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REVIEW

Chemokines and Their Receptors in Rheumatoid Arthritis

Future Targets?

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease leading to joint destruction (1). In RA, migration of leukocytes into the synovial tissue (ST) occurs. These leukocytes and other cells in the ST, particularly RA ST fibroblasts, produce mediators of inflammation, including chemokines (1). Chemokines, currently numbering more than 50, are chemotactic cytokines that are important in recruitment of leukocytes and angiogenesis. They exert chemotactic activity toward a variety of cell types (2–7). Some chemokines, particularly CXC chemokines containing the ELR motif, are angiogenic. The last few years have seen a rapid development of studies aimed at targeting proinflammatory chemokines or their receptors in RA and animal models of RA (8,9). This review summarizes many of the important developments in this field. Based on the number of recently published studies, it is likely that the next few years will bring several new preclinical and clinical trials targeting chemokines.

Chemokines

Chemokines have been classified into 4 supergene families based on the location of cysteine residues (Figure 1). The 4 groups are CXC, CC, C, and CX_3C chemokines (10–12). A relatively new classification system was introduced in 2000, in which chemokines are considered as chemokine ligands, and each chemokine has been assigned a designation of CXCL, CCL, XCL, or CX3CL1 (Figure 1) (10–12). In this report, both the former and new nomenclature are noted.

CXC chemokines have 2 conserved cysteines separated by 1 unconserved amino acid (9,13) (Figure 1). CXC chemokines classically were thought to be involved in the chemotaxis of neutrophils. Many chemokines may have arisen from reduplication of ancestral genes (13). Hence, CXC chemokines that act on neutrophils are clustered on chromosome 4q12–13 (13). However, some genes of more newly discovered CXC chemokines that recruit lymphocytes tend to be located away from the major clusters (13). This diversification may reflect functional specialization. Another function of CXC chemokines is to modulate angiogenesis (7,14,15). In general, chemokines containing the ELR motif, such as interleukin-8 (IL-8)/CXCL8, epithelial neutrophil–activating peptide 78 (ENA-78)/CXCL5, growth-related oncogene α (GROα)/CXCL1, and connective tissue activating peptide III (CTAP-III)/CXCL7, promote angiogenesis. Conversely, ELR-lacking chemokines, including platelet factor 4 (PF-4)/CXCL4, interferon-γ–inducible 10-kd protein (IP-10)/CXCL10, and monokine induced by IFNγ (Mig)/CXCL9, inhibit neovascularization (7,15,16). Contrary to this paradigm, the ELR-lacking stromal cell–derived factor 1 (SDF-1)/CXCL12 is angiogenic (5,16).

CC chemokines have adjacent cysteine residues (Figure 1) (8,13). These chemokines classically were thought to induce monocyte chemotaxis, although many members of this group may also recruit other cell types such as lymphocytes. As with CXC chemokines, many of the genes encoding CC chemokines are clustered, in this case on human chromosome 17q11.2 (4,8,13). Genes of CC chemokines recruiting lymphocytes are not found in this cluster in general.

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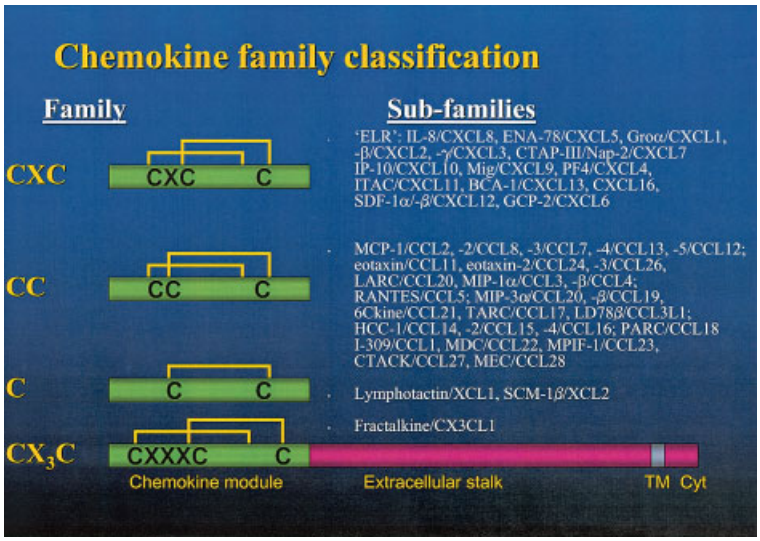

