



Structural biology of chemokine receptors

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ABSTRACT

Chemokine receptors are G protein-coupled receptors that mediate migration and activation of leukocytes as an important part of a protective immune response to injury and infection. In addition, chemokine receptors are used by HIV-1 to infect CD4 positive cells. The structural bases of chemokine receptor recognition and signal transduction are currently being investigated. High-resolution X-ray diffraction and NMR spectroscopy of chemokines indicate that all these peptides exhibit a common folding pattern, in spite of its low degree of primary-sequence homology. Chemokines' functional motifs have been identified by mutagenesis studies, and a possible mechanism for receptor recognition and activation is proposed, but high-resolution structure data of chemokine receptors is not yet available. Studies with receptor chimeras have identified the putative extracellular domains as the major selectivity determinants. Single-amino acid substitutions in the extracellular domains produce profound changes in receptor specificity, suggesting that motifs in these domains operate as a restrictive barrier to a common activation motif. Similarly HIV-1 usage of chemokine receptors involves interaction of one or more extracellular domains of the receptor with conserved and variable domains on the viral envelope protein gp 120, indicating a highly complex interaction. Elucidating the structural requirements for receptor interaction with chemokines and with HIV-1 will provide important insights into understanding the mechanisms of chemokine recognition and receptor activation. In addition, this information can greatly facilitate the design of effective immunomodulatory and anti-HIV-1 therapeutic agents.

Keywords: Chemokines, G-protein coupled receptors, Inflammation, HIV-1

Abbreviations used:

MIP, macrophage inflammatory protein; MCP, monocyte chemoattractant protein; RANTES, regulated upon activation normal T expressed and secreted; IL-8, interleukin 8; SDF, Stromal-derived factor; DARC, Duffy antigen receptor for chemokines; MGSA, melanoma growth stimulating activity; PF-4, platelet factor 4; NMR, nuclear magnetic resonance; HIV, human immunodeficiency virus.

INTRODUCTION

Chemokine receptors are G protein-coupled receptors that have classically been viewed as transducers of leukocyte chemoattractant peptides denoted as chemokines. Most of these peptides are secreted by many cell types in response to inflammatory stimuli (reviewed by Hedrick & Zlotnik 1996; Baggiolini 1998; Luster 1998; Locati & Murphy 1999). Activation of chemokine receptors triggers an inflammatory response by inducing migration of leukocytes from the circulation to the site of injury and/or infection. However, chemokine and chemokine receptor-knock out experiments on mice have demonstrated that these molecules also play pivotal roles in angiogenesis, hematopoiesis, brain and

heart development (Ma *et al*, 1998; Zou *et al*, 1998; Nagasawa *et al*, 1996; Broxmeyer & Kim 1999; Baird *et al*, 1999). Furthermore, chemokine receptors have been identified as key coreceptors in the entry of HIV-1 in CD4⁺ cells (Feng *et al*, 1996; Deng *et al*, 1996; reviewed by Berger *et al*, 1999), thereby playing a major role in HIV-1 transmission and pathogenesis.

Chemokines are peptides 70-120 residues long that are classified into four classes according to the location of the Cys residues at the N-terminus (Table 1). The CXC class consists of chemokines with a pair of Cys separated by a single residue. The most prominent members of this class are interleukin-8 (IL-8), stromal derived factor-1 (SDF-1), g-interferon inducible protein-10 (IP-10), platelet factor-4 (PF-

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4), neutrophil activating protein-2 (NAP-2) and melanoma growth stimulating activity (MGSA). The CC class chemokines have two adjacent Cys and include macrophage inflammatory protein-1 (MIP-1a, MIP-1b), regulated upon activation of normal T expressed and secreted (RANTES), monocyte chemoattractant protein-1 (MCP-1). The CX3C class chemokines contains two Cys separated by three residues and are represented by fractalkine/neurotactin. The C-class chemokines contain a single Cys and are represented by lymphotactin/ATAC/SCM. Chemokine receptors are grouped according to their binding selectivity to chemokines. Thus CXCR1 binds selectively IL-8, CXCR4 binds SDF-1 and CXCR5 binds B cell-attracting chemokine 1 (BCA1). CXCR2, CXCR3, and CCRs are

promiscuous and bind several chemokines. For example, CCR5 binds MIP-1a, MIP-1b and RANTES. Importantly, CXCR4 and CCR5 have been identified as the major coreceptors for the entry of HIV-1 strains, and chemokine binding blocks HIV-1 infection *in-vitro*. This review focuses on the structural and functional insights of chemokines and their receptors and the challenges that need to be confronted in this field in the new millennium.

