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REVIEW ARTICLE

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Chemokines and chemokine receptors in the pathogenesis of systemic sclerosis

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Abstract Activation of the immune system and increased synthesis of extracellular matrix proteins by fibroblasts are hallmarks in the pathogenesis of systemic sclerosis (SSc). The mechanisms that initiate the accumulation of inflammatory cells are still unknown. Chemokines are a family of small molecules that are divided into subfamilies according to the position of NH_2-terminal cysteine motif. A new nomenclature for chemokines recently has been introduced in an attempt to overcome the confusion resulting from a number of different names for the same chemokines. Recent data indicate that chemokines, and in particular MCP-1 (CCL2), might be involved in the pathogenesis of SSc at different levels. MCP-1 is highly upregulated in skin specimens from SSc patients compared with those from healthy controls. Dermal fibroblasts release MCP-1, which is able to induce and perpetuate the migration of inflammatory cells into the skin. Interestingly, data from animal models, as well as from in vitro studies, indicate that MCP-1 might also be involved in the increased synthesis of extracellular matrix proteins, by either direct or indirect mechanisms. In conclusion, chemokines represent interesting candidates for target-directed therapies for SSc. This concept has to be confirmed by further studies using animal models for SSc and other fibrotic diseases.

Key words Chemokine receptors · Chemokines · Monocyte chemoattractant protein-1 (MCP-1) · Systemic sclerosis (SSc)

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Introduction

Systemic sclerosis (SSc) is a generalized fibrotic disease affecting the skin and various internal organs. The histopathological hallmark of SSc is an accumulation of extracellular matrix proteins that occurs in later stages of the disease, and results in a tightening of the skin in most patients. The extracellular matrix proteins are synthesized by dermal fibroblasts, which appear to be activated in patients with SSc. Current research focuses on the mechanisms that lead to the activation of SSc fibroblasts. Possible explanations include clonal selection of fibroblasts with a high rate of synthesis for extracellular matrix proteins, primary genetic changes, and paracrine activation of fibroblasts by neighboring cells.[1]

Inflammatory infiltrates can be found in early disease stages. Together with microvascular changes, these infiltrates often occur before fibrosis of the skin is clinically and histologically detectable. They are located primarily in perivascular areas and are T-cell-dominated.[2] Dermal fibroblasts that are located adjacent to inflammatory infiltrates produce higher amounts of procollagen mRNA.[3] Thus, inflammatory cells might contribute to the activated phenotype of SSc dermal fibroblasts either by direct cell–cell contact, or by the release of profibrotic cytokines such as transforming growth factor-β (TGF-β)[4] or platelet-derived growth factor (PDGF).[5] We have recently shown that 5-lipoxygenase is overexpressed in the skin of SSc patients, and that fibroblasts from SSc patients produce higher amounts of the proinflammatory leukotriene B4.[6] However, the mechanisms that initiate the migration and activation of inflammatory cells in the early pathogenesis of SSc remain to be elucidated.

Chemokines and chemokine receptors: structure and classification

Chemokines are a family of small molecules with a molecular weight of 8–12 kDa. At present, the human chemokine

system is known to include approximately 50 chemokines and 20 G protein-coupled receptors. Based on structural and genetic similarities, chemokines can be divided into four subfamilies.[7] While most chemokines contain at least four cysteine amino acids in conserved positions, the NH_2-terminal cysteine motifs differ between subfamilies. In the CXC chemokine family, the two cysteines at the N-terminus are separated by a variable amino acid. Genes from this family usually cluster at human chromosome 14q12–21. Chemokines of the CC family have adjacent cysteines close to the N-terminus, and their genes cluster (with some exceptions) at 17q11.2–12. Lymphotactin defines the C chemokine family with only two cysteines, one of which is located at the N-terminus. The CX_3C chemokine fractalkine has a typical chemokine domain at its N-terminus except for the presence of three amino acids between the first two cysteines. Fractalkine has a mucin-like stalk, and is the only chemokine that is membrane-bound.[8] A more recent classification system distinguishes inflammatory/inducible chemokines from constitutive/homoeostatic chemokines according to their physiological properties, including the sites of expression and cellular distribution (Table 1).

