

Chapter 6

HIV Coreceptors and Their Roles in Leukocyte Trafficking During Neuroinflammatory Diseases

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6.1 Overview

Due to the increasing resistance of HIV-1 to antiretroviral therapies, there has been much emphasis on the discovery and development of alternative therapeutics for HIV-1-infected individuals. The chemokine receptors CXCR4 (Bleul et al. 1996a; Feng et al. 1996; Nagasawa et al. 1996; Oberlin et al. 1996) and CCR5 (Alkhatib et al. 1996; Deng et al. 1996; Dragic et al. 1996) were identified as target molecules from the time their role as coreceptors for HIV-1 entry into leukocytes was first discovered 10 years ago. Initial studies focused on the use of the chemokine ligands, or altered derivatives, of CXCR4 and CCR5 to prevent the entrance of HIV-1 into immune cells (Schols 2006). While these studies showed some initial promise, there was evidence of significant caveats to their use, including selection of alternative coreceptor utilizing strains (Marechal et al. 1999; Mosier et al. 1999) and the potential to cause inflammatory side effects. These data prompted the development and study of small molecule inhibitors of CXCR4 and CCR5, which have also been used to examine the roles of these molecules in a variety of inflammatory and infectious diseases.

Since their discovery as HIV-1 coreceptors, expression of CXCR4 and CCR5 has been detected on diverse leukocyte populations and has been shown to influence the promotion, maintenance, and regulation of inflammation (Bleul et al. 1996b; D'Apuzzo et al. 1997; Granelli-Piperno et al. 1996; Klein and Rubin 2004; Mohle et al. 1998; Sozzani et al. 1997; Wu et al. 1997). The trafficking of CXCR4- and CCR5-expressing leukocytes occurs in a wide range of diseases with diverse etiologies that affect a variety of tissue sites, including the central nervous system (CNS). Because most antiretroviral therapies are unable to efficiently cross the blood–brain barrier (Boffito et al. 2006), the CNS retains special status as a potential viral reservoir during HIV-1 infection. It is therefore imperative to consider how

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targeting HIV-1 coreceptors impacts on leukocyte trafficking into the CNS, as it could influence the incidence, progression and severity of advanced HIV-1 infection, including the development of HIV-1-associated neurological diseases. For example, as HIV-1-infected immune cells are believed to bring virus in to the CNS (Fischer-Smith and Rappaport 2005), enhancement of leukocyte trafficking into the CNS could lead to increases in CNS viral replication and dissemination. Alternatively, increased parenchymal entry of immune cells might promote the induction of demyelinating disease via enhanced entry of myelin specific T cells into the CNS (Hellings et al. 2002; Holz et al. 2000; Lunemann et al. 2004; Muraro et al. 2002). Additionally, the prevention of leukocyte trafficking by receptor antagonism could lead to increased susceptibility to lethal opportunistic infection, as was observed in multiple sclerosis (MS) patients treated with natalizumab, a humanized monoclonal antibody against α 4-integrin, an adhesion molecule shown to be essential for the migration of leukocytes into the CNS (Adelman et al. 2005). In either scenario, the effects on CNS leukocyte trafficking resulting from CXCR4 and CCR5 antagonism could be especially detrimental when applied to an immunocompromised patient population such as one infected with HIV-1.

The following chapter will discuss the current data relating to the role of CXCR4 and CCR5 in leukocyte trafficking in the CNS. Included is a discussion of the contributions of CXCR4 and CCR5 to neuroinflammatory diseases caused by infection with either HIV-1 or WNV and by autoimmune mechanisms such as in MS. To gain preliminary insight into how altering leukocyte trafficking patterns in the CNS could affect disease outcome, studies utilizing animal models of autoimmune and virologic CNS diseases and receptor antagonism or deficiency will be covered. Finally, the potential benefits and/or hazards of targeting CXCR4 and CCR5 in the context of HIV infection will be addressed.

6.2 Leukocyte Trafficking into the CNS

The movement of leukocytes out of the blood and into diseased tissue is the hallmark of inflammation. The coordination of this movement begins in the secondary lymphoid tissue where leukocytes are primed with information regarding antigen specificity, become activated, and up-regulate molecules that can direct their infiltration into specific target tissues. The general sequence of events leading to leukocyte entrance into inflamed tissue is a well-defined process involving selectins, integrins and chemokines (Butcher and Picker 1996). The first step in this sequence involves a family of molecules that bind sialylated carbohydrates known as selectins, which are expressed on the activated endothelium and mediate the “capture and rolling” of lymphocytes along the vascular wall. The rolling motion of a lymphocyte is converted to firm adhesion by the combined action of integrins and chemokines. Integrins are expressed by lymphocytes, and endothelial cells express their carbohydrate ligands. Integrins undergo conformational changes in response to activation of G-protein-coupled chemokine receptors, which are also expressed by trafficking

lymphocytes (Johnston and Butcher 2002; Springer 1994). Chemokine ligands, which comprise a large family of proteins that generally direct lymphocyte extravasation into tissues, thus accomplish directed homing of lymphocytes. Although the molecular patterns required to enable trafficking from the circulation into the CNS are incompletely understood, three important molecules, intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1 (Laschinger and Engelhardt 2000), and P-selectin (Piccio et al. 2002) appear to play essential roles in this process. ICAM-1, VCAM-1 and P-selectin are all expressed by activated endothelial cells during induction of disease or by treatment with the inflammatory mediators tumor necrosis factor alpha (TNF- α), CD40 ligand (CD40L), lipopolysaccharide (LPS) and IL-1 β (Ubogu et al. 2006). After the CNS endothelium is activated, the expression of P-selection and ICAM-1 mediate the capture of circulating T cells through interactions with P-selectin glycolipid (PSGL)-1, and lymphocyte-function-associated antigen (LFA)-1 (integrin α L β 2), respectively (Biernacki et al. 2001; Bullard et al. 2007; Piccio et al. 2002). Firm adhesion of trafficking T cells depends upon ICAM-1, while monocytes utilize VCAM-1, although both share a common receptor, very-late antigen (VLA)-4 (Floris et al. 2002).

