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REVIEW ARTICLE

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Chemokines in synovial inflammation in rheumatoid arthritis: basic and clinical aspects

Abstract Rheumatoid arthritis (RA) is a chronic, multisystem autoimmune disease characterized by persistent synovitis. Since chemotactic cytokines (chemokines) may play critical roles in the recruitment of leukocytes in RA, analyses of chemokines and their receptors should provide insight into events in synovial inflammation in RA. The production of chemokines is regulated by cytokines such as tumor necrosis factor (TNF)-α produced in the inflamed joint, suggesting that the efficacy of anti-TNF-α therapy is mediated at least partly by the reduction of chemokine production. Chemokines have a role in joint inflammation not only by inducing leukocyte chemotaxis, but also by activating immune cells and angiogenesis. The pathogenesis of RA has been shown to be mediated by Th1-type T cells, because Th1-related chemokine receptors are preferentially expressed on cells in synovial fluid and synovial tissue. Accordingly, antichemokine therapy may be important as a possible new approach to therapeutic intervention in RA.

Key words Angiogenesis · Chemokine · Chemotaxis · Rheumatoid arthritis (RA) · Th-1

Introduction

Chemotactic cytokines (chemokines), a family of homologous serum proteins of 7–16 kDa, direct leukocyte migration into regions of inflammation. More than 40 structurally related human chemokines are known. Chemokines can be subdivided into four distinct supergene families, such as CXC, CC, C, and CX3C chemokines, based on the location of conserved cysteine residues.[1,2] In the CXC subfamily the first two cysteines in the moleclule are separated by an amino acid, and in the CC subfamily the first cysteines are adjacent. CXC chemokines chemoattract mainly neutrophils, while CC chemokines exert their effect essentially on monocytes and T cells.[3] Chemokines act on target cells through chemokine receptors which are single-chain, seven-helix membrane-spanning receptors that are coupled to G proteins.[4] The chemokine receptors comprise two major groups: the CC chemokine receptors 1–10 (CCR1–10) that bind CC chemokines, and the CXC chemokine receptors 1–6 (CXCR1–6) that bind CXC chemokines.[5] Since chemokines often share receptors and cells express a number of receptor types, chemokines exhibit multiple overlapping activities. Thus, the interaction of chemokines and their receptors is thought to have a critical role in the pathogenesis of various inflammatory and immune diseases.

Rheumatoid arthritis (RA) is a chronic disease that is characterized by inflammation of the joints and concomitant destruction of cartilage and bone. Joint parenchymal lesions in patients with RA are characterized by synovial hyperplasia, with an increased number of resident fibroblasts and infiltrating immune cells.[6] Synovial fluids in RA are abundant in neutrophils, monocytes, macrophages, lymphocytes, and dendritic cells, which contribute to the pathogenesis of the disease through the release of degradative enzymes, prostaglandins, reactive oxygen species, and proinflammatory cytokines.[7] The migration of cells into the inflamed joint is mediated by chemokines released by the activated cells in the joint. There are several lines of evidence indicating that chemokines accumulating in synovial tissue contribute to the pathogenesis of synovitis.[8] In this review, we focus on the role of chemokines in the pathogenesis of synovial inflammation in RA, and discuss the therapeutic strategy targeting chemokines for this disorder.

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Production and expression of chemokines

The human CXC and CC chemokines, which have been reported to be involved in RA, and their corresponding

Table 1. Chemokines involved in RA and their corresponding receptors

CXC chemokines	
Interleukin-8 (IL-8/CXCL8)	CXCR1 and 2
Growth-related gene product α (Gro-α/CXCL1)	CXCR2
Epithelial–neutrophil activating protein-78 (ENA-78/CXCL5)	CXCR2
Interferon-γ inducible protein-10 (IP-10/CXCL10)	CXCR3
Monokine induced by interferon-γ (MIG/CXCL9)	CXCR3
Stromal-derived factor-1 (SDF-1/CXCL12)	CXCR4
CC chemokines	
Monocyte chemoattractant protein-1 (MCP-1/CCL2)	CCR2
Macrophage inflammatory protein-1α (MIP-1α/CCL3)	CCR1 and 5
Macrophage inflammatory protein-1β (MIP-1β/CCL4)	CCR5
Regulated upon activation normally T cell expressed and secreted (RANTES/CCL5)	CCR1 and 5
Thymus and activation-regulated chemokine (TARC/CCL17)	CCR4
Macrophage-derived chemokine (MDC/CCL22)	CCR4

receptors are shown in Table 1. Synovial fluid from RA patients contains a variety of chemokines, including IL-8/CXCL8, GRO-α/CXCL1, ENA-78/CXCL5, IP-10/CXCL10, SDF-1/CXCL12, MIG/CXCL9, MCP-1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4, and RANTES/CCL5.[8] Since the synovial tissue of RA patients expresses these chemokines at high levels, both synovial lining cells and infiltrating leukocytes are thought to be their sources.[8] Among these cells, macrophages and fibroblasts in the synovial tissue are a major source of chemokines.[9–11] Synovial fluid mononuclear cells of RA patients also express these chemokines.[8] Increased expression of mRNA of IL-8/CXCL8/CXCL8, GRO-α/CXCL1, MCP-1/CCL2, MIP-1α/CCL3, and MIP-1β/CCL4 is found in synovial fibroblasts when they are stimulated with IL-1.[12]

Chemokine receptors

Since leukocyte migration towards inflammatory lesions is directed by the interaction of chemokines with their receptors, the regulation of chemokine receptor expression represents a pivotal means of controlling inflammatory responses at the disease site in RA. The mRNA expression of CC chemokine receptors CCR-1, CCR-2, CCR-3, and CCR5 is up-regulated in the joints of arthritic mice.[13] Immunostaining of T cells in the synovial fluid of RA patients shows higher expressions of CXCR3 and CCR5, a receptor for IP-10/CXCL10 and MIG/CXCL9, and a receptor for MIP-1α/CCL3, MIP-1β/CCL4, and RANTES/CCL5 than the levels in blood.[14] High expression of CXCR4 is found on synovial T cells of RA patients with high expession of SDF-1/CXCL12, a ligand for CXCR4, on synovial endothelial cells.[15] The expression of chemokine receptors in an RA joint may be regulated by cytokines. For example, IL-2, IL-4, IL-10, and IL-12 can enhance CC chemokine receptor expression and chemotactic responsiveness to MCP-1/CCL2 and RANTES/CCL5 on T lymphocytes.[16] Treatment of blood T cells with IL-2 induces the expression of CCR1, CCR2, CCR5, and CXCR3, and cells become responsive to the appropriate inflammatory chemokines.[17] The expression of CXCR4 is up-regulated by IL-15[11] and transforming growth factor (TGF)-β.[15] We recently showed that interferon-γ maintains the expression of IL-8/CXCL8 receptors CXCR1 and CXCR2 on T lymphocytes.[2] These results suggest that cytokines may play an important role in regulating chemokine receptor expression on T cells and in sustaining the function of these cells in response to the chemokines. CC chemokine receptors on monocytes/macrophages are also up-regulated in RA joints.[18] Thus, the expression of chemokine receptors on immune cells can be differentially regulated under different pathophysiological conditions, which is crucial in determining their recruitment to inflamed RA joints.

Clinical significance of chemokines in rheumatoid arthritis

Migration

Neutrophil migration

Neutrophils, which constitute more than 90% of the cellular components in RA synovial fluids in RA,[19] have been shown to play an important role in synovial inflammation. In RA pannus there are relatively few neutrophils, but these cells have been identified at the cartilage–pannus junction.[20] IL-8/CXCL8 is thought to be the most critical chemokine for neutrophils in rheumatoid synovitis. Several studies have shown elevated levels of IL-8/CXCL8 protein and mRNA in synovial fluids and the synovial cells of RA patients.[21–24] The IL-8/CXCL8 level in synovial fluid is correlated with leukocyte counts in the synovial fluid.[22,23] Rheumatoid synovium highly expresses IL-8/CXCL8.[25] Since the production of IL-8/CXCL8 is induced by a variety of cells when stimulated with IL-1 and tumor necrosis factor (TNF)-α, IL-8/CXCL8 may mediate the effects of IL-1 and TNF-α. As well as IL-8/CXCL8, GRO-α/CXCL1 and ENA-78/CXCL5, which can also induce neutrophil chemotaxis, are expressed in monocytic cells in the synovial lining layer of RA pannus.[8,26]

Monocyte migration

Monocytes are thought to play an important role in the joint inflammation of RA.[27] Monocyte infiltrates are predominant in RA joints, and their products such as cytokines and enzymes amplify the inflammatory responses and destroy connective tissue. Monocytes may be recruited to the inflamed joint in response to chemotactic agents produced at the foci of inflammation. Most of the CC-chemokines can induce the chemotaxis of monocytes. High levels of MCP-1/CCL2 are detected in the synovial fluid of RA patients, and synovial cells highly express MCP-1/CCL2 mRNA.[28,29] MCP-1/CCL2 mRNA is expressed in synovial fibroblasts stimulated with IL-1 and TNF-α.[29] Recently, Hayashida et al. reported that MCP-1/CCR2 is a major monocyte chemokine released by fibroblasts.[30] MIP-1α/CCL3 has been found in RA joints, and was shown to be twice as high in active than in inactive RA.[31] MIP-1β/CCL4 and RANTES/CCL5 have also been detected in inflamed joints of RA.[26]

Lymphocyte migration

Although most of the tissue damage associated with rheumatoid synovitis is mediated by activated macrophages and synoviocytes, it has been suggested that tissue-infiltrating lymphocytes are involved in the inflammatory response.[32] Activated T lymphocytes release cytokines such as IFN-γ, IL-4, and IL-6,[33–36] which further activate lymphocytes and induce the production of autoantibodies such as rheumatic factor. T cells can express most known chemokine receptors, and this expression depends on maturation, activation, and functional differentiation. Various chemokines, including IL-8/CXCL8,[37] MCP-1/CCL2,[38,39] MIP-1α/CCL3, MIP-1β/CCL4,[40,41] RANTES/CCL5,[42] and SDF-1/CXCL12,[11] have been determined to be lymphocyte chemoattractants. RANTES/CCL5 is produced primarily by T cells and induces chemotaxis of T cells. MIP-1α/CCL3 preferentially attracts CD8+ T cells,[39,41] while RANTES/CCL5 preferentially attracts CD4+ T cells.[42] MIP-1α/CCL3 and RANTES/CCL5 are able not only to be chemotactic, but also to activate T lymphocytes and promote T cell proliferation.[43] IL-8/CXCL8, GRO/CCL1, IP-10/CXCL10, and SDF-1/CXCL12 belong to the CXC chemokine family, and have chemotactic activity for T lymphocytes.[36,44] Although various T cell chemoattractants such as MCP-1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4, RANTES/CCL5, Gro-α/CXCL1, and IL-8/CXCL8 have been detected in inflamed joints of RA patients,[26,36,37,39–42,44] there have been few studies concerning their roles in lymphocyte involvement in RA. The synovial fluid of RA patients contains chemotactic activity for T lymphocytes, and combinations of anticytokine antibodies such as anti-IL-15 and anti-IL-8/CXCL8,[45] anti-MCP-1/CCL2, and anti-IL-8/CXCL8, or anti-MIP-1α/CCL3 and anti-IL-8/CXCL8[46] partially inhibit the chemotactic activity. SDF-1/CXCL12–CXCR4 interactions were shown to play important roles in T cell accumulation in RA synovium.[11,15] These findings indicate that various chemoattractants may be involved in lymphocyte recruitment in inflamed joints of RA.

Angiogenesis

Angiogenesis, which is the growth of new blood vessels from preexisting vessels and capillaries, has a critical role in the inflammation and pannus formation of RA joints. Angiogenesis is regulated by an opposing balance of angiogenic and angiostatic factors.[47] Some CXC chemokines promote, but others inhibit, angiogenesis. The CXC chemokine subfamily that contain a ELR (glutamyl–leucyl–arginyl) motif, such as IL-8/CXCL8, NAP-2/CXCL7, ENA-78/CXCL5, and Gro-α/CXCL1, is responsible for angiogenic responses, whereas CXC chemokines lacking the ELR motif, such as IP-10/CXCL10 and MIG/CXCL9, are reported to be angiostatic, demonstrating the diverse roles that chemokines can play in this process.[47] Although IL-8/CXCL8, Gro-α/CXCL1, and MIG/CXCL9 have been shown to be expressed in the synovial tissue,[26] the role and balance of the CXC chemokines in RA joints in angiogenesis are unclear. The CC chemokine MCP-1/CCL2 can also contribute to angiogenesis, inducing chemotaxis of human endothelial cells.[48]

Th1 and Th2

Although the triggering agent of RA is still uncertain, lymphokines released by activated CD4+ T lymphocytes play a pivotal role in the inflammatory process of this disorder. CD4+ T lymphocytes can be subdivided into two distinct populations, Th1 and Th2, by the spectrum of cytokines produced by these cells.[49] Th1 cells generate IL-2, interferon-γ, and TNF-β, and promote cell-mediated immunity, whereas Th2 cells generate IL-4, IL-5, IL-6, and IL-10, and play a role in humoral immunity and allergic diseases.[50] There is evidence that a balance of different cytokine producers is crucial for an effective immune response and the outcome of infectious and autoimmune diseases.[51,52] In RA, a Th-1 response has been shown to be predominant because lymphocytes in synovial fluids from RA patients produce excessive amounts of interferon-γ and IL-2.[7]

Great attention has recently been paid to the possibility of distinguishing Th1 from Th2 cells on the basis of the differential expression of chemokine receptors. Chemokines have a role in effector and amplification mechanisms of polarized Th1- and Th2-mediated immune responses, and their receptors might serve as targets for selective modulation of T cell-dependent immunity. CXCR3 and CCR5 are expressed on Th1-type cells, whereas CCR4, CCR8, and to a lesser extent CCR3 are expressed on Th2-type T cells, and have been shown to be responsible for Th1- and Th2-dominant immune responses, respectively.[53–55] These observations suggest that the differential expression of chemokine receptors may be useful for discriminating pathogenic Th1 and Th2 cells in allergic and autoimmune diseases such as RA. Both CXCR3- and CCR5-expressing Th1 cells are more abundant in synovial fluid, synovial fluid cells, and synovial tussue than in peripheral blood.[14,56,57] We found increased expression of CXCR3 on peripheral blood CD4+ T cells of RA patients (Fig. 1).

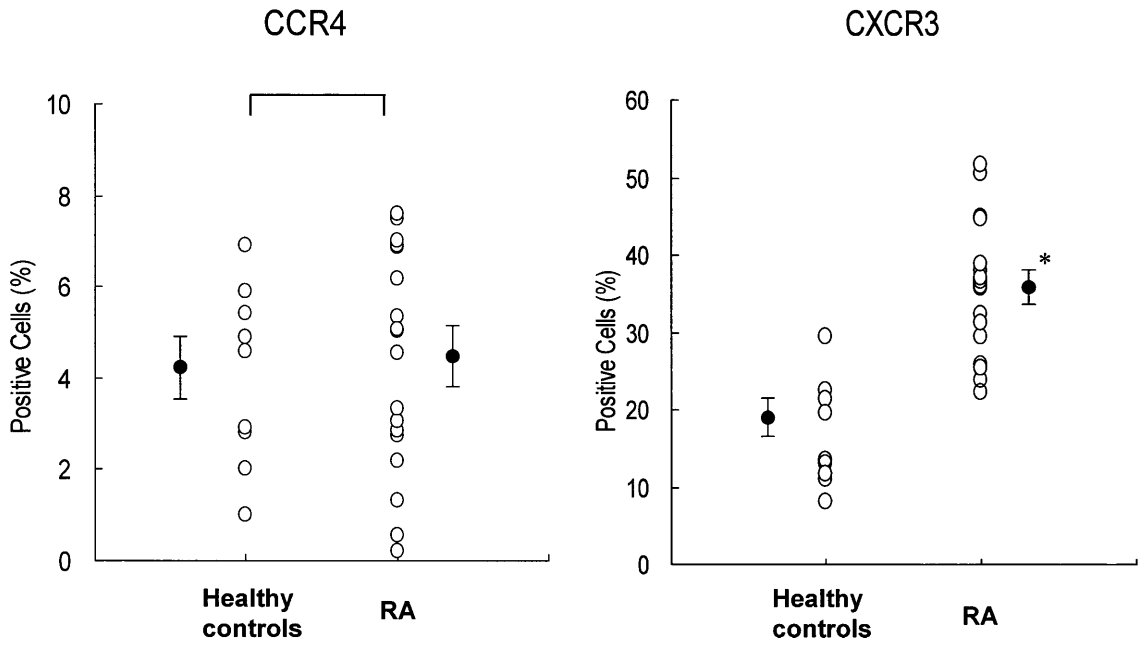

Fig. 1. Expression of CCR4 and CXCR3 on CD4+ T cells of peripheral blood from RA patients (n = 17) and healthy controls (n = 9). Results are expressed as mean ± SE

On the other hand, CCR4-expressing Th2 cells were at very low lovels in both synovial fluid and peripheral blood T cells[57] (Fig. 1). Concomitantly, ligands for CXCR3 (IP-10/CXCL10 and MIG/CXCL9) and for CCR5 (MIP-1α/CCL3, MIP-1β/CCL4, and RANTES/CCL5) are preferentially expressed in inflamed joints of RA patients,[58] suggesting that these chemokines may participate in the selective recruitment of CXCR3+ and CCR5+ T cells, and that the pathogenesis of RA is mediated by Th1-type immunity (Fig. 2). Our recent report showed that peripheral blood CD4+ T cells from patients with active systemic lupus erythematosus (SLE) express increased CCR4, indicating that SLE is a Th2-dominant disease.[59] Thus, the determination of Th1 and Th2 preferences might give us a new insight into the pathogenesis of collagen vascular diseases.

A 32 base pair deletion allele in the CCR5 gene (*CCR5 Δ32 allele*) is found at high frequencies in healthy Caucasian populations. Significantly larger populations of RA patients carrying the deletion allele are negative for IgM rheumatoid factor compared with those who are homozygous for the normal allele, and the population of *CCR5 Δ32* allele carriers has fewer swollen joints than those homozygous for the normal allele.[60] Gomez-Reino et al.[61] also reported that no RA patients had the monozygous *CCR5 Δ32 allele*. However, since a recent report showed two RA patients with a homozygous deletion in the CCR5 gene,[62] further studies are necessary to elucidate whether CCR5 has an important role in Th1-related inflammation in RA.

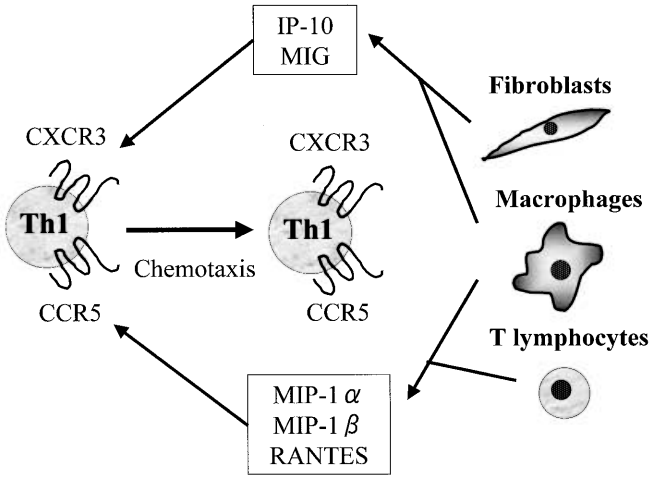

Fig. 2. Th1 response in synovial inflammation of RA

Protease-induced T cell chemotaxis

Serine proteases such as thrombin,[63,64] neutrophil cathepsin G,[65] mast cell chymase,[66] and endothelin[67] have been shown to have chemotactic activity for leukocytes. The enzymatic activity of these proteases seems to be necessary for this activity because the activity is decreased by treatment with their specific inhibitors. Recently, we found that CD13/aminopeptidase N has chemotactic activity for T lymphocytes,[68] and plays a role in lymphocyte migration to RA inflamed joints (unpublished observations). Aminopeptidase N (E.C.3.4.11.2) is a membrane-bound metalloprotease, and was shown to be identical to CD13,[69] a 150-kDa cell-surface glycoprotein, which was originally used as a marker for subpopulations of hematopoietic cells.[70] CD13/aminopeptidase N is widely distributed in the outer cell membrane of a variety of mammalian cells such as monocytes/macrophages and fibroblasts.[71–73] We found increased activity and expression of CD13/aminopeptidase N in syn-

ovial fluid and synovial tissue of RA patients, and increased expression of CD13/aminopeptidase N mRNA in synovial fibroblasts isolated from synovial tissues of RA patients. Therefore, we next examined the significance of CD13/aminopeptidase N in lymphocyte involvement in inflamed RA joints, and found that the activity of CD13/aminopeptidase N in synovial fluid significantly correlated with the number of lymphocytes in the synovial fluid, and moreover the chemotactic activity to lymphocytes detected in synovial fluid from RA patients is decreased by pretreatment with bestatin, a specific inhibitor of CD13/aminopeptidase N. These results indicate that CD13/aminopeptidase N may be an important lymphocyte chemoattractant in RA.

Conclusions

Clinical trials of a therapy for RA to block the biological activity of proinflammatory cytokines such as TNF-α using antibodies or soluble receptors demonstrate marked improvement in both clinical and laboratory data, which comfirm the critical role of the proinflammatory cytokines in the pathogenesis of RA. An important mechanism of action of anti-TNF-α treatment in RA is the diminished recruitment of inflammatory cells from the blood to the synovial joint, namely chemotaxis. A reduction in the serum concentration of IL-8/CXCL8 and MCP-1/CCL2 is observed in RA patients treated with anti-TNF-α antibody.[74] The expression of IL-8/CXCL8 and Gro-α/CXCL1 in synovial tissue is reduced in RA patients receiving anti-TNF-α therapy.[75] Thus, in RA, anti-TNF-α therapy reduces the production of synovial chemokines, resulting in a reduction of migration of inflammatory cells into RA joints. The therapeutic effect of methotrexate or leflunomide also results at least partly from suppressing the chemotactic properties of peripheral blood neutrophils from RA patients.[76] Injection of MCP-1/CCL2 receptor antagonist[77] and neutralizing monoclonal antibody to MCP-1/CCL2[8] reduces the severity of chronic arthritis in an animal model of arthritis. Histologically, mononuclear cell infiltration is absent in subsynovium of mice treated with the MCP-1/CCL2 antagonist, but the effect of the antagonist of MCP-1/CCL2 is reversible because the swelling symptoms return when the antagonist treatment is stopped. A polyclonal antibody to RANTES/CCL5[78] and MetRANTES,[13] an antagonist for CC chemokine receptor, ameliorates symptoms in animals with adjuvant-induced arthritis. A specific CXCR4 antagonist inhibits the severity of joint inflammation in collagen-induced arthritis.[79] Accordingly, antichemokine receptor therapy may be important as a possible new approach to therapeutic intervention.

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