

## The roles of chemokines in leukocyte recruitment and fibrosis in systemic sclerosis

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### 1. ABSTRACT

Systemic sclerosis (SSc, scleroderma) is an autoimmune disease characterized by excessive extracellular matrix deposition and vascular injury in the skin and other visceral organs. Although the pathogenesis remains unclear, interactions among leukocytes, endothelial cells, and fibroblasts are likely to be central to the pathogenesis of the disease. Chemokines mediate the leukocyte chemotaxis and migration through endothelia into the organ tissues, leading to the interaction between leukocytes and fibroblasts. While amounts of literatures reported chemokine abnormalities in SSc, which might explain the altered accumulation of effector leukocyte subsets in the affected tissues. Among various chemokines, monocyte chemoattractant protein-1 (MCP-1/CCL2) likely has the most critical role for tissue fibrosis in SSc. Although therapeutic effect for targeting MCP-1 has been demonstrated in mouse models of SSc or fibrotic disorders, it is unknown whether this strategy is effective in human clinical trials. Here recent data will be reviewed on the pathogenic role of chemokines and their receptors in SSc.

### 2. PATHOGENESIS OF SYSTEMIC SCLEROSIS

SSc is an autoimmune rheumatic disease characterized by fibrosis and vascular injury in the skin and internal organs. The etiology and pathogenesis of SSc remains unknown. Three facets of the disease typify the clinical feature of SSc: excessive collagen deposition, vascular damage, and immunological activation (1-3). In addition, these clinical features are likely to be interrelated each other.

Tissue fibrosis such as skin sclerosis and pulmonary fibrosis is the most characteristic feature of SSc. Tissue fibrosis results from excessive accumulation of collagens and other extracellular matrix proteins. Fibroblasts from SSc patients show augmented collagen synthesis (4, 5) probably due to both intrinsic abnormalities and increased stimulation by profibrogenic cytokines from other cells and fibroblasts themselves. Among various cytokines, transforming growth factor (TGF)-beta, platelet derived growth factor (PDGF), connective tissue growth factor (CTGF), interleukin (IL) -4, and IL-6 have been

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considered to play central roles in this process of SSc (6-11).

Clinical manifestations of vascular abnormality include Raynaud's phenomenon, digital ulcers, nailfold bleeding, pulmonary hypertension, and renal crisis (12). The typical vascular involvement is proliferation of the vascular intima, which results in inadequate blood flow. Since Raynaud's phenomenon usually antedates to other clinical symptoms and induce tissue hypoxia, endothelium injury may be the first event of the disease. Then, profibrogenic cytokines produced by platelets and inflammatory cells may induce the tissue fibrosis.

Immunological activation in SSc is likely a key inducer of vascular abnormalities and tissue fibrosis. Amounts of findings in SSc patients or SSc mouse model have demonstrated that tissue infiltrating immune cells including T cells and cytokines released from these cells are critical for the development of tissue fibrosis (13-15). Furthermore, recent studies have shown that B cell abnormalities are detected in SSc patients and mouse model of SSc (9, 16-19).

### 3. THE ROLE OF IMMUNE CELLS

In patients with SSc, mononuclear cell infiltration is found in the skin before any histological evidence of skin fibrosis (20). Histological analysis of the skin demonstrates that perivascular infiltration of mononuclear cells is associated with increased collagen synthesis in the surrounding fibroblasts in patients with early stage of SSc (21, 22). These infiltrates consist of T cells, macrophages, mast cells, and few B cells (20, 23, 24). Especially, the perivascular T cell infiltrate consists mainly of activated T cells ( $CD3^+CD4^+HLA-DR^+\alpha\text{beta T cell receptor}^+$ ) (25, 26). Consistent with this, serum levels of T cell-derived cytokines and cell surface molecules are increased in SSc patients. That is, serum levels of IL-2 and soluble form of IL-2 receptor, CD4, and CD8 are elevated (27, 28). Accumulation of monoclonal and oligoclonal  $\alpha\text{beta T}$  cells in the scleroderma skin may be explained by antigen-driven proliferation of T cells (29). Additionally, oligoclonal  $\gamma\text{delta T}$  cells are also increased and activated in SSc skin lesions (29). In the lung of SSc patients,  $CD8^+$  T cells and oligoclonal  $\gamma\text{delta T}$  cells are increased (30, 31). Since  $\gamma\text{delta T}$  cells show preferential adhesion to fibroblasts (32), they may influence the development of tissue fibrosis.  $\gamma\text{delta T}$  cells also bind preferentially to endothelial cells and show significant cytotoxicity (33), which may be related to the vascular injury in this disease. A recent study has demonstrated that B cell infiltration and the expression of B cell-related genes are augmented in the lesional skin of SSc (23). Circulating B cells are activated in patients with SSc (9, 17, 34), in consistent with hypergammaglobulinemia and autoantibody production.

