The chemokine/chemokine-receptor family: potential and progress for therapeutic intervention

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The chemokines are a large superfamily of chemotactic cytokines that are utilized to direct the trafficking and migration of leukocytes within the immune system. The chemokines mediate their activity through a large family of G-protein-coupled receptors, and thus are highly tractable as therapeutic targets. Exciting advances have been made in the field within the past year, not the least of which is the disclosure of potent antagonists of several chemokine receptors. Several CCR5 antagonists have demonstrated potent antiviral activity and may represent novel therapeutic agents for the treatment of AIDS. In addition, new biological insights have been gained from the demonstration that the targeting of cells to inflammatory sites is tissue specific, such that different chemokine/chemokine-receptor pairs are utilized in recruitment of T-lymphocytes to the skin and to the intestine. Also, utilization of neutralizing antibodies to the CXCR3 ligand Mig in murine allograft transplantation models has demonstrated the importance of CXCR3 in orchestrating T-cell-mediated tissue rejection.

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Abbreviations

CCR	CC receptor
CXCR	CXC receptor
GPCR	G-protein-coupled receptor
MCP	monocyte chemotactic protein
SDF	stromal-derived factor
SLC	secondary lymphoid-tissue chemokine
SP	spiropiperidine
TM	transmembrane

Introduction

Although the trafficking and selective accumulation of leukocyte subsets are essential components of host response to injury, insult and infection, these same processes are also responsible for the pathophysiology of inflammatory and autoimmune diseases, as well as rejection in transplantation. Evidence has accumulated over the past decade that much of this cell migration is regulated by a superfamily of about 50 small proteins called chemotactic cytokines, or chemokines [1,2]. Strikingly, individual chemokines act on distinct cell types. For example, IL-8 principally recruits and activates neutrophils [3,4], whereas the actions of MIG are restricted to lymphocytes [5]. Because the goal of therapeutic intervention is disease modification with minimal impact on normal host defense, the specificity offered by the chemokines make them extremely attractive targets. Modulation of chemokine activity may also allow regulation of other physiological functions, as these mediators also play roles in hematopoiesis [6,7], angiogenesis [8,9], differentiation and development [10–15]. Paradoxically, the chemokines and their receptors are used by intracellular pathogens, most notably HIV and *Plasmodium vivax*, to faciliate entry and transmission and may represent novel antiviral and antiparasitic targets [16–18].

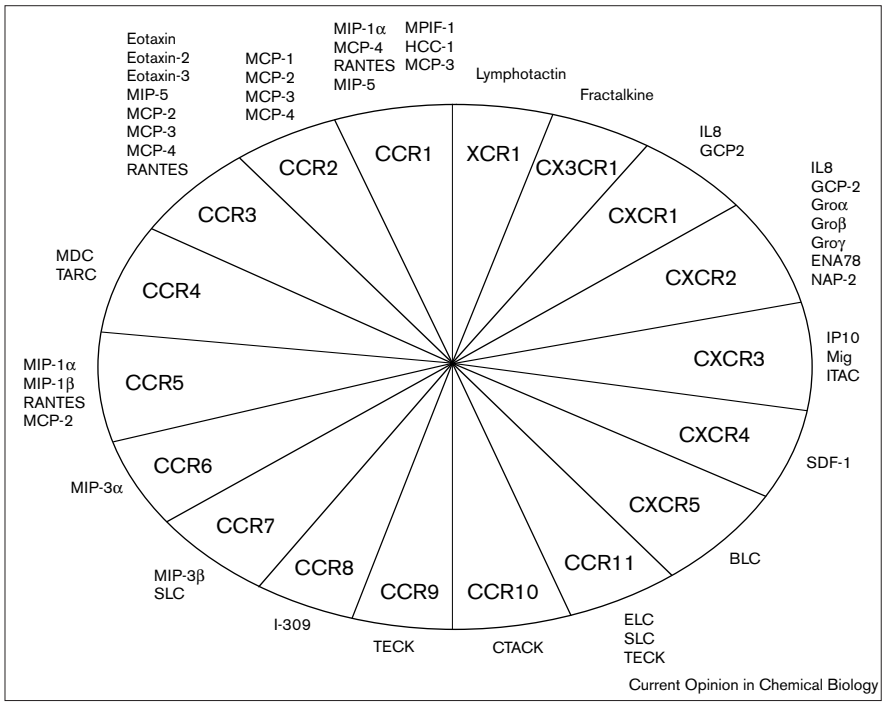
Classically, the chemokine superfamily is defined by four conserved cysteines that form two disulfide bonds, and can be structurally subdivided into two major branches on the basis of the spacing of the first cysteine pair. Chemokines in which these residues are adjacent, such as RANTES and MIP-1α, form the CC subfamily, and those in which they are separated by a single amino acid, such as IL-8 and IP-10, comprise the CXC subfamily. Additional variants of these motifs exist — there is at least one chemokine in which the cysteines are separated by three residues (CX_3C), and one that lacks the first cysteine in the pair (C).