The CNS is protected from immune cell intrusion by a highly specialized system of microvasculature known as the blood–brain barrier (BBB) (Lucas et al. 2006). The BBB is comprised of a network of various cell types and modifications that contribute to maintaining its immune-privilege status including endothelial cells joined by tight junctions, their encasement by pericyte-embedded basement membranes and an additional barrier comprised of glial cell foot-processes. These modifications create an additional area through which leukocytes must exit to gain parenchymal entry known as the perivascular space, which is unique to the CNS (Ballabh et al. 2004). Under normal conditions, this formidable barrier is effective at limiting the trafficking of leukocytes from the blood into the CNS parenchyma. However, when the BBB is compromised, immune cells are able to gain access to the CNS. The extent of BBB penetration and leukocyte entrance depends upon disease etiology (Frohman et al. 2006). Despite differences in the extent of parenchymal entry during infectious or autoimmune diseases, infiltrating leukocytes first accumulate in the perivascular spaces. The perivascular infiltrate is therefore the cardinal lesion associated with all neuroinflammatory diseases. Control of immune infiltration of the CNS poses a unique dichotomy in which its limitation is greatly desired for the treatment of MS, but might also be detrimental during infectious disease where leukocytes are required to clear pathogens. Understanding the specific mechanisms regulating the trafficking of leukocytes across the blood–brain barrier is therefore paramount in developing therapies that prevent or promote inflammation within the CNS.

The HIV-1 coreceptors CXCR4 and CCR5 bind to ligand members of a family of molecules known as chemokines, or chemotactic cytokines. While the hallmark function of these small proteins is the direction of leukocyte trafficking, they can also participate in cellular events such as activation and costimulation (Bajetto et al. 2001a). Members of the chemokine family can be classified as either homeostatic or inflammatory based on their temporal expression (Charo and Ransohoff 2006; Kim 2005). Although traditionally the CNS had been thought to be protected from immune acti-

vation, it is now known that a variety of chemokines and their receptors are expressed in the CNS, both constitutively and during inflammatory diseases, including CXCR4, CCR5 and their ligands (Bajetto et al. 2001b; Cartier et al. 2005).

Classically categorized as a homeostatic chemokine, the ligand for CXCR4, known as CXCL12 or SDF-1, can be detected in the quiescent CNS at the microvasculature and by subpopulations of neurons (Krumbholz et al. 2006; Stumm et al. 2002). CXCR4 is also expressed constitutively throughout the CNS and can be found on endothelial cells, oligodendrocytes, microglia, and neurons (Klein and Rubin 2004). In the absence of inflammation, CXCL12 and CXCR4 direct the movement and proliferation of and recognition between a variety of resident neural cells types during CNS development (Lu et al. 2002; Pujol et al. 2005; Stumm et al. 2003). Neurophysiologic roles for these molecules in the adult CNS have not been established. During inflammatory conditions, however, expression of CXCL12 is altered (McCandless et al. 2006; McCandless et al. 2008a), suggesting that its role in the CNS is not limited to a developmental one. Because CXCR4 is ubiquitously expressed on leukocytes, it is likely that BBB expression of CXCL12 affects the trafficking of leukocytes, including CD4+, CD8+ T cells and macrophages (Bleul et al. 1996b; Klein and Rubin 2004). Much of the work studying inflammatory-induced CXCL12 has been done in the context of autoimmune neuroinflammation, such as MS and its murine model experimental autoimmune encephalomyelitis (EAE), during which endothelial cells, neurons, microglia, and astrocytes have all been shown to increase their expression of CXCL12 (Ambrosini et al. 2005; Calderon et al. 2006; Krumbholz et al. 2006; McCandless et al. 2006; McCandless et al. 2008a). In complement, increased numbers of CXCR4-expressing T cells and macrophages traffic to and infiltrate the CNS during MS, leading to demyelination and neuronal injury (Frohman et al. 2006; Prat and Antel 2005). More recent studies have examined the roles of CXCL12 and CXCR4 during encephalitis due to neurotropic viruses, such as the West Nile virus (WNV) and in the context of neuroAIDS (Langford et al. 2002; McCandless et al. 2008b; Peng et al. 2006). These studies have focused on CXCL12 expression by various BBB constituents including endothelial and glial cells and collectively support the notion that CXCR4 plays an important role in regulating the trafficking of leukocytes into the CNS parenchyma.

Unlike CXCR4, expression of CCR5 and its four ligands, CCL3, -4, -5 and -8, only occurs during inflammatory states. CCR5 may be expressed by activated T cells, macrophages, microglia and astrocytes (Bleul et al. 1997; Granelli-Piperno et al. 1998; Qin et al. 1998; Wahl et al. 1999), depending on the disease context. In most neuroinflammatory diseases, including neuroAIDS and MS, CCL3, -4, and -5 are expressed within inflammatory lesions (McManus et al. 1998; Simpson et al. 1998; Van Der Voorn et al. 1999) while CCR5 is expressed by infiltrating mononuclear cells (Simpson et al. 2000; Sorensen et al. 1999), suggesting a role for these molecules in the recruitment of immune cells during inflammation. While most of this data is correlative, several recent studies utilizing mice with targeted deletion of CCR5 or examining cohorts of patients with natural mutations of CCR5 or its ligands have begun to shed light on the differential roles of CCR5 in the trafficking mononuclear cell subsets in to the CNS (Table 6.1). While initial interpretations

Table 6.1 Role of CCR5 in neuroinflammatory disorders

	Disease	Effect of CCR5 deletion/ polymorphism	Reference
Murine models	EAE	No effect	Tran et al. (2000)
	Chronic MHV	Decreased macrophage infiltration and demyelination	Glass et al. (2001)
		Acute MHV	Decreased CD4+ T cell trafficking into the CNS, impaired viral clearance; No effect on CD8+ T cell trafficking into the CNS
	LCMV	Enhanced fatality with delayed CD8+ T cell infiltration	
	MS	No effect; CCR5 Δ 32 not protective	Bennetts et al. (1997)
Human diseases	Flavivirus infection	Delayed onset of disease	Barcellos et al. (2000)
		Decreased severity of disease	Schreiber et al. (2002)
		Associated with early death	Gade-Andavolu et al. (2004)
		No effect on disease	Kantarci et al. (2005)
		No association with MS	Ristic et al. (2006)
		Associated with MS	Favorova et al. (2006)
		Protective role	Otaegui et al. (2007)
		Decreased severity of disease	Van Veen et al. (2007)
		Increased risk of symptomatic WNV infection	Glass et al. (2006)
Increased risk of symptomatic WNV infection	Lim et al. (2008)		
Associated with tickborne encephalitis (TBEV)	Kindberg et al. (2008)		

EAE experimental autoimmune encephalomyelitis, *MHV* murine hepatitis virus, *LCMV* lymphocytic choriomeningitis virus, *MS* multiple sclerosis, *WNV* West Nile virus, *TBEV* tick-borne encephalitis virus

suggested a simple “summon and response” relationship, it has now become apparent that CCR5-expressing leukocytes may be involved in both clearance of infection and in the counter-regulation of T cell responses that might lead to postinfectious inflammatory sequelae.

