blood-brain barrier (Nottet *et al*, 1996; Persidsky *et al*, 1997). 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). One study suggests that heterozygosity for the CCR5 Δ32 allele does not reduce the risk of developing HIV-1 dementia (Barroga *et al*, 1997). However, further studies are required to determine whether individuals that express higher levels of CCR5, or other coreceptors, on cells of the immune or central nervous systems are at increased risk for developing HIV-1 encephalopathy. Elucidating the role of

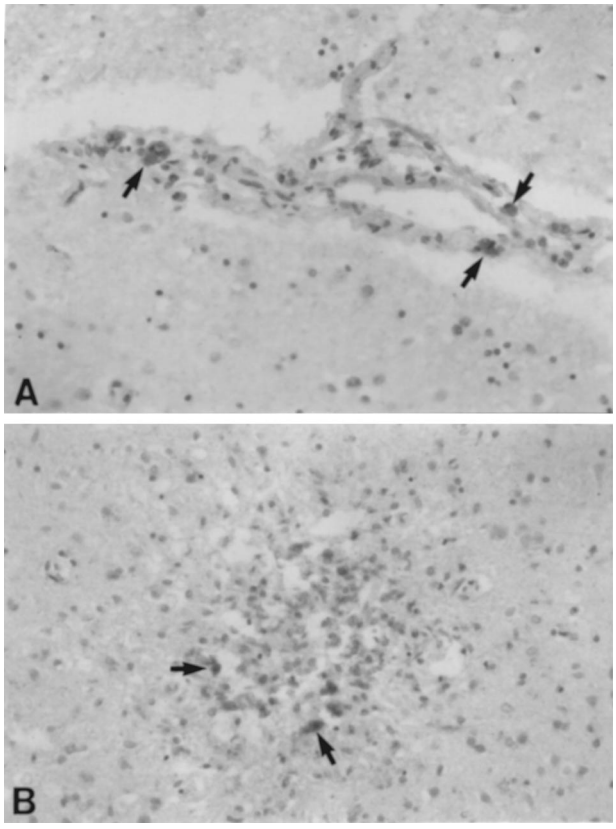

Figure 2 Immunostaining of CCR5 in brain tissue from AIDS patients. (**A**) CCR5-positive perivascular mononuclear cells and macrophages (arrows) in white matter. (**B**) CCR5-positive cells (arrows) in a microglial nodule. Original magnification, $\times$200. From Vallat *et al* (1998) with permission from American Journal of Pathology.

cytokines and other host factors that determine the pattern and level of CCR5 expression in the brain of AIDS patients are important areas for future investigation.

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, such as neuronal dysfunction or apoptosis induced by soluble forms of the HIV-1 gp120 Env protein (Lipton and Gendelman, 1995) or chemokines (Hesselgesser *et al*, 1998; Zheng *et al* 1999a,b). Apoptosis of neurons and astrocytes is induced by HIV-1 infection *in vitro* (Shi *et al*, 1996; Öhagen *et al*, 1999; Power *et al*, 1998; Zheng *et al*, 1999a,b) and has been demonstrated in autopsy brain tissues from children and adults with AIDS (Shi *et al*, 1996; Vallat *et al*, 1998 and references therein). Neurons are not directly infected by HIV-1, suggesting that neuronal apoptosis is likely to be induced by soluble factors. Soluble HIV-1 gp120 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). Some HIV-1 viruses which use CXCR4, in addition to CCR5 or CCR3, are more cytopathic in primary brain cultures *in vitro* (Öhagen *et al*, 1999; Zheng *et al*, 1999a,b). Furthermore, replacement of the Env with an X4 Env is sufficient to confer the ability to induce apoptosis in primary human neurons *in vitro* to an otherwise non-apoptosis-inducing HIV-1 virus (Öhagen *et al*, 1999). These findings are consistent with the observation that X4 HIV-1 viruses are generally more cytopathic than R5 HIV-1 viruses in infected as well as uninfected bystander T cells (Herbein *et al*, 1998 and references therein). However, CXCR4 usage is neither necessary nor sufficient to cause apoptosis in primary human neurons (Öhagen *et al*, 1999; Zheng *et al*, 1999a,b). Moreover, 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). SDF-1, the CXCR4 ligand, is expressed in the brain (Bleul *et al*, 1996, and references therein) and can induce signaling and chemotaxis in human neurons *in vitro* (Hesselgesser *et al*, 1997; Zheng *et al*, 1999a,b). 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, other studies suggest that effects of SDF-1 on neurons can be pro-apoptotic at least in some settings (Hesselgesser *et al*, 1998; Zheng *et al*, 1999a,b). 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*, ??). A recent study which demonstrates that a neurovirulent SHIV (an SIV-HIV-1 chimera that contains the *tat*, *rev*, *vpu*, and *env* genes of HIV-1 in a genetic background of SIV) causes AIDS, neurologic disease, and renal disease in rhesus macaques is also consistent with a role of CXCR4 in neurologic disease (Liu *et al*, 1999). 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.