Chemokines induce their effects by binding to an array of G-protein-coupled receptors (GPCRs), some of which are specific and interact with a single chemokine, whereas others — the so-called shared receptors — bind multiple ligands within, but not between, the CC or CXC branches [19]. For example, CCR8 binds only the CC chemokine I-309 [4,20,21], whereas CCR1 interacts with the CC chemokines MIP-1α, RANTES, and monocyte chemotactic protein (MCP)-3 [22,23], and CXCR3 with the CXC chemokines IP-10, Mig, and I-Tac [24–26]. The current roster consists of 11 CC receptors (CCR1–11), five CXC receptors (CXCR1–5), one CX_3C receptor, and one C receptor (Figure 1) [19]. In addition, there are two highly promiscuous non-signaling receptors or binding proteins, DARC [27] and D6 [19,28,29]. Chemokines can be specific, and interact with only one receptor type (e.g. I-309 interacts only with CCR8), or they can be pleiotropic and bind to multiple receptors (e.g. RANTES can bind to CCR1, 3 and 5 [22,23,30–32]).

As knowledge of the size and complexity of the chemokine system has increased, so has the concern about redundancy: the inhibition of a single element in the network might be compensated for by other components rendering efficacy of therapeutic intervention problematic. Fortunately, studies with knockouts, antibodies and other inhibitors have demonstrated that although redundancy does exist there is considerable specificity as well. The specificity arises from spatial and temporal control of chemokine production, as well as regulation of receptor expression on particular leukocyte subtypes. For example, while CXCR3 and CCR8 are

Figure 1

The chemokine/chemokine-receptor family. Chemokine receptors whose ligands have been identified are shown within the wheel, with their known chemokine ligands are listed peripherally.



both expressed by T cells, the former is restricted to cells with the Th1 phenotype, and the latter to lymphocytes with a Th2 phenotype [33–35]. These observations suggest that antagonism of CXCR3 will be useful in modulating Th1-mediated responses such as transplant rejection, and that CCR8 antagonists might be effective in Th2 orchestrated diseases, such as asthma. Indeed, studies in animal models are consistent with these hypotheses [36,37].