6.3 NeuroAIDS

Neurological disease associated with HIV-1 infection results from primary replication within the CNS, which generally occurs during advanced stages of the disease when viral isolates reportedly expand their coreceptor usage from CCR5 to

CXCR4. However, viruses that replicate within the CNS primarily infect cells via CCR5, suggesting a distinctive role for this receptor in the biology of neuroAIDS. Consistent with this, CCR5 has been detected on HIV-infected macrophages within both the CNS and the peripheral blood in individuals with advanced disease. Additionally, T cell-tropic viruses that traffic in and out of the brain during progressive HIV-1 disease may play a greater role in HIV-1-associated neuropathogenesis than macrophage-tropic viruses, which have been shown to induce less neurotoxicity (Zheng et al. 1999). As the accumulation of macrophages within the CNS has been correlated with encephalitis and dementia (Marcondes et al. 2008), it is possible that CXCR4 and/or CCR5 play roles in the physiologic turnover of macrophages within the CNS. In this section, we will discuss the evidence implicating CXCR4 and CCR5 in the trafficking of leukocytes into the CNS during HIV-induced neuroinflammatory diseases. As infection and replication of HIV-1 and other viruses in brain macrophages and microglia represent the principal reservoir and vehicle for viral dissemination in nonlymphoid tissues, understanding the mechanisms that promote the infiltration of these cells is essential for developing targeted therapies aimed at depleting this reservoir.

6.3.1 *Human Studies*

The first insights into the essential role of macrophages in the neurodissemination of HIV came from studies examining chemokine receptor expression on postmortem specimens from patients with HIV encephalitis. In a majority of these specimens, both CXCR4 and CCR5 were detected on brain macrophages and microglia within inflammatory lesions, which also exhibited staining for HIV-1 antigen (Bonwetsch et al. 1999; Sanders et al. 1998; Vallat et al. 1998). The overall frequency of CCR5-expressing perivascular macrophages was positively correlated with severity of HIV-1-induced neurologic disease, whereas the frequency of CXCR4-expressing macrophages did not correlate with disease severity (Vallat et al. 1998). CCR5 expression was also significantly enhanced in HIV-1-specific CD8 T cells taken from cerebrospinal fluid versus peripheral blood of HIV-infected patients without neurologic disease (Shacklett et al. 2004). Ligands for these receptors (CXCL12, CCL2, -3, -5) were found to be expressed by activated astrocytes (Peng et al. 2006; Sanders et al. 1998), suggesting that turnover of macrophages may increase during neuroinflammatory states. Characterization of chemokine receptors found on T lymphocytes and monocytes in brain sections from subjects with various neuroinflammatory diseases, however, revealed that CCR1 and CCR5 are present on perivascular and parenchymal monocytic cells whereas only CCR5 was present on parenchymal macrophages (Trebst et al. 2003). These findings suggest that CCR5-expressing mononuclear cells, macrophages, and microglia contribute to progression of neurologic disease of individuals with AIDS by promoting virus entry and replication within the CNS.

As CCR5 was also found to be expressed by normal microglia in nonencephalitis brain specimens, this receptor may play a role in the physiologic turnover of these

cells, although this remains a topic of much debate (Ajami et al. 2007; Mildner et al. 2007). Using a coculture of endothelial cells and astrocytes that models several aspects of the human blood–brain barrier, Weiss et al. (1999) examined the mechanism whereby the HIV-derived factor Tat, a protein that activates viral gene expression (Deng et al. 2002), may facilitate monocyte transmigration. HIV-1 Tat induced significant expression of CCL2 by astrocytes and upregulated expression of CCR5 on human monocytes (Weiss et al. 1999). Although they demonstrated that transmigration across an *in vitro* BBB model could be inhibited by antiCCL2 antibodies, others have shown that antiCCR5 and antiCCR1 also both abrogate monocyte migration in similar models suggesting that a variety of inflammatory processes could augment monocyte migration into the CNS (Ubogu et al. 2006).

6.3.2 *Macaque Model*

The macaque model of neuroAIDS using simian immunodeficiency virus (SIV) has confirmed that viruses utilizing CCR5, such as SIV(mac)251, can cause primary disease in the CNS via the infiltration of SIV-infected mononuclear cells. Similar to patients with HIV-encephalitis, macaques with SIV encephalitis exhibit elevated CNS levels of CCL3–5 and perivascular infiltrates expressing CXCR4 and CCR5. Within these infiltrates, CCR5 localized specifically to cells within microglial nodules (McManus et al. 2000; Westmoreland et al. 1998). This model has further demonstrated that CCR5 expression on blood monocytes and brain microglia and/or macrophages distinguishes animals that develop encephalitis from those that do not (Marcondes et al. 2008). Recently, IL-15 treatment of SIVmac251-infected macaques was associated with decreased percentages of CCR5-expressing CD4+ T cells within the peripheral blood (Mueller et al. 2008). IL-15 had previously been reported to improve the survival and effector function of HIV- and SIV-specific CD8+ T cells and to up-regulate CCR5 on human CD4+ T cells. Because IL-15 treatment of acute SIV infection of rhesus macaques also led to an increase in the viral set point and acceleration of disease, the authors speculated that the decreased percentages of peripheral CCR5-expressing CD4+ T cells might indicate that the trafficking of these cells into tissue increases infection. Interestingly, one of the IL-15-treated animals developed neuropathological signs of early SIV encephalitis with increased mononuclear cell infiltrates and parenchymal microglial nodules, suggesting that increased expression of CCR5 on virally infected mononuclear cells promotes viral entry and neuropathology (Mueller et al. 2008).