Microarray analysis revealed that no genes are differentially expressed in SSc fibroblasts and normal fibroblasts (23), suggesting exogenous fibroblast activation by immunocompetent cells. Infiltrating mononuclear cells

in the skin express both TGF-beta and PDGF (35, 36). Macrophages can produce various cytokines including IL-1, tumor necrosis factor (TNF)-alpha, interferon (IFN)-gamma, IL-6, TGF-beta, and PDGF that can regulate inflammation and tissue fibrosis (14). Activated macrophages produce IL-1 and TNF-alpha that are important to induce some chemokine and adhesion molecule expression. In both skin and lungs, T cells appear to play a role in fibrosis through activation of monocytes and direct release of profibrogenic cytokines. An emerging hypothesis for the pathogenesis of fibrotic disorders suggests that an imbalance between the type 1 helper T cell (Th1) and type 2 helper T cell (Th2) cytokines leads to abnormal responses to tissue injury. Th2 cytokines including IL-4, IL-6, and IL-13 stimulate the synthesis of collagen by human fibroblasts (37-39). By contrast, Th1 cytokines such as IFN-gamma and TNF-alpha suppress collagen production by fibroblasts in vitro (39). Therefore, in general, a relative shift to Th2 from Th1 cytokines can induce tissue fibrosis. In patients with SSc, T cells infiltrating the skin or lung demonstrate predominantly Th2 profile (10, 29, 40-43). A shift from Th2 to Th1 response correlates with improvement in skin fibrosis in SSc (44). Human mast cells are shown to be a rich source of chemokines (45), as well as a number of cytokines, growth factors and mediators.

Thus, certain leukocyte subsets and their secreted cytokines appear to be involved in the development of tissue fibrosis of SSc via stimulating collagen synthesis from fibroblasts.

### 4. MECHANISM OF LEUKOCYTE RECRUITMENT

The physiology of leukocyte recruitment through endothelial cells has led to a concept of multistep cascades involving cell adhesion molecules and chemokines. Essentially, four steps can be discerned (46-48). Step 1 involves the initial contact of the leukocytes with the endothelial cells. This is a transient step, characterized by "rolling" processes that are predominantly mediated by selectins. In step 2, rapid activation ("triggering") of integrin molecules takes place, in which chemokines and chemokine receptors are involved. The activated integrin molecules result in "firm adhesion", after which the cell may start "diapedesis". During these processes, chemokine and chemokine receptors regulate the selective recruitment of certain subsets of leukocytes in cooperation with adhesion molecules.

Among various adhesion molecules, intercellular adhesion molecule-1 (ICAM-1) has been considered to have the most important role in the pathogenesis of SSc (49). Several previous studies have shown that SSc fibroblasts exhibit increased surface ICAM-1 expression and augmented potential to bind with T cells (50-52). The augmented collagen mRNA expression is found on fibroblasts that are localized adjacent to dermal blood vessels within the lesional skin of SSc patients, suggesting the direct cellular interaction between fibroblasts and T cells (22, 53). In human dermal fibroblasts, increased

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**Table 1.** Relevant chemokines in systemic sclerosis and other fibrotic disorders

Group	Systematic name	Colloquial name	Receptor usage	
CC	CCL2	MCP-1	CCR2	
	CCL3	MIP-1alpha	CCR1, 5	
	CCL4	MIP-1beta	CCR5	
	CCL5	RANTES	CCR1, 3, 5	
	CCL7	MCP-3	CCR1, 2, 3	
	CCL17	TARC	CCR4	
	CCL18	PARC	CCR3	
	CCL21	SLC	CCR7	
	CCL22	MDC	CCR4	
	CCL27	CTACK	CCR10	
CXC	CXCL1	GRO-alpha	CXCR2	
	CXCL2	GRO-beta	CXCR2	
	CXCL3	GRO-gamma	CXCR2	
	CXCL5	ENA-78	CXCR2	
	CXCL8	IL-8	CXCR1, 2	
	CXCL9	MIG	CXCR3	
	CXCL10	IP-10	CXCR3	
	CXCL11	I-TAC	CXCR3	
	CXCL12	SDF-1	CXCR4	
	CX3C	CX3CL1	Fractalkine	CX3CR1