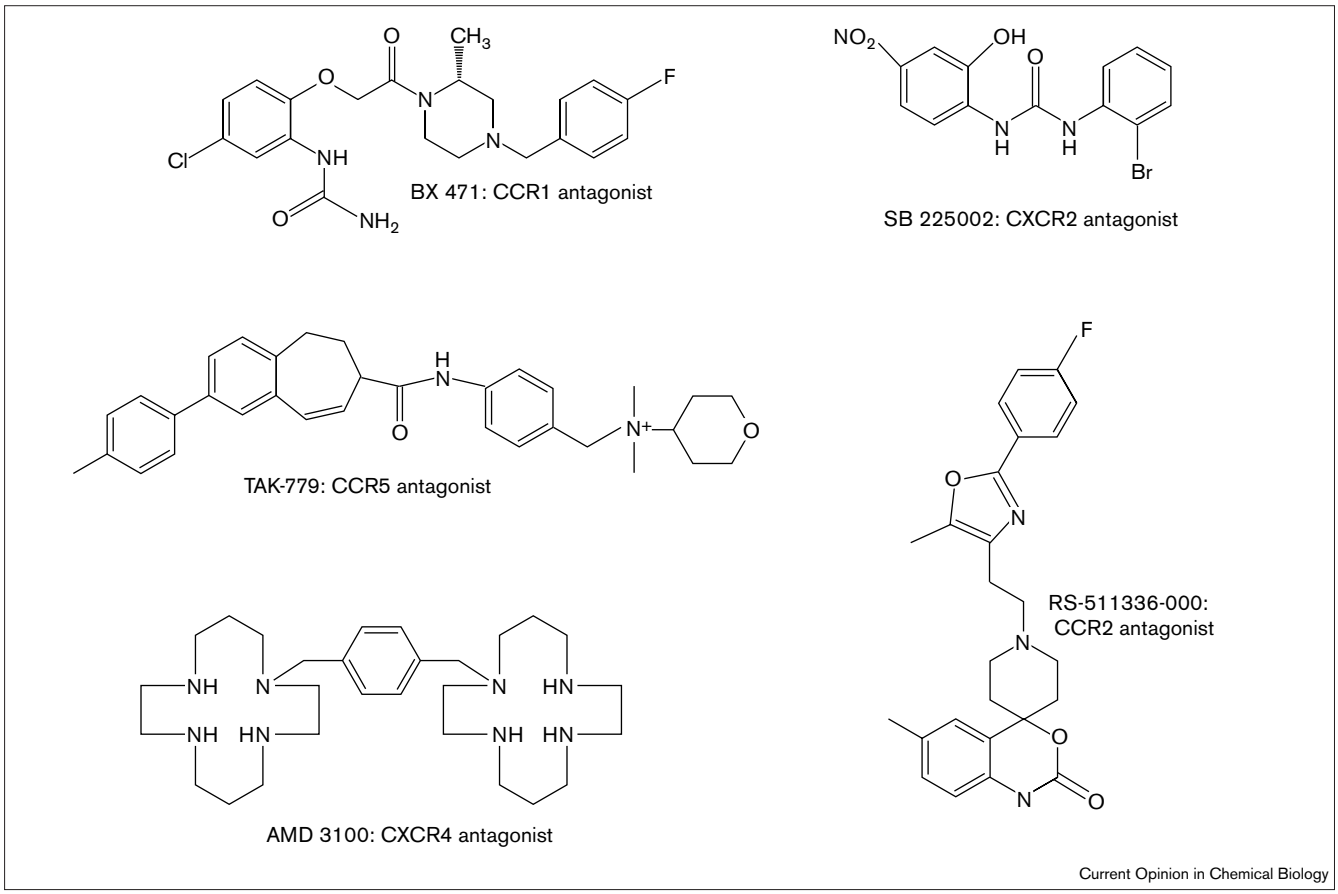
Our challenge, if we are to effectively exploit the therapeutic potential embodied in the chemokine system, is to identify the specific ligands and receptors that are essential and rate limiting in a given disease setting, and develop reagents to inhibit their interactions. Chemokine receptors are particularly attractive targets because they are GPCRs, a protein class for which there is a long history of developing small, non-peptidyl antagonists. In this review we discuss information that has emerged over the past year defining and validating therapeutic targets, as well as advances in the development of inhibitors of chemokine-receptor interactions.

Non-peptidyl, small-molecule antagonists of chemokine receptors

This past year has seen a substantial increase in reports of non-peptidyl small-molecule antagonists of chemokine receptors. Although much of the information still comes from the patent literature, presentations that provide more detailed descriptions of potencies and functional properties of such inhibitors are being made at national and international meetings, and papers are beginning to appear in peer-reviewed journals. High-affinity antagonists have been reported for CCR1, CCR2, CCR5, CXCR2, and CXCR4 [38,39,40•,41••,42–45].

