Chemokines and Their Receptors: Drug Targets in Immunity and Inflammation

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Abstract

The chemokine system coordinates leukocyte migration in immunity and inflammation and is implicated in the pathogenesis of many human diseases. Although several successful strategies have been identified to develop drugs targeting chemokines and their receptors, this has not yet resulted in many new therapeutics. This is likely due to a complexity of the chemokine system, which was not initially appreciated, that is characterized by redundancy, pleiotropy, and differences among species. Nevertheless, our understanding of chemokine biology is continuing to grow and several promising drugs are currently being tested in late-stage clinical trials. In this review, we examine the role of chemokines in health and diseases and discuss strategies to target the chemokine system.

INTRODUCTION

Chemokines are small cytokines with selective chemoattractant properties, coordinating the homeostatic circulation of leukocytes as well as their movement to sites of inflammation or injury. Dysregulated expression of chemokines and their receptors is involved in the development of many human diseases, including autoimmune and chronic inflammatory diseases as well as immunodeficiency and cancer (1, 12, 23). As a consequence, there has been considerable effort in both the public and private sectors to develop drugs to modulate the activities of chemokines and their receptors. As a result, chemokine-receptor antagonists have already made their way into the clinic. In this review, we examine the role of chemokines and their receptors in immunity and inflammation and discuss various strategies being taken to develop drugs to modulate the chemokine system in vivo.

The approximately 50 chemokines and 20 receptors identified to date are classified into four families on the basis of the pattern of the first two of four cysteine residues of the ligand (**Figure 1**). The large CC chemokine family (for chemokine nomenclature, see Reference 2) consists of chemokines with the first two cysteine residues adjacent to each other, whereas the CXC family is characterized by the presence of a single amino acid residue between the first two cysteines. Fractalkine (CX3CL1) is the only member of the CX3C chemokine family, in which three amino acid residues separate the first two cysteines. Finally, two highly related chemokines, both binding to the XCR1 receptor, belong to the XC family, characterized by the absence of two cysteine residues.

Given the bewildering number of chemokine ligands, it has also been useful for some to classify chemokines into inflammatory or homeostatic molecules based on their patterns of expression and associated function. Inflammatory chemokines are produced by activated cells and recruit leukocytes to inflamed tissues, whereas homeostatic chemokines are constitutively produced and involved in maintaining homeostatic leukocyte trafficking as well as the architecture of secondary lymphoid organs. However, this distinction is somewhat artificial as some chemokines fall into both categories depending on the biological context.

Chemokines exert their chemotactic functions by binding to chemokine receptors, seven-transmembrane-spanning proteins coupled to heterotrimeric G protein, i.e., G protein–coupled receptors (GPCRs). Chemokine binding to chemokine receptors dissociates $G\alpha_I$, the $G\alpha$ most commonly associated with those receptors, and $G\beta\gamma$ subunits of the heterotrimeric G proteins, leading to calcium flux and activation of the phosphatidylinositol 3-kinase (PI3K) and the small Rho GTPases signaling pathways, among others (3). Consistent with G_i association, the majority of chemokine responses are inhibited by treatment with pertussis toxin (PTx) (4). Nevertheless, in some circumstances, PTx cannot completely block chemokine-induced responses owing to chemokine receptor association to G proteins other than G_i, such as $G_{q/11}$ or G_{16} (3). Thus, depending on their coupling to distinct G proteins, chemokine receptors may initiate distinct signal transduction pathways and exert several biological functions (**Figure 2**).

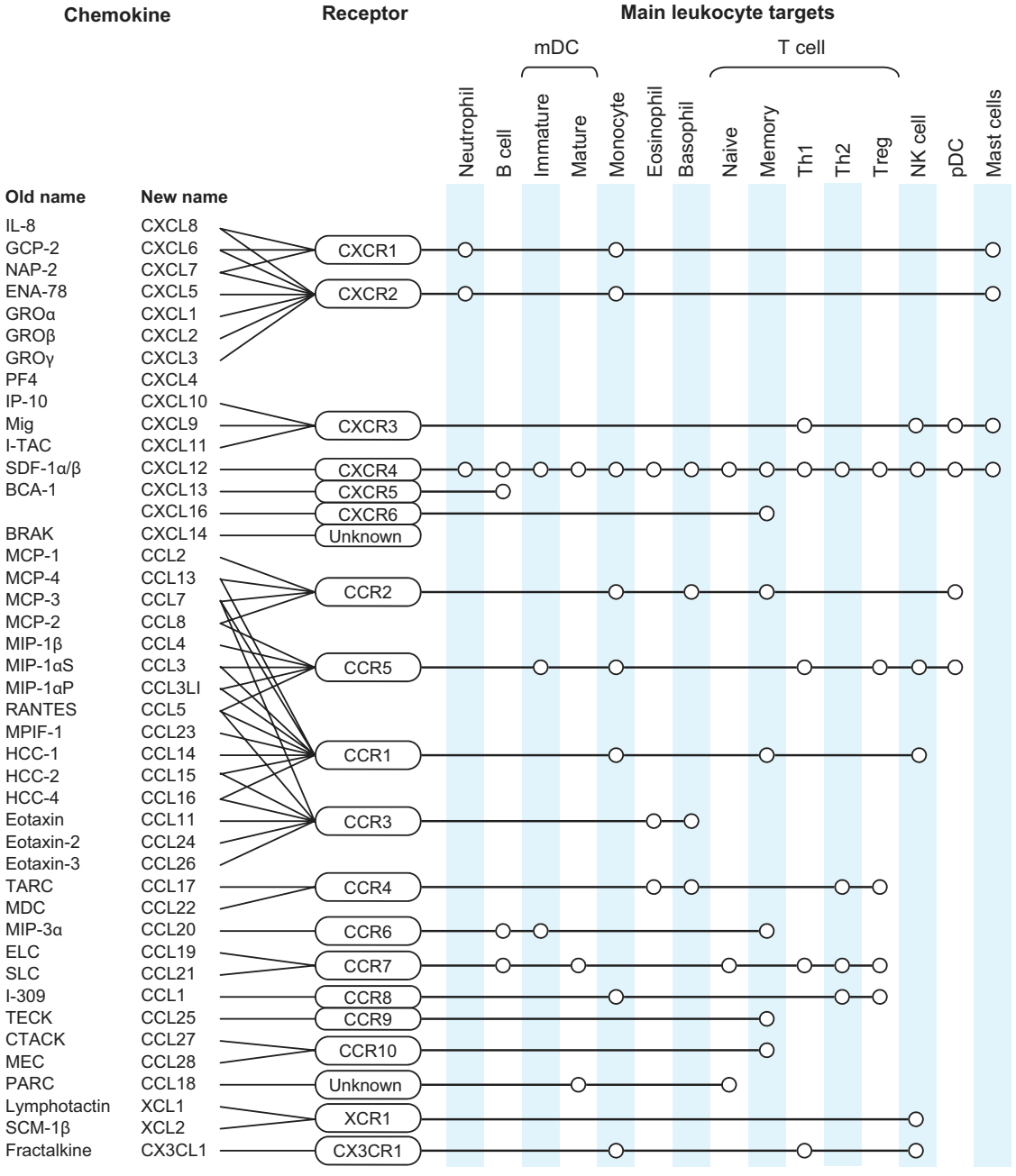
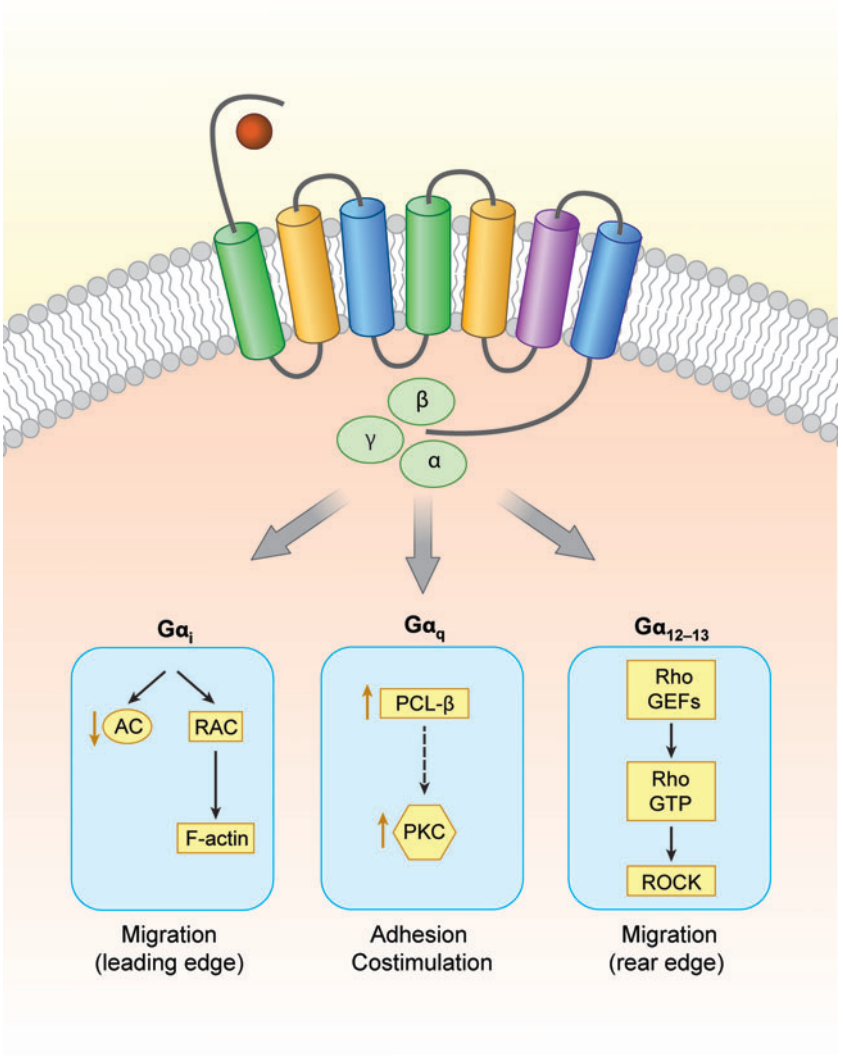