Chemokines bind to their receptors on the surface of target cells. These receptors are members of the 7-transmembrane-domain receptor family, and are coupled to heterotrimeric G_i proteins.[9] While there are numerous reports addressing the biological effects mediated by chemokine receptors, the majority of molecular mechanisms and signaling transduction pathways initiated by the binding of chemokines are still under investigation. To induce chemotactic migration of target cells, the release of the βγ subunits from the heterotrimeric G_i protein is required. Among other pathways, the βγ subunits directly activate phosphoinositide-specific phospholipase C (PLC) isoenzymes, leading to inositol-1,4,5-trisphosphate formation, and a transient rise in the concentration of intracellular free calcium. Another characteristic feature of most chemokine receptors (except CXCR 4) is the induction of short and transient signals. The rapid termination of receptor activity is achieved by phosphorylation at multiple sites of the cytoplasmic COOH-terminus, homologous and heterologous desensitization, and subsequent internalization. It is noteworthy that the chemokine/chemokine receptor system is highly redundant in that chemokine receptors bind several different chemokines, and chemokines bind to several different receptors. This redundancy is physiological, and is not caused by in vitro artifacts, since it involves high-affinity interactions between chemokines and different receptors.

Table 1. Classification of chemokines according to their physiological properties.[7] For new nomenclature see Tables 2 and 3

	Chemokine(s)	Receptor
Homeostatic	CTACK, MEC	CCR10
	SDF-1	CXCR4
	BCA-1	CXCR5
	SLC, ELC	CCR7
	TECK	CCR9
	DC-CK1	Unknown
Inflammatory	I-TAC, MIG, IP-10	CXCR3
	CXCL16	CXCR6
	RANTES, MIP-1α, MCP-2, MCP-3	CCR1
	MCP-1, MCP-2, MCP-3, MCP-4	CCR2
	Eotaxin-1, Eotaxin-2, Eotaxin-3, RANTES, MCP-2, MCP-3, MCP-4, MEC	CCR3
	RANTES, MIP-1α, MIP-1β, MCP-2	CCR5
	I-309	CCR8
	Fractalkine	CX_3CR1
Homeostatic and inflammatory	MDC, TARC	CCR4
	LARC	CCR6

CTACK, cutaneous T cell-attracting chemokine; MEC, mucosae-associated epithelial chemokine; SDF-1, stromal cell-derived growth factor 1; BCA-1, B cell-activating chemokine-1; SLC, secondary lymphoid tissue chemokine; ELC, Epstein Barr virus-induced receptor ligand chemokine; TECK, thymus-expressed chemokine; DC-CK1, dendritic cell chemokine 1; I-TAC, interferon-inducible T cell α-chemoattractant; MIG, monokine induced by γ interferon; IP-10, interferon-inducible protein 10; CXCL16, CXC ligand 16; RANTES, regulated on activation normal T cell expressed and secreted; MIP, macrophage inflammatory protein; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine; TARC, thymus- and activation-regulated chemokine; LARC, liver- and activation-regulated chemokine

One problem in chemokine research is the fact that a certain chemokine has been discovered by different groups at the same time, and they have published using different names. The confusion resulting from this mixture of names for chemokines has led to a proposal for a new chemokine nomenclature.[10] The new chemokine nomenclature uses CC, CXC, XC, and CX_3C to define the chemokine family, and "L" as an abbreviation for (chemokine receptor-) ligand, followed by a number to designate the specific chemokine. As an example, the monocyte chemoattractant protein-1 (MCP-1)/monocyte chemotactic and activating factor (MCAF)/lymphocyte-derived chemotactic factor (LDCF) is now named CCL2. Tables 2 and 3 give an overview of the new nomenclature of the CC and CXC chemokine/receptor families.

Function of chemokines: more than just the migration of inflammatory cells

The main target cells of chemokines are bone marrow-derived cells, and by far their best characterized function is the control of basal and inflammatory trafficking.[7,11] This function includes microanatomical movement, e.g., during hematopopoiesis to attract precursor cells to specific sites in bone marrow and thymus to facilitate adequate cell development, as well as macroenvironmental movement, e.g., migration of T lymphocytes from the bone marrow and thymus to the spleen and lymph nodes.

During inflammation, sentinel cells at the inflammatory focus release chemokines and generate a chemotactic gradient to surrounding blood vessels. Chemokines presented on endothelial cells next to the inflammatory focus induce the activation of integrins, resulting in arrest of the leukocytes that express the corresponding chemokine receptors. Leukocyte migration along the chemotactic gradient then requires the sensing of subtle differences in the concentration

Table 2. Proposed new nomenclature for CXC chemokines.[10] Only the main receptors for each chemokine are listed, although some chemokines bind to additional receptors