A common feature of most of these compounds, regardless of structural class, is the presence of a basic group, suggesting an interaction with a negatively charged residue in the receptor. Results from site-directed mutagenesis experiments with CCR2 and CCR5 support this hypothesis. The most potent CCR2 antagonists are the spiropiperidines (SPs), and structure/activity relationship studies clearly show a requirement for the basic nitrogen in these compounds [40•]. Most chemokine receptors contain two acidic residues in the upper third (extracellular side) of transmembrane helix (TM)7, D284 and E291 in CCR2. Elimination of the negative charge at position 291 by substitution with alanine (E291A) or glutamine (E291Q) decreased the affinity of the SPs by >180-fold, but had only minor effects on the binding of the chemokine MCP-1. In contrast, the D284A or D284N mutations did not alter compound affinity. Some of the SPs also have significant affinity for the biogenic amine receptors, in part because of an interaction between the basic nitrogen in the ring system of the SP and an aspartic acid residue in TM3. Interestingly, modeling indicates that the critical aspartic acid residue occupies the same spatial position in this receptor as does E291 in CCR2, implying that the mode of binding of the SPs may be the same in both receptors [40•]. TAK-779 (Figure 2) is an antagonist of CCR5 that

Figure 2



Structures of some recently reported chemokine receptor antagonists.

contains a quaternary nitrogen [41••]. As was observed with CCR2, an acidic residue in TM7, E283, appears to be important for interaction of the compound, again suggesting a similar binding motif [46•]. Interestingly, TAK-779 has affinity for CCR2 as well, most probably reflecting the significant sequence homology between the two receptors [41••]. Additional mutagenesis of CCR5 suggests that TAK-779 binds in a pocket in the upper third of the transmembrane region bounded by TM1, 2, 3, and 7 [46•], a motif and location used by antagonists of other GPCRs [47].

Because the interactions between antagonists and receptors appear to be similar across the GPCR superfamily, inhibitors developed for other family members have provided and will continue to provide starting points for inhibitors of the chemokine receptors. These receptors represent the largest subfamily of GPCRs and potentially provide a rich source of structural information. Structure/activity relationships for small-molecule inhibitors developed across the chemokine receptors should not only help foster rational design of agonists/antagonists, but also help to refine the molecular models for the GPCRs.

New findings on the role of chemokines and their receptors in immune system homeostasis, inflammatory processes and autoimmunity

Several chemokines are known to induce the rapid activation of cell-surface integrins and the triggering of firm adhesion of T-lymphocytes on vascular endothelium under flow conditions. These chemokines include stromal-derived factor (SDF)-1, secondary lymphoid-tissue chemokine (SLC), and MIP3β [48]. SDF-1 signaling through CXCR4 leads to rapid adhesion of $CD34^+$ progenitor cells under flow [49], and appears to control the retention of T- and B-lymphocytes as well as myeloid precursors in the bone marrow [50,51]. In contrast, activation of CCR7, the receptor for SLC, is required for firm adhesion of T-lymphocytes, but not B-lymphocytes in the high endothelial venules of secondary lymphoid tissue [52–54]. Thus, CCR7 appears to be involved in the regulation of the trafficking and flow of T-cells through secondary lymphoid tissue. However, MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium [55].

A rather remarkable finding is that the recognition and triggering of T-lymphocyte adhesion on the vascular

endothelial surface appears to occur in a tissue-dependent manner in peripheral tissues. Thus, T-lymphocytes targeted for recruitment to the skin express CCR4, and its ligand, thymus- and activation-regulated chemokine (TARC), is expressed in cutaneous vascular endothelium, but not in intestinal vascular endothelium [56••]. In addition, CCR4-expressing T-lymphocytes are enriched in tissues from various dermatides compared with their levels in the peripheral blood. In contrast, intestinal vascular endothelial cells express the chemokine TECK (thymus-expressed chemokine), and T-lymphocytes bearing its receptor, CCR9, are prominent in intestinal inflammatory disorders [57•].