Structure and Function of Chemokines

Significant progress has been made in solving the molecular structure of chemokines at high resolution by X-ray crystallography and NMR spectroscopy (Clore & Gronenborn 1995; Crump *et al*,

TABLE 1

Class	Receptor	Ligands	Expression
CC	CCR1	RANTES; MIP-1a; MIP-5; MCP-3; MPIF-1; Lkn-1	Mo, T, NK; iDC; Neutrophils
	CCR2	MCP-1,2,3,4,5	Mo; T(act) †; NK(act)
	CCR3	RANTES; eotaxin; eotaxin-2,3; MCP-2,3,4,5	Eosinophils, basophils,
	CCR4	TARC; MDC	Th1, Tc2, NK, iDC
	CCR5	RANTES; MIP-1a,b	Mo; Th1; Tc1, iDC
	CCR6	MIP-3a/LARC	T cells; iDC;
	CCR7	ELC, SLC	T cells, Mo, mDC
	CCR8	I-309, TARC, MIP-1a	Th2
	CCR9(D6)	MIP-1a,b; RANTES; MCP-1,2,3,4; eotaxin; TECK	T cells
CXC	CXCR1	IL-8; GCP-2	Neutrophils
	CXCR2	IL-8; GROa,b,g; NAP-2; GCP-2; ENA-78	Neutrophils
	CXCR3	IP-10; MIG; ITAC	T(act); Th1; Tc2
	CXCR4	SDF-1a,b	Widely expressed
	CXCR5	BCA-1	B cells
CX3C	CX3CR1	Fractalkine	Mo, NK, TI
C	XCRI	Lymphotactin	T cells
Other	DARC	IL-8; RANTES; MCP-2,3; MIP-5	Erythrocytes

† Activated.

Abbreviations.

T, T-lymphocyte; Th, T helper cells; Mo, monocytes/macrophages; NK, natural killer; iDC, immature dendritic cells; mDC, mature dendritic cells; Tc, cytotoxic T cells; lkn, leukotactin; TARC, T cell and activation-related chemokine; LARC, liver and activation-related chemokine; SLC, secondary lymphoid tissue chemokine; ELC, Epstein-Barr virus-induced receptor-induced chemokine; MDC, monocyte-derived chemokine; NAP, neutrophil-activating peptide; GRO, growth-related oncogene; ENA, epithelial cell-derived neutrophil-activating factor; GCP, granulocyte chemoattractant protein; MIG, monokine-induced gamma interferon; IP10, gamma-interferon-inducible protein-10; ITAC, interferon-inducible T-cell a-chemoattractant; BCA, B cell-attracting chemokine; MPIF, myeloid progenitor inhibitory factor; I-309, inducible 309; TECK, Thymus-expressed chemokine.

1998b; Shao *et al.*, 1998; Skelton *et al.*, 1999). We have learned that regardless of the low degree of sequence homology among the chemokines, they exhibit a common folding pattern in which the polypeptide chain is folded into three antiparallel β -strands overlaid by an α -helix. This structure is stabilized by intramolecular disulfide bonds (Fig 1). In most cases, the N-terminus preceding the first Cys is unstructured in the crystal or NMR structures, and therefore the spatial orientation of these residues is unknown. Although chemokines exhibit similar tertiary structures, their quaternary structures, as revealed by X-ray crystallography and NMR spectroscopy are different, forming dimers and tetramers. The functional significance of the quaternary structure remains to be determined as chemokines at physiological concentrations are mostly monomers and fully active. Consistent with this view, monomeric analogs of chemokines are as