CXC chemokine/receptor family		
Systematic name	Human ligand	Chemokine receptor(s)
CXCL1	GROα/MGSA-α	CXCR2 > CXCR1
CXCL2	GROα/MGSA-β	CXCR2
CXCL3	GROγ/MGSA-γ	CXCR2
CXCL4	PF4	Unknown
CXCL5	ENA-78	CXCR2
CXCL6	GCP-2	CXCR1, CXCR2
CXCL7	NAP-2	CXCR2
CXCL8	IL-8	CXCR1, CXCR2
CXCL9	MIG	CXCR3
CXCL10	IP-10	CXCR3
CXCL11	I-TAC	CXCR3
CXCL12	SDF-1α/β	CXCR4
CXCL13	BLC/BCA-1	CXCR5
CXCL14	BRAK/bolekine	Unknown
CXCL15	Unknown (mouse ligand: lungkine)	Unknown

GRO, growth-regulated oncogene; MGSA, melanoma growth stimulating activity; PF4, platelet factor 4; ENA-78; epithelial-derived neutrophil attractant-78; GCP-2, granulocyte chemotactic protein-2; NAP-2, neutrophil-activating protein-2; IL-8, interleukin-8; BLC, B lymphocyte chemoattractant; BRAK, breast and kidney. For other abbreviations, see Table 1

Table 3. Proposed new nomenclature for CC chemokines.[10] Only the main receptors for each chemokine are listed, although some chemokines bind to additional receptors

CC chemokine/receptor family		
Systematic name	Human ligand	Chemokine receptor(s)
CCL1	I-309	CCR8
CCL2	MCP-1/MCAF	CCR2
CCL3	MIP-1α	CCR1, CCR5
CCL4	MIP-1β	CCR5
CCL5	RANTES	CCR1, CCR3, CCR5
(CCL6)	Unknown	Unknown
CCL7	MCP-3	CCR1, CCR2, CCR3
CCL8	MCP-2	CCR3
(CCL9/10)	Unknown	Unknown
CCL11	Eotaxin	CCR3
(CCL12)	Unknown	CCR2
CCL13	MCP-4	CCR2, CCR3
CCL14	HCC-1	CCR1
CCL15	HCC-2/Lkn-1/MIP-1δ	CCR1, CCR3
CCL16	HCC-4/LEC	CCR1
CCL17	TARC	CCR4
CCL18	DC-CK1	Unknown
CCL19	MIP-3β/ELC/exodus-3	CCR7
CCL20	MIP-3α/LARC/exodus-1	CCR6
CCL21	6Ckine/SLC/exodus-2	CCR7
CCL22	MDC/STCP-1	CCR4
CCL23	MPIF-1	CCR1
CCL24	MPIF-2/Eotaxin-2	CCR3
CCL25	TECK	CCR9
CCL26	Eotaxin-3	CCR3
CCL27	CTACK/ILC	CCR10

MCAF, monocyte chemotactic and activating factor; Lkn-1, leukotactin-1; LEC, liver-expressed chemokine; STCP-1, stimulated T cell chemotactic protein; MPIF, myeloid progenitor inhibitory factor; CTACK, cutaneous T cell-attracting chemokine; ILC, interleukin-11 receptor alpha-locus chemokine; HCC, hemofiltrate CC chemokine. For other abbreviations, see Table 1

of chemokines. Originally, this was thought to happen via the accumulation of chemokine receptors at the leading edge of migrating cells. However, chemokine receptors remain distributed uniformly within the plasma membrane during migration.[12] Instead, cell polarization is characterized by an asymmetric distribution of intracellular signaling molecules to the leading edge of migrating cells.[13] However, it remains to be elucidated how the chemotactic gradient at the cell surface is transferred to the distribution of intracellular signaling molecules.

During the last few years, it has become clear that leukocyte chemotaxis and lymphocyte development are by no means the only biological activity of chemokines. Since an impaired angiogenesis is a key feature of SSc, it is important to know whether chemokines influence the formation of new vessels. The structural domain of the CXC chemokine family determines their angiogenic potential.[14] The NH_2 terminus of CXC family members such as IL-8 (CXCL8) and growth-related oncogenes (GRO) α, β, and γ (CXCL1-3) contain three amino acid residues (Glu–Leu–Arg), the so-called ELR motif. These ELR(+) CXC chemokines have strong chemotactic effects on endothelial cells in vitro and induce corneal neovascularization in vivo. In contrast, ELR(−) CXC chemokines, including platelet factor 4 (CXCL4) and monokine induced by IFN-γ (MIG, CXCL9), are potent inhibitors of angiogenesis. Interestingly, the angiogenic activity of ELR(+) CXC chemokines in vivo could be observed in the absence of leukocyte infiltration, indicating a direct role of these chemokines in angiogenesis.[15]

