Annu. Rev. Pharmacol. Toxicol. 2002. 42:469–99

STRUCTURE, FUNCTION, AND INHIBITION OF CHEMOKINES

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Key Words X-ray, NMR, mechanism, agonist, antagonist, G protein–coupled receptor

■ **Abstract** Chemokines are the largest family of cytokines in human immunophysiology. These proteins are defined by four invariant cysteines and are categorized based on the sequence around the first two cysteines, which leads to two major and two minor subfamilies. Chemokines function by activating specific G protein–coupled receptors, which results in, among other functions, the migration of inflammatory and noninflammatory cells to the appropriate tissues or compartments within tissues. Some of these proteins and receptors have been implicated or shown to be involved in inflammation, autoimmune diseases, and infection by HIV-1. The three-dimensional structure of each monomer is virtually identical, but the quaternary structure of chemokines is different for each subfamily. Structure-function studies reveal several regions of chemokines to be involved in function, with the N-terminal region playing a dominant role. A number of proteins and small-molecule antagonists have been identified that inhibit chemokine activities. In this review, we discuss aspects of the structure, function, and inhibition of chemokines.

INTRODUCTION

Chemokines are a family of small proteins that are defined by four conserved cysteine residues. These proteins activate G protein–coupled receptors and induce cells to migrate through a concentration gradient. The role of chemokines is to promote accumulation of these cells at the source of chemokine production. Some chemokines are homeostatic in nature and are constitutively produced and secreted. These homeostatic proteins serve a variety of functions. For example, they direct the trafficking of lymphocytes to lymphoid tissues. They are also involved in immune surveillance and function to localize T or B cells with antigen (on the surface of antigen-presenting cells) in the lymphatic system (1). Other chemokines are considered inflammatory and are only produced by cells during infection or a pro-inflammatory stimulus. The role of inflammatory chemokines is to induce the migration of leukocytes to the injured or infected site. In addition, inflammatory chemokines activate the cells to mount an immune response and initiate wound

healing (1). While we have very neatly described these proteins by their functional role, in fact, their roles are more complicated and depend on the physiological context.

While cellular chemotaxis, or the migration of cells through a concentration gradient, is the best-known function of chemokines, these proteins also serve other roles. Many excellent reviews have recently been written on the signal transduction mechanism (2) and function (3–6) of chemokines, which cover their additional roles. In this review, we focus predominantly on the structure, interactions with their receptors, and inhibition of chemokines and their receptors.

We also note that there has been a recent change in the nomenclature of chemokines (7). To promote the use of this new nomenclature, we have used the new designations, but have included the former chemokine names in parentheses.

FUNCTION

Chemokine activity is initiated by the chemokine agonist binding to a specific G protein–coupled receptor. A two-step model has been proposed for activation of the receptor, wherein the main body of the chemokine agonist specifically recognizes and binds the receptor in the first step. This is followed by a conformational change in the chemokine that is presumed to be largely due to the flexible N terminus (8). The conformational change allows the N terminus to make the necessary interactions with the receptor that leads to receptor activation. Activation of the chemokine receptor is followed by exchange of bound GDP for GTP in the α subunit of the G proteins. The G proteins disassociate from the receptor and activate several effector molecules downstream, which results in a cascade of signaling events within the cytoplasm of the cell (2). This sequence of events results in diverse physiological processes including leukocyte migration and trafficking, leukocyte degranulation, cell differentiation, and angiogenesis or angiostasis (5, 6, 9). Although chemokines are traditionally associated with the development and response of the immune system, examples exist that indicate a broader role. Based on knock-out studies of mice, the chemokine CXCL12 (SDF-1α) or its receptor, CXCR4, have equivalent phenotypes. Both suffer from impaired fetal development of the cerebellum, the cardiac septum, gastric vasculature, and B-cell lymphopoesis. These mice die either in utero or at birth (10, 11).

Chemokines have proved central to the process of extravasation of leukocytes, which includes multiple steps involving interactions of adhesion molecules and the chemoattractant function of these proteins (12, 13). Both B- and T-cell maturation involves several chemokines at different stages of development (5, 14, 15). Chemokine systems involved in B-cell maturation include CXCL12 (SDF-1α)/CXCR4 (11), CXCL13 (BCA-1), and CCL20 (MIP-3α) (6). Chemokine agonists and receptors that have been observed to regulate T-cell maturation include CCL2 (MCP-1), CCL3 (MIP-1α), CCL5 (RANTES), and the receptors CCR2 and CCR5 (5).

Central to immunity and surveillance by the immune system is the migration of dendritic cells (DCs) to tissues and lymph nodes. Several chemokines regulate the migration of monocytes and immature dendritic cells, which express chemokine receptors such as CCR1, CCR2, CCR5, CCR6, CCR7, and CXCR2 (16–19). Chemokine receptor expression is regulated on these DCs. Inflammatory chemokines promote recruitment and localization of DCs to sites of inflammation and infection. Upon exposure to maturation signals, DCs undergo a chemokine receptor switch, with downregulation of inflammatory chemokine receptors followed by induction of CCR7. This allows immature DCs to leave tissues and to localize in lymphoid organs (due to CCR7 agonists), where antigen presentation takes place (20, 21).

Chemokine biology is intrinsically linked to the activities of other cytokines. An example of how chemokines modulate the immune response is evident from the function of CCL2 (MCP-1) on T-helper-cell polarization (22). As a response to challenges to the host, type 1 and type 2 T-helper (TH1 and TH2)-cells secrete cytokines that enhance cell-mediated and humoral immunity, respectively. However, CCL2 (MCP-1)-deficient mice are unable to mount TH2 responses and thus synthesize extremely low levels of IL-4, IL-5, and IL-10. These mice cannot achieve the immunoglobulin E subclass switch that is characteristic of TH2 responses. Because wild-type mice (Balb/c) are normally susceptible to *Leishmania major* owing to the TH2 response, the CCL2 (MCP-1)-deficient mice, which can only mount a TH1 response, are far more resistant to *Leishmania major* infection.

The ELR^+ α-chemokines, CXCL8 (IL-8), CXCL7 (NAP-2), CXCL1 (MGSA/GRO-α), and CXCL5 (ENA-78) promote angiogenesis, whereas interferon-inducible α-chemokines that lack the ELR motif such as CXCL4 (PF4), CXCL10 (IP-10), and CXCL9 (Mig) inhibit angiogenesis (23, 24). The ability of the CC chemokines CCL19 (ELC) and CCL21 (SLC) to inhibit angiogenesis and attract immune effector cells is under investigation for antitumor therapy (25).

STRUCTURE

Primary Sequence

Chemokines are defined by four invariant cysteine residues that form disulfide bonds. The first cysteine in the sequence forms a covalent bond with the third, and the second and fourth cysteines also form a disulfide bond. The chemokine family is subclassified on the basis of the local sequence at the first two cysteines (Figure 1). Chemokine agonists that have an intervening amino acid between the first two cysteines are subclassified as CXC or α-chemokines. If the first two cysteines are adjacent to each other, the chemokines are known as CC or β-chemokines. Another subfamily, the CX3C or the γ-chemokine, possesses only one protein in its category and is defined by three intervening residues between the first two cysteines. The CX3C chemokine is unusual because it is part of a cell surface receptor. [Recently CXCL16 was discovered to be part of a receptor (26).]

CX3CL1 (fractalkine) forms the N-terminal domain of the receptor neurotactin and is followed by a series of mucin-like domains, a transmembrane helix, and a short cytoplasmic domain (27). Recombinant CX3CL1 (fractalkine) in the absence of the remaining receptor residues is chemotactic (28). Full-length CX3CL1 (fractalkine) and its receptor CX3CR1 function as adhesion molecules (29).