Figure 1

Human chemokines and their receptors. The old and the new names, the specific receptor(s), and the main leukocyte target(s) are reported for each chemokine.

Figure 2

Multiple signaling pathways induced by GPCRs. Chemokine receptors are seven-transmembrane molecules coupled to heterotrimeric G proteins. The β- and γ-subunits are assembled into βγ dimers that act as functional units. The α-subunits bind guanine nucleotides, being active when GTP is bound. The G proteins are usually classified by the nature of their α-subunit—α_s, α_i, α_q, and $\alpha_{12/13}$—into four classes. Chemokine receptors can signal through different Gα-protein families, leading to distinct transduction pathways and biological effects. $G\alpha_i$ coupling regulates gradient sensing and F-actin polymerization at the leading edge of a migrating cell. On the other side, the "backness" signaling depends on chemoattractant-induced $G\alpha_{12/13}$ activation, resulting in actin-myosin contraction. A few reports indicated that the coupling of chemokine receptor to $G\alpha_{q/11}$ leads to enhanced cell adhesion and activation (17).



The orchestration of leukocyte migration in lymphoid organs and inflamed tissues depends on the ability of chemokines to regulate two distinct processes: leukocyte extravasation and selective migration inside tissues. With the exception of CXCL16 and CX3CL1, which are integral membrane proteins, all chemokines are secreted basic proteins that bind to negatively charged glycosaminoglycans (GAGs) (5). GAGs are generally attached to proteins on cell surfaces or in the extracellular matrix, forming structures called proteoglycans. Thus, when released by endothelial cells or other parenchymal cells, chemokines tend to remain concentrated and immobilized at tissue sites. Upon binding to chemokine receptors on leukocytes, chemokines induce an intracellular signaling cascade that results in a rapid increase in integrin binding

avidity, leading to firm adhesion of the leukocyte to the endothelium and transmigration (6, 7). Within tissues, chemokines coordinate directed migration of leukocytes in specific areas (8), although it is not yet clear whether this is the consequence of chemotaxis toward a soluble or surface-bound gradient or enhanced random motility (chemokinesis) promoted inside the tissue by chemokine-induced signaling (9–11).

CHEMOKINES IN INNATE AND ADAPTIVE IMMUNITY

Recruitment of neutrophils and monocytes into inflamed tissues is mainly directed by two subfamilies of chemokines: the CXC chemokine subfamily, characterized by a glutamate-leucine-arginine (ELR) motif at the N–terminus, and the CC subfamily of monocyte chemoattractant proteins (12). Production of ELR^+ CXC chemokines, such as CXCL8, is induced by inflammatory stimuli, including bacterial lipopolysaccharide (LPS), interleukin (IL)-1, and tumor necrosis factor (TNF)-α, and causes attraction and activation of neutrophils in injured tissues. In addition, these chemokines have a clear role in angiogenesis and wound repair (13). Among the monocyte chemoattracting proteins, including CCL2, CCL7, CCL8, and CCL13, CCL2 and its receptor CCR2 have a nonredundant role in monocyte trafficking into inflamed tissues and seem to be involved in mobilizing monocyte precursors from the bone marrow and in regulating specific aspects of adaptive immune responses (12).

Adaptive immunity starts with the encounter between naïve antigen-specific T cells and dendritic cells (DCs) loaded with cognate antigen in secondary lymphoid organs. This encounter is driven by CCR7 and its ligands—CCL19 and CCL21—which act as a bridge between innate and adaptive immune responses (14). To meet and prime T lymphocytes after antigen capture in tissue, DCs must leave these periphery sites and migrate into the T cell area of lymphoid tissues. During maturation in inflamed tissue, DCs downregulate expression of chemokine receptors constitutively expressed on immature cells, such as CCR1, CCR5, and CCR6, and upregulate expression of CCR7, which drives DCs into the afferent lymphatic vessels and the lymph node (LN) (15).