ICAM-1 expression induced by IL-4 parallels the increase in ICAM-1-dependent T cell adhesion (54). Furthermore, a recent study indicates that ICAM-1 expression contributes to the development of skin fibrosis in tight-skin mice, a model of SSc, especially via ICAM-1 expressed on skin fibroblasts (55).

## 5. CHEMOKINES IN SSC

### 5.1. Chemokines

Chemokines, a large family of small (7-15 kDa), structurally related heparin-binding proteins, are soluble mediators that were originally identified because of their chemotactic properties (56, 57). Chemokines and their receptors predominantly regulate target-cell selectivity as chemokine receptor expression is specific to certain leukocytes subsets (56, 58). However, chemokines have also other functions such as the regulation of angiogenesis, vascular proliferation, and fibrosis (59) that may contribute to manifestations of SSc. Based on the arrangement of their N-terminal cysteine residues, chemokines are classified as CC, CXC, C, and CX3C chemokines. Recently, significant abnormalities of chemokine expression have been found in SSc (49, 60, 61) (Table 1).

### 5.2. Monocyte chemoattractant protein 1/CCL2

MCP-1 is produced by macrophages, fibroblasts, endothelial cells and other cells. MCP-1 is a predominant chemoattractant and activator of monocytes and T cells. In addition to its chemoattractant activities, this chemokine induces Th2 cell polarization (62) and stimulates collagen production by fibroblasts via specific receptors and endogenous upregulation of TGF-beta expression. The latter results in autocrine and/or juxtacrine stimulation of collagen gene expression (63).

Promoter polymorphism in the MCP-1 gene is associated with the susceptibility and MCP-1 expression of skin fibroblasts in SSc (64). Serum levels and spontaneous production levels by peripheral blood mononuclear cells of

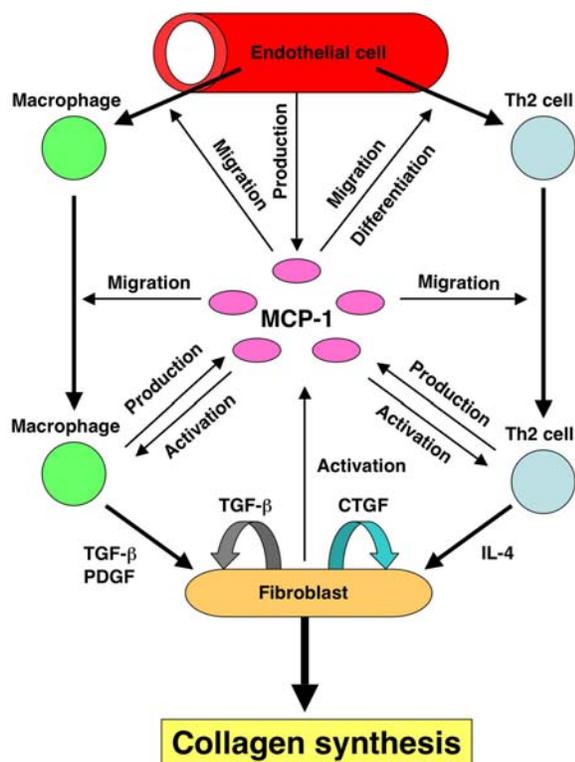
MCP-1 were elevated in SSc patients compared with normal controls (65). Immunohistochemical analysis showed that MCP-1 was strongly expressed in the epidermis, inflammatory mononuclear cells, and endothelial cells in the sclerotic skin of SSc patients, but not expressed in any normal skin (65). Consistent with this report, a critical role of MCP-1 was revealed in transendothelial leukocyte migration using an in vitro assay system (66). This study suggests that SSc fibroblasts promote leukocyte migration across endothelial cell monolayers through an MCP-1-dependent mechanism.