6.4 CNS Autoimmunity

MS, a chronic demyelinating disease of the CNS, is the most common cause of non-traumatic disability among young adults (Frohman et al. 2006). At the cellular level, MS is mediated by myelin-specific CD4+ T cells that destroy oligodendrocytes

and trigger a cascade of inflammatory events leading to recruitment of additional immune cells that induce progressive demyelination and, ultimately, axon destruction. Clinically, MS is characterized by variations in the progression of disease over time, which has led to three clinical classifications of the disease. Relapsing-remitting MS (RRMS), the most common presenting form of MS, is a stable condition interrupted by recurrent attacks of temporary neurological disability. RRMS often evolves into secondary-progressive MS (SPMS), in which patients no longer remit but rather progressively deteriorate and accrue neurological disability. Primary-progressive MS (PPMS) follows a continuously declining course beginning at disease onset. In MS, it is generally accepted that acute inflammatory lesions begin with breakdown of the BBB (McFarland and Martin 2007), leading to the formation of perivascular infiltrates of mononuclear cells, parenchymal penetration by myelin-specific T cells, and subsequent recruitment and activation of monocytes/microglia that cause demyelination and axonal damage (Hauser et al. 1986; Huseby et al. 2001; Prat and Antel 2005; Ransohoff et al. 2003).

A variety of proinflammatory chemokines, including ligands of both CXCR4 and CCR5, are detected within the CNS of individuals with autoimmune neuroinflammatory diseases. Thus, in patients with MS and in mice with EAE, CCL3–5 are elevated within the CNS and CSF and treatments that decrease the levels of these ligands lead to decreased infiltration of mononuclear cells and diminished disease (Eltayeb et al. 2007; Fischer et al. 2000; Glabinski et al. 2002, 2000; Irony-Tur-Sinai et al. 2006; McCandless et al. 2006). CXCR4 and CCR5 ligands, however, exhibit differential roles in the pathophysiology of CNS autoimmunity, which can be traced to their cellular sources and location. CXCL12, which is expressed by the CNS microvasculature, regulates BBB immune privilege while CCL3–5 is expressed by glial cells and promotes the migration of leukocytes into the CNS parenchyma. In this section, we will discuss the roles of CXCR4 and CCR5 in CNS autoimmune diseases, drawing from studies utilizing the EAE model as well as tissue samples derived from MS patients.

6.4.1 *CXCL12 and CXCR4*

At many tissue sites, CXCL12 expression increases during autoimmune disease and CXCR4 participates in the localization, proliferation and activation of effector leukocytes at inflamed tissues sites (Garcia-Vicuna et al. 2004; Nagase et al. 2001; Nanki and Lipsky 2000). AMD3100, a bicyclam specific antagonist of CXCR4 signaling (De Clercq 2003; Hatse et al. 2002), has been employed to analyze the role of this receptor in a variety of biological processes, as targeted deletion of either CXCL12 or CXCR4 leads to embryonic lethality due to defects in the development of multiple organ systems (Ma et al. 1998; Zou et al. 1998). Amelioration of disease in a variety of murine models of autoimmunity has been accomplished via chronic treatment with AMD3100 (Lukacs et al. 2002; Matthys et al. 2001), suggesting that CXCR4 activation is required during the expression of certain autoimmune diseases. During CNS autoimmunity, activation of CXCR4 may be necessary

for the development of myelin-specific T cells as use of mutant chemokine ligands that antagonize CXCR4 decreased EAE by inhibiting the sensitization phase of the disease, leading to decreased activation of encephalitogenic T cells (Kohler et al. 2008). The role of CXCL12 and CXCR4 within the CNS during autoimmunity, however, is more complicated.

CSF derived from patients with neuroinflammatory diseases contains elevated levels of CXCL12 (Giunti et al. 2003). In studies using CNS tissues derived from both mice and humans with and without neuroinflammatory diseases, BBB expression of the CXCL12 β isoform has been observed to be dynamic, exhibiting alterations in its level and location depending on the disease context (McCandless et al. 2008a, b, 2006). In normal CNS tissues CXCL12 β expression occurs along the abluminal surfaces of CNS endothelial cells where leukocytes, which ubiquitously express the receptor CXCR4, engage it when attempting to enter the CNS. The polarized expression of CXCL12 β acts to localize mononuclear cells to perivascular spaces during neuroinflammation, thereby limiting their entry into the CNS parenchyma. This subcompartment retention, which is analogous to the role of CXCL12 β in lymphoid compartments, could be an integral component of CNS protection from the pathologic consequences of lymphocyte-induced glial cell activation. Consistent with this, in both humans and mice with CNS autoimmune disease, BBB expression of CXCL12 β increases and relocates across the inflamed venules, allowing the egress of leukocytes from CNS perivascular spaces into the parenchyma, leading to glial activation and demyelination. This altered pattern of CXCL12 expression at the BBB was shown to be highly specific for MS, occurring in 10–100% of venules within inflammatory lesions in postmortem CNS specimens. Other diseases affecting the CNS such as viral encephalitis, CNS lymphoma and Alzheimer's disease did not show altered CXCL12 expression at the BBB (McCandless et al. 2008a).

Alteration in homeostatic CXCL12 expression also correlated with increased astrocyte expression of CXCL12 within the glial limitans (Calderon et al. 2006; McCandless et al. 2008a). Recent studies have implicated the cytokine interleukin (IL)-1 β in the regulation of CXCL12 expression and location within the CNS. Exposure to IL-1 β , as well as myelin basic protein (MBP) induces CXCL12 expression by astrocytes *in vitro* (Calderon et al. 2006). Administration of IL-1 β , but not TNF- α , to naïve mice induced CXCL12 relocation in approximately 90% of vessels, and mice with targeted deletion of the IL-1R do not relocate CXCL12 at the microvasculature after immunization with MOG (McCandless et al., 2009). Consistent with this, mice with targeted deletion of IL-1R are resistant to EAE (Matsuki et al. 2006). Further studies will determine the mechanisms of this effect with regard to the infiltration of CXCR4-expressing mononuclear cells.

Use of a phospho-specific antibody against the ligand-activated form of CXCR4 also revealed an association between relocation of CXCL12 and activation of CXCR4 within luminal leukocytes (McCandless et al. 2008a). These data suggest that aberrant expression of CXCL12 at the BBB could contribute both to leukocyte entry into and egress from perivascular spaces. Consistent with this, administration of AMD3100 to mice with EAE leads to widening of inflammatory lesions, increased demyelination and worsened clinical disease. These results demonstrate

a critical role for CXCL12 β in regulating the trafficking of lymphocytes through the perivascular space during CNS autoimmunity.