T cells enter LNs via high endothelial venules (HEVs), which are specialized postcapillary venules found in lymphoid organs and made up of cuboidal endothelial cells. This process is directly controlled by CCR7 expressed on naïve T cells and its ligands CCL19 and CCL21 (14), which are produced by DCs and HEV, respectively. CXCL12-CXCR4 interactions also participate during T cell extravasation to secondary lymphoid organs, although this process seems to be more relevant for memory T cells (16). In addition to their role as chemoattractants, during T cell activation by DCs, CXCL12 and CCL5 chemokines may deliver costimulatory signals, enhancing T cell proliferation and cytokine production (17).

After priming, activated T cells must leave the LN T cell area and exert their functions. One of the initial events is therefore the downregulation of CCR7 and the upregulation of other chemokine receptors specific for the target tissue. Some $CD4^+$ T cells upregulate expression of CXCR5, and therefore become directed to the follicle, where they provide help to B cells (8). Conversely, other activated T cells are recruited in peripheral tissues to fight invading pathogens (18).

Primed T lymphocytes differentiate into effector cells that are specialized in their ability to act against different classes of pathogens. For example, type 1 and type 2 helper (Th1 and Th2) lymphocytes secrete cytokines enhancing cell-mediated and humoral immunity, respectively. Chemokine receptors are differentially expressed on effector Th cells (19). In particular, CCR5 and, to a lesser extent, CXCR3 predominate on Th1 cells, whereas CCR4 and CCR8 are preferentially found on Th2 cells. Chemokines attracting Th1 cells are induced by interferon (IFN)-γ and suppressed by IL-4, which, in contrast, induces chemokines preferentially attracting Th2 cells.

T cell priming induces the generation of long-lived memory T cells. Two different populations of memory T cells have been identified based on distinction in the expression of CCR7 (20). $CCR7^+$ memory T cells—termed central memory (T_{CM})—have the capacity to migrate into lymphoid tissue, but, because of the simultaneous expression of inflammatory chemokine receptors, they can also reach sites of inflammation. In contrast, $CCR7^-$ memory cells—termed effector memory (T_{EM})—do not have LN homing potential because they do not express CCR7. CCR7 is the key LN homing chemokine receptor and its expression permits effector and memory T cells that have migrated into inflamed peripheral tissues to reenter the lymphoid compartment via the afferent lymph (21). This migration of activated T cells from the tissue back to the LN may have important implications for the generation and control of immune responses.

B cell entry into secondary lymphoid tissues and homing to lymphoid follicles depend on CCR7, CXCR4, and CXCR5 (8). CXCR5 is upregulated during B cell maturation in the bone marrow and is expressed by all mature B cells. Upon antigen-receptor triggering, B cells upregulate CCR7 expression and thus relocate from the follicle to the B/T-zone, where they find and interact with antigen-specific T cells that help them to produce antibodies. At this stage, activated antibody-secreting plasma cells relocate to the red pulp of the spleen and the medullary cords of LNs driven by the simultaneous downregulation of CCR7 and CXCR5 and upregulation of CXCR4. CXCR4 expression is also pivotal for plasma cell homing to the bone marrow.

If lymphocyte entry and homing into secondary lymphoid organs are mainly controlled by the precise balance of chemokine gradients in the organs and chemokine receptor expression in T and B cells, the interaction between a chemoattractant lipid and its receptor regulates lymphocyte egress from the thymus and lymph nodes (8). Binding of sphingosine-1-phosphate (S1P)—produced through sphingosine kinase-mediated phosphorylation of sphingosine—to the $S1P_1$ receptor (a GPCR) is required for lymphocyte egress from lymphoid organs, as shown by the fact that S1P1-deficient T and B cells are unable to exit from secondary lymphoid tissue (22).

CHEMOKINES IN DISEASE

The attraction of leukocytes to sites of inflammation and infection is an essential component of the host response to disease. Chemokines and chemokine receptors are an integral part of this process and have been implicated in the pathophysiology of many infectious and inflammatory diseases (**Table 1**) (1, 12, 23). Although chemokines are

Table 1 Chemokines and their receptors implicated in disease

Disease	Key cell	Chemokine	Chemokine receptor
Acute inflammation			
Myocardial infarction stroke	Neutrophil	CXCL1, CXCL2, CXCL8	CXCR1, CXCR2
Ischemia-reperfusion	Monocyte	CCL2	CCR2
Autoimmune			
Atherosclerosis	Monocyte Th1 cell	CCL2, CCL5, CX3CL1, CXCR2 CXCL9, CXCL10, CXCL11	CCR2, CCR5, CX3CR1 CXCL2 CXCR3
Multiple sclerosis	Monocyte Th17 cell	CCL2, CCL4, CCL4, CCl5 CXCL10, CCL21	CCCR1, CCR2, CCR5 CXCR3, CCR7
Rheumatoid arthritis	Monocyte Th1 cell Neutrophil	CCL2, CCL3, CCCL4, CCL5 CXCL9, CXCL10, CXCL12 CXCL1, CXCL5, CXCL8	CCR1, CCR2, CCR5 CXCR3, CXCR4 CXCR1, CXCR2
Psoriasis	Th1/17 cell Neutrophil	CCL4, CCL20, CCL27 CXCL9, CXCL10 CXCL1, CXCL2, CXCL8	CCR5, CCR6 CXCR3 CXCR2
Type I diabetes	Th1/CD8	CXCL9, CXCL10	CXCR3, CCR4
Crohn's	Gut homing Th1	CCL28	CCR9
Chronic hepatitis	CD8	CCL3, CCL4 CXCL9, CXCL10	CCR5 CXCR3
Lupus	Plasmacytoid DC B cells	CXCL9, CXCL10, CXCL11 CXCL13	CXCR3 CXCR5
Transplantation			
Allograft	CD8 T cell	CCL3, CCL4, CCL5 CXCL9, CXCL10	CCR5 CXCR3
Allergic inflammation			
Asthma	Th2 cell Eosinophil Mast cell	CCL17, CCL22, CCL1 CCL11, CCL26 CXCL10	CCR4, CCR8 CCR3 CCR3, CXCR3
Atopic dermatitis	Skin homing Th2 cells	CCL1, CCL13, CCL17, CCL18, CCL27	CCR4, CCR8, CCR10
Cancer			
Growth	Malignant cell	CXCL12	CXCR4
Metastasis: Lymph node		CCL19, CCL21 CXCL9, CXCL10, CXCL11	CCR7 CXCR3
Gut		CCL25	CCR9
Skin		CCL17, CCL28	CCR4, CCR10
Stem cell:	Stem cell		
Mobilization		CXCL12	CXCR4
Engraftment		CXCL12	CXCR4