One of two exceptions to the four-cysteine paradigm is the C or δ-chemokine, in which the polypeptide has only two of the four cysteines. The same gene encodes both members of this subfamily, which share different splice sites and differ in only two amino acids. There are also a few chemokines from the β-chemokine subfamily that possess six cysteines, all of which are also disulfide bonded. Over 40 chemokines have been identified in humans (30) (Figure 1). The sequence identities between chemokines vary from less than 20% to over 90%. It is important to note that each chemokine also has a secretion sequence. In some instances, the native protein has been N-terminal sequenced, but in most cases the boundary between the signal sequence and the mature protein has been determined by computational methods that identify secretion sequences. This review concerns itself only with the mature protein.

The designation of chemokines into different families that are based primarily on variations in sequences between the first two cysteines has some interesting biochemical differences. One difference involves the binding of chemokines to receptors. Receptors that interact with multiple chemokines do so only with chemokines belonging to the same subfamily. Competitive binding for receptors across subfamilies is rare.

Secondary structure

The chemokine topology consists of an elongated N terminus that precedes the first cysteine (Figure 2A). This extended N terminus has no particular structural features and in most cases is unobservable in high-resolution structural studies. Following the first two cysteines is a loop of approximately ten residues, which, in many cases, is succeeded by one strand of a 3_{10} helix. The region of the structure between the second cysteine and the 3_{10} helix is known as the N loop and plays an important functional role. The single-turn 3_{10} helix is succeeded by three β-strands and a C-terminal α-helix. Each secondary structural unit is connected by turns known as the 30s, 40s, and 50s loops, which reflects the numbering of residues in the mature protein. In addition to having important roles in connecting secondary structures, the 30s and 50s loops possess the latter two of the four cysteines characteristic of the family. The first two cysteines following the N-terminal region limit the flexibility of the N terminus, owing to the disulfides with the third cysteine on the 30s loop and the fourth cysteine in the 50s loop, respectively. Despite the presence of the two cysteines following the N terminus, NMR dynamics studies indicate that the flexibility of the N loop is greater than the flexibility of other regions of the protein (excluding the N and C termini). This flexibility may play a role in the mechanism of chemokine receptor binding and/or activation (31, 32).

TABLE 1 List of chemokines with known three-dimensional structures and their receptors

			Method of structure determination and oligomerization state	
Subfamily	**Chemokine**	**Receptor**	**X-ray**	**NMR**
CXC	MGSA/GRO-α (CXCL1)	CXCR2	—	Dimer (33)
	Gro-α (CXCL2)	CXCR2	—	Dimer (34)
	PF4 (CXCL4)	Unknown	Tetramer (35)	Tetramer (36)
	NAP-2 (CXCL7)	CXCR2	Tetramer (37)	Monomer (38)
	IL-8 (CXCL8)	CXCR1, CXCR2	Dimer (39)	Dimer (40)
	SDF-1α (CXCL12)	CXCR4	Dimer (41)	Monomer (8)
CC	I-309 (CCL1)	CCR8	—	Monomer (42)
	MCP-1 (CCL2)	CCR2, CCR10	Dimer (43)	Dimer (44)
	MIP-1α (CCL3)	CCR1, CCR5	—	Monomer (45)
	MIP-1β (CCL4)	CCR5	—	Dimer (46)
	RANTES (CCL5)	CCR1, CCR3, CCR5	—	Dimer (47)
	MCP-3 (CCL7)	CCR1, CCR2, CCR3, CCR10	—	Monomer (48)
	MCP-2 (CCL8)	CCR2,	Monomer (49)	—
	eotaxin-1 (CCL11)	CCR3	—	Monomer (50)
	HCC-2 (CCL15)	CCR1	—	Monomer (51)
	MPIF-1 (CCL23)	Unknown	—	Monomer (52)
	exotaxin-2 (CCL24)	CCR3	—	Monomer (53)
	vMIP-II	Multiple	Dimer (54)	Monomer (55)
CX_3C	Fractalkine (CX_3CL1)	CX3CR1	Dimer (56)	Monomer (57)

Tertiary structure

The three-dimensional structures of several chemokines have been determined by X-ray crystallography and/or NMR (Table 1). The experimentally determined monomeric three-dimensional fold of all chemokines is identical (Figure 2B). The β-strands that follow the N loop and 3_{10} helix are positioned antiparallel to each other and form a β-pleated sheet. Each β-strand is linked to the next by a flexible type I or III turn (30s and 40s loops) of 3 to 4 residues in length. The 30s loop is particularly important for the activity of many chemokines. The third β-strand is connected by a type III turn (50s loop) to the C-terminal α-helix. This C-terminal α-helix is oriented at approximately 75° to the plane of the β-sheet.

The core of the chemokine structure is stabilized largely by the two disulfides and by hydrophobic interactions from one side of the C-terminal helix and a portion of the β-sheet. Alanine mutagenesis of any of the cysteines of CXCL8 (IL-8) leads to loss of structure and function. However, the presence of two covalent links (disulfides from homocysteine, penicillamine, or selenocysteine)

in CXCL8 retained the structure but had different functional effects depending on which cysteines were modified. Modification of the disulfide bond between Cys-9 and Cys-50 by other disulfide-forming groups had small effects on function, but perturbation of the disulfide between Cys-7 and Cys-34 led to a dramatic reduction in potency (58). In another study, the disulfide pattern in CXCL8 (IL-8) was rearranged and the resulting molecule was characterized by X-ray crystallography and by functional studies (59). An unnatural disulfide between Cys-9 and Cys-38 was created by a Cys50Ala mutant to destroy the natural disulfide between Cys-9 and Cys-50, and a Glu38Cys mutant allowed the unnatural disulfide to be generated. The resulting variant had a root mean square deviation of only 1.4 Å from the wild-type protein, but with significantly reduced binding affinities to CXCR1 and CXCR2. All of this suggests that the disulfide between Cys-7 and Cys-34 either participates in receptor binding or is necessary to constrain different regions of the chemokine involved in receptor binding. The results with the disulfide between Cys-9 and Cys-38 indicate that relatively subtle differences in structure can lead to dramatic effects on biological activity.

The deleterious effect of destroying disulfide bonds is not universal for chemokines. In contrast to CXCL8 (IL-8), CXCL10 (IP-10) retains activity after its disulfides are reduced with dithiothreitol and subsequently modified with iodoacetamide or S-methyl-methanethiosulfonate (60). For tetrameric CXCL7 (PF4), reduction of the two disulfides induces a dimer $\Leftrightarrow$ monomer transition. The dimer retains the chemokine structure, but the equilibrium is mostly shifted to an unfolded monomeric form (61).

In the C subfamily, there is only one disulfide between Cys-11 and Cys-48. The presence of a single disulfide in XCL1 (lymphotactin) is apparently not sufficient to stabilize its structure. Attempts at structural studies for both chemically synthesized (E. J. Fernandez & E. Lolis, unpublished data) or recombinant (T. Handel, personal communication) XCL1 were unsuccessful owing to unfolded protein. Interestingly, both proteins were active in chemotaxis assays. More recently, XCL1 (lymphotactin) containing eight sites of O-linked glycosylation was chemically synthesized (62). The presence of these glycosylation sites may stabilize the three-dimensional fold of the protein and allow structural studies to proceed. It is also possible that this protein may be stabilized in vivo only in the presence of other molecules (including its receptor).

Four of the six cysteines of the 6Ckines have the same disulfide pattern as the CC chemokine family. The additional disulfide bond occurs between a cysteine near the end of the N loop and a cysteine on the C-terminal α-helix. This disulfide in I309 (CCL1) replaces van der Waals interactions at the core with a covalent bond that is essential for proper folding but is not necessary for biological activity (42).