CXCL12 has also been implicated in the initial myelination of the CNS. During development, the migration, proliferation and differentiation of oligodendrocyte precursor cells (OPCs) that populate the spinal cord, hindbrain and basal forebrain with mature oligodendrocytes is accomplished via the synergistic effects of growth factors and chemokines, including CXCL12 (Benveniste and Merrill 1986; Dziembowska et al. 2005; Franklin 2002; Frost et al. 2003; Hinks and Franklin 1999; Kadi et al. 2006; Redwine and Armstrong 1998; Tsai et al. 2002; Wu et al. 2000). Studies examining spinal cord myelination in embryonic mice with targeted deletion of CXCR4 suggest that it may control the survival and migration of OPCs in this CNS region (Dziembowska et al. 2005). Interestingly, studies of a variety of CXCR4-expressing cell types have indicated that CXCL12 may synergize with platelet-derived growth factor (PDGF), transforming growth factor (TGF)- β 1 or insulin-like growth factor (IGF)-1 in promoting CXCR4-mediated localization, proliferation, survival and maturation (Akekawatchai et al. 2005; Avecilla et al. 2004; Basu and Broxmeyer 2005; Kadi et al. 2006; Kortesisidis et al. 2005; Lataillade et al. 2000; Sanders et al. 2000). Thus the presence of growth factor receptors on OPCs may enable migration and proliferation during the initial myelination of the CNS according to the precise location and timing cues provided by other factors. During inflammation, however, new cues in the form of Th1 cytokines and chemokines may be required to trigger these developmental attributes.

CXC chemokines may also play a role in remyelination during CNS autoimmunity. Recently, subpopulations of cells expressing markers of OPCs (NG2, O4) have been identified within the adult mammalian brain (Dawson et al. 2003). Studies examining the expression of chemokines within MS lesions, however, have been controversial with some investigators detecting the *in situ* expression of CXC receptors 1–3 on proliferating oligodendrocytes while others have not (Filipovic et al. 2003; Omari et al. 2006, 2005). These investigations did not include antiCXCR4 in the panel of chemokine receptor antibodies used in their immunohistochemical analyzes. Studies inducing EAE in mice with targeted deletion of CXCR2 did not appear to uncover any enhancement in disease susceptibility or progression in these mice. However this single study focused on the role of CXCR2-expressing neutrophils in EAE, so extensive analyzes of demyelination and recovery in comparison with wild-type mice were not performed (Abromson-Leeman et al. 2004). Further studies on the roles of CXCR2 and CXCR4 are clearly warranted as these chemokines may play differential roles in various aspects of the remyelination process.

6.4.2 CCR5 and Its Ligands

The role of CCR5 in the trafficking of leukocytes during CNS autoimmune diseases is poorly understood. Studies indicate that under noninflamed, physiologic states, few T cells enter the CNS and there is minimal CNS engraftment of blood-derived

monocyte precursors of microglia (Ajami et al. 2007; Mildner et al. 2007). However, in disease states where the BBB is disrupted, expression of a variety of chemokines, including CCL5, is increased, as is the trafficking of antigen-specific lymphocytes and a subpopulation of monocytes that differentiate into ramified parenchymal microglia (Mildner et al. 2007). Thus, CCR5 may be important for both the trafficking of autoreactive lymphocytes and the demyelinating process believed to be initiated and maintained by activated monocytes and microglia within the CNS parenchyma.

In support of this, active MS lesions contain CCR5 expressing infiltrating T cells comprised of members of both the adaptive (CD4+, CD8+) and innate ($\gamma\delta$ TCR+) arms of immunity (Rinaldi et al. 2006). While autoreactive CD4+ and CD8+ T cells have been shown to play roles in CNS demyelinating diseases in murine models, $\gamma\delta$ T cells have recently come to the forefront as important participants in the initial induction of EAE (Lees et al. 2008; Odyniec et al. 2004; Smith and Barnum 2008; Szalai and Barnum 2004; Szalai et al. 2005). Interestingly, $\gamma\delta$ TCR+T cell lines derived from MS patients, compared to lines derived from healthy control subjects, expressed lower levels of CCR5 but higher levels of ligand (CCL5), suggesting the presence of an autoregulatory loop (Murzenok et al. 2002). Additional CCR3 and CCR5 expressing cells present within MS lesions are foamy macrophages and activated microglia (Balashov et al. 1999; Simpson et al. 2000; Sorensen et al. 1999). In a study by Trebst et al. (2003), 70% of CSF CD14+ monocytes derived from MS patients during exacerbations were found to express CCR1 and CCR5, regardless of the stage of disease, versus <20% of circulating monocytes. CCR1/CCR5 expressing monocytes were found in perivascular infiltrates and at demyelinating edges of lesions. While early lesions contained CCR1/CCR5-expressing monocytes and CCR1/CCR5 negative microglia, those examined at later stages contained macrophages that expressed only CCR5. The authors suggest that a subset of CCR1+/CCR5+ blood monocytes traffic into the CNS where, in the presence of ligands they are retained and, which upon further activation, down-regulates CCR1 and upregulates CCR5. A recent study identified four patterns of demyelination in active MS lesions. In all four of these patterns, infiltrating monocytes coexpress CCR1 and CCR5. The characteristics of pattern II lesions suggested a primary inflammatory mechanism of myelin injury, while pattern III lesions showed features consistent with oligodendrocyte degeneration. In pattern II lesions, the number of cells expressing CCR1 significantly decreased while CCR5 increased in late active compared with early active demyelinating regions. In striking contrast, numbers of cells expressing CCR1 and CCR5 were equal in all regions of pattern III lesions (Mahad et al. 2004).

Support for the role of CCR5 in the trafficking of CD4 T cells and macrophages during CNS autoimmunity also comes from studies using viral models of demyelination. Intracranial infection of the coronavirus mice with mouse hepatitis virus (MHV) results in an immune response-mediated demyelinating disease that serves as another model MS. During MHV-induced demyelination, CD4+ T cells amplify demyelination by attracting macrophages into the CNS following viral infection by mechanisms that are not yet understood. In studies using mice with targeted deletion

of CCR5, virus-specific CD4⁺ T cells were unable to traffic into the CNS which led to higher CNS viral titers early during infection (Glass and Lane 2003b). CCR5 is not, however, required for the trafficking of virus-specific CD8⁺ T cells in this model as there were no differences in trafficking patterns of CCR5^{-/-} CD8⁺ T cells. On the contrary, loss of CCR5 led to increased activity of virus-specific, cytotoxic CD8 T cells and eventual viral clearance that was no different from wild-type mice (Glass and Lane 2003a). However, the authors did observe a significant decrease in the extent of macrophage recruitment and demyelination during the chronic demyelinating phase of MHV CNS disease. Analysis of chemokine receptor expression on infiltrating macrophages and microglia revealed that most of them expressed CCR5. This was one of the first studies implicating CCR5 in the trafficking of macrophages into the CNS during a neuroinflammatory disease.