(*Continued*)

Table 1 (*Continued*)

Disease	Key cell	Chemokine	Chemokine receptor
HIV			
R5-tropic	Macrophage; activated T cell	CCL3, CCL4, CCL5	CCR5
X4-tropic	T cell	CXCL12	CXCR4

clearly important for the ability of the host to control infections, they can also be detrimental in certain inflammatory diseases, such as asthma, atherosclerosis, rheumatoid arthritis, and multiple sclerosis (MS), where inflammatory cells are recruited into tissue sites causing an inflammatory infiltrate, which results in tissue damage. The chemokine/chemokine receptor axis participates in the pathophysiology of these diseases by leading to the pathologic accumulation and activation of leukocytes in affected tissues. In such disorders, it has been suggested that chemokines and their receptors could be used as therapeutic targets for controlling pathologic inflammation. The following section highlights the role of chemokines and their receptors in different types of inflammatory diseases where distinct chemokine systems have been implicated in distinct types of immunopathology, such as acute injury, autoimmune inflammation, transplantation, and allergic inflammation. In addition, chemokines and their receptors may play important roles in tumor formation and metastasis and have a uniquely important role in HIV infection; these areas are also discussed as they represent important pharmacologic targets for therapeutic intervention.

Acute Injury

The host response to acute infection or injury consists of an exuberant neutrophilic inflammatory response. As is often the case in inflammation, this is a double-edged sword, and this robust protective response can also be deleterious to host tissue. Host response to many acute tissue insults, such as ischemia, induces a neutrophil-rich inflammatory response that significantly contributes to tissue injury. For example, ischemia-reperfusion injury is thought to contribute to many important pathologic conditions, including acute myocardial infarction, stroke, shock, and acute respiratory distress syndrome. Therapies aimed at blocking neutrophil influx into such tissues are being evaluated as an attractive new strategy for these important disorders. CXCR1 and CXCR2 are the main neutrophil chemokine receptors in humans (24). Although also expressed on subsets of effector T cells and monocytes, CXCR1 and CXCR2 are most highly and uniformly expressed on neutrophils. Preclinical studies in which CXCR1 and CXCR2 are blocked have shown efficacy in inhibiting neutrophil influx and tissue damage in models of ischemia-reperfusion injury and are currently being evaluated in the clinic (25–28). Specific inhibition of neutrophil influx into tissues will likely have broad clinical appeal if effective, as the neutrophil has been implicated not only in ischemia-reperfusion injury but also in the pathogenesis of a wide range of diseases, including MS, asthma, inflammatory bowel disease, and psoriasis.

Autoimmunity

Infiltration of IFN-γ-secreting $CD4^+$ and $CD8^+$ effector T cells and activated macrophages into tissues is characteristic of Th1-type inflammation. This type of cell-mediated inflammatory response is thought to play an important pathophysiological role in many prominent human diseases, including Type 1 diabetes mellitus, atherosclerosis, Crohn's disease, and solid organ allograft rejection. Recently, it has been shown from murine studies that a newly described subset of CD4 Th cells that secrete IL-17, called Th17 cells, may also play important roles in autoimmune diseases (29). This has been seen in experimental autoimmune encephalitis (EAE), a murine model of MS, and in a murine model of collagen-induced arthritis. Interfering with chemokine receptors on Th1 and Th17 cells and/or activated macrophages would have the advantage of potentially benefiting a number of the disorders mentioned above. In fact, inhibiting T cell trafficking with antibodies to adhesion receptors expressed on T cells has shown promise in the clinic for several Th1-type inflammatory disorders. Antibodies specific for αL integrin (CD11a) (e.g., Odulimomab and Efaluzimab), which together with CD18 forms the LFA-1 complex, have shown promise for graft-versus-host disease, transplant rejection, and psoriasis (30). Further, Natalizumab, a mAb to α4 integrin chain that blocks the binding of both α4b1 (VLA-4) to VCAM-1 on brain infiltrating T_H1 cells and α4β7 to MadCAM-1 on gut infiltrating T_H1 cells, is very efficacious in Crohn's disease (31) and MS (32). However, blocking all α4-mediated leukocyte trafficking led to an increased susceptibility to a deadly opportunistic CNS infection caused by reactivation of a clinically latent JC polyomavirus infection. Natalizumab therapy illustrates the promise and perils of inhibiting leukocyte trafficking. Inhibiting T cell entry into the brain halted the entry of encephalogenic $CD4^+$ T cells and the ensuing autoimmune destruction of the myelin sheath. However, inhibiting the entry of another subset of T cells, most likely a subset of $CD8^+$ T cells, led to reactivation of JC virus.

A greater understanding of the specific T cell subsets that are pathogenic in a given disease and a deeper understanding of the specific infectious diseases held in check by this subset will lead to more effective and better-tolerated therapies. In this regard, organ and T cell subset-specific chemokine receptors remain attractive candidates for certain organ-specific diseases. For example, the CCL25-CCR9 interaction is believed to play an important role in guiding the entry of T cells into the small intestine (33) and therefore represents an attractive target for inflammatory bowel disease. Clinical trails are currently underway to examine the effectiveness of a CCR9-specific small-molecule antagonist in inflammatory bowel disease. Likewise, CCL27-CCR10 and CCL17- or CCL22-CCR4 interactions play an important role in T cell entry into the skin (34) and therefore represent attractive combinatorial targets for inflammation of the skin, such as psoriasis and atopic dermatitis. In addition, in some chronic inflammatory diseases, such as rheumatoid arthritis, ectopic lymphoid neoorganogenesis occurs locally (i.e., in the synovium) and is postulated to contribute to the perpetuation and intensity of smoldering local inflammation. Lymphotoxin-induced expression of CXCL13 and CCL21 play an important role in this process and may represent novel therapeutic targets to attenuate local inflammation through the dissolution of these ectopic lymphoid aggregates (35, 36).

The activated macrophage also plays an important role in many autoimmune-mediated disease processes, such as MS, rheumatoid arthritis, and atherosclerosis. Thus modulating the trafficking patterns of macrophages may also attenuate inflammation in these chronic inflammatory diseases. Macrophage subsets are less well defined than T cell subsets, although recent studies have identified a $CCR2^{hi}CX3CR1^{lo}$ inflammatory subset and a $CCR2^{lo}CX3CR1^{hi}$ homeostatic subset (37, 38). Of note, preclinical studies in models of atherosclerosis (39), MS (40), and rheumatoid arthritis (41) have revealed that blocking CCR2 was effective in attenuating disease. Therapies aimed at blocking the interaction of CCR2 with its ligands are currently being evaluated in the clinic. On a cautionary note, however, inhibiting monocyte trafficking may also have deleterious side effects similar to inhibiting T cell trafficking that need to be considered and evaluated. Recently, it has been shown that CCR2 plays a critical role in microglial precursor trafficking into the brain in a model of Alzheimer's disease (42). In the absence of microglial recruitment into the brain, beta-amyloid was not cleared as effectively, leading to increased levels of toxic beta-amyloid levels, which resulted in injury to blood vessels and early mortality.