Role of MCP-1 and CCR2 in delayed hypersensitivity and inflammatory models

The chemokine MCP-1 is expressed in most inflammatory conditions and its expression correlates with the influx of monocytes to inflammatory sites. Deletion of either MCP-1, or its primary receptor CCR2, results in attenuation of the recruitment of monocytes to multiple inflammatory stimuli, suggesting that CCR2 plays a major role in monocyte recruitment. In fact, the development of disease is attenuated when MRL-Fas(lpr)mice, in which multi-organ autoimmune disease spontaneously develops, are backcrossed to MCP-1 –/– mice [58].

In addition to its role in monocyte trafficking, however, CCR2 appears to play a role in modulation of the cytokine secretion pattern and in the Th-1/Th-2 balance of the inflammatory response in multiple experimental paradigms. Thus, in both mycobacterial-antigen and schistosomal-antigen elicited granuloma models, the concentration of interferon γ in the draining lymph nodes is decreased in CCR2 –/– mice, suggesting an attenuation of the Th-1 polarized response [59•]. A similar apparent switch in Th-1 to Th-2 type cytokine profiles occurs in Cryptococcal and Leishmania infection in CCR2 –/– mice [60,61•]. In addition, mycobacterial-antigen and schistosomal-antigen elicited granulomas in mice that are induced to overexpress MCP-1 by adenoviral-mediated transfection also showed an apparent Th-1 to Th-2 phenotypic switch [62]. In contrast, MCP-1 –/– mice are deficient in the ability to mount Th-2 responses [63]. Although the mechanism of these effects is not understood, it is clear that chemokines and their receptors will modulate the inflammatory process in ways not totally defined by their cell-trafficking properties.

CCR5- and CXCR3-expressing T-lymphocytes in inflammatory lesions in rheumatoid arthritis and multiple sclerosis

T-lymphocytes expressing CCR5 and CXCR3 are enriched in rheumatoid arthritis synovial tissue and in active lesions in multiple sclerosis compared with in the peripheral circulation [64,65]. Although previous data suggested that there were no differences in the incidence and progression of rheumatoid arthritis in the CCR5 Δ32 null population, recent data suggest that the progression, but not incidence, of multiple sclerosis is reduced in patients bearing the CCR5 Δ32 allele [66]. These data further suggest that CCR5 antagonists may have beneficial therapeutic effect in multiple sclerosis, and that CXCR3 antagonists may prove beneficial in both MS and rheumatoid arthritis.

Role of chemokines and their receptors in allograft rejection

It has previously been recognized that the survival of heterotopic heart allografts is prolonged in CCR1 –/– mice, and that lower doses of cyclosporine are required to completely protect allograft survival in these mice. It has now been shown that a similar phenotype is observed in both CCR2 –/– and CCR5 –/– mice [67]. In addition, antibodies to CCR5 attenuate the development of graft-versus-host disease in a murine model, although it is not clear if this effect is due to neutralization of CCR5 or depletion of CCR5-expressing cells [68].

More pronounced and dominant effects, however, are observed by utilizing neutralizing antibodies to the CXCR3 ligand Mig. The recruitment of T-lymphocytes into class II MHC disparate skin allografts and consequent graft rejection are completely attenuated with αMIG therapy [69••]. In addition, the attenuation of graft rejection in IFNγ –/– mice is reversed by injection of Mig into the grafted tissue. Neutralization of Mig also attenuates rejection in a heterotopic heart allograft model (RL Fairchild, unpublished data), and the same phenotype has been observed in the CXCR3 –/– mouse (C Gerard, personal communication). Thus, attenuation of the activity of CXCR3 and its ligands has a major effect on the development of the immune response to foreign antigen.