CCR5 is among a group of chemokine receptors expressed by T cells that are essential for their effector functions during Th1 inflammatory responses. Studies indicate that cytokines that contribute to the development of autoreactive T cells up-regulate their expression of CCR5 and enhance their encephalitogenic properties (Bagaeva et al. 2003) and that DT390-RANTES-SRalpha, a recombinant immunotoxin, prevents EAE via decreasing the numbers of CCR5⁺-infiltrating cells within the CNS (Jia et al. 2006). Consistent with this, increased percentages of CCR5 peripheral blood mononuclear cells (PBMCs) can be found in the blood and CSF of MS patients versus healthy controls or patients with other types of neurological diseases (Martinez-Caceres et al. 2002b). PBMCs that express CCR5 include lymphocytes, monocytes and myeloid-derived dendritic cells (mDCs), which are major stimulators of T cells. Comparisons of CCR5 mRNA on PBMCs derived from patients with different forms of MS revealed significantly increased expression in those derived from PPMS versus SPMS, RRMS and control patients (Jalonen et al. 2002), suggesting that synthesis of CCR5 within leukocytes increases with severity of MS. CD209⁺ CCR5⁺ dendritic cells are abundant in nonlesional gray matter in multiple sclerosis and may thus play a role in the activation of autoreactive T cells within the CNS parenchyma, leading to exacerbations (Cudrici et al. 2007).

Both memory T cells and mDCs have been shown to exhibit increased expression of CCR5 and CCL5 in the blood and CSF of MS patients (Pashenkov et al. 2002). CSF memory T cells, which could potentially differentiate into effector cells via antigen encounter derived from MS patients, express disproportionately high levels of CCR5 when compared with peripheral blood mononuclear cells (Sorensen et al. 1999, 2002; Zang et al. 2000). During relapse, CD4 and CD8 cells within the CSF are enriched for CCR5 while during remission, CCR5 alone is reduced in CSF CD4 T cells suggesting that CCR5 expression on those particular cells is a marker of disease activity (Misu et al. 2001). Treatment with immunosuppressive drugs alters the numbers of CCR5-expressing T cells in the periphery. Both cyclophosphamide and glatiramer therapy, for example, reverse the increase in the percentages of IFN- γ -producing CCR5 expressing T cells in MS patients (Allie et al. 2005; Karni et al. 2004). In addition, treatment with methylprednisolone can decrease the percentages of CCR5-expressing CD4 T cells in the peripheral blood (Martinez-Caceres et al. 2002a). IL-12 stimulates myelin-reactive T cells to up-regulate the beta-chemokine receptor, CCR5, in correlation with the

acquisition of central nervous system-infiltrating and encephalitogenic properties (Bagaeva et al. 2003). In contrast, in vitro treatment of T cells with IFN- β inhibits expression of CCL3 and CCL5 mRNA, and surface expression of their receptor CCR5, suggesting that the mechanism of IFN-beta treatment of MS lies in impairment in T cell trafficking (Zang et al. 2000). Consistent with this, treatment with IFN- β is associated with a decrease in CCR5-expressing mononuclear cells in the peripheral blood (Teleshova et al. 2002). Similarly, Sorensen and Sellebjerg (2001) found that T cells in the peripheral blood of SPMS patients exhibited lower levels of CCR5, which may have left this compartment and trafficked into the CNS (Sorensen and Sellebjerg 2001). These studies suggest that targeting CCR5 might be useful for the treatment of CNS autoimmune diseases and, in support of this, peripheral administration of anti-CCL3 antibodies has been shown to prevent recurrence of autoimmune anterior uveitis in Lewis rats (Manczak et al. 2002). However, genetic approaches have not been as conclusive in that CCL3- and CCR5-deficient mice are both fully susceptible to EAE (Tran et al. 2000) and studies on patients with the *CCR5* Δ 32 allele, which encodes a truncated, nonfunctional protein, reveal an unclear relationship between CCR5 activity and MS severity.

Approximately a dozen genome screenings of MS cohorts and multiplex MS families have evaluated the relationship between the presence of the *CCR5* Δ 32 allele and disease onset, severity and outcome of MS. The majority of these studies suggest that while decreased activation of CCR5 does not confer protection from MS (Bennetts et al. 1997), it may delay disease onset and attenuate recurrent disease activity (Kantor et al. 2003; Sellebjerg et al. 2000). In addition, patients with the *CCR5* Δ 32 allele have been observed to have more benign clinical courses with smaller lesion volumes, lower black hole ratio on MRI and higher percentages of lesions with signs of remyelination (Kaimen-Maciel et al. 2007; Schreiber et al. 2002). Similar findings have been reported for other CCL5 polymorphisms including the low-producer alleles CCL5-403*G and CCR5+303*G, which were associated with reduced risk of severe axonal loss and reduced T2 hyperintense and T1 hypointense lesion volumes on MRI, respectively (van Veen et al. 2007). In contrast, high-producer alleles CCL5-403*A and CCR5+303*A were associated with a worse clinical disease course and early age at onset. Similarly, a study of *CCR5* Δ 32 allele carriage in a Spanish population revealed a statistically significant difference between the study group and the control group for the carriers of at least one deleted allele in which the allele was more frequent in the control group, suggesting a possible protective effect of this deletion against MS (Otaegui et al. 2007). Most of these authors concluded that CCR5 antagonism may attenuate disease activity in MS or may provide a therapeutic target to modulate inflammatory demyelination (Sellebjerg et al. 2000). Several studies have refuted these findings, suggesting instead that the *CCR5* Δ 32 polymorphism is either not a major determinant of susceptibility for MS (Kantarci et al. 2005; Ristic et al. 2006; Sellebjerg et al. 2007; Silversides et al. 2004) or is positively associated with MS (Favorova et al. 2002), especially in patients with worsened outcome including primary progressive disease and early death (Gade-Andavolu et al. 2004; Pulkkinen et al. 2004). The use of novel CCR5 antagonists in various animal models of MS may be necessary to address the role of CCR5 in the neuropathogenesis of MS.