Transplantation

Organ transplantation is often the only effective treatment for the large number of patients with end-stage kidney, liver, heart, or lung disease. Although a transplant is often life saving, it ushers in a new set of problems and potential diseases for the organ recipient. These include increased risk of infection (including opportunistic infections), organ ischemia-reperfusion injury, acute rejection, and chronic organ dysfunction from chronic rejection. These processes involve recruitment of leukocytes into the transplanted organs, with neutrophil recruitment dominating infections and ischemia-reperfusion injury, and lymphocytes and monocyte recruitment involved in rejection. Chemokine expression has been shown to be important in all of these processes and thus represents a potential therapeutic target to prevent these complications of transplantation (43).

Organ ischemia-reperfusion injury, acute rejection, and chronic rejection lead to increased expression of multiple chemokines that are surprisingly similar in all organs after transplantation (43). However, organ-specific differences do exist so one cannot generalize the response to all transplanted organs (44). In the limited studies looking at chemokine expression following human organ transplantation, the expression of CXCL8 has been associated with ischemia-reperfusion injury (45), whereas CCL2, CCL3, CCL4, CCL5, CXCL9, CXCL10, and CXCL11 have been associated with acute rejection (46). In chronic rejection of lungs, CXCL8, CCL5, CXCL9, CXCL10, CXCL11, and CCL2 are upregulated and may be predictive of the development of this complication (47, 48). Preclinical studies using chemokine receptor-deficient mice have indicated that the chemokine system plays an important role in allograft rejection (49, 50). These studies have revealed important roles for CXCR3, CCR1, CCR2, CCR5, and CCR7 in acute allograft rejection and graft tolerance, although some controversy remains as to the effectiveness of inhibiting a single chemokine receptor.

Allergy

Allergic inflammation is characterized by the tissue infiltration of IL-4-, IL-5-, and IL-13-secreting T_H2 cells; eosinophils; and mast cells. This type of immune response is thought to have evolved to defend the host against parasitic infections (51). Increasing evidence suggests that this response also underlies the pathological immune response in several allergic diseases that are now epidemic in developed countries, such as asthma, food allergies, and atopic dermatitis (52). Because parasitic infections have essentially been eradicated in developed countries, inhibiting the trafficking of T_H2 cells, eosinophils, and mast cells into tissues should be very safe. The challenge then is to identify the specific chemoattractants that control the trafficking of these immune cells. The chemokine receptor CCR3 was initially thought to be such a molecule; however, subsequent studies have revealed that although it is expressed on all eosinophils, it is only present on a minority of T_H2 cells (53, 54). CCR3-deficient mice have a profound defect in eosinophil migration, but T_H2 cell trafficking appears to be intact and CCR3-deficient mice can mount a tissue-specific allergic response (55, 56). Thus, CCR3 blockade appears to be a viable approach to block eosinophil trafficking but not T_H2 cell trafficking. T_H2 cell trafficking is under the control of chemoattractant receptors (57) and STAT6-inducible genes (58). Thus T_H1 cells preferentially utilize CXCR3, CXCR6, and CCR5, whereas T_H2 cells preferentially use CCR4, CCR8, and the lipid PGD2 chemoattractant receptor CRTh2 (59). It remains to be seen, however, if inhibition of these or other combinations of chemoattractant receptors specifically expressed on T_H1 versus T_H2 cells can selectively inhibit the trafficking of either T cell type. Further complexity is added to the system as certain important GPCRs, such as BLT1, the receptor for the chemoattractant leukotriene B4, is expressed and functional on effector T cells, including T_H1 and T_H2 cells and CD8 T cells (60, 61). Another complexity is the differential utilization of chemoattractants at different microanatomic sites within a pathological lesion. For example, in the asthmatic lung, BLT1 plays an important role in attracting T_H2 cells from the interstitium into the airway (61), whereas in Crohn's disease CCL20 is highly expressed by follicle-associated epithelium well situated to recruit $CCR6^+$ T_H1 cells into the mantle zone of gut-associated lymphoid follicles (62, 63). Mast cells and basophils, important effector cells in allergic inflammation, also express specific chemokine receptors, such as CCR2, CCR3, CXCR1, CXCR3, and CXCR4 (64). In a murine model of asthma, CCR3 was shown to play a critical role in the positioning of eosinophils and mast cells (55). Whether mast cell and basophil chemokine receptors are targets for modulating allergic inflammation remains to be determined.

HIV-1

Chemokine receptors are obligate co-receptors for HIV-1 infection and are required for the virus to enter cells (65). By facilitating entry into cells, these receptors determine viral tropism. CXCR4 is a coreceptor for strains of HIV-1 that infect T cell lines (T-tropic strains), and CCR5 is a co-receptor for HIV-1 isolates that infect macrophages and activated T cells (M-tropic strains). CCL3, -4, and -5, which are

CCR5 ligands, and CXCL12, the specific CXCR4 ligand, block the entry of M-tropic and T-tropic HIV-1, respectively, into cells. The importance of chemokine receptors in the pathophysiology of HIV-1 infection became apparent when it was discovered that a polymorphic variant of CCR5 could explain the observation that certain persons who are at high risk for HIV-1 infection remain uninfected (66, 67, 68). In persons who are homozygous for a 32–base pair deletion in the gene for CCR5, a functional CCR5 protein cannot be synthesized, and such persons are not found in HIV-1-positive cohorts. In addition, cells from persons with this mutation cannot be infected with HIV-1 in vitro (69). Furthermore, in persons who are heterozygous for the mutation, the rate of progression of HIV-1 infection is slower than in those without the mutation (66). Although the molecular mechanism of the fusion event is not known, HIV glycoprotein 120, CD4, and a chemokine receptor associate on the cell membrane before HIV enters the cells (70, 71, 72). The facts that persons with the Δ32 CCR5-null mutation are resistant to HIV infection and are apparently healthy and that chemokines can inhibit HIV entry into cells lead to considerable interest in targeting CCR5 as a new form of antiviral therapy.

Cancer

Although chemokine receptors are predominantly expressed on leukocytes, they are also expressed at lower levels on many other cell types. On tumor cells, these receptors are found at increased levels and they may participate in metastasis, tumor growth, and survival. Furthermore, tumors secrete chemokines with angiogenic activity, which may play a role in maintaining an adequate blood supply to the tumor. Surveys of tumor cells have found that CXCR4 and CCR7 are the most widely expressed chemokine receptors on tumors, although others, such as CXCR3, CCR9, and CCR10, have also been detected (73). CCR7 and CXCR3 may promote metastasis to LNs, whereas CCR10 and CCR9 may promote metastasis to the skin and gut, respectively (74). CXCR4 effects on cancer cells seem to be more complex, with evidence suggesting that CXCR4 signaling may be involved in tumor growth, survival, and vascularization, in addition to guiding the movement of cancer cells expressing this ubiquitously expressed primordial chemokine receptor (75). In addition, CXCR4 plays an important role in stem cell localization within its bone marrow niche and is being explored therapeutically as means to mobilize stem cells prior to transplantation and ensuring their engraftment following transplantation (76).