Cultured SSc fibroblasts display increased expression of MCP-1 mRNA and protein, compared to fibroblasts from control skin (67-69). Furthermore, stimulation with PDGF significantly augmented MCP-1 expression in dermal fibroblasts from SSc patients compared with that from normal controls (67, 70). Increased MCP-1 expression on SSc fibroblasts stimulated the chemotaxis of mononuclear cells (70). Although MCP-1 has no direct effects on dermal fibroblasts, it contributes to fibrosis in patients with SSc by inducing the differentiation of IL-4-producing T cells (71). Exogenously administered MCP-1 stimulates autoinduction of MCP-1 mRNA in cultured skin fibroblasts from SSc patients, but not fibroblasts from normal controls (67). CCR2, the ligand of MCP-1, is upregulated in the skin of patients with active SSc, which is consistent with a key role of MCP-1-CCR2 axis in SSc pathogenesis (72). Further, CCR2 expression on SSc fibroblasts appears to regulate the expression of MCP-1, suggesting potential autocrine regulation of key profibrotic properties via a MCP-1/CCR2 loop in SSc (72). MCP-1 upregulated the mRNA levels of alpha1 (I) collagen and decorin in normal human skin fibroblasts, whereas those of fibronectin and biglycan were not significantly changed (73).

In human SSc, mRNA expression of MCP-1 is most augmented among 4507 genes when bronchoalveolar lavage (BAL) cells from SSc lung were compared with controls (74). Consistent with this, protein levels of MCP-1 are increased in BAL fluids from SSc patients with lung inflammation and in sera from SSc patients with pulmonary fibrosis (65, 74).

Mast cell is suggested to be important regulator of tissue fibrosis in SSc. Stem cell factor, a mast cell growth factor, is overexpressed in dermal fibroblasts from SSc patients (75, 76). Stem cell factor stimulates mast cells to increase the production of MCP-1, which in turn stimulates fibroblasts to produce more collagen (77, 78).

Experiments in animal models also support the role of MCP-1 in tissue fibrosis. In murine sclerodermatous graft-versus-host disease (GVHD) model, increased expression of MCP-1 precedes the development of skin and lung fibrosis (79). In a rat model of bleomycin-induced pulmonary fibrosis, mRNA and protein levels of MCP-1 are elevated (80). Administration of anti-MCP-1 neutralizing antibody reduced skin sclerosis in bleomycin-treated mice (73). Anti-MCP-1 gene therapy attenuated pulmonary fibrosis in bleomycin-treated mice (81). Mice



**Figure 1.** The estimated role of MCP-1 in the development of tissue fibrosis in systemic sclerosis. MCP-1 augments recruitment of macrophages and T cells into the tissues. MCP-1 chemoattracts macrophages to the fibroblasts, and TGF-beta and PDGF produced by activated macrophages stimulate collagen synthesis from fibroblasts. Additionally, MCP-1 preferentially induces the differentiation for Th2 rather than Th1 cells. Th2 cells migrated to the fibroblast induce the collagen production from fibroblasts via secreting IL-4. In turn, activated fibroblasts produce MCP-1, leading to the amplification loop for the development of tissue fibrosis.

deficient for CCR2 are protected from fluorescein isothiocyanate-induced and bleomycin-induced lung fibrosis (82, 83). In one of those studies, it has been demonstrated that MCP-1-CCR2 interactions limited prostaglandin E2 production in alveolar epithelial cells leading to fibrogenesis after injury, suggesting the regulatory role of MCP-1 for epithelial cells (82). CCR2 signaling plays a key role in bleomycin-induced pulmonary fibrosis by regulating fibrogenic cytokine expression and fibroblast responsiveness to TGF-beta (84). TGF-beta production by pulmonary fibroblasts was dependent on endogenous MCP-1 synthesis in the mouse model of pulmonary granuloma (85).

TGF-beta and subsequent CTGF or basic fibroblast growth factor (bFGF) application cause persistent skin fibrosis, although an injection of either TGF-beta, bFGF, or CTGF can induce just a transient fibrosis (8, 86, 87). In this SSc model, the mast cell number was significantly but transiently increased at the early phase,

whereas the number of macrophages continued to increase (88). In the lesional skin, serial injection of CTGF following TGF-beta remarkably increase MCP-1 mRNA expression compared with those after a single injection of TGF-beta or CTGF, suggesting the role of MCP-1 for tissue fibrosis.