Quaternary structures

Chemokines are known to oligomerize at the high concentrations required for structural analyses by both X-ray crystallography and NMR, or in the presence of

physiological molecules such as heparin. They display a variety of homo-oligomeric structures that may play a functional role in vivo. Although the general consensus is that the chemokine functional unit is the monomer (see section below), the different quaternary structures found for the CXC, CC, and CX3C chemokines could explain the strict recognition of receptors within a subfamily. CXCL8 (IL-8) forms a dimer in both the crystal form and in solution (Table 1). The tetrameric CXCL4 (PF4), a CXC chemokine, can be considered to be a dimer of CXC dimers (Figure 2B). However, while CXCL12 (SDF-1α) is a dimer similar to CXCL8 (IL-8) in crystalline form (41, 63), it is monomeric when studied by NMR (8). The CC chemokines MIP-1β (CCL4) and CCL5 (RANTES) are dimeric, both in solution and crystalline form (Figure 2B). The viral CC chemokine vMIP-II, like CXCL12 (SDF-1α), crystallizes as a dimer (54) but is monomeric in solution studies (55). CX3CL1 (fractalkine) is monomeric in solution studies (57), but it associates into a unique dimeric structure in the crystal (56) (Figure 2B).

While the overall monomeric topology of chemokines is similar, dimers of different subfamilies adopt different quaternary conformations (Figure 2B). Initially, it was hypothesized that the different quaternary structure could explain the rare cross-reactivity among the various chemokine subfamilies (55). The dimer interface of CXC chemokines is made up of residues largely from the first β-strand (Figure 2B). The association of two monomers produces a dimer that resembles a much smaller version of the major histocompatibility proteins, in that two helices pack against a β-sheet. The subunit interface of CC dimers is entirely different and is composed of residues from part of the extended N terminus (Figure 2B). The CX3C chemokine, (CX_3CL1) fractalkine, is a monomer in solution studies by NMR, but crystallizes as a dimer that is unlike CXC or CC dimers. In general, CXC and CX_3C chemokines tend to dimerize into a more globular structure, whereas CC chemokines associate into a relatively elongated structure. One exception to this trend is the CC chemokine CCL2 (MCP-1), which crystallizes as both a CXC-like and CC-like dimer (43). Human chemokines activate distinct sets of receptors within subfamilies with no overlap in receptor specificity across these subfamilies. Human herpesvirus-8 vMIP-II dimerizes as other CC chemokines, but it interacts with receptors from all chemokine receptor subfamilies (64, 65). It has been suggested that vMIP-II might be able to assume multiple quaternary structures that allow the protein to interact with receptors from the different subfamilies (55). The different quarternary structures could very nicely explain the rare cross-reactivity in receptor activation among the various chemokine subfamilies.

The two different modes of dimerization in chemokines that distinguish CXC from CC chemokines are attributed to differences in electrostatic surface topology between the two subfamilies. There are more hydrophobic residues (40%–80%) in the first β-strand of the CXC chemokines compared to the CC chemokines (Figure 1) (23, 66, 67). The CXC chemokines are thus more inclined to dimerize utilizing the first β-strand as the interface between the monomers, which thereby

decreases the exposure of the apolar sidechains of the residues in this region to the surrounding aqueous environment. It is interesting that, despite the relative decrease in overall number of hydrophobic residues, the CC chemokines have more residues with aromatic sidechains than the CXC chemokines in this region (Figure 1). In most CC chemokines, the residues in the immediate vicinity of the first two cysteines are hydrophobic, especially those that have been observed to associate as dimers—for example, CCL4 (MIP-β), CCL5 (RANTES), and vMIP-II. It should be noted, however, that the dimer interface of CC chemokines is derived from a combination of both hydrophobic and electrostatic interactions.

The dissociation constants for oligomeric chemokines are usually in the micromolar range and are always greater than the measured physiological concentrations of chemokines in the serum, which are in the nanomolar range (68, 69). Maximal activity also occurs at nanomolar concentration in vitro, which suggests that all chemokines are active as monomers. Whether any of the oligomeric molecules have any function in vivo remains to be determined. It is interesting to note, however, that chemokines aggregate in the presence of physiological molecules, such as glycosaminoglycans (GAGs) that include heparin and proteoglycans (70). This aggregation state may protect these small proteins from proteolysis, provide a mechanism for a chemotactic gradient by releasing chemokines that have been secreted, participate in recognition and presentation of specific chemokines to their receptors for maximal activity, or increase the association constant for oligomers. In support of a role for chemokine oligomerization in receptor activation, G protein–coupled receptors have recently been observed to be active as dimers (2, 71). The role of oligomeric chemokines continues to be investigated.

The hypothesis that chemokines are active as monomers is supported by engineered monomeric chemokines such as CXCL8 (IL-8) in which the backbone amide of Leu-28 is methylated to prevent dimerization and yet retains full biological activity (72). A CCL2 (MCP-1) Pro8Ala mutant, which cannot dimerize, is also biologically active, whereas a mutant lacking residues 2 to 8 functions as a competitive inhibitor of wild-type CCL2 (MCP-1) (73). CCL3 (MIP-1α) has also been shown to be biologically active as a monomer (74). This implies that the quaternary oligomeric conformations of chemokines may be required for functions other than receptor binding. Therefore, structural and mutational studies of chemokines for receptor binding and biological activities have been analyzed in the context of the monomeric species.

BINDING MECHANISM

The three-dimensional structure of the chemokine agonist-receptor complex has still not been determined, and the structural epitopes that dictate the activity of the chemokine family have been proposed from structural and mutational analyses of chemokines and their receptors.

Chemokines

The extended N terminus of all chemokines studied to date is believed to activate the receptor subsequent to recognition and binding. For some chemokines, for example, CXCL12 (SDF-1α) and CCL5 (RANTES), the first N-terminal residue is critical for activity. In CXCL12 (SDF-1α), cleavage of the N-terminal lysine results in total loss of activity, yielding a potent antagonist (8). In CCL5 (RANTES), if the N-terminal serine is preceded by a methionine (75) or is modified by aminooxypentane (AOP-RANTES) (76), the resulting molecules are potent antagonists. Cleavage of the two N-terminal residues of CCL11 (eotaxin-1) and CCL5 (RANTES) by the cell surface dipeptidase CD26 also results in potent antagonists (77, 78). In many chemokines that induce activation and migration of neutrophils, a three-residue motif of Glu-Leu-Arg (the ELR motif) in the extended N terminus preceding the first cysteine is critical for activity (23). Likewise, for the CCR2-binding CCL2 (MCP-1), the entire 10-residue N terminus preceding the first cysteine is involved in receptor binding and activation. Deletion of the N-terminal glutamate results in a marked reduction in activity, and deletion of the first two residues results in conversion from an agonist to an antagonist (79, 80). Truncation of the first N-terminal residue of CCL2 (MCP-1) leads to a mutant protein that acquires a novel activity toward eosinophils. These cells become chemotactic in response to the mutant chemokine, mobilize cytosolic free Ca^{2+} changes, and induce actin polymerization, presumably through CCR3 (81). An exception to this strong reliance on the N-terminal sequence is CXCL5 (ENA-78), which is a potent stimulator of neutrophils and is a member of the ELR^+ [residues 11 to 13 for CXCL5 (ENA-78)] subfamily of CXC chemokines. It is interesting that truncation mutants do not have a simple pattern that can explain the different levels of activity. The order of activity [CXCL5(9–78) or CXCL5(5–78) > CXCL5 > CXCL5(10–78)] indicates that the presence of 2 or 5 residues prior to the ELR^+ motif is more optimal than the wild-type protein (82).

The N-loop region that follows the first two cysteines and connects the N terminus to the β-sheet region through the single turn of a 3_{10} helix is the major receptor-binding site, and the sequence therein confers receptor specificity. In CXCL8 (IL–8) residues YSKPF (13–17) confer slightly greater specificity toward CXCR1 over CXCR2 (83–85). In CXCL1 (MGSA/GRO-α), the residues LQGI (15–18) confer specificity only to CXCR2. Switching these regions in CXCL8 (IL-8) and CXCL1 (MGSA/GRO-α) results in a reversal of receptor binding and activation by the chimeric proteins (86). In SDF-α (CXCL12), the sequence RFFESH (12–17) confers specificity to CXCR4. A chimeric molecule generated by replacing the N terminus and N-loop region of CXCL1 (MGSA/GRO-α) with that of SDFα (CXCL12) results in a CXCR4 agonist with only sevenfold less potency than wild-type CXCL12 (SDF-1α) (8).