STRATEGIES TO CONTROL THE CHEMOKINE SYSTEM

Blocking pathological leukocyte recruitment into tissues would be beneficial to persons suffering from inflammatory and autoimmune diseases. As already mentioned, monoclonal antibodies that inhibit leukocyte adhesion, such as those targeting the integrin lymphocyte function-associated antigen (LFA-1) or the α4 integrin subunit of the very late antigen-4 (VLA-4), have been successfully used to treat MS and Crohn's disease (30–32). Considering the major role chemokines play in directing leukocyte migration and the greater selectivity of their expression compared to

integrins, they represent attractive targets for therapeutic intervention. In addition, chemokine receptors are GPCRs and, thus, potential targets for small-molecule antagonists accessible from the extracellular milieu, an approach that appears not possible to use to interfere with other cytokine systems or adhesion receptors, such as the integrins and selectins.

The strategy of inhibiting inflammation and immunity by interfering with the chemokine system has been exploited during evolution from viruses to humans, as shown by the existence of non-signaling or "silent" chemokine receptors acting as decoys and scavengers (77). These silent receptors compete with signaling chemokine receptors for interaction with ligands, thereby preventing cell activation. Although it is currently difficult to envisage a potential use of such scavengers in the clinic, their physiological role indicates that the chemokine system represents an ideal target to fight inflammatory diseases.

Theoretically, the chemokine system can be inhibited either by blocking chemokine-receptor binding or by inhibiting second messengers induced upon receptor triggering (78). Among the intracellular targets, the phosphatidylinositol-3′-kinase γ (PI3Kγ) is a very attractive candidate for therapeutic intervention in autoimmunity and inflammation. *Pi3kcg-/-* mice show defective migration of neutrophils and macrophages, reduced chemoattractant-induced neutrophil respiratory burst, and impaired adaptive immune responses (79–82). Recently, selective and orally deliverable inhibitors of PI3Kγ have been described and successfully used to block inflammation in mouse models for rheumatoid arthritis and systemic lupus (83, 84). Although the efficacy and safety of these type of inhibitors in patients are not yet proven, these data provide important proof-of-concept that PI3Kγ can be inhibited selectively to suppress inflammation. In addition to this promising strategy, the vast majority of pharmaceutical approaches have been directed toward inhibiting the interaction of chemokines with their GPCRs. Several approaches are being pursued simultaneously, including antibodies to chemokines or their receptors, small-molecule inhibitors of chemokine receptors, modified chemokine antagonists, and inhibitors of chemokine presentation or higher-order structure (**Figure 3**).

Antibodies

Although therapeutic monoclonal antibodies (mAbs) now represent one of the major products of the pharmaceutical industry to inhibit specific aspects of immune cell function, their use to interfere with the chemokine system was not initially explored; instead initial efforts emphasized small, orally deliverable molecules. However, the recent success with the mAb approach has led to a flurry of activity to generate specific antichemokine or chemokine-receptor mAb therapies.

Neutralizing mAbs against chemokines have been successfully used to inhibit leukocyte migration in animal models of MS (85), inflammatory arthritis (86), asthma (87) and in cardiac (88), lung, small bowel, and pancreatic islet (89) allograft models. In the case of human research, a mAb developed to neutralize CXCL8 (ABX-IL8, Abgenix) has been used to inhibit neutrophil and monocyte infiltration in the lungs of patients suffering from chronic obstructive pulmonary disease (COPD). Treatment

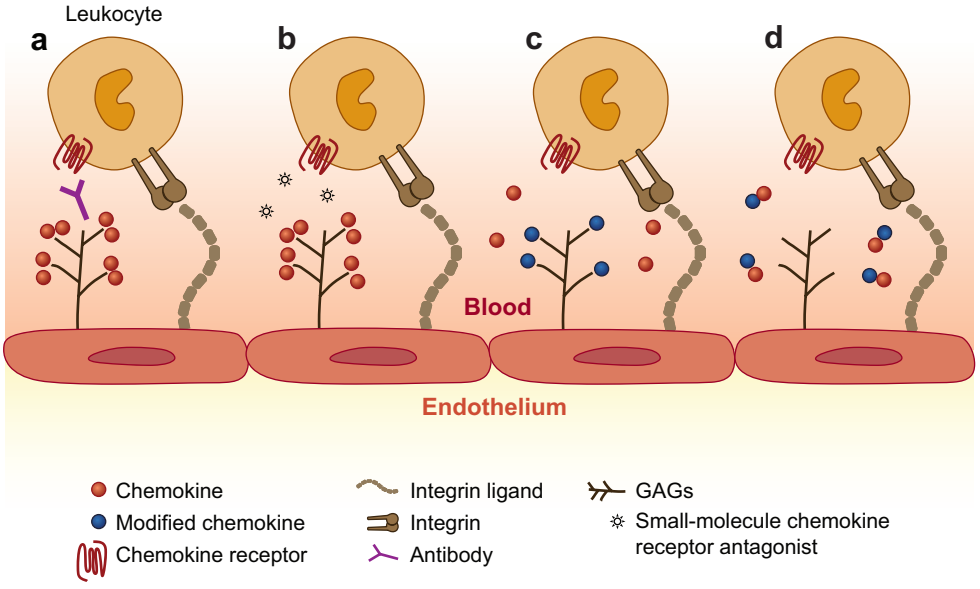


Figure 3

Different strategies to control the chemokine system. (*a*) Recruitment of leukocyte into inflamed tissues can be blocked with antibodies against chemokines or chemokine receptors, (*b*) chemokine receptor small-molecule antagonists, (*c*) nonfunctional chemokines that bind to GAGs but do not activate the receptors, (*d*) and nonfunctional chemokines that either inhibit GAG binding or inhibit higher order structures (oligomers) needed for presentation to chemokine receptors on GAGs. In addition, inhibitors of chemokine receptor signal transduction can be used (not shown).

reduced the severity of dyspnea but patients did not show any improvement in lung function or health status (90). The antibody has been also tested for psoriasis and rheumatoid arthritis and, due to disappointing results, its development has been stopped.

A humanized mAb against the CCR2 chemokine receptor (MLN1202, Millennium) is currently being tested in Phase II clinical trials for rheumatoid arthritis, atherosclerosis, MS, and scleroderma. Likewise, a humanized mAb against the chemokine CXCL10 (MDX-1100, Medarex) is in Phase I trails for ulcerative colitis.