Chemokine receptors and HIV

The chemokine receptors CCR5 and CXCR4 are required for HIV to infect its target cells [70–76]. The entry mechanism as currently understood is an ordered process in which the viral envelope protein, gp120, following interaction with the host cell-surface protein CD4, undergoes a conformational change enabling it to bind to the appropriate chemokine receptor, CCR5 for M-tropic or R5 strains, and CXCR4 for T-tropic or X4 strains. This second interaction produces a further conformational change in gp120, activating gp41 and thereby initiating fusion with the cell membrane and viral entry [77–83]. Substantial human genetic evidence supports the hypothesis that antagonism of CCR5 will have antiviral activity. An allele of CCR5 that has a 32 base pair deletion causes early termination of translation and lack of surface expression. Individuals homozygous for Δ32-CCR5 are resistant to infection by HIV, whereas those who are heterozygous become infected but have delayed disease progression [84–88]. Importantly, the health of Δ32-CCR5 homozygotes appears unimpaired implying that CCR5 antagonists will be without mechanism-of-action-based side effects. On the basis of these findings, efforts have been focused on

the development of antagonists, particularly of CCR5, as antiviral agents.

The first reported non-peptidyl, small-molecule, antagonist of CCR5 is TAK-779 [41••]. This compound binds to CCR5 with an IC_{50} of 1 nM, as measured by competition against RANTES, and to CCR2 with an IC_{50} of 27 nM. It has little affinity for other chemokine receptors. More importantly, it inhibits fusion and viral entry with IC_{50}s of 2–100 nM depending on the assay used. Although poor oral bioavailability is likely to complicate clinical use, TAK-779 has shown that small antagonists of CCR5 can have antiviral activity. A second CCR5 antagonist, Schering C (structure undisclosed), was presented at the 7th Conference on Retroviruses and Opportunistic Infections [89•]. It has an IC_{50} for the CCR5 receptor of 1 nM, appears to be a more potent antiviral than TAK-779, is orally bioavailable, and should enter phase I clinical trials during the second half of 2000. Additional CCR5 antagonists have been described by Merck, including the 4-(carbamoyl)-piperidines the most potent of which have affinities for the receptor of <1 nM and moderate antiviral activities with IC_{95}s of 100–200 nM as measured in peripheral blood mononuclear cell spread assays [90•,91•].

The bicyclams are a series of highly potent and selective inhibitors of X4-tropic HIV-1 replication and are now known to be CXCR4 antagonists [44,45]. The most efficacious of these is AMD 3100 (Figure 2), an antagonist of replication with IC_{50}s of 2–50 nM against a variety of X4 strains, which is currently in clinical trials [92]. A variety of peptide antagonists of CXCR4 have been described, the most potent of which is the 18 amino acid cationic peptide T22 ([Tyr5,12,Lys7]-polyphemusin II), and its analogs T134 and T140 all of which have IC_{50}s of less than 10 nM in antiviral assays [93]. Studies with blocking CXCR4 monoclonal antibodies suggest that these peptides bind to the amino terminus and two of the extracellular loops of CXCR4 [94].

Conclusions

As described above, just as our understanding of the complexity and pleiotropy of the chemokine/chemokine-receptor family continues to advance, so does our understanding of the multifactorial roles played by this family in the maturation, differentiation, homing, activation and trafficking of leukocytes within the immune system and in response to inflammatory stimuli. It is an enormous challenge to understand how the temporally and spatially complex pattern of secretion of chemokines and other cytokines translates into the orchestrated movement of leukocytes throughout the body. It is an even larger challenge to understand the role that these mechanisms play in the pathogenesis of human autoimmune and chronic inflammatory diseases. However, the development of selective chemokine receptor antagonists appears to have great potential to provide novel therapeutic agents for the treatment of these disorders and for the therapy of HIV, in addition to providing tools to increase our understanding of the underlying biology of the immune system.

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- •• of outstanding interest

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