In a recent study of CCL11 (eotaxin-1), alanine-scanning mutagenesis of the N-terminal region and N loop identified four categories of residues necessary for activity. One category was defined by mutants that retained wild-type binding

affinity and activity. A total of four residues were found in the N-terminal region and in the N loop. The second category revealed three mutants that retained wild-type affinity but with reduced activity. In the third category, substitution of individual residues at two positions of the N terminus or two positions at the N loop resulted in significant lowering of binding affinities but still retained measurable activities. The fourth category included only one mutant that had little activity but with 20-fold reduced affinity for CCR3; this mutation substituted alanine for Phe-11, the aromatic group following the second cysteine (87).

Chemically synthesized peptides of the N terminus and N loop display reduced activity at 10- to 1000-fold higher concentrations relative to the wild-type full-length protein. This validates the importance of not only these regions, but also of other regions of the chemokine in maintaining wild-type activities (88–93).

The type I or type III turn connecting the first and second β-strands has also been implicated in receptor binding. For example, in CCL2 (MCP-1), the residues Ser-34 and Lys-35 are critical for activity (94, 95). Because chemokines, with a few exceptions, have a positively charged residue at this position, it is possible that the 30s loop is involved in a fundamental electrostatic interaction with the receptor that brings the agonist to the cell surface or receptor. Other residues, for example those on the N loop, presumably determine receptor specificity.

Following the 30s loop is the second β-strand, which has a significant number of cationic residues. In most CXC and CC chemokines, the C terminus of the second β-strand has a lysine or arginine. This region of the chemokines is speculated to be the glycosaminoglycan (GAG) binding site (96–99). Little is known about the involvement of the third β-strand in chemokine activity. The C-terminal α-helix has been shown to modulate the activity of at least three chemokines, CCL2 (MCP-1) (79), CXCL1 (MGSA/GRO-α) (100), and CXCL12 (SDF-1α) (89), but in general it is not believed to be involved in receptor activation.

An alternative approach toward defining receptor-binding epitopes includes the characterization of the electrostatic surface potential of the chemokine, an approach that has been used to probe the growth hormone agonist-receptor system (101). "Hot-spots" that drive agonist-receptor interactions on the chemokine surface include an apolar bulge (Region 1 in Figure 3). The exposed aromatic sidechain underlying this bulge is either a phenylalanine or tyrosine in most CC chemokines and some CXC chemokines, and it has been experimentally determined to be important for interactions with the receptor (8, 87, 102). Changes in the surface area of this region brought about by mutating residues that contribute to the bulge result in lowered binding affinities of the chemokine agonist for its receptor, particularly for CCL5 (RANTES) and CXCL12 (SDF-1α) (8, 102). Another region that modulates chemokine activity for CXC chemokines is an intense positive charge of a crevice as indicated in Region 2 of Figure 3.

The interactions between chemokines and their receptors are normally considered in the context of a 1:1 stoichiometric complex. As discussed previously, chemokines interact with other molecules that may affect their oligomeric state and/or activity. For example, cell surface and soluble glycosaminoglycans bind

positively charged regions on the protein. This may result in enhanced local concentrations of chemokines, which may explain the alteration in activities observed in the presence of heparin. A recent study shows that the strong positive potential on CXCL12 (SDF-1α) (Region 2 in Figure 3) is the heparin binding site. The heparin moiety was docked into the positive potential of the CXCL12 (SDF-1α) dimer and the resulting molecular model of the CXCL12 (SDF-1α) heparin complex suggested that heparin could stabilize the dimer under physiological concentrations of CXCL12 (SDF-1α) (99). CXCL8 (IL-8), CCL5 (RANTES), CCL2 (MCP-1), and CCL3 (MIP-1α) were also observed to form higher order molecular multimers in the presence of heparan sulfate, chondroitin sulfate, and dermatan sulfate (70). The effect of soluble versus cell surface GAGs on CXCL8 (IL-8), CCL5 (RANTES), CCL2 (MCP-1), and CCL3 (MIP-1α) was compared by the activities mediated by the receptors CXCR1, CXCR2, and CCR1 (103). While chemokines were observed to have enhanced activities when bound to cell surface GAGs, the soluble GAG-chemokine complexes were unable to bind the receptor, resulting in diminished biological activity (104). Such chemokine-matrix interactions may be necessary for the chemokine to provide directional information to ensure correct agonist-receptor docking. Beside GAGs, another example of tethering chemokines to the cell surface for proper presentation to cells is based on the molecular complex formed between CXCL12 [SDF-1α and fibronectin (105)]. Conceptually, fibronectin-bound CXCL12 (SDF-1α) induces directed migration of T cells that is independent of a soluble spatial chemokine gradient.

Chemokine Receptors

Chemokine receptors are embedded in the lipid bilayer of the cell surface and possess seven-transmembrane domains (7TM) (Figure 4). These receptors have been designated CX3CR1 through 6, CCR1 through 11, XCR1, and CX_3CR1 based on whether they bind chemokines from the CXC, CC, C, or CX_3C chemokine

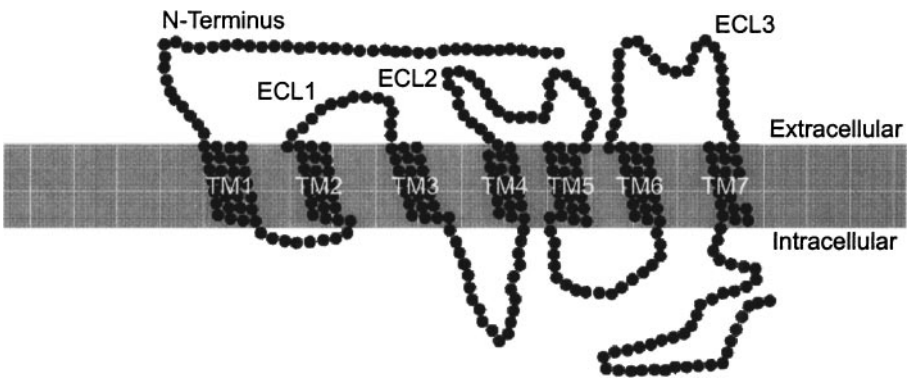

Figure 4 Topology of a typical chemokine receptor.

subfamilies, respectively (7). The prototypical GPCR, rhodopsin, has only recently been characterized by X-ray crystallography (106). This receptor reacts to a photon of light, and it is not clear whether using rhodopsin for modeling chemokine agonist-receptor interactions is valid.

Mutational analyses of the chemokine receptors have identified specific regions that interact with the agonists. The growing consensus is that the chemokine recognition and receptor activation sites are distinct. Mutagenesis of chemokine receptors indicates that binding sites are spread throughout the polypeptide. Presumably, these residues are in close proximity in the folded receptor. CXCR1 has a binding site at Asp-11. When substituted by glutamine or lysine (the corresponding residue in CXCR2), binding of CXCL8 (IL-8) is unaffected. However, substitution to an alanine results in loss in binding. It is possible, therefore, that mutations Asp11Glu and Asp11Lys preserve the binding interactions with CXCL8 (IL-8) that are also favorable for signaling, whereas the shortened alanine sidechain in the Asp11Ala mutant results in a cavity in the binding surface of CXCR1, which eliminates binding (107). Arg-280 in extracellular loop (ECL) 3 is critical for agonist binding. Other residues implicated in agonist binding and/or signal transduction are Arg-199, Arg-203, Asp-265, and Glu-275 (107, 108).