Small Molecules

In contrast to adhesion molecules and cytokine receptors, GPCRs can be blocked by small-molecule antagonists. It is therefore not surprising that pharmaceutical research has mainly adopted this strategy to interfere with the chemokine system in an attempt to block leukocyte recruitment in inflammatory diseases. The discovery in 1996 that CCR5 is an obligate co-receptor for HIV-1 entry into human cells (91–93) intensified interest in the chemokine field. Since then, numerous chemokine receptor antagonists

belonging to different chemical series have been patented. Indeed, once the secrets of the chemistry of chemokine receptor antagonists were solved (94), the identification of small molecules able to inhibit specific chemokine receptors increased.

Most chemokine receptors bind several ligands, and small molecules that block the binding site for one specific chemokine may be unable to inhibit binding of other ligands. Targeting the receptor transmembrane helices has proved to be an effective strategy in the case of CCR5 (95), CCR1 (96) and CXCR1/CXCR2 (97). However, several molecules identified in vitro as potent antagonists of human chemokine receptors demonstrated very weak efficacy in mice, and thus, nonrodent animal models also have been chosen for preclinical studies.

The most successful and advanced chemokine receptor antagonists are those designed to block the two HIV-1 co-receptors, CCR5 and CXCR4. The first small CCR5 antagonist with antiviral activity that was described was TAK-799 (Takeda), which binds to a cavity formed between transmembrane helices 1–3 and 7 near the extracellular part of CCR5 (98). However, it showed variable antiviral activity and poor oral bioavailability. In a preclinical model, an oral CCR5 inhibitor was effective at decreasing the plasma viral load in simian HIV (SHIV)-infected macaques (99) and was also able to protect them from vaginal SHIV challenge (100). New CCR5 antagonists are being tested in clinical trials and, among the most promising ones, UK-427857 (Pfizer), now named maraviroc, demonstrated that it is well tolerated in healthy volunteers and reduces viral load in HIV-infected persons; it is now in Phase III clinical trials for the treatment of HIV-1 infection and was recently approved by the U.S. Food and Drug Administration for the use in people with advanced HIV who are not responding to other medications. Thus, CCR5 antagonists show promise as a new class of antiviral therapy that targets HIV entry into cells and may add to the armamentarium of reverse transcriptase inhibitors and protease inhibitors for the treatment of HIV infection, and is the first antichemokine therapy approved for use in the clinic.

Effective CCR5 inhibitors are expected to be useful therapeutic drugs in transplant rejection as well (101). In agreement with data obtained with CCR5-deficient mice (102), in vivo treatment with the CCR5 inhibitor TAK-799 significantly prolonged pancreatic islet allograft survival in transplant models (103). Interestingly, patients homozygous for CCR5Δ32 have longer survival of renal transplants (104) but higher incidence of ischemic-type biliary lesions following liver transplantation (105) than in the control groups, underlining the importance of validating which receptor-ligand pairs are crucial in specific pathological conditions.

The most advanced CXCR4 antagonists belong to the class of bicyclams and AMD3100 is currently the most potent molecule of the series. AMD3100 has a potent in vitro antiviral activity against X4 and R5/X4 HIV-1 viruses and inhibits CXCL12-induced calcium signaling and chemotaxis. During Phase I clinical trials, single-dose intravenous AMD3100 administration was found to increase the peripheral leukocyte counts owing to the mobilization of hematopoietic ($CD34^+$) stem cells from the bone marrow into the blood circulation (106). AMD3100 has recently entered Phase III clinical trials as a stem cell mobilizing agent in patients with non-Hodgkin's lymphoma or multiple myeloma. In addition, AMD3100 is being considered for preserving myocardial function through the mobilization of stem cells (107).

Another promising orally deliverable drug is the CCR9 small-molecule inhibitor Traficet-EN (ChemoCentryx) that was well tolerated and appeared effective in Phase II clinical trails for Crohn's disease and is now being evaluated in a Phase III clinical trail.

Modified Chemokines

In many chemokines, truncation at the N terminus abrogates their signaling activity while preserving their binding properties, and thus, N-terminally truncated chemokines have been designed to function as chemokine receptor antagonists. Similarly, extension of the chemokine N terminus has proven to be a valuable approach to generate antagonists: Starting with Met-RANTES (methionine-CCL5) (108) and AOP-RANTES (109), several other N-terminally modified CCL5 were produced and, among them, PSC-RANTES has been successfully used to prevent vaginal SHIV transmission in primates (110).

Inhibiting Chemokine Presentation and Higher-Order Structure

As already mentioned, the low-affinity interaction of chemokines with GAGs is critical for leukocyte recruitment into inflamed tissues (111). GAGs provide a substrate for chemokine presentation to their receptors on passing leukocytes. In addition, GAGs serve to locally restrain chemokines to their site of production and allow for the generation of a directional gradient that can be sensed by leukocytes (112). GAGs also protect chemokines from proteolytic cleavage (113) and play an important role in facilitating chemokine oligomerization, which is a requirement for the in vivo function of at least some chemokines (RANTES and IP-10) (114, 115).

Several human pathologies, such as cancer and atherosclerosis, are accompanied by qualitative and quantitative changes in GAG expression (116) and it is tempting to speculate that this modification of the tissue environment may be involved in dysregulated leukocyte recruitment. Indeed, several lines of evidence suggest that the chemokine-GAG interactions are not only driven by electrostatic forces but may be specific, and may therefore represent potential drug targets.

Although retaining their chemoattractant properties in vitro, chemokines mutated in their GAG-binding sites have been shown to be unable to recruit cells in vivo (114), indicating the relevance of these interactions in controlling chemokine bioavailability. Interestingly, in the case of one CCL5 GAG mutant (RANTES-[^{44}AANA^{47}]), an in vivo dominant negative inhibition of the wild-type (WT) ligand has been reported, suggesting that the mutant may form nonfunctional heterodimers with the WT protein (116). Interfering with chemokine oligomerization has also been shown to inhibit the function of IP-10 in vivo but not in vitro (115), suggesting that chemokine oligomerization also plays an important role in chemokine presentation and function in vivo and represents a therapeutic target in addition to the chemokine-GAG interaction. Inhibiting chemokine oligomerization versus GAG binding maybe a more attractive approach as it will allow for more specificity and selectivity.

The elusive nature of the sequence specificity of a specific GAG-chemokine interaction together with problems related to carbohydrate chemistry represent a major obstacle in the development of molecules that can selectively block chemokine-GAG interactions. Although not specific, heparin prevents interaction of chemokines with proteoglycans and it is possible that part of heparin's antiinflammatory properties seen in animal models and clinical trials (117–119) may be related to its ability to inhibit the chemokine-GAG interaction.

DIFFICULTIES IN TAKING CHEMOKINES FROM THE BENCH TO THE BEDSIDE

Pharmaceutical research has faced several problems when trying to translate success in preclinical animal models of chemokine inhibition to success in the clinic to treat human disease. In addition to the classical problems of pharmacokinetics and toxicity, and the over reliance on animal models that do not accurately predict a response in human disease, problems related to relatively unique properties of the chemokine system, including redundancy, pleiotropy, and speciation, have emerged as stumbling blocks (**Figure 4**).