active as the wild type (Rajaratnam *et al.*, 1997; Lowman *et al.*, 1997). A genetic approach has been used to probe the functional significance of the structural motifs of chemokines. An IL-8 mutant with truncation up to the first Cys in the N-terminal domain fails to activate IL-8 receptors in neutrophils and functions as an antagonist (Hebert *et al.*, 1991; Clark-Lewis *et al.*, 1991; Moser *et al.*, 1993). Experiments with chimeras of IL-8 with related chemokines and residue substitutions have identified the Glu-Leu-Arg motif in the N-terminal domain of IL-8, MGSA, and NAP-2 as essential for activation of CXCR1 and CXCR2 (Clark-Lewis *et al.*, 1991). On the other hand, His18 and Phe21, corresponding to the N-loop of IL-8, are required for binding and ligand-binding selectivity to the receptor (Suetomi *et al.*, 1999). None of these residue substitutions perturbed the overall tertiary structure of IL-8 as demonstrated by NMR spectroscopy, further supporting the argument that these residues are interacting with the receptor. Similar studies with SDF-1 revealed that Lys 1 and Pro 2 are required for activation of CXCR4, and residues 12-17 corresponding to the N-loop of SDF-1 are important for binding as demonstrated by domain-swapping experiments between SDF-1 and IP-10 and MGSA (Crump *et al.*, 1998a). Mutagenesis experiments with the CC chemokine RANTES revealed that selective residues within the N-loop determine the ligand-binding specificity toward CC receptors. Arg 17 is important for RANTES binding to CCR1, Phe 12 for binding to CCR3, and Phe 12 and Ile 15 for binding to CCR5 (Pakianathan *et al.*, 1997), thus forming distinct but overlapping binding domains. On the other hand, Pro 2, Asp 6, and Thr 7 near the N-terminus of RANTES are required for activation of CCR1, Pro 2 and Tyr 3 for CCR3, and Tyr 3 and Asp 6 for CCR5. The main conclusion of these studies is that chemokines exhibit two partially-overlapping domains, an N-terminal domain required for activation of the receptor (Fig 2), and a solvent-exposed N-loop that dictates the ligand selectivity and provides most of the binding energy.

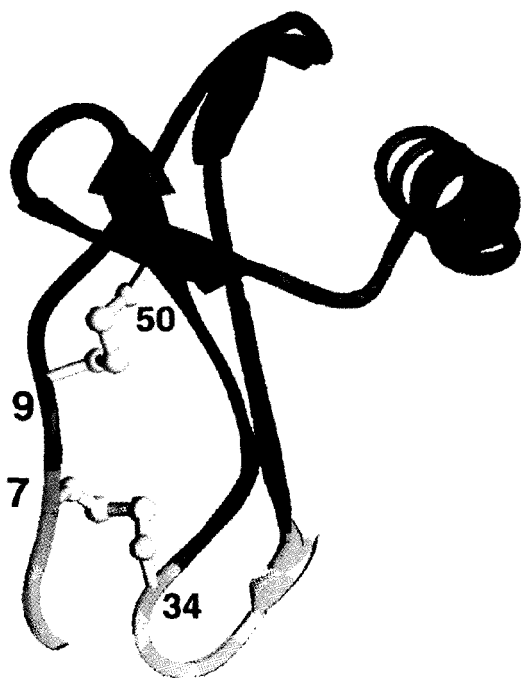


Fig 1. The structure of IL-8 consists of three antiparallel beta strands overlaid by an alpha helix. The two disulfide bonds (between aminoacids 7-34 and 9-50, respectively) are indicated.

The ready interconversion of chemokine ligand-selectivity by residue substitutions and the common structural fold of chemokines support a "scaffold hypothesis" *i.e.*, the core structure of chemokines serves as a scaffold from which functionally-important residues are optimally oriented to interact with the chemokine receptors. The most compelling evidence for this hypothesis is the conversion of PF4 and IL-10 into IL-8 receptor agonist by residue substitutions (Clark-Lewis *et al*, 1993; 1994). Despite these advances, major questions remain to be addressed in order to understand the mechanisms of recognition of chemokine by the receptors, namely,

1. What are the structures of chemokines bound to the receptors?
2. What residues in chemokines coordinate with the receptors?
3. What are the structural and functional relationships between chemokines and HIV-1 envelope glycoprotein (gp120), as both bind and activate chemokine receptors?