CXCR1 and CXCR2 are significantly different in sequence at the N terminus. CXCR1 is unable to bind most ELR^+ chemokines other than CXCL8 (IL-8) and GCP-2 (CXCL6) (109, 110). It is possible that the differences at the N termini of these two receptors and the differences among the ELR^+ chemokines lead to different sites of interaction of CXCL8 (IL-8) and CXCL6 (GCP-2) with CXCR1, and CXCL8 (IL-8), CXCL6 (GCP-2), CXCL1 (MGSA/GRO-α), and CXCL7 (NAP-2) with CXCR2. Unlike CXCR1, CXCR2 binds CXCL8 (IL-8) through the N terminus and ECL1. CXCL7 (NAP-2), however, exhibits a different binding pattern and interacts exclusively with ECL1, whereas MGSA/GROα (CXCL1) interacts with the N terminus but not ECL1 (111). According to these studies, residues Glu-7, Asp-9, and Glu-12 are important for binding and activation, while Katancik et al. have identified Lys-108, Asn-110, and Lys-120 as being critical for signaling only (112). This confirms the results of an earlier study of ELR^+ chemokines that showed that the determinants of high affinity binding and those of receptor activation are distinct for CXCR2 (113).

The interactions of CXCR4 with its agonist CXCL12 (SDF-1α) result from residues in the N terminus and ECL2. The three N-terminal residues Glu-14, Glu-15, and Tyr-21 of CXCR4 are of particular importance for binding CXCL12 (114, 115). Residues in ECL2, especially the acidic sequence of Glu-179, Ala-180, Asp-181, and Asp-182, are critical for activation of CXCR4 (114). CXCL12 (SDF-1α) has a high positive potential, and a significant amount of this positive charge is localized in Region 2 (Figure 3). It is possible that the interactions of the positive charge on CXCL12 (SDF-1α) with the negatively charged regions on CXCR4 contribute to the association of these two proteins. CXCR4 is also the syncytium-inducing HIV-1 strain (X4 strain) coreceptor (116). Negatively charged residues of CXCR4 also appear to be involved in interactions with the basic V3 loop

of gp120, the envelope glycoprotein of X4 HIV-1 strains (117). Mutagenesis of specific residues on the CXCR4 N terminus, ECL1, and ECL2 identified glutamate and aspartate residues, specifically Glu-15, Glu-32, Asp-97, Asp187, and Asp-193, as being important for interactions with X4 HIV-1 gp120 (117, 118). Other residues such as Asn-11, Arg-30, and Arg-188 have also been identified as binding determinants for HIV-1 gp120. Based on chimeric studies that involved replacing ECL2 of CXCR2 with the corresponding loop from CXCR4, the observation was made that the CXCR4 ECL2 was able to confer the HIV-1 coreceptor function to the chimeric CXCR2 (119). Since both CXCL12 (SDF-1α) and the V3 loop domain of X4 HIV-1 gp120 that interacts with CXCR4 have a high positive potential, and since the interacting domains of CXCR4 are mostly negatively charged, it is likely that the interactions between CXCR4 and these molecules are driven by charge complementarity.

Charge potential also appears to regulate CCL2 (MCP-1) interactions with CCR2. The DYDY motif, which includes residues Asp-25 and Asp-27 of the CCR2 N terminus, presumably interacts with the basic cluster on the chemokine formed by the residues Arg-24 and Lys-49 (95). In the model that is proposed, the remainder of the CCR2 N terminus aligns itself along a hydrophobic groove on the surface of CCL2 (MCP-1). This orients the signaling residues, Tyr-13 and the N terminus of CCL2 (MCP-1), to make the necessary interactions with CCR2. CCL2 (MCP-1) also interacts with ECL1 of CCR2, specifically Asn-104 and Glu-105, which contributes to high-affinity binding, and with His-100, which is critical for activation of the receptor (120).

Biophysical studies using NMR techniques on domains of CCR3 and the agonist CCL11 (eotaxin-1) indicate that the receptor N terminus is the major determinant of agonist binding (32). Interactions between ECL2 and CCL11 (eotaxin-1) were inferred from the observation that the mixture of eotaxin-1 and peptides derived from ECL2 precipitated from solution, unlike peptides from ECL1 and ECL3, which showed no indication of binding to the chemokine.

The CCR5 N terminus is also important for recognition and binding to its chemokine agonists. Truncation of the N-terminal region progressively decreased binding affinity for the agonists CCL5 (RANTES) and CCL4 (MIP-1β). Asp-2, Tyr-3, Tyr-10, Asp11, Glu-18, and Lys-26 are specifically important for binding as deduced from alanine-scanning mutants (121–123). The two disulfides, which are present in all chemokine receptors, are critical for binding to CCL4 (MIP-1β). It is interesting that the absence of the cysteines reduces but does not eliminate the use of CCR5 by HIV-1 strains (R5 HIV-1) that infect cells through this receptor (124). There are other regions of CCR5 involved in agonist interactions, such as ECL2, which is important for proper signaling subsequent to binding (125). Additionally, the second and seventh transmembrane (TM) domains play a role both in binding and/or signaling. The second TM domain includes a Thr-X-Pro (residues 82–84) motif. This motif introduces a kink in the helical domain, presumably due to destabilization of the α-helix by the proline. The Pro84Ala mutant has lower binding affinity for the chemokines CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5

(RANTES), and MCP-2 (CCL8) and displays virtually no activity. Mutating Thr-82 to an alanine results in impaired activation of the receptor but with wild-type affinity for the agonists (126). TM7 is required for binding as alanine mutants in this region and chimeric receptors that replace the CCR5 TM7 with the corresponding region from CCR1 have impaired activity in response to CCL4 (MIP-1β). Met-287 of TM7 is critical for this CCR5 activity, although none of the chimeras or mutants showed any defect as R5 HIV-1 coreceptors for R5 strains (127).

Posttranslational modifications of chemokine receptors have been observed to affect activity. Sulfation of tyrosines, particularly Tyr-3, in the CCR5 N terminus is essential for proper activity of the receptor, both in binding to chemokines and in R5 HIV-1 coreceptor activity (128). CXCR4 is also posttranslationally modified by N-linked glycosylation of Asn-11 and Asn-176. This modification is reported to maintain high-affinity agonist binding (129). Both glycosylation and sulfation of the receptor CCR2B has been observed. Tyr-26 is sulfated in the CCR2B N terminus. Mutation of this residue to alanine results in diminished activities toward CCL2 (MCP-1) (130). In the same study, N-linked glycosylation was also confirmed as treatment of cells expressing wild-type CCR2B by N-glycosidase F resulted in a reduction of the observed molecular weight of the receptor from 50 kDa to 45 kDa.

DISEASE AND INHIBITION

Inhibitors of chemokine activities have been developed from monoclonal antibodies, mutants of chemokines, natural proteins encoded by viral genomes, and small molecules identified by screening programs. Other technologies that are being developed to combat chemokine-mediated human diseases include gene therapy, antisense inhibitors, and ribozymes. Page limitations preclude us from discussing all of these potential therapies. This section focuses on viral proteins and small organic molecules. Many of the neutralizing monoclonal antibodies are described in catalogs of companies that sell these molecules for research purposes. The mutant inhibitory chemokines have already been discussed and are not included in this section.