Chemokines orchestrate leukocyte recruitment in tissues and their biological effects are the consequence of the specific properties of the attracted cells (**Table 2**). For example, CXCL8 is involved in acute injury because it attracts into tissues neutrophils and monocytes, which express the specific receptor for the chemokine and are the major players of acute inflammation. The chemokine system is however characterized by redundancy (120). Every cell population has several receptors for various chemokines, and tissues typically produce several chemoattractant molecules in response to inflammatory stimuli (**Figure 4*a***). When considering the crucial role of leukocytes in fighting invading pathogens, the redundancy of the chemokine system is not surprising, but it represents a serious concern for the development of drugs that interfere with a specific chemokine or receptor. Thus, in an inflammatory context, blocking one specific receptor/ligand, even with a potent inhibitor, may result in lack of beneficial effects because of the large number of chemokines expressed by tissues and the redundancy in chemokine functions (**Table 2**).

As already discussed, chemokines mediate other biological activities besides cellular recruitment, and, in addition, each chemokine is active on various cell populations (**Figure 4*b***). The pleiotropic effects of the chemokine-receptor interactions may represent major obstacles for every therapeutic strategy, in particular, when homeostatic cytokines or broadly expressed receptors, such as CXCL12 and its receptor CXCR4, are chosen as drug targets.

Although the development of several chemokine and chemokine receptor knockout mice has provided key information to our understanding of chemokine biology, it is clear that the mouse may not always represent the optimal model for chemokine-related human biology. As is the case for most of the genes involved in immunity, chemokines and their receptors are under positive selection pressure—chemokines are among the eight most rapidly changing proteins—and the gene organization of human and mouse chemokine clusters is very different (121). The lack of correlation is

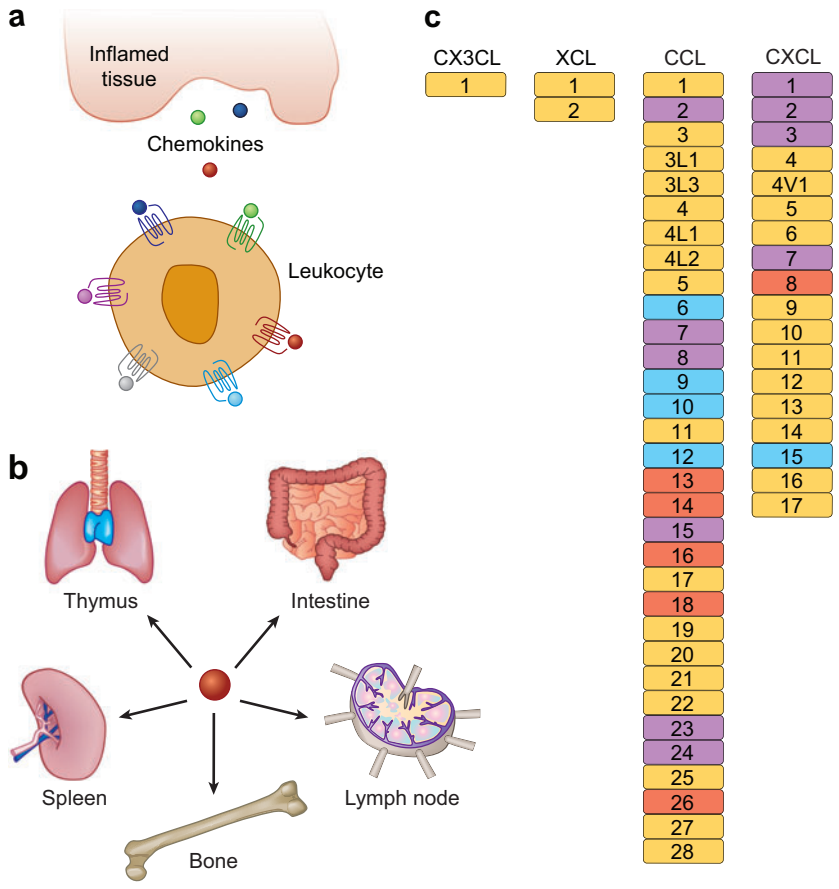


Figure 4

Some critical properties of the chemokine system. Redundancy is illustrated in part *a*. Inflamed tissues produce a variety of chemokines, and leukocytes typically express several chemokine receptors. Thus, approaches aimed to therapeutically block one chemokine or one receptor may be inefficient because other chemokine-receptor pairs may drive leukocytes into the tissue. Pleiotropy, illustrated in panel *b*, may represent an obstacle to therapy. The same chemokine can be involved in several physiological processes and have many cellular targets. In this case, it would be necessary to block one specific function while leaving the others unaffected. Finally, the lack of complete correlation between the mouse and human chemokine system is schematically illustrated in panel *c*. Yellow: human chemokines having functional equivalents in mice; red: chemokines existing in humans only; blue: chemokines existing in mice only; purple: chemokines having different functions in the two species.

evidenced by the fact that some chemokine genes that exist in one species do not exist in the other (**Figure 4*c***). As a consequence of the clustered organization and distinct evolution of the inflammatory chemokine genes, a single chemokine in one species may have more than one ortholog or may lack structural and functional equivalents in the other species.

Table 2 Key chemokine systems in inflammation

Chemokine	Key target cell	Type of inflammation
CXCL1, CXCL2, CXCL8 CCL2	Neutrophil Monocyte	Acute Inflammation
CCL2, CCL4, CCL5, CX3CL1 CXCL9, CXCL10, CXCL11 CCL3, CCL4, CXCL9, CXCL10 CXCL13	Monocyte Th1 cell CD8 T cell B cells	Th1-type Inflammation (e.g., chronic hepatitis)
CCL17, CCL22, CCL1 CCL11, CCL26 CCL2, CCL11, CXCL10 CXCL13	Th2 cell Eosinophil Mast cell B cell	Th2-type Inflammation (e.g., Asthma)
CCL20	Th17, DC	Th17-type Inflammation (e.g., MS)

CONCLUSIONS

Much has been learned about the chemokine system in the past 20 years since their discovery as highly inducible genes and as chemotactic factors secreted from activated cells (23). We now understand that this complex system orchestrates the precise movement of leukocytes that is necessary to generate and deliver immune and inflammatory responses to specific anatomic sites. We have also begun to decode this system as it relates to pathology, and we now appreciate that this family of chemotactic cytokines is intimately involved in the pathogenesis of many important human diseases. Chemokine receptors are considered among the most druggable targets in the immune system, leading the pharmaceutical industry to invest heavily in the field. Although the initial expectation of quickly and easily identifying many antichemokine blockbuster drugs may have been unrealistic, several chemokine receptor antagonists and antichemokine and chemokine receptor antibodies are currently in the clinic. A CCR5 antagonist for the treatment of HIV-infected patients will likely be the first drug targeting the chemokine system to reach patients. We believe that additional useful drugs can and will be found as we gain a deeper understanding of the complex biology of the chemokine system.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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