Thus, MCP-1 may play an important role in the induction of fibrosis via its direct effect on fibroblasts together with its indirect effect mediated by a number of cytokines released from leukocytes recruited into the lesional tissues (Figure 1).

### 5.3. Other CC chemokines

Macrophage inflammatory protein-1alpha (MIP-1alpha), MIP-1beta and regulated upon activation normal T cell expressed and secreted (RANTES/CCL5) mainly chemoattract T cells and monocytes. Serum levels and spontaneous production levels by peripheral blood mononuclear cells of MIP-1alpha, and MIP-1beta were elevated in SSc patients compared with normal controls (65). Elevated serum MIP-1alpha levels correlated with pulmonary fibrosis, suggesting the potential role of this chemokine in the development of pulmonary fibrosis (65). Consistently, the other group reported that BAL fluid MIP-1alpha levels were higher in SSc patients with alveolitis than those in SSc patients without alveolitis (89). Expression of abundant mRNA and protein levels of RANTES has been demonstrated in the skin of SSc patients, but not in the skin of healthy controls (90, 91). RANTES are increased in BAL fluids from SSc patients (89). MIP-1alpha and RANTES mRNA levels are also increased in the BAL cells of patients with sarcoidosis and hypersensitivity pneumonitis, which can lead to pulmonary fibrosis (92). Elevated cutaneous mRNA of MIP-1alpha and RANTES precedes the development of dermal and pulmonary fibrosis in a murine sclerodermatous GVHD model (79). In Korean patients, there was significant evidence of genetic interaction between the RANTES gene polymorphism and increased risk of SSc (93).

Pulmonary and activation-regulated chemokine (PARC/ CCL18) is preferentially expressed in the lung of healthy individuals (94). Elevated serum PARC levels correlated with pulmonary fibrosis and more sensitively reflected the pulmonary fibrosis activity than did other serum markers of pulmonary fibrosis in SSc (95). Interestingly, levels of PARC protein and mRNA are elevated in the lungs of patients with hypersensitivity pneumonitis, idiopathic pulmonary fibrosis, asthma, and lung sarcoidosis (96-98), suggesting the roles of PARC in the development of immune-mediated fibrotic lung diseases. Spontaneous PARC production by BAL cells was negatively correlated with lung vital capacity, whereas there was a positive correlation of PARC concentrations with BAL neutrophil and eosinophil counts (99). The increase in the frequency of PARC-positive alveolar macrophages and the expression levels of PARC were found in SSc patients with fibrotic lung diseases (99). It has been demonstrated that the PARC stimulates collagen mRNA and protein production in cultured human dermal and lung fibroblasts (100).

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Serum MCP-3 levels were elevated in patients with SSc, and correlated with the extent of skin sclerosis and the severity of pulmonary fibrosis (101). Another group has demonstrated overexpressed MCP-3 in early-stage SSc and tight-skin mice, suggesting a potential mediator of dermal fibrosis in SSc (102). Serum cutaneous T-cell attracting chemokine (CTACK/CCL27) levels were significantly increased in patients with SSc compared with normal controls (103). Expression of CTACK was augmented in the sclerotic skin of SSc patients (103). Serum levels of monocyte-derived chemokine (MDC/CCL22) and thymus and activation-regulated chemokine (TARC/CCL17), both are chemotactic for Th2 cells, were increased in SSc patients compared to controls (104). Elevated serum levels of TARC were shown during pathogenesis of bleomycin-induced pulmonary fibrosis, and neutralization of TARC attenuated pulmonary fibrosis (105).