Figure 1. Chemokine family classification. IL-8 = interleukin-8; ENA-78 = epithelial neutrophil–activating peptide 78; Groα = growth-related oncogene α; CTAP-III = connective tissue activating peptide III; Nap-2 = neutrophil-activating peptide 2; Mig = monokine induced by interferon-γ (IFNγ); PF4 = platelet factor 4; ITAC = IFN-inducible T cell α chemoattractant; BCA-1 = B cell activating chemokine 1; SDF-1α = stromal cell–derived factor 1; GCP-2 = granulocyte chemotactic protein 2; MCP-1 = monocyte chemoattractant protein 1; LARC = liver and activation-regulated chemokine; MIP-1α = macrophage inflammatory protein 1α; TARC = thymus and activation–regulated chemokine; HCC-1 = hemofiltrate CC chemokine 1; PARC = pulmonary and activation-regulated chemokine; MDC = macrophage-derived chemokine; MPIF-1 = macrophage procoagulant-inducing factor 1; CTACK = cutaneous T cell–attracting chemokine; MEC = mucosae-associated epithelial chemokine; SCM-1β = single C motif 1β; TM = transmembrane domain; Cyt = cytoplasmic domain. Adapted, with permission, from ref. 12.

C chemokines and CX_3C chemokines have been described based on the position of cysteine residues (Figure 1) (1). The C family contains lymphotactin/XCL1 and single C motif 1β (SCM-1β)/XCL2, while the CX_3C family contains fractalkine/CX3CL1.

CXC chemokines. My group and several others have detected IL-8/CXCL8 in high quantities in the synovial fluid (SF), ST, and sera of patients with RA (17–20). Subsynovial macrophages, ST lining cells, and endothelial cells express IL-8/CXCL8 (18,21,22). We have shown that ST macrophages constitutively produce IL-8/CXCL8 (18). Unlike macrophages, ST fibroblasts require exogenous stimuli such as IL-1, tumor necrosis factor α (TNFα), or lipopolysaccharide (LPS) to produce IL-8/CXCL8 (18,23). Regulation of IL-8/CXCL8 production from RA ST fibroblasts appears to be controlled by NF-κB (24). Kraan et al showed that IL-8/CXCL8 was increased in the involved joints compared with the uninvolved joints of patients with RA (25). In a study by Taylor et al, TNFα blockade in patients with RA resulted in decreased ^{111}In-labeled granulocyte migration into affected joints as well as a reduction in the expression of IL-8/CXCL8 in the ST (26). Chemokine networks exist in the RA joint, so that RANTES/CCL5, monocyte chemoattractant protein 1 (MCP-1)/CCL2, or SDF-1/CXCL12 up-regulates RA ST fibroblast expression of IL-8/CXCL8, indicating that chemokines can regulate the expression of other chemokines in the joint (27). A study by Patterson et al showed binding of labeled IL-8/CXCL8 to RA ST neutrophils (28). However, although a main function of IL-8/CXCL8 may be to recruit neutrophils, it has been increasingly difficult to demonstrate an association between SF neutrophil counts and IL-8/CXCL8 levels. An alternative paradigm may be that a prominent function of RA joint IL-8/CXCL8 is to mediate angiogenesis. Indeed, we have shown that IL-8/CXCL8 is a potent mediator of angiogenesis in the RA joint (14,22).

ENA-78/CXCL5 is a chemotactic factor for neutrophils and is angiogenic (9,15,22,29). We have shown

that the ENA-78 levels observed in RA SF are much higher than those in SF from patients with osteoarthritis (OA) (30). RA ST fibroblasts constitutively produce ENA-78/CXCL5, and this production is augmented by IL-1, TNFα, or IL-18 (30,31). ENA-78/CXCL5 accounts for a portion of the angiogenic activity observed in RA ST (22).

GROα/CXCL1 is a potent neutrophil chemoattractant found in RA SF and ST (17,23,32,33). GROα/CXCL1 is inducible by TNFα and IL-1β in RA ST fibroblasts (33,34). GRO induces expression of interstitial collagens by RA fibroblasts (32).

CTAP-III/CXCL7, which is derived from human platelets (35), is angiogenic. CTAP-III/CXCL7 found in RA ST extracts induces synthesis of glycosaminoglycans and growth of RA ST fibroblasts (36). Cytokines such as IL-1β, basic fibroblast growth factor, and epidermal growth factor synergize with CTAP-III/CXCL7 in inducing glycosaminoglycan synthesis (36).

Granulocyte chemotactic protein 2 (GCP-2)/CXCL6 was recently identified as being up-regulated in RA ST fibroblasts exposed to Toll-like receptor 2 (TLR-2) ligands (37). Concentrations of GCP-2/CXCL6 were up-regulated in RA SF compared with OA SF.

IP-10/CXCL10 is up-regulated in RA SF compared with OA SF (38,39). IP-10/CXCL10 serum levels in rheumatoid factor–positive individuals were higher than those in control (OA or trauma) patients (38). Serum IP-10/CXCL10 levels were reported as being similar to normal in one study (40) but increased in another study (38). RA ST macrophages and fibroblasts immunostain for IP-10/CXCL10, and RA ST expresses greater amounts of IP-10/CXCL10 messenger RNA (mRNA) than does OA ST (39,41). TWEAK (human TNFα-like weak inducer of apoptosis) induces IP-10/CXCL10 production from RA ST fibroblasts (42). Moreover, the adhesion molecules CD11b and CD18 and intracellular adhesion molecule 1 (ICAM-1) are required for IP-10/CXCL10 induction on RA ST fibroblasts (39). Functionally lacking the ELR motif, IP-10/CXCL10 is angiostatic (7). Overall, IP-10/CXCL10 may recruit Th1 cells into RA ST (39).

Mig/CXCL9 levels were also elevated in RA versus control SF and present in RA ST, particularly in macrophages (38,43). Like IP-10/CXCL10, Mig/CXCL9 is angiostatic (7). PF-4/CXCL4 was reported to be elevated in the serum of RA patients (44). PF-4/CXCL4 is also angiostatic (45). SDF-1/CXCL12 specifically binds to CXCR4 and has been implicated in B cell/T cell/monocyte recruitment into the RA ST, as well as in mediating angiogenesis (46–50). Endothelial SDF-1/CXCL12 is involved in SF T cell adhesion to ICAM-1 (46). SDF-1/CXCL12 plays a role in CD4+ T cell recruitment into the RA ST (49). T cells are able to migrate beneath RA ST fibroblasts in vitro, a process termed pseudoemperipolesis. Pseudoemperipolesis may serve as a marker of T cell accumulation within ST (50).

Both SDF-1/CXCL12 and CXCR4 are expressed by CD68+ macrophages in the RA ST. Using a SCID mouse engrafted with human ST in vivo, Blades et al showed that when the graft was injected with SDF-1/CXCL12, human U937 monocytoid cells were recruited to the ST (48). SDF-1/CXCL12 was a more potent stimulus for monocyte recruitment than even the potent stimulus TNFα.

SDF-1/CXCL12, despite lacking the ELR motif, promotes angiogenesis (16,51). RA ST fibroblasts produce SDF-1/CXCL12 under hypoxic conditions. ST fibroblast-derived SDF-1/CXCL12 accumulates and is immobilized on heparin sulfate molecules of endothelial cells, where it can promote angiogenesis and inflammation (52). RA plasma contains more SDF-1/CXCL12 than does control plasma (53). SDF-1/CXCL12 accounts for a portion of the angiogenic activity observed in RA SF, as determined by the Matrigel plug rodent in vivo angiogenesis assay (52).

B cell recruitment has recently gained support as a potential target in RA. Burger and coworkers showed that RA ST fibroblasts support spontaneous migration of B cells beneath them via an SDF-1/CXCL12 and CD106 vascular cell adhesion molecule 1 (VCAM-1)–dependent mechanism (47). B cell activating chemokine 1 (BCA-1/CXCL13) binds CXCR5, which is expressed on circulating mature B cells and a subset of memory CD4+ T lymphocytes (54). Shi and colleagues (55) and Takemura and coworkers (56) reported that BCA-1/CXCL13 is expressed in RA ST in follicular dendritic cells in germinal centers. Endothelial cells and fibroblasts in the ST also express BCA-1/CXCL13 (56). Hence, BCA-1/CXCR5 and SDF-1/CXCL12 interactions may also recruit B cells into the inflamed joint.

CC chemokines. MCP-1/CCL2 is a potent chemoattractant for monocytes (57), and our group previously reported that MCP-1/CCL2 levels were higher in RA SF than in OA SF (57). MCP-1/CCL2 is detectable in the sera of RA patients. Stimuli for RA ST fibroblast MCP-1/CCL2 production include IL-1, TNFα, and IFNγ (23,57–61). Hypoxia decreases IL-1–induced RA ST fibroblast MCP-1/CCL2 induction (62). Messenger RNA for MCP-2/CCL8 has recently been detected in RA ST fibroblasts stimulated by TLR-2 ligands (37).

Quantities of this chemokine were larger in RA SF than those in OA SF.

Macrophage inflammatory protein 1α (MIP-1α)/CCL3 chemoattracts a variety of cells, including lymphocytes, monocytes, basophils, and eosinophils (4,8). We previously observed that RA MIP-1α/CCL3 levels were higher than were OA SF levels (63). SF mononuclear cells and ST fibroblasts produce MIP-1α/CCL3 mRNA and protein (23,63). MIP-1α/CCL3, produced by CD44 T cells, is augmented by IL-15 stimulation (64). SF neutrophils produce more MIP-1α/CCL3 than do RA peripheral blood neutrophils (65). MIP-1β/CCL4 gene expression was observed in RA SF T cells (66). We have shown that TNFα and IL-1 induce RA ST fibroblast MIP-1α/CCL3 production (63). MIP-1β/CCL4, in contrast to many of the previously described chemokines, appears to be up-regulated in OA SF compared with RA SF (67).

MIP-3α/CCL20 is a CC chemokine that chemoattracts T cells, B cells, monocytes, and immature dendritic cells. Large amounts of MIP-3α/CCL20 have been detected in RA SF and RA ST. In RA ST, MIP-3α–producing cells were often located in proximity to immature dendritic cells, suggesting the capacity to recruit these cells (68). In the ST, mainly infiltrating macrophages and ST lining cells expressed MIP-3α/CCL20. RA ST fibroblasts produced this chemokine in response to cytokines, including TNFα, IL-1, IL-17, or IL-18 (69–71). MIP-3α is potently chemotactic for monocytes (70).

RANTES/CCL5 chemoattracts lymphocytes and monocytes as well as other cell types (8,72,73). RA ST fibroblasts produce RANTES/CCL5 mRNA upon stimulation with TNFα, IL-1, or IFNγ (23,73–75). Thrombin also activates RA ST fibroblast RANTES/CCL5 production via protease-activated receptor 1 (PAR-1) (76). RANTES/CCL5 mRNA has also been detected in RA peripheral blood and SF T cells, as well as in the lining layer and macrophages in RA ST (66,73). RANTES/CCL5, as well as MCP-1/CCL2, can participate in cytokine networks by inducing RA ST fibroblasts to produce IL-8/CXCL8 and IL-6 (27).

Lymphoid aggregates are often present in inflamed RA ST. Buckley recently described immunohistologic expression of Epstein-Barr virus–induced gene 1 ligand chemokine (ELC)/CCL19, a B cell–attracting chemokine, in RA ST (77). It may be that this chemokine along with the CXC chemokines SDF-1/CXCL12 and BCA-1/CXCL13 contribute to B lymphocyte recruitment in RA synovitis.

C and CX_3C chemokines. Lymphotactin/XCL1 is expressed in CD8+ T cells and is significantly increased in CD4+,CD28− T cells from RA versus normal peripheral blood (78). Blaschke et al demonstratd that in RA ST, lymphotactin/XCL1 was mainly localized in CD4+ sublining cells (78). Incubation of lymphotacin/XCL1 with RA ST fibroblasts resulted in down-regulation of matrix metalloproteinase 2 (MMP-2) production. Lymphotactin/XCL1 stimulated the transmigration of the CD45RO+/CD45RB− T cells. These T lymphocytes preferentially accumulate in vivo in the joints of patients with RA (79).

Fractalkine/CX3CL1 plays a dual role, being chemotactic for monocytes and lymphocytes and also serving as a cellular adhesion molecule (80,81). Our group has observed high levels of soluble fractalkine/CX3CL1 in RA SF (81). In the peripheral blood and SF, mainly monocytes expressed this chemokine. In RA ST, macrophages, fibroblasts, endothelia, and dendritic cells expressed fractalkine/CX3CL1 (81). Fractalkine/CX3CL1 stimulation of RA ST fibroblasts resulted in up-regulation of MMP-2 levels (81). Fractalkine/CX3CL1 may contribute to the accumulation of CX_3CR+ T cells in RA ST (27).

Fractalkine/CX3CL1 has recently received much attention in the field of atherosclerosis. When the fractalkine/CX3CL1 receptor CX3CR1 was deleted in mice, atherosclerosis development was greatly reduced (82). Similarly, in another study in humans, a CX3CR1-M280/I249 polymorphism was associated with reduced cardiovascular risk (83). My laboratory has also shown that fractalkine/CX3CL1 is angiogenic (84). Because accelerated atherosclerosis is an integral part of RA, it is likely that targeting fractalkine/CX3CL1 or its receptor may be useful in cardiovascular disease as well as RA.

Chemokine receptors

Chemokines mediate their effects via 7-transmembrane domain receptors that are a subset of G protein–coupled receptors (GPCRs) (13). As shown in Table 1, there is a great deal of redundancy and binding promiscuity between chemokine ligands and their receptors. Chemokine receptors have been associated with various subtypes of autoimmune inflammation. Thus, RA, which is considered a Th1 disease, is associated with CXCR3 and CCR5 (85,86). Conversely, asthma is considered a Th2 disease and is associated with CCR3, CCR4, and CCR8 (13,85).

Among CXC chemokine receptors, CXCR1 and CXCR2 are expressed on RA macrophages and neutrophils (28). We and other investigators have shown CXCR3 to be expressed in RA joints and perhaps to be an important receptor in homing of leukocytes into the

Table 1. Human chemokine receptors*

Receptor	Ligand
CXCR1	IL-8/CXCL8, GCP-2/CXCL6
CXCR2	IL-8/CXCL8, GROα/CXCL1, GROβ/CXCL2, GROγ/CXCL3, CTAP-III/NAP-2/CXCL7, ENA-78/CXCL5, GCP-2/CXCL6
CXCR3	IP-10/CXCL10, Mig/CXCL9, ITAC/CXCL11, PF-4/CXCL4
CXCR4	SDF-1/CXCL12
CXCR5	BCA-1/CXCL13
CXCR6	CXCL16
CCR1	MIP-1α/CCL3, RANTES/CCL5, MCP-3/CCL7, MIP-5; HCC-1/CCL14, HCC-2/CCL15, HCC-4/CCL16; LD78β/CCL3L1, MPIF-1/CCL23
CCR2	MCP-1/CCL2, MCP-3/CCL7, MCP-4/CCL13; HCC-4/CCL16
CCR3	Exotaxin/CCL11, exotaxin-2/CCL24, exotaxin-3/CCL26; RANTES/CCL5, MCP-2/CCL8, MCP-3/CCL7, MCP-4/CCL13; HCC-2/CCL15, MEC/CCL28
CCR4	TARC/CCL17, MDC/CCL22
CCR5	MIP-1α/CCL3, MIP-1β/CCL4; LD78β/CCL3LI, RANTES/CCL5; MCP-2/CCL8, HCC-1/CCL14
CCR6	MIP-3α/CCL20
CCR7	MIP-3β/CCL19, 6Ckine/SLC/CCL21
CCR8	I-309/CCL1
CCR9	TECK/CCL25
CCR10	CTACK/CCL27, MEC/CCL28
CX3CR1	Fractalkine/CX3CL1
XCR1	Lymphotactin/XCL1, SCM-1β/XCL2
DARC	Duffy antigen; binds some CC and CXC chemokines

*IL-8 = interleukin-8; GCP-2 = granulocyte chemotactic protein 2; GROα = growth-related oncogene α; CTAP-III = connective tissue activating peptide III; NAP-2 = neutrophil-activating peptide 2; ENA-78 = epithelial neutrophil–activating peptide 78; Mig = monokine induced by interferon-γ (IFNγ); ITAC = IFN-inducible T cell α chemoattractant; PF-4 = platelet factor 4; SDF-1 = stromal cell–derived factor 1; BCA-1 = B cell activating chemokine 1; MIP-1α = macrophage inflammatory protein 1α; MCP-3 = monocyte chemoattractant protein 3; HCC-1 = hemofiltrate CC chemokine 1; MPIF-1 = macrophage procoagulant-inducing factor 1; MEC = mucosae-associated epithelial chemokine; TARC = thymus and activation–regulated chemokine; MDC = macrophage-derived chemokine; SLC = secondary lymphoid tissue chemokine; TECK = thymus-expressed chemokine; CTACK = cutaneous T cell–attracting chemokine; SCM-1β = single cysteine motif 1β; DARC = Duffy antigen receptor for chemokines. Adapted, with permission, from ref. 12.

RA ST (85,86). CXCR3 has been shown to mark a subset of T lymphocytes. Ruth and colleagues reported that most T lymphocytes in RA SFs were CXCR3+ (87). CXCR3 is expressed by endothelial cells, some dendritic cells, and T cells in the RA ST (87,88). CXCR3 has also been found on RA ST mast cells (41). CXCR4 is present on a variety of cell types. Transforming growth factor β1 induces CXCR4 expression on RA ST T cells (46).

Among CC chemokine receptors, CCR5 showed strong expression on RA ST fibroblasts and ST T lymphocytes (85). CCR5 has been shown in several studies to be present on mononuclear cells in SF from patients with RA (27,86,89,90). We demonstrated lymphocyte chemokine receptor expression in RA, which may play a role in inflammatory cell recruitment into the joint. Our data suggest that CCR4 and CCR5 may be critical to this process (87).

Recently, CCRL2, which codes for a putative 7-membrane GPCR, has been identified on RA SF neutrophils and some macrophages (91). Inflammatory products present in RA SF activate this receptor, indicating that CCRL2 may be involved in RA pathogenesis. The gene for this receptor is located on chromosome 3, in close proximity to other chemokine receptors.

We compared chemokine receptor expression on peripheral blood, SF, and ST monocyte/macrophages in RA. We observed that monocytes mainly express CCR1 and CCR2 in normal and RA peripheral blood (92). In contrast, CCR3 and CCR5 are up-regulated in RA SF. The differential expression of these receptors suggests that CCR1 and CCR2 may be involved in monocyte recruitment from the circulation, while CCR3 and CCR5 may be important in monocyte retention in the joint (92).

Other chemokine receptors may also be involved in RA. CCR6, a receptor for MIP-3α/CCL20, was detected on infiltrating white blood cells in the RA ST (69). XCR1, the lymphotactin/XCL1 receptor, was immunolocalized to ST lymphocytes, macrophages, and fibroblasts.

Regarding CX_3C chemokines, the fractalkine/CX3CL1 receptor was present on RA SF CD3+ lymphocytes and CD14+ macrophages. In RA ST, macrophages and dendritic cells were the predominant cells bearing CX_3CR1 (81). CX_3CR1 has been implicated in both monocyte recruitment and lymphocyte recruitment in the RA joint (81,93).

Chemokines may also bind to the Duffy receptor. DARC binds both CC and CXC chemokines and was originally described on red blood cells. A recent study showed that RA ST endothelium expressed DARC (94).

Chemokine targeting strategies in experimental arthritis

Temporal expression of chemokines in arthritis models. As a prelude to determining which chemokines to target, the temporal expression of chemokines has been determined in animal models. For instance, ENA-78/CXCL5, MCP-1/CCL2, and MIP-1α/CCL3 were assessed during the course of rat adjuvant-induced arthritis (AIA), a model for human RA, in order to differentiate between chemokines involved in the early events or later stages of the disease (95). Levels of both ENA-78/CXCL5 and MIP-1α/CCL3 in the sera and joint homogenates of rats showed an early increase, preceding the onset of clinical symptoms. In contrast, MCP-1/CCL2 appeared to be involved mainly in the later phase of AIA (96). Another group of investigators observed increased RANTES/CCL5 and MIP-1α/CCL3 production in murine collagen-induced arthritis (CIA) compared with that in normal mice (97). Determining the time course of chemokine expression during arthritis development should aid in pinpointing therapeutic intervention points in disease.

CXC chemokines. In the last several years, several studies targeting chemokines or their receptors experimentally have been published. Because a mouse homolog of IL-8/CXCL8 has not been identified, many of these studies were performed in rabbits. A neutralizing antibody to IL-8/CXCL8 prevented neutrophil infiltration into rabbit LPS/IL-1–induced arthritis (98). Similarly, an antibody to IL-8/CXCL8 inhibited LPS- or monosodium urate crystal–induced leukocyte infiltration into rabbit knee joints (99,100). A nonpeptide oral antagonist of the IL-8/CXCL8 receptor, CXCR2, inhibited acute IL-8/CXCL8– or LPS-induced arthritis and chronic antigen (ovalbumen)-induced arthritis in rabbits (101). In LPS-induced rabbit arthritis, elevated SF GRO levels preceded knee joint leukocyte infiltration, which was inhibited 54% by GRO-specific neutralizing antibody, 48% by anti–IL-8, and 70% by a combination of both antibodies (102).

Preventative anti–MIP-2/GROα/CXCL1 in a mouse homolog resulted in delayed onset and severity of murine CIA (96). Finally, AMD3100, an antagonist of the SDF-1/CXCL12 receptor CXCR4, inhibited CIA preventively and therapeutically in IFNγ-deficient DBA/1 mice (103). Matthys et al used IFNγ-deficient DBA/1 mice in order to hasten the course of CIA compared with that in non–cytokine-deficient animals. Thus, these data suggest that targeting CXC chemokines and their receptors may have an impact on experimental arthritis and potentially on RA.

In an interesting study, Salomon et al administered IP-10/CXCL10 naked DNA vaccine to rats induced to develop AIA (104). Rats developed protective immunity against AIA. Moreover, self-specific anti–IP-10/CXCL10 or rabbit anti-rat IP-10/CXCL10 adoptively transferred disease suppression. These results suggest IP-10/CXCL10 may be a target in RA.

In another study, my laboratory used an anti–ENA-78/CXCL5 polyclonal antibody strategy preventively in rat AIA. The severity of AIA was attenuated, as determined by joint swelling and circumference. This antibody was unable to reverse the disease course when injected during the further development of arthritis, suggesting that to be effective, antibody must be given early in the course of arthritis development (105).

PF-4/CXCL4 is angiostatic and inhibits endothelial cell proliferation (106). An antiinflammatory peptide of PF-4/CXCL4 successfully inhibited mouse CIA when administered therapeutically (106). Although the mechanism of action of this peptide was unclear, interestingly, circulating IL-1 levels decreased with treatment. Hence, unlike most of the other published studies, in the study by Wooley et al, administration of a chemokine rather than blocking of a chemokine's actions was beneficial. Although not examined in this study, the angiostatic role of PF-4/CXCL4 may have played a role in the effect of this chemokine on CIA, because other angiogenesis inhibitors are effective in this model.

Very few studies have examined arthritis in gene-deficient mice. However, Terkeltaub and coworkers observed that CXCR2 gene–deficient mice exhibited leukocyte-deficient inflammatory responses in a murine subcutaneous air pouch model injected with urate crystals (107). These same animals showed less severe Lyme arthritis than did normal mice in another study (108). Because mice, in contrast to humans, express only CXCR2, not both CXCR1 and CXCR2, the relevance to human disease of deleting only CXCR2 remains unclear.

CC chemokines. A number of studies have targeted MIP-1α/CCL3 and MCP-1/CCL2. Passive immunization with anti–MIP-1α/CCL3 delayed the onset and reduced the severity of murine CIA (96). A 67–amino acid sequence of MCP-1/CCL2, which acts as an MCP-1/CCL2 antagonist in vivo, abrogated arthritis in MRL/*lpr* autoimmune mice (109). Injection of neutralizing MCP-1/CCL2 resulted in reduced macrophage numbers and arthritis in rats with CIA (110). Bindarit, an indazolic derivative inhibitor of TNFα and MCP-1/CCL2,

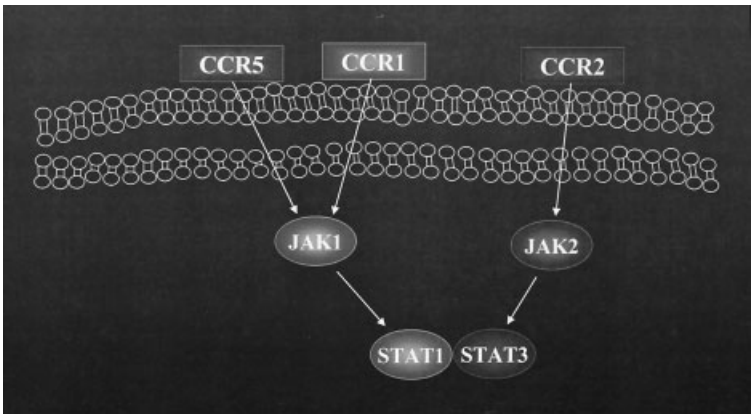

Figure 2. Hypothesized CC chemokine receptor signaling in joints of rats with adjuvant-induced arthritis. STAT1 = signal transducer and activator of transcription 1.

inhibited rat AIA (111). Injection of anti–MCP-1/CCL2 preventively resulted in decreased influx of ^{111}In-labeled T cells into the rat streptococcal cell wall antigen–induced arthritic joint (112).

Administration of Met-RANTES, a CCR1/CCR5 antagonist, reduced mouse CIA (113). Likewise, anti-RANTES/CCL5 reduced mouse CIA (114). A CCR5 nonpeptide antagonist, when used preventively, inhibited mouse CIA and migration of leukocytes to the joints (115). This inhibitor, however, also blocked binding of ligand to CXCR3, making it difficult to determine its precise mechanism (116). KE-298 (2-acetylthiolmethyl-4-[4-methylphenyl]-4-oxobutanoic acid), an antirheumatic drug, reduced MCP-1/CCL2 and RANTES/CCL5 production and also reduced the severity of rat AIA (117). This drug also suppressed production of these chemokines by IL-1– stimulated RA ST fibroblasts. Very surprisingly, a recent study showed that a CCR5 gene–deficient mouse developed CIA to the same extent as wild-type mice (118). The reason for the discrepant data using CCR5-deficient mice is unclear. Nonetheless, most available data in other models appear to indicate CCR5 is a reasonable therapeutic target in RA.

Manipulation of the CCR2 receptor has produced varied outcomes. Antibody-mediated blockade of CCR2 during initiation of CIA in mice resulted in markedly improved clinical signs of arthritis, while blockade during disease progression (therapeutic administration) worsened the clinical and histologic signs of arthritis (119). Moreover, in a recent study, CCR2 gene–deficient mice developed severe, aggressive CIA compared with wild-type mice (118). Interestingly, when lethally irradiated wild-type mice received bone marrow transplants from CCR2 gene–deficient mice, the transplanted mice developed frank CIA. In contrast, wild-type mice transplanted with wild-type bone marrow did not develop CIA, suggesting that the expression of CCR2 in the hematopoetic compartment is a critical component of CIA development (118). Hence, it appears that targeting CCR1/CCR5 may be useful in experimental arthritis. Results for CCR2 blockade are much less clear at this time and await further studies.

My laboratory has examined chemokine receptor expression and function in rat AIA (120). Using real-time polymerase chain reaction, we showed significant up-regulation of CCR1, CCR2, CCR5, and MIP-1β/CCL4 mRNA on postadjuvant injection day 18, coincident with peak joint inflammation. Increases in tyrosine phosphorylation of CCR1 on days 14, 18, 21, and 24, of CCR2 on days 14 and 18, and of CCR5 on days 14, 18, and 21 were observed in rats with AIA compared with nonarthritic rats. The joint signaling intermediates, JAK-1, signal transducer and activator of transcription 1 (STAT-1), and STAT-3, were associated with CCR1 and were tyrosine phosphorylated on days 14 and 18. CCR2 was associated with phosphorylated JAK-2, STAT-1, and STAT-3 on day 18. CCR5 was associated with STAT-1 and STAT-3 on days 18 and 21 and correlated with JAK-1 phosphorylation and binding on day 18. Although JNK signaling has been shown by other investigators to be important in RA synovium, it appears not to be important in CCR5 signaling in rat AIA joints. By immunohistologic analysis, CCR5, phosphorylated STAT-1, and phosphorylated STAT-3 were detected on ST lining cells, macrophages, and endothelial cells in inflamed rat joints on day 18. Although the majority of

phosphorylated STAT-1 and CCR5 were on ST lining cells and macrophages, phosphorylated STAT-3 was predominantly expressed on endothelial cells (see Figure 2). Up-regulation and activation of chemokine receptors may play a role in macrophage and endothelial infiltration in rat AIA joints.

Combined chemokine blockade. Several studies have addressed the blocking of multiple chemokines. One study showed that a combination of MCP-1/CCL2 and GROα/CXCL1 inhibition with chemokine antagonists resulted in more reduced arthritis than MCP-1/CCL2 blockade alone in MRL-Fas^{lpr} mice with AIA (121). In another study, rat AIA was diminished by DNA vaccination with chemokine-encoding DNA vaccines to MCP-1/CCL2, MIP-1α/CCL3, or RANTES/CCL5 (122). These studies do not address the possible toxicities associated with combining blocking agents, which potentially could be problematic in human trials.

Chemokine targeting strategies in human RA

As a prelude to chemokine targeting in humans, one study has shown decreased severity and incidence of RA in patients with the CCR5 Δ32 allele, which codes for a nonfunctional CCR5 allele (123,124). Another study showed that this allele was found less frequently in patients with RA (125). These studies underscore the idea that targeting CCR5 in humans may be beneficial in RA.

Regarding direct targeting, antibodies to IL-8/CXCL8, ENA-78/CXCL5, and GROα/CXCL1 at least partially neutralized RA SF–derived chemotactic activity for neutrophils in vitro, and antibodies to MIP-1α neutralized RA SF-derived chemotactic activity for monocytes (33,67). Among drugs currently available for RA therapy, anti-TNFα reduced synovial expression of IL-8/CXCL8 and MCP-1/CCL2 in patients with RA (26).

One trial examined the use of anti–IL-8/CXCL8 in human RA. Results of this trial were not published, but the compound was apparently not developed further. Because no published data exist, it is difficult to comment on this therapeutic agent.

Recently, a CCR1 small molecule antagonist was examined in a phase Ib 2-week study. This inhibitor resulted in a marked decrease in ST macrophages posttherapy (126). Although this study was designed mainly to examine the effect of the inhibitor on RA synovial inflammation, one-third of the 16 patients fulfilled the American College of Rheumatology 20% criteria (127) for improvement after therapy. These results suggest CCR1 may be a beneficial target in RA.

Conclusion

Although superficially one may conclude that blocking chemokines or their receptors is futile due to redundancies in the chemokine/chemokine receptor systems, careful analysis suggests that blockade of specific chemokine/chemokine receptors may be valuable. However, several caveats must be considered. First, as with many animal models, there are more reports of animal studies using preventative regimens than reports of studies using therapeutic regimens, as would be used in RA patients, again lessening the relevance to human disease. Second, many compounds targeting chemokine receptors are species specific, hence resulting in difficulties translating these compounds to human use. Third, cleavage of some chemokines by metalloproteinases may result in antagonist rather than agonist effects on their receptors, potentially making targeting of chemokines difficult. Nonetheless, after reviewing the existing data, it seems that there is sound rationale for targeting CXC chemokines, CC chemokines, and fractalkine/CX3CL1. Among chemokine receptors, CXCR3 and CCR5 appear to be likely target candidates. Preliminary results from a small human trial involving targeting CCR1 were promising. Thus, evidence is mounting that targeting chemokines may be useful therapy, either alone or adjunctively, in RA.

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