Viral chemokine homologues

There are two types of viral proteins that inhibit chemokines. One type belongs to the chemokine family that may have been pirated from a mammalian genome and mutated to confer benefits to the virus (131). These molecules have subverted the chemokine system either to attract the appropriate target cells to infect or to inhibit the host immune response mediated by chemokine receptors. An example of this is the Kaposi's sarcoma-associated herpesvirus-8 (HHV8) genome, which encodes three chemokine-like molecules that interact with a variety of human chemokine receptors. These three chemokines—viral macrophage inflammatory protein-I, -II, and -III (vMIP-I, vMIP-II, and vMIP-III)—antagonize several human

chemokine receptors and activate others (65, 132–134). vMIP-I and vMIP-II share extensive homology (>70%) with each other. While vMIP-I is a CCR8 agonist and antagonizes other receptors, such as CCR3 and CCR5 (135), vMIP-II has broad spectrum antagonist activities on CXCR4, CCR1, CCR2, CCR5, CCR10, CX3CR, and XCR1 (65, 132, 136–138). Additionally, vMIP-II is an agonist for CCR3 (132). Its activity on CCR8 remains unresolved because both agonist (133) and antagonist (139, 140) activities have been reported. It is interesting to note that HHV-8 also encodes a constitutively active GPCR (141), and vMIP-II is an inverse agonist of this receptor (142). How vMIP-II interacts with so many receptors is still under active study (143). vMIP-II has the same chemokine topology as the human homologues and is a monomer in solution studies, but it is a CC dimer in the crystal (54, 55). vMIP-III is only 37% homologous to vMIP-I and vMIP-II and is a CCR4 agonist (134). The three-dimensional structures of vMIP-I and vMIP-III are yet to be determined.

Cytomegalovirus encodes two α-chemokines, vCXC-1 and vCXC2. vCXC1 attracts human neutrophils and is almost as potent as IL-8. It preferentially activates CXCR2 but not CXCR1 (131, 144). Activities of vCXC2 have not yet been reported.

MC148 is a CC chemokine from the human poxvirus *Molluscum contagiosum* virus (MCV). MC148 potently interfered with chemotaxis of human monocytes, lymphocytes, and neutrophils in response to a large number of CC and CXC chemokines with diverse receptor specificities. Evidence that the viral protein binds to human chemokine receptors was obtained by competition binding and calcium mobilization experiments. MC148 has been reported to interact with at least the following CC and CXC receptors: CCR1 and/or CCR5, CCR2, CCR8, CXCR1 and/or CXCR2, and CXCR4 (145, 146). A more recent study, however, indicates that MC148 interacts exclusively with CCR8, and not with any of the receptors listed above (140).

Other viral protein inhibitors

The second category contains viral proteins that inhibit chemokine activities and has no relationship in sequence or structure to chemokines. The cowpox virus secretes a 35-kDa protein, vCCI, with broad CC chemokine binding affinities (147). The mechanism of inhibition is believed to be different from other inhibitors that bind directly to chemokine receptors. The crystal structure of vCCI describes a unique β-sandwich fold with an exposed patch of negatively charged residues (148). It is believed that many CC chemokines interact with vCCI at this patch and are, therefore, prevented from binding their natural receptors.

Vaccinia viruses (VV) and other orthopoxviruses, such as *Ectromelia* virus (EV), also secrete chemokine binding proteins (vCKBP) that selectively bind chemokines. vCKBP from VV binds CC chemokines with high affinity but not CXC or C chemokines. The results of receptor binding experiments show that vCKBP binds CC chemokines with different affinities: CCL3 (MIP-1α) > CCL11

(eotaxin) > CCL5 (RANTES) > CCL2 (MCP-1) > CCL1 (I309) (149, 150). The murine γ-herpesvirus also secretes a 44-kDa protein, hvCKBP, that has broad chemokine binding capabilities. It binds and inhibits the activities of chemokines from the CXC, CC, C, and CX3C subfamilies (151). hvCKBP has a strong affinity for CXCL8 (IL-8), moderate affinity for CXCL1 (GRO-α) and CXCL10 (IP-10), and no apparent affinity for CXCL13 (BCA-1) and CXCL12 (SDF-1α). In the CC subfamily, hvCKBP interacts strongly with all the chemokines tested: CCL5 (RANTES), CCL3 (MIP-1α), CCL2 (MCP-1), and CCL13 (MCP-4). hvCKBP also interacts with CX3CL1 (fractalkine), but has low affinity for XCL1 (lymphotactin). All of these interactions occur with the monomer of the chemokine. Moreover, hvCKBP binds these chemokines in the presence of heparin and heparan sulfate, which implies that its binding site on chemokines is independent of the GAG binding site. These viral proteins present model structures with potential therapeutic applications in reducing chemokine-associated ailments such as inflammation.

Small-Molecule Antagonists of Chemokine Receptors

Animal models have implicated a number of chemokines and their receptors in disease (152). In addition, the presence of increased expression of chemokines or their receptors in diseased tissues suggests a pathophysiological role for these molecules. The only disease that is known to require the chemokine system in humans is AIDS.

Given the success the pharmaceutical industry has had with small-molecule antagonists of G protein–coupled receptors using high-throughput screening techniques, this method is most likely to lead to a compound that can be optimized into a molecule that can enter clinical trials (153). Small-molecule antagonists exist for seven of the eighteen chemokine receptors. We review the application of these small molecules in studying the pathophysiology of chemokines and their receptors.

One of the first nonpeptide small-molecule receptor antagonists described, [N-(2-hydroxy-4-nitrophenyl)-N′-(2-bromophenyl)urea (SB 225002)], is an inhibitor of ELR^+ chemokine-receptor interactions (154) (Figure 5A). This compound has >150-fold selectivity for CXCR2 over CXCR1, despite the sequence similarity

Figure 5 Examples of small-molecule chemokine receptor antagonists. (*A*) CXCR2 antagonist N-(2-hydroxy-4-nitrophenyl)-N′-(2-bromophenyl)urea (154). (*B*) CCR1 antagonist 4-hydroxypiperidine analog (157). (*C*) CCR1/CCR3 antagonist UCB35625 (158). (*D*) CCR3 antagonist (S)-methyl-2-napthoylamino-3-(4-nitrophenyl)propionate (159). (*E*) CCR2B antagonist (160). (*F*) CCR5 antagonist N,N-dimethyl-N-[4-[[[2-(4-methylphenyl)-6,7-dihydro-5H-benzocyclohepten-8-l] carbonyl]amino]benzyl]tetrahydro-2H-pyran-4-aminium chloride (161). (*G*) CXCR4 antagonist AMD3100 (162).

A
B
C
D
E
F
G

of these two receptors. Only one murine IL-8 receptor with similar homology to CXCR1 and CXCR2 has been identified (155). Gene-deletion studies reveal that the mice suffer from an increase in B cells and display splenomegaly. This receptor is also the major mediator of neutrophil chemotaxis (156). In human studies, it has not been possible to distinguish between the roles of CXCR1 and CXCR2. Studies with SB 225002 show that human neutrophil chemotaxis is predominantly mediated by CXCR2. Coadministration of IL-8 and SB 225002 in rabbits, which express two IL-8 receptors, inhibited margination of neutrophils in a dose-dependent manner. These studies suggest that while CXCR2 is the neutrophil chemoattractant, CXCR1 is responsible for production of superoxide radicals and degranulation of neutrophils (154).

A clear association with a human disease is not known for CCR1, although some of the agonists [CCL3 (MIP-1α) and CCL5 (RANTES)] for this receptor appear at inflammatory sites, particularly for multiple sclerosis and rheumatoid arthritis. CCL3 (MIP-1α) was found to play a role in murine experimental immune encephalomyelitis, an in vivo animal model of multiple sclerosis (163). RANTES seems to be upregulated in rheumatoid arthritis (164). To provide additional tools to examine the role of CCR1 in human disease with the potential to develop therapeutically useful treatments, a high-throughput screen identified 4-hydroxypiperidine compounds with a Kd as low as 40 nM (Figure 5B). This compound was specific for CCR1 and inhibited Ca2+ mobilization by CCL3 (MIP-1α) (157).