Many chemokines are expressed in the brain and mediate normal responses involved in inflammation such as recruiting specific populations of leukocytes across the blood-brain barrier and inducing chemotaxis and cell migration (reviewed in Glabinski and Ransohoff, 1999). Chemokines such as MIP-1α, MIP-1β, RANTES, MCP-1, IP-10, and IL-8 are expressed by activated microglia and reactive astrocytes (Ransohoff *et al*, 1993; Godiska *et al*, 1995; Berman *et al*, 1996; Sasseville *et al*, 1996; Peterson *et al*, 1997; Conant *et al*, 1998; McManus *et al*, 1998; Sanders *et al*, 1998; Oh *et al*, 1999). Expression of MIP-1α, MIP-1β, and MCP-1 (Schmidtmayerova *et al*, 1996; Conant *et al*, 1998; Sanders *et al*, 1998), and possibly IP-10 and IL-8 (Sanders *et al*, 1998), as well as immune activation markers (e.g. HLA-DR, TNF-α, IFN-γ) is increased in the brain of AIDS patients with dementia, mostly by activated but uninfected cells. Increased MCP-1 in brain and cerebrospinal fluid (CSF) has been shown to be associated with an increased risk of HIV-1 dementia (Conant *et al*, 1998; Cinque *et al*, 1998), raising the possibility that increased expression of MCP-1 may contribute to the increased trafficking of mononuclear cells in the CNS of AIDS patients. Increased expression of MIP-1α, MIP-1β, RANTES, MCP-3, and IP-10 has been shown to correlate with SIV encephalitis in a primate model (Sasseville *et al*, 1996). Together, these observations suggest that the level of coreceptor expression and immune activation are likely to impact HIV and SIV disease progression in the CNS. Chemokines have been implicated in the pathogenesis of other acute and chronic infectious or inflammatory disorders (reviewed in Ransohoff, 1997; Glabinski and Ransohoff, 1999; Hesselgesser and Horuk, 1999). For example, during multiple sclerosis attacks, CSF levels of IP-10, Mig, and RANTES are elevated and brain expression of corresponding chemokine receptors (CCR5 and CXCR3, and to a lesser extent CCR1 and CCR3) is increased (Sørensen *et al*, 1999). Increased expression of MIP-1α, MCP-1, and RANTES has been demonstrated in experimental

allergic encephalomyelitis (EAE), a mouse model for multiple sclerosis (Ransohoff *et al*, 1993; Godiska *et al*, 1995; Berman *et al*, 1996). Immunomodulators that regulate the expression or effects of chemokines in the brain is an active area for drug development.

Future directions and therapeutic approaches
Important areas for future investigation include understanding the role of chemokine receptors in mediating virus entry and disease pathogenesis in the CNS. It will be important to elucidate mechanisms that regulate the expression and functional activity of HIV coreceptors, to define the expression of coreceptors on different cell types in the CNS, and to determine the role of individual variation in the expression of chemokines and chemokine receptors in host susceptibility to neurologic disease. Understanding the normal functions of chemokine receptors in the CNS and their role in other acute and chronic inflammatory and neurodegenerative disorders is another goal of future studies. HIV 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 (reviewed in Michael and Moore, 1999). HIV-1 infection via CCR5 can be inhibited by RANTES derivatives such as AOP-RANTES and other single agents (Simmons *et al*, 1997; Mack *et al*, 1998). Infection via CXCR4 can be inhibited by small molecules such as bicyclam derivatives (AMD3100) and other new drugs (Schols *et al*, 1997; Donzella *et al*, 1998). Other therapeutic strategies targeted at inhibition of HIV-1 coreceptors, such as other small molecule antagonists, immunomodulatory therapies, and gene therapies, are also being developed (reviewed in Cairns and D'Souza, 1998; Berson and Doms, 1998; Dimitrov *et al*, 1998). Understanding the role of chemokines and chemokine receptors in HIV-1 infection of the CNS will be important for elucidating mechanisms of HIV-1 replication and disease pathogenesis in the CNS and advancing their development as therapeutic targets.

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