### 5.4. CXC chemokines

CXC chemokines with ELR (glutamic acid-leucine-arginine) motif between the N-terminus and the first cysteine attract neutrophils (106). ELR<sup>+</sup> CXC chemokines include IL-8 (CXCL8), growth-regulated oncogene-alpha (GRO-alpha/CXCL1), GRO-beta/CXCL2, GRO-gamma/CXCL3, and epithelial-neutrophil activating protein-78 (ENA-78/CXCL5). The distribution of polymorphism of the IL-8, CXCR1, and CXCR2 genes has been shown in patients with SSc (107). In Korean patients, there is significant evidence of genetic interaction between the IL-8 gene polymorphism and increased risk of SSc (93). Elevated serum IL-8 and GRO-alpha levels have been reported in SSc patients (108). Levels of IL-8 protein are increased in SSc skin biopsy specimens, and elevated levels occur more commonly in skin biopsy specimens taken from patients with disease duration of less than 1 year (109). SSc dermal fibroblasts produce more IL-8 than do normal fibroblasts in vitro (110). IL-8 is also overexpressed in lung tissues and possibly promotes angiogenesis of pulmonary fibrosis (111). IL-8 is also increased in BAL fluids from patients with SSc (89). It has been demonstrated that IL-8 is secreted by alveolar macrophages and lung fibroblasts in patients with lung fibrosis (112, 113). Serum GRO-alpha correlates with the involvement of internal organs such as pulmonary fibrosis (108).

CXC chemokines without ELR motif chemoattract CXCR3-expressed Th1 cells. These chemokines include monokine induced by IFN-gamma (MIG/CXCL9), interferon-gamma-inducible protein (IP-10/CXCL10), IFN-inducible T cell chemoattractant (I-TAC/CXCL11), and stromal cell-derived factor 1 (SDF-1/CXCL12). Serum levels of IP-10 were increased in SSc patients compared to controls (104). Systemic treatment with I-TAC attenuates bleomycin-induced lung fibrosis, via inhibiting vascular remodeling (114). The loss of CXCR3, the ligand for IP-10, MIG, and I-TAC leads to insufficient IFN-gamma production after bleomycin-induced lung injury and fails to antagonize the development of tissue fibroproliferation (115).

SDF-1 and its receptor CXCR4 regulate steps in new vessel formation. SDF-1 and CXCR4 were

upregulated in the skin of patients with early SSc, and progressively decreased, with the lowest expression in the latest phases of SSc (116). Microvascular endothelial cells from SSc patients with diffuse skin involvement expressed significantly higher amounts of SDF-1 in the early stage of disease, with a progressive reduction of SDF-1 and CXCR4 in later stages (116). On the surface of cultured microvascular endothelial cells from SSc patients with diffuse skin sclerosis, SDF-1 and CXCR4 colocalized in polarized areas, suggesting that they are activated in vivo and that they are under strict genetic control to retain capping function (116).

### 5.5. CX3C chemokines

Fractalkine/CX3CL1 expressed on endothelial cells mediates activation and adhesion of leukocytes expressing its receptor, CX3CR1. Soluble fractalkine exhibits chemotactic activity for CX3CR1-expressing leukocytes. In consistent with this, fractalkine was strongly expressed on endothelial cells in the affected skin and lung tissues (117). Soluble fractalkine levels were significantly elevated in sera and were associated with the existence of digital ischemia, and severity of pulmonary fibrosis (117). Numbers of CX3CR1-expressing cells including monocytes/macrophages were increased in the lesional skin and lung tissues from SSc patients with diffuse cutaneous involvement (117). These results suggest that fractalkine augments the recruitment of CX3CR1-expressing mononuclear cells into the affected tissue of SSc, leading to inflammation and vascular injury.

### 5.6. Chemokine and fibrocytes

Recent studies have demonstrated that fibrocytes expressing both leukocyte (CD45) and mesenchymal (collagen I) antigens have critical roles in the mouse model of pulmonary fibrosis, vascular wall remodeling, pulmonary hypertension, and wound healing (118-121). Human fibrocytes express the chemokine receptors such as CCR3, CCR5, CCR7, and CXCR4 (119, 122, 123). By contrast, mouse fibrocytes express CCR2, CCR7, and CXCR4 (119, 122-124). Anti-CXCL12 neutralizing antibody inhibited intrapulmonary recruitment of circulating CXCR4<sup>+</sup> fibrocytes and attenuated lung fibrosis in the bleomycin-induced pulmonary fibrosis mouse model (119). A population of fibrocytes that express CCR7 and that are distinct from the CXCR4-expressing fibrocytes has also traffic to the lungs of bleomycin-treated mice (119). However, the number of CCR7<sup>+</sup> fibrocytes found in the fibrotic lung is markedly lower than the number of CXCR4<sup>+</sup> fibrocytes (119). Fibrocytes isolated from the mouse lungs expressed CCR2 and migrated to MCP-1 and MCP-5/CCL12 