Even when specific cell types are associated with a particular disease, it can be difficult to develop a single therapeutic molecule that targets the associated cell type because multiple chemokine agonists and receptors can be involved in regulating the activity of these cells. In these diseases, it may be necessary to administer multiple antagonists targeting the different chemokine agonists and receptors. For example, in human asthma, the eosinophil, a cell that expresses CCR1, CCR3, and CXCR2, is required for bronchial hyperactivity. While CCR3 is the major chemokine receptor expressed in this cell, CCR1 and/or CXCR2 may also play a minor role in recruiting eosinophils into the lungs. Sabroe et al. describe an aminopiperidine derivative, UCB35625 (Figure 5C), which antagonizes the effects of chemokine-mediated activity of both CCR1 and CCR3 (158). However, significantly higher concentrations of UCB35625 are required for chemokine displacement than for inhibition of receptor function. In competition-binding studies the compound is unable to sufficiently displace the CCR1 and CCR3 bound chemokines for an IC_{50} to be determined. It was concluded that the compound binds to a site that may slightly overlap with the chemokine binding sites of both receptors but that its mechanism of inhibition is based on its ability to block receptor activation. Furthermore, it is speculated that the compound interacts with a region that undergoes a conformational change upon ligand binding. [It should be noted that with the CCR3-specific inhibitor, SK&F-L-45523, there is a large discrepancy between the IC_{50} for Ca^{2+} mobilization (9 nM) and for receptor binding (800 nM) (159).]

It would be interesting to compare the dual-specific chemokine antagonist UCB35625 with a specific CCR3 antagonist in the presence or absence of the

high affinity CCR1 antagonist 4-hydroxypiperidine derivative in animal models of human diseases. A CCR3-specific nonpeptide antagonist has recently been described (SB-328437) (159) (Figure 5D). The IC_{50} of this molecule based on competitive displacement of ^{125}I-CCL11 (eotaxin-1) on human eosinophils is 4.5 nM. Although the pharmacodynamics and pharmacokinetics would need to be defined in all animal studies, the strategy of comparing the two antagonists described above might help in determining the relative contributions of CCR1 and CCR3 in asthma or other allergic diseases.

A CCR2B antagonist has been described by Forbes et al. (160) (Figure 5E). Antagonists of CCR2 have been studied as potential therapeutic agents for diseases such as atherosclerosis (165) and rheumatoid arthritis (166). This antagonist has a K_i of 50 nM for CCR2. It also interacts with CCR5 but with lower specificity and a K_i of 4.26 μM.

Antagonists also exist to the two major HIV-1 coreceptors, CCR5 and CXCR4. CCR5 is the coreceptor for nonsyncytium HIV-1 strains (categorized as R5 strains), while CXCR4 is the coreceptor for the syncytium-inducing strains (X4 strains). CCR5 is a particularly attractive target for inhibiting HIV-1 entry because there is a natural mutation in some humans that involves a 32-base pair deletion and generates a nonfunctional receptor that does not result in any ill effects (167). Virtually all of the individuals that are homozygous for this mutation are resistant to HIV-1 infections. The implication of this observation is that R5 strains are responsible for the initial infection and are associated with the asymptomatic stage of the disease. During the course of infection, the R5 strains mutate to X4 strains, which immediately precedes the full onset of AIDS (168).

Whether antagonism of CCR5 will decrease R5 virus titers in the serum and delay AIDS is not known. CCR5 antagonists have been developed to address this issue (Figure 5F) (168). A number of studies already indicate that CCR5 antagonism could alter the course of the disease. In vivo assays of human PBL-SCID mice infected with R5 strains suggest that inhibition of CCR5 by modified RANTES antagonists may accelerate the conversion to X4 strains (169). In vitro studies also indicate that a substantial number of chemokine receptors can be coreceptors for HIV-1, although in vivo CCR5 and CXCR4 are thought to be the major coreceptors (6, 116, 170). Inhibition of both receptors may give rise to other strains that may alter the pathophysiology of this disease as these receptors are likely to be expressed on different types of cells.

A number of small-molecule inhibitors of X4 HIV-1 strains were synthesized that were later discovered to target CXCR4 when this receptor was identified as an HIV-1 coreceptor (162, 171, 172). AMD3100 (Figure 5G) has been injected in human volunteers and is well tolerated, but it is unlikely to be approved for drug use owing to pharmacokinetic parameters (173). Analogues of AMD3100 with better oral availability have been developed (174). The concept of using CXCR4 as a drug target has been validated by in vitro experiments of DeClerq and colleagues, who showed that the X4 isolates could be converted to the less pathogenic R5 strains by AMD3100 (175). As mentioned above, gene deletion of either CXCL12

(SDF-1α) or CXCR4 in mice is fatal to the fetus. The effect of long-term inhibition of CXCR4 activity in adults is not known. Whether inhibition of CXCR4 delays the onset of AIDS will not be known until full clinical trials are initiated and analyzed, as not all patients infected with HIV-1 sero-convert from R5 strains but still suffer from the known symptoms of AIDS.

Other Inhibitors

In addition to the antagonists of chemokine receptors listed above, the U.S. patent database contains descriptions of small-molecule antagonists to CXCR1, to the MCP-1 receptor (presumably CCR2), and to other molecules described in this section. It will not be long before other small-molecule antagonists will be available. The challenge will be to identify therapeutic applications for these antagonists aimed at curing or ameliorating the effects of human diseases.

CONCLUSIONS

As the physiological properties of chemokines become known, it will be necessary to understand the molecular basis of these functions. There are only a handful of known chemokine three-dimensional structures, although there are over 40 chemokines identified in humans. The three-dimensional structure of a chemokine agonist-receptor complex remains to be determined. The pharmacological implications of characterizing chemokine structures are vast. These proteins are associated with wound healing, proper functioning of the immune system, angiogenesis/angiostasis, metastasis, organ development, and lymphoid trafficking, among other biological processes. Improper functioning of the chemokine-mediated signaling system can lead to diseases such as inflammation and autoimmunity. Also, the chemokine system is associated with HIV-1 pathophysiology and may be associated with contagious diseases such as herpesvirus-8 associated Kaposi's sarcoma and many other viral diseases. Development of small-molecule antagonists, partial agonists, and agonists may be aided by model systems that are based on a thorough understanding of the mechanism of binding and activation involved in the chemokine agonist-receptor system. Determination of the GPCR rhodopsin structure by X-ray crystallography is encouraging and could lead to a model system for crystallizing the chemokine GPCRs complexed to their cognate agonists or antagonists. Such a structural approach could be used to convert lead compounds into small-molecule antagonists with increased potency, efficiency, absorption, distribution, and lowered metabolism, elimination, and toxicity; these benefits would make the use of these molecules in therapeutics more attractive.

ACKNOWLEDGMENT

We are grateful to Marc Deller for assistance with the figures and for critical reading of the manuscript.