Molecular Architecture of Chemokine Receptors

In contrast to the chemokines, no high-resolution structures of chemokine receptors or indeed of any G protein-coupled receptor are available. This is

mainly due to the difficulties involved in crystallizing membrane proteins. The structure of chemokine receptors has been probed exclusively by conventional genetic experiments. Sequence analysis of all chemokine receptors reveals a high degree of sequence homology in the transmembrane regions but high variability in the extramembrane domains (Fig 3). This structural feature has greatly facilitated the construction of functional receptor chimeras, which have allowed identifying the motifs determining ligand-binding selectivity. Early experiments showed that the exchange of the extracellular N-terminal domains between CXCR1 and CXCR2 switched the ligand binding selectivity (La Rosa *et al*, 1992). Furthermore, replacement of the N-terminus of mouse CXCR2, which does not bind IL-8, by the corresponding N-terminus of CXCR1 confers binding to IL-8 (Suzuki *et al*, 1994). Similar experiments on CCR2 (Montecarlo & Charo, 1997), and the promiscuous chemokine receptor "Duffy antigen" (DARC) (Lu *et al*, 1995) that binds CC and CXC chemokines, have shown that the N-terminus dictates the ligand-binding selectivity. On the other hand, ligand selectivity for CCR1 and CCR5 is determined by the third and second extracellular loops respectively (Samson *et al*, 1997). These observations are consistent with the view that extracellular

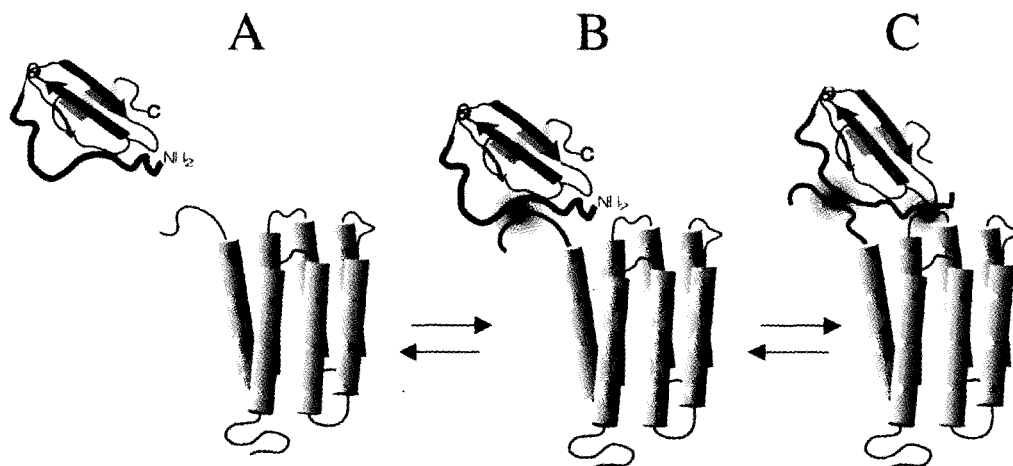


Fig 2. Model of chemokine receptor-ligand interaction. A. Receptor is in an inactive conformation. B. Ligand binds to the N-terminus of the receptor, leading to a conformational change. C. The conformational change allows for ligand binding to the activation domain of the receptor.



Fig 3: Residue homology among chemokine receptors. The percentage of residue conservation is indicated in shades of gray. Darker represents higher degree of conservation (scale on the right). CCR5 amino acid sequence is used as reference.

regions of chemokine receptors are the major determinants of ligand-binding selectivity. Conversely, the binding and activation sites in the receptor may be determined by other motifs common to all chemokine receptors. The traditional approach to identifying the binding and activation sites involves applying Ala scanning mutagenesis. Interestingly, Ala substitution of residues in the N-terminus of CXCR1 did not affect binding to IL-8, however the N-terminus synthetic peptide of CXCR1 bound IL-8 with low affinity (Leong *et al*, 1994). This suggests that the N-terminus of the receptor adopts a configuration that allows interaction of IL-8 with the polypeptide backbone rather than with specific side chains. This is not unprecedented, as most of the binding interface of CD4 to HIV-1 gp120 occurs via main-chain atoms (Kwong *et al*, 1998). Further Ala substitutions in the extracellular loops in CXCR1 have shown that Arg 200 and Arg 203 in the second extracellular loop and Asp 265 in the third extracellular loops are major determinants for binding to IL-8. Therefore, it is likely that the ligand-binding pockets of chemokine receptors

are delineated mainly by their extracellular domains. The major questions that remain to be addressed are:

1. What is the tertiary structure of chemokine receptors?
2. Where is the activation domain on the receptor?
3. What is the role of transmembrane regions in binding and activation of the receptor?