Recently, it has been suggested that the chemokine MCP-1 (CCL2) is involved in collagen turnover.[16] In a murine model of crescentic nephritis, the expression of MCP-1 accompanied the influx of inflammatory cells and the development of fibrosis. Blocking the function of MCP-1 by the administration of anti-MCP-1 antibodies resulted in a decreased deposition of type I collagen in the kidney. Similarly, in bleomycin-induced pulmonary fibrosis, MCP-1 is expressed in a time-dependent manner after exposure to bleomycin.[17] Other functions of chemokines include the control of cell proliferation, alteration of the expression of adhesion molecules, and regulation of the interactions between HIV and target cells.[18]

Chemokines and SSc: evidence for a prominent role of MCP-1 in the pathogenesis of SSc

A number of groups have reported abnormalities in the expression pattern of chemokines in SSc. Early studies focused on IL-8 (CXCL8), showing increased serum levels of IL-8 in a subset of patients with SSc, Raynaud's phenomenon without skin involvement, and eosinophilic fasciitis compared with healthy controls.[19,20] Immunohistochemistry using anti-IL-8 antibodies revealed a constitutive synthesis of IL-8 in keratinocytes and macrophages, whereas endothelial cells expressed IL-8 more intensively in SSc than in healthy control skin biopsies.[21] In addition, IL-8 appears to play a role in the development of lung fibrosis, since

bronchoalveolar lavage fluids from SSc patients with and without alveolitis showed higher levels of IL-8 than control subjects.[22,23] Alveolar macrophages are the predominant source of IL-8 in the lung. Alveolar macrophages secreting IL-8 can be found consistently more often in subjects with fibrosing alveolitis than in subjects without fibrosing alveolitis.[24] IL-8 binds to two related receptors, CXCR-1 and CXCR-2. Recently, Renzoni et al.[25] described an association between SSc and two polymorphisms in the CXCR-2 gene. The functional significance of this finding is unclear, and has to be analyzed in future studies.

MIP-1α (CCL3) and MIP-1β (CCL4) are chemokines that have also been suggested to contribute to the pathogenesis of pulmonary fibrosis. Serum levels of MIP-1α and MIP-1β, as well as bronchoalveolar lavage levels of MIP-1α, are increased in patients with SSc, and this increase correlates with the presence of alveolitis and pulmonary fibrosis, respectively.[22,26]

In addition, we have recently shown that the CC chemokine RANTES (CCL5) is expressed in the epidermis of SSc patients, whereas no expression could be detected in healthy control skin. Skin biopsies from nonfibrotic sites showed a higher percentage of cells expressing RANTES than did skin biopsies from fibrotic skin areas. Interestingly, expression of RANTES could not be detected in esophageal specimens from the same patients, indicating that RANTES might be involved predominantly in the pathogenesis of cutaneous lesions.[27]

Results from a number of studies suggest that MCP-1 (CCL2) plays a prominent role in the pathogenesis of SSc. Numerous groups have shown that MCP-1 is expressed abundantly in skin biopsies from SSc patients on the mRNA and protein level, whereas only minimal expression could be detected in healthy skin.[26,28–30] Interestingly, expression of MCP-1 was also found at nonfibrotic skin sites, indicating that MCP-1 might contribute to early stages of the disease.[28] Cell types expressing MCP-1 in SSc skin included fibroblasts, keratinocytes, endothelial cells, and inflammatory cells.

Experiments with blocking anti-MCP-1 antibodies revealed that cultured fibroblasts from SSc patients promote the migration of mononuclear leukocytes by the release of biologically active MCP-1.[28,31] Whether MCP-1 is also directly involved in other pathogenic steps of SSc, such as the accumulation of extracellular matrix proteins, is currently a subject of controversy. We could not show any effects of recombinant MCP-1 on the synthesis of the N-terminal propeptide of type I procollagen (PINP) by dermal fibroblasts, as assessed by real-time PCR and radioimmunoassay, a finding, which was confirmed by the experiments of Galindo et al.[29] On the other hand, Yamamoto et al.[32] used Northern blot hybridization and quantification by densitometry, and found up to a five-fold increase in α1(I) collagen mRNA after the incubation of dermal fibroblasts with recombinant MCP-1. Possible explanations for these findings include different cell sources, since Yamamoto et al. used outgrowth cultures, whereas other groups obtained fibroblasts from biopsies by enzymatic digestion.