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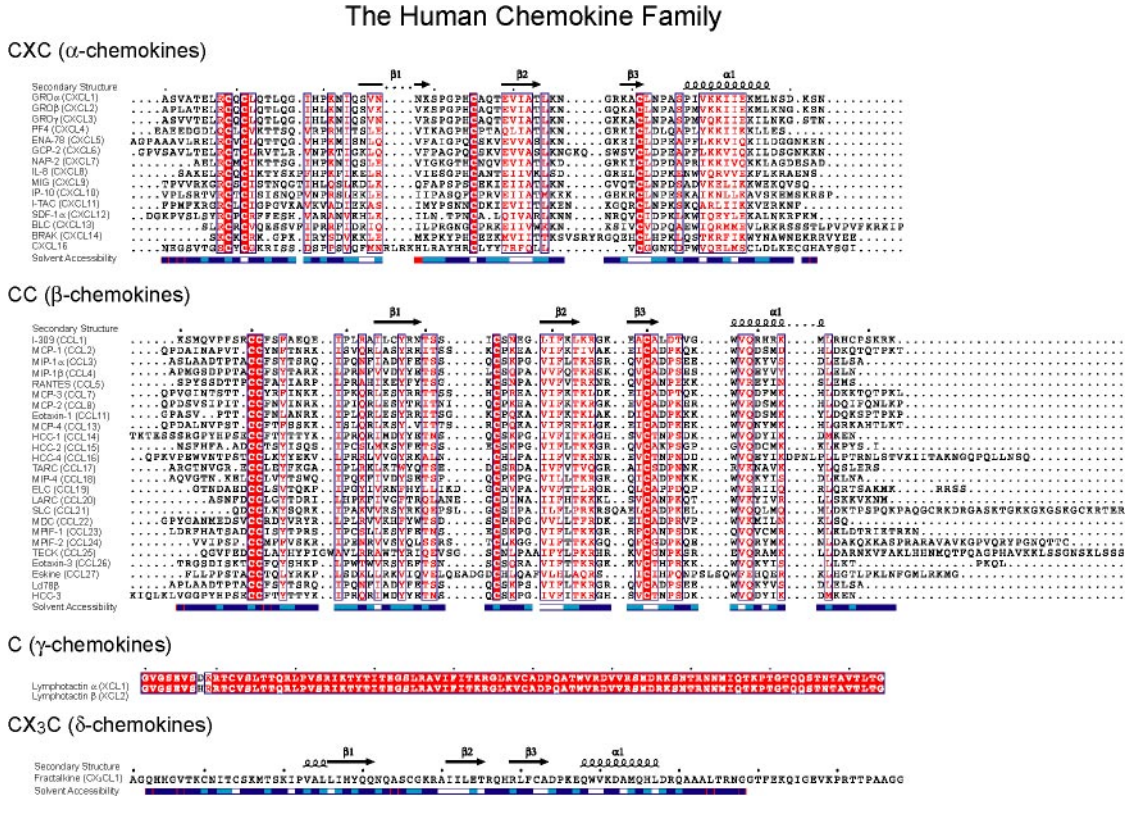

Figure 1 Primary sequence alignment of human chemokines known to date. Residues with a red background are identical in the alignment, while those with red letters are homologous in each of the chemokine subfamilies. Above each alignment is the predicted secondary structure based on a prototype in the subfamily. Beneath each alignment is the predicted solvent accessibility for the chemokine monomer; the dark blue indicates a residue with a fully exposed sidechain, light blue indicates partial exposure, and white indicates that the residue is buried.

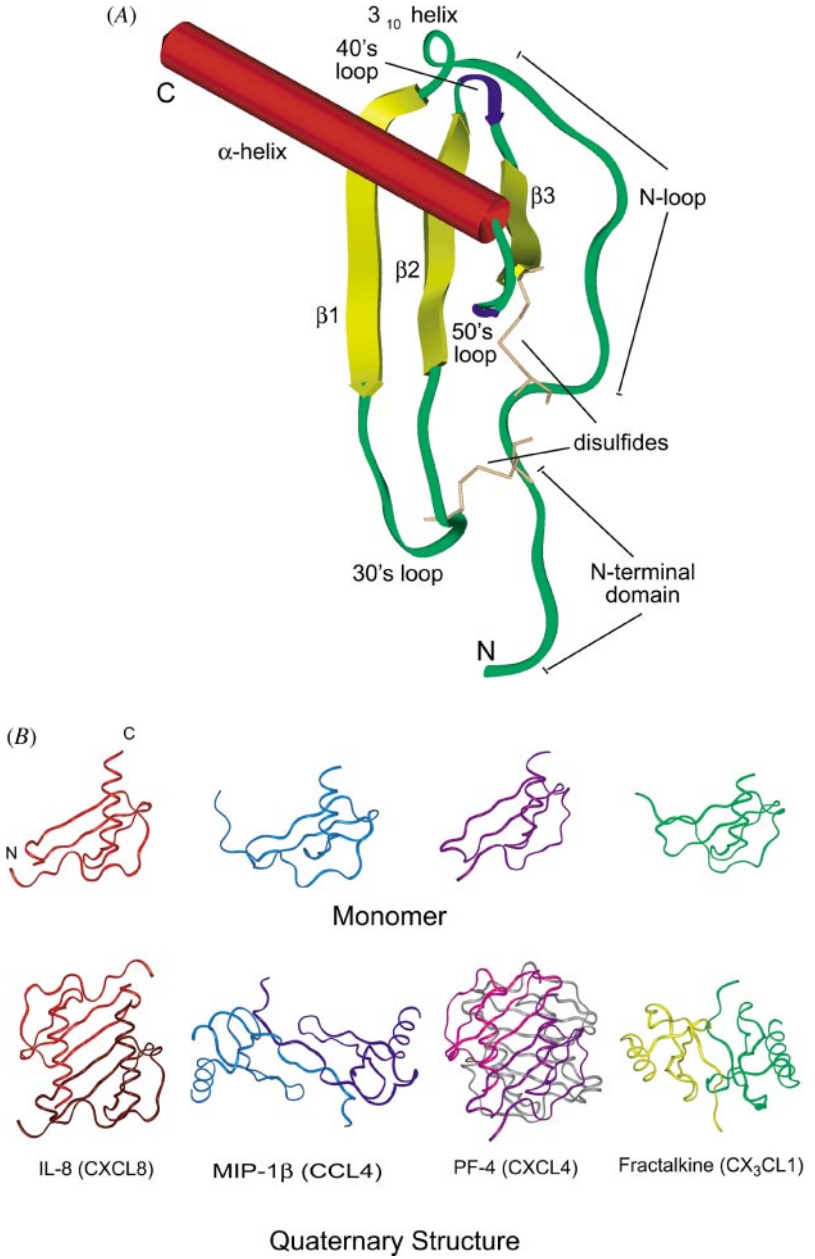

Figure 2 Ribbon trace of the chemokine Ca atoms. (*A*) Monomer of the prototypical chemokine CXCL8 (IL-8) with the various secondary structure elements labeled accordingly. (*B*) Monomers (*on top*) and the corresponding quaternary structures (*below*) of the four chemokines, IL-8 (CXCL8), MIP-1β (CCL4), PF4 (CXCL4), and fractaline (CX_3CL1). Monomers are colored in different shades.

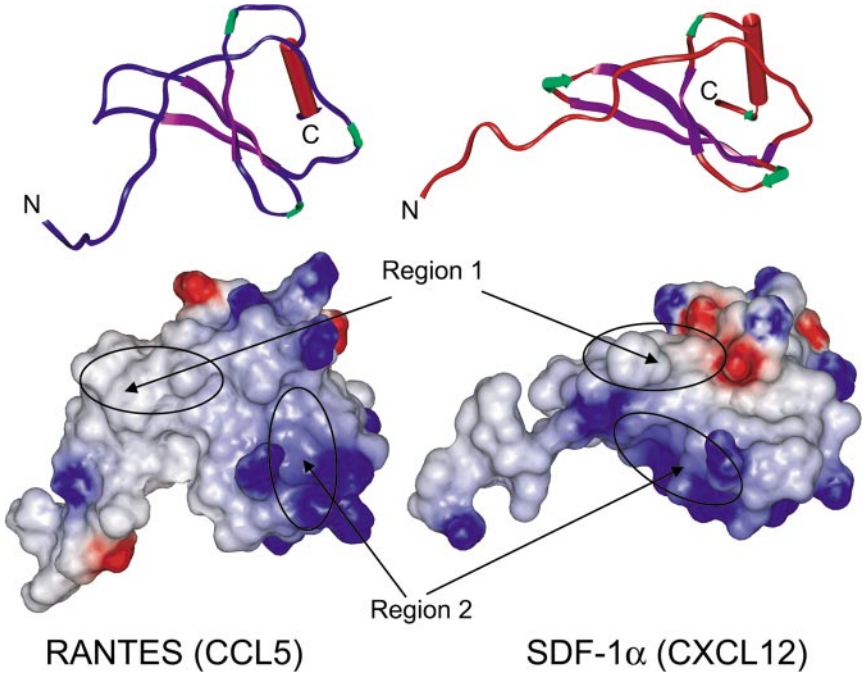

Figure 3 Electrostatic potential map of the chemokines RANTES (CCL5) and SDF-1α (CXCL12). The monomeric structure of each chemokine is shown above its potential map.