6.5 West Nile Virus Encephalitis

WNV is a neurotropic flavivirus now endemic within the Northern hemisphere that cycles between ornithophilic mosquitoes and natural bird reservoirs but may also incidentally infect humans and other vertebrate animals (Campbell et al. 2002; Glaser 2004; Williams 2004). While most WNV infections are asymptomatic or manifest as a mild, flu-like illness, potentially fatal neuroinvasive infections, including meningitis, encephalitis and anterior myelitis, occur in the elderly or immunocompromised. In the CNS, WNV targets cortical, midbrain, cerebellar and spinal cord neurons leading to their injury or death (Fratkin et al. 2004; Hunsperger and Roehrig 2006; Samuel et al. 2006; Shrestha et al. 2003). The high incidence of WNV neuroinvasive disease in patients on antiT cell therapies (Katz and Bianco 2003; Kleinschmidt-DeMasters et al. 2004) and in mice with T cell deficiencies (Shrestha et al. 2006; Wang et al. 2006, 2003a, b) indicate that, similar to other neurotropic viruses, the clearance of WNV within the CNS relies heavily on cell-mediated immune responses that promote the migration and effector functions of T cells into the CNS parenchyma. Experiments in mice have established that some chemokines and their receptors have essential roles in directing leukocytes to the CNS to clear WNV from infected neuronal cells. CCL3–5, chemokines that all bind the chemokine receptor CCR5, are strongly induced in the CNS after WNV infection (Glass et al. 2005, 2006; Klein et al. 2005; Shirato et al. 2004), and targeted deletion of CCR5 is associated with depressed leukocyte trafficking, increased viral burden and enhanced mortality (Glass et al. 2005). WNV encephalitis is associated with the early expression of CXCL10 by virally infected neurons that proceeds in a caudal to rostral direction within the CNS with significantly higher levels detected in the cerebellum by day 5 postinfection (Klein et al. 2005). Loss of CXCL10 was associated with decreased recruitment of WNV-specific CD8 T cells into the CNS, high CNS viral loads and enhanced mortality. The identification of CXCL12 as a key regulator of leukocyte trafficking at the BBB led to recent studies evaluating the role of this chemokine in the migration of virus-specific T cells during WNV encephalitis.

6.5.1 *CXCL12 and CXCR4*

The discovery that CXCL12 serves to prevent excessive immune cell entry by localizing infiltrating immune cells to perivascular spaces led to the hypothesis that BBB expression of CXCL12 might also prevent infiltrating virus-specific T cells from entering the CNS during infections with neurotropic viruses. Indeed, analysis of human postmortem specimens from patients who have succumbed to WNV revealed that CXCL12 maintains its polarity at the BBB (McCandless et al. 2008a, b) and that infiltrating T cells remained primarily sequestered within perivascular spaces with little parenchymal entry in most CNS regions (McCandless et al. 2008a, b). Using a murine model of lethal WNV encephalitis, the authors demonstrated that

CXCL12 mRNA and protein at the BBB are down-regulated during the course of WNV encephalitis and that the decreased levels of CXCL12 are associated with a concomitant decrease in the numbers of perivascular T cells and an increase in the numbers of parenchymal T cells. In addition, administration of a CXCR4 antagonist early in the course of CNS infection led to increased parenchymal penetration of WNV-specific CD8+ T cells, enhanced viral clearance and improved survival from 10 to 50%. Interestingly, the augmented numbers of infiltrating CD8+ T cells was associated with decreased glial cell activation, suggesting that T cell entry into the CNS for the purpose of viral clearance does not necessarily lead to inappropriate immune activation and pathology. It is also probable that virus-specific T cells that can efficiently eliminate a pathogen trigger mechanisms for efficient T cell egress from or elimination within the CNS versus T cells that gain entry into the CNS but do not encounter their antigens. Further studies are necessary to identify the molecules that regulate pathways of T cell exit.

The demonstration that CXCR4 inactivation leads to increased leukocyte entry poses a dilemma for the use of CXCR4 antagonists in patients infected with HIV-1. While it suggests that CXCR4 antagonism might promote the entry of virus-specific T cells, it also raises the issue of whether the entry of HIV-infected monocyte might also be enhanced, promoting the CNS as a reservoir of virus. Because HIV-specific CD8 T cell responses are defective in chronic HIV infection (Trabattoni et al. 2004), CXCR4 antagonism in the setting of HIV infection may therefore tip the balance in favor of extensive HIV neuroinvasion and acceleration of HIV encephalitis. Further preclinical evaluations that focus on the CNS may be warranted before novel CXCR4 antagonists are approved as antiinfectives for patients with HIV-1.

6.5.2 CCR5

The role of CCR5 in viral infections of the CNS has been studied using a variety of viral models including MHV, lymphocytic choriomeningitis (LCMV) and WNV (de Lemos et al. 2005; Glass and Lane 2003b; Glass et al. 2005; Nansen et al. 2000). As observed in CNS autoimmune diseases, CCR5 expression is essential for the trafficking of CD4+ T cells in all of these viral models. In addition, impaired trafficking of CD8+ T cells and macrophages was observed in LCMV- and WNV-infected mice with targeted deletion of CCR5. In all circumstances, CCR5-deficient mice had increased viral burdens and, in the cases of LCMV and WNV, adoptive transfer of splenocytes from virally-infected CCR5+/+ mice into infected CCR5-/- mice increased leukocyte accumulation in the CNS and increased survival rates to approximate those observed in infected CCR5+/+ control mice. These studies indicate a critical role for CCR5 in antiviral immune responses within the CNS and suggest that targeting CCR5 to prevent infection with HIV-1 could have dire consequences if patients become infected with neurotropic viruses.

In support of this, several recent studies have evaluated the clinical outcomes observed in flavivirus-infected patients that carry the *CCR5Δ32* allele. One group

has now published two reports that associate deficiency of CCR5 with symptomatic WNV infection. In one report, which examined WNV infection and carriage of *CCR5Δ32* in cohorts of patients from Colorado and Arizona, loss of CCR5 was associated with increased neuroinvasive disease (Glass et al. 2006). In a meta-analysis of the Colorado and Arizona cohorts plus two additional cohorts from Illinois and California, homozygosity for the *CCR5Δ32* allele was found to be higher in patients with symptomatic WNV infection (Lim et al. 2008). The discrepancy in the severity of WNV infection associated with *CCR5Δ32* homozygosity between the two studies was blamed on underpowering as the small sample sizes were unable to show such an association for a gene found in populations of European descent with allelic frequencies ranging from 0 to 0.29 (McNicholl et al. 1997). In addition, neither study demonstrated any impact of *CCR5Δ32* homozygosity on age incidence in symptomatic, WNV-infected patients.

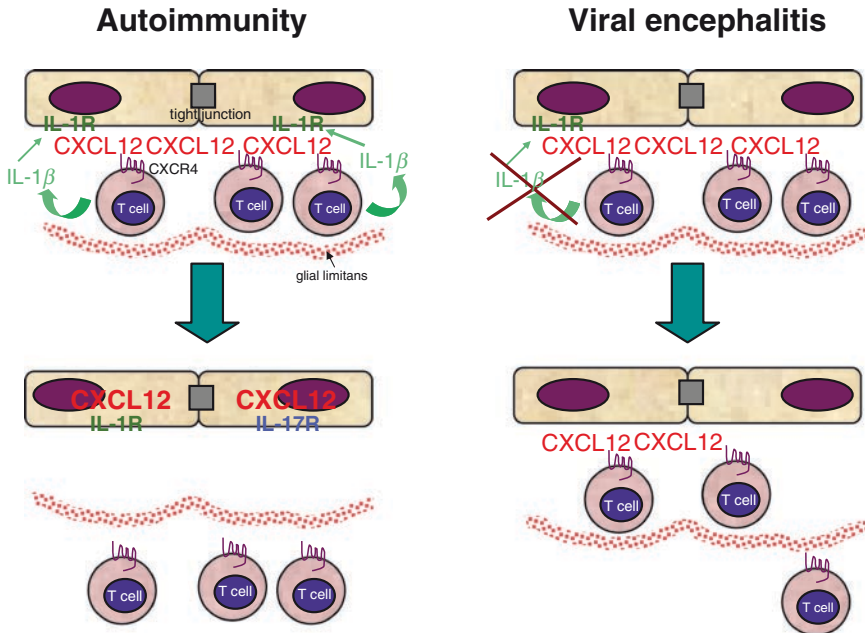


Fig. 6.1 Role of CXCL12 in T cell trafficking at the BBB. Depicted are proposed models for CXCL12-mediated regulation of T cell egress from perivascular spaces in the context of autoimmune and WNV encephalitides. In autoimmune disease, CXCR4-expressing T cells express the cytokine IL-1β (McCandless et al., 2009), which binds its receptor, IL-1R, on endothelial cells. This induces the intracellular uptake of CXCL12 via unknown mechanisms, allowing T cells to exit from the perivascular space. In the case of WNV encephalitis, IL-1β is not expressed within the CNS (Cheeran et al. 2005; Kong et al. 2008) and T cells remain localized to perivascular spaces until late in the course of disease when CXCL12 levels are down-regulated and T cells begin to enter the parenchyma (McCandless et al. 2008b)

Carriage of the *CCR5*Δ32 allele, however, may be a risk factor for severe infections with flaviviruses, in general. Kindberg et al. (2008) performed *CCR5*Δ32 genotyping among Lithuanian patients with tick-borne encephalitis (TBE), an often fatal infection caused by the TBE flavivirus (TBEV). As with WNV, TBEV in most individuals induces a self-limited, febrile illness with influenza-like symptoms while certain individuals, for unknown reasons, develop severe meningoencephalitis. In the Kindberg study, a significant increase in *CCR5*Δ32 allele prevalence was observed in patients with TBE compared with nonTBE aseptic meningoencephalitis subjects and healthy subjects were seronegative for TBE. Carriage of the *CCR5*Δ32 allele was also associated with increased clinical severity of disease, although individuals with *CCR5*Δ32 were not members of the group with the most severe symptoms (Kindberg et al. 2008). Thus, there are likely to be additional risk factors for severe TBE neuroinvasive disease that are currently unknown.

6.6 Conclusions

Coreceptor antagonism for the prevention of widespread cellular entry has been considered an attractive approach to halt the progression of HIV-1 ever since the discovery that carriage of *CCR5*Δ32 confers resistance to initial infection with HIV-1. However, the multifunctional role of HIV-1 coreceptors suggests that additional insights are required to identify unforeseen hazards of pharmacological chemokine receptor inactivation. This appears to be particularly important in the case of CXCR4 antagonism, which leads to increased trafficking of mononuclear cells into the CNS, potentially impacting on the CNS viral reservoir. While few studies have directly addressed how HIV-1 infection itself impacts on leukocyte CNS entry and no studies have implicated CXCR4 in this process; the studies outlined in this chapter strongly suggest that HIV-1 enters the CNS within mononuclear cells and that this migration is enhanced during CXCR4 antagonism. Because HIV-infected macrophages and microglia have also been implicated in the inflammatory-mediated destruction of neurons (Adamson et al. 1996; Dawson et al. 1993; Giulian et al. 1996; Nottet et al. 1995; Persidsky et al. 2000), CXCR4 antagonists could potentially increase both the incidence and severity of neuroAIDS. Thus, studies examining the impact of CXCR4 antagonism on CNS viral loads and neuronal injury are warranted prior to the approval and implementation of this new class of antiHIV drugs.

Use of CCR5 antagonists, on the other hand, might limit HIV-1 neuroinvasion and/or alter the immunopathology ascribed to CCR5-expressing mononuclear cells that enter the CNS. As outlined above, CCR5 is involved in the CNS trafficking of HIV-infected mononuclear cells and plays a role in glial cell activation and amplification of inflammatory responses. Thus, inactivation of CCR5 could prove to be efficacious in slowing the progression of HIV-1 infection. These speculations, however, have not undergone rigorous evaluation in animal models of neuroAIDS. Thus, the effect of newly approved CCR5 antagonists on HIV infection with the CNS is currently unknown and will therefore be determined empirically. If CCR5

antagonism does prove to be efficacious for the prevention of neuroAIDS, based on the virologic studies in animal models described above, its use would not be recommended if alternative immunotherapeutic agents that promote CD8 T cell-mediated clearance of HIV-1 were developed and implemented. Therefore, use of CCR5 antagonists will need to be continually reevaluated through animal model experimentation as novel therapeutic targets emerge and are translated into new drug treatments for HIV-infected patients.

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