Chemokine Receptors as Coreceptors for Entry of HIV-1

The HIV-1 entry is initiated by fusion of the viral envelope with the plasma membrane of the target cell. Tropism of a given HIV-1 strain for a CD4⁺ cell is dictated by the specificity of its envelope gp120 to a particular chemokine receptor (reviewed by Doranz *et al*, 1997; Moore *et al*, 1997; Berger *et al*, 1999). T-tropic (X4) viruses primarily infect T-cell lines using CXCR4, while M-tropic (R5) viruses mainly infect macrophages and primary T cells using CCR5. Dual tropic (R5X4) viruses infect cells using either CCR5 or

CXCR4. The importance of CCR5 in HIV-1 transmission was highlighted by the observation that homozygous individuals with a 32-bp deletion in the CCR5 gene are highly resistant to HIV-1 infection (Dean *et al*, 1996). Furthermore, lymphocytes of these individuals do not support entry of R5 virus, indicating that CCR5 is required for HIV-1 infection. CXCR4 has been implicated in HIV-1 progression in late stages of infection (Schuitemaker *et al*, 1992), whereas the role of other chemokine receptors including CCR2B, CCR3, CCR8 and CX3CR1 in HIV-1 transmission and pathogenesis has not yet been established, although these receptors appear to mediate the entry of some HIV-1 isolates *in-vitro*.

Elucidation of the crystal structure of a CD4-HIV-1 envelope gp120-neutralizing antibody complex (Kwong *et al*, 1998) has provided detailed insight into the gp120 domains that may be important for binding to chemokine receptors. On the basis of studies with neutralizing antibodies and residue substitutions by site-directed mutagenesis guided by the molecular structure of gp120, the presumed binding site for chemokine receptors has been mapped to both conserved and variable domains in this highly polymorphic envelope glycoprotein (Rizzuto *et al*, 1998).

The gp120 binding sites on chemokine receptors were initially investigated by the construction of chemokine-receptor chimeras. Chimeras of the human CCR5 and its homologous murine CCR5 (mCCR5), which does not support HIV-1 infection, have shown that any single human extracellular domain substitution in mCCR5 confers coreceptor activity to the mutant mCCR5 for some HIV-1 isolates (Picard *et al*, 1997). However, the entry of different HIV-1 strains shows different requirements for extracellular domains. These findings indicate the complexities of the interactions of gp120 with chemokine receptors. Further mutagenesis studies have indicated that deletion of the CCR5 N-terminus down to Thr 16 did not affect the entry of some R5 viruses, but deletions beyond Cys 20 impaired coreceptor activity of the mutant receptors (Hill *et al*, 1998). Extensive Ala substitution on the extracellular regions of

CCR5 have identified residues, including Asp 2, Asp 11, Glu 18, Tyr 14, Tyr 15, Asn 93 and Pro 183, as important for the entry of R5 viruses (Rabut *et al*, 1998; Dragic *et al*, 1998, Kuhmann *et al*, 1997). However, cells over-expressing some of these mutant receptors became susceptible to HIV-1 infection (Dragic *et al*, 1998). A possible explanation for these observations is that CCR5 exists in various conformational states at equilibrium, with one or more conformational states favoring the interaction with HIV-1 (Lee *et al*, 1999). A mutation may reduce those conformational states that interact with HIV-1, which could be overcome by overexpression of the mutant CCR5. Other chemokine receptors could potentially act as coreceptors but they are unable to reach a conformational state favoring interaction with HIV-1. However, mutations could shift the equilibrium in favor of those conformational states that do interact with HIV-1. Support for this view is provided by the observation that G protein-coupled receptors, including chemokine receptors, exist in at least two different conformations. In the absence of agonists, an inactive conformation is favored over an active one, which is usually undetectable, although readily revealed in cells over-expressing the receptor (Akhter *et al*, 1997). Furthermore, artificial or natural mutations in different domains of G protein-coupled receptors have been shown to shift the equilibrium in favor of the active conformation, often leading to pathological conditions (Rao and Oprian, 1996). Similarly, individuals bearing natural mutations of CCR5 exhibit different degrees of susceptibility to HIV-1 (Carrington *et al*, 1999). These properties of chemokine receptors have precluded assigning the binding sites for HIV-1 by simple residue substitutions in the extracellular domains of coreceptors. Moreover, we still cannot rule out that the HIV-1 site could be located within the transmembrane domains of the coreceptor. Unfortunately, few studies have focused on probing the role of transmembrane domains on the recognition of HIV-1. It is possible that the gp120-binding site is located within the conserved

transmembrane domains and that access to this site is restricted in most chemokine receptors by extracellular motifs functioning as a steric barrier. This view is supported by experiments with several G protein-coupled receptors indicating that transmembrane domains form part of the sites for agonists and antagonists (Liaw *et al*, 1997; Yang *et al*, 1997; Cho *et al*, 1995). Most importantly, recent studies have mapped conserved hydrophobic domains on gp120 as sites for chemokine-receptor binding (Rizzuto *et al*, 1998), suggesting that the gp120 hydrophobic domain may interact with the conserved hydrophobic transmembrane domains of chemokine receptors. The steric barrier could be the major determinant of coreceptor usage by different HIV-1 strains. This barrier could be removed by domain or residue substitutions. Indeed, in contrast to the wild type CXCR4, a mutant CXCR4 (D187V) can mediate the entry of R5 isolates (Wang *et al*, 1998), suggesting that access to the binding site for R5 viruses on CXCR4 was restricted by steric hindrance and unmasked by the mutations.

Chemokines inhibit the entry of HIV-1 *in vitro*, and individuals with mutations in the SDF-1 gene that appears to increase the levels of this chemokine exhibit a prolonged onset of AIDS (Winkler *et al*, 1998). It is argued that binding of chemokines to the coreceptors blocks the binding of the viral gp120. The mechanism of this blockage is unknown, however it appears that receptor coupling to G proteins is not required for HIV-1 entry or chemokine inhibition. For instance, target cells treated with the *Pertussis* toxin, which uncouples the chemokine receptor to Gi, as well as with cells expressing a CCR5 mutant lacking Asp-Arg-Tyr, a motif required for interaction with G proteins, still supported the entry of HIV-1 (Doranz *et al*, 1997). The current view is that the binding of chemokines and gp120 involves overlapping receptor determinants as CCR5 mutants (Y14N) do not bind MIP-1b or mediate HIV-1 fusion (Kuhmann *et al*, 1997). On the other hand, CCR5 mutants with substitutions of extracellular Cys residues do not bind chemokines but still mediate the entry of HIV-1 (Blanpain *et al*, 1999). Further studies

on the mechanisms of chemokine inhibition of HIV-1 entry should provide a chemokine-based strategy to develop an anti-HIV-1 therapy. In fact, a RANTES derivative has been synthesized, aminooxypentane (AOP) RANTES, that is a potent antagonist of both, RANTES binding and HIV-1 infection (Simmons *et al*, 1997).

The challenging questions to address in the future are:

1. What are the residue pairs at the interface between chemokine receptor and gp120?
2. How does the interaction of gp120 and the chemokine receptor initiate the fusion of the virus membrane with the plasma membrane of the target cell?
3. What is the molecular structure of the gp120-chemokine receptor complex?

The overexpression of functional G protein-coupled receptors in heterologous hosts and novel crystallization procedures for membrane proteins (Landau and Rosenbusch, 1996) should be applied to chemokine receptors in order to unveil the mechanisms of recognition of chemokines and HIV-1. This effort will likely allow a rational structure-based approach to the design of anti-inflammatory agents as well as anti-HIV-1 therapy.

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