It has to be emphasized that these results do not exclude an *indirect* role of MCP-1 in the development of fibrosis. The profibrotic effects of MCP-1 may require interactions with other cell types, such as Th2 cells. In skin specimens of patients with SSc, Mavalia et al.[33] found a predominance of Th2 cells, and there is increasing evidence that Th2 cytokines contribute to the deposition of collagen in fibrotic diseases such as SSc.

Interestingly, MCP-1 has been shown to induce neoangiogenesis in animal models independently from accompanying inflammatory infiltrates.[34] These data suggest that MCP-1 might also contribute to the dysbalanced angiogenesis in the skin of SSc patients.

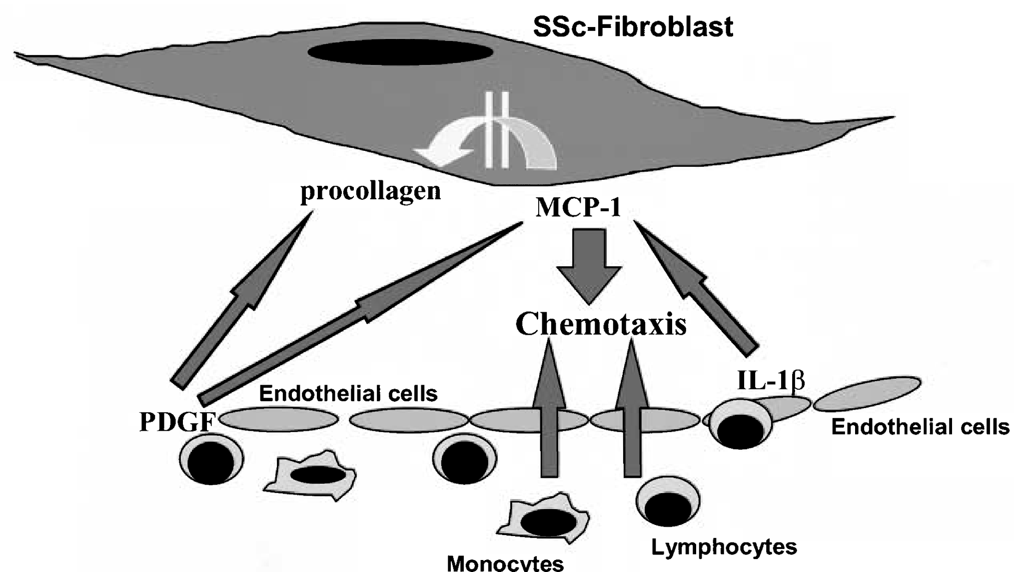

Fig. 1. Proposed function of monocyte chemoattractant protein (*MCP-1*) (CCL2) in the pathogenesis of systemic sclerosis (*SSc*). Dermal fibroblasts act as sentinel cells by the release of MCP-1 in the early pathogenesis of SSc. Platelet-derived growth factor (PDGF) and interleukin-1β (*IL-1β*) trigger in part the synthesis of MCP-1 in dermal fibroblasts, resulting in a further increase in the expression of MCP-1. MCP-1 attracts mononuclear cells across the endothelial cell barrier into the dermis, but most likely does not directly induce the synthesis of procollagen

Conclusion and perspectives

The CXC chemokine IL-8, and in particular the CC chemokine MCP-1, have been characterized as important molecules in the pathogenesis of systemic sclerosis (Fig. 1). MCP-1 is released from dermal fibroblasts and is able to initiate the migration of mononuclear cells through endothelial cell monolayers. Dermal fibroblasts might therefore act as sentinel cells in the early pathogenesis of systemic sclerosis by triggering the accumulation of perivascular infiltrates. Whether MCP-1 is also directly involved in other pathogenic pathways, such as vascular changes and the development of fibrosis, has not yet been clarified.

The involvement of chemokines in a variety of pathological processes has made them interesting candidates for target-directed therapies. In general, the action of chemokines can be blocked by chemokine-specific neutralizing antibodies, chemokine receptor antagonists, or the inhibition of intracellular signaling pathways, which are activated by binding chemokines to their respective receptors.[35] The proof of the concept that antagonizing chemokines is a valid therapeutic approach has been shown in animal models for a variety of diseases. As an example, the administration of an N-terminal truncated MCP-1 analog that exerts antagonistic effects on the MCP-1 receptor CCR2 prevents the onset of arthritis in the MRL-*lpr* mouse model of rheumatoid arthritis.[36] Furthermore, recent progress in pharmacological research has led to the development of potent and specific chemokine receptor antagonists, which can be administered orally.[37] However, before MCP-1 or other chemokines can be considered as therapeutic targets in SSc, their functional importance has to be proven in animal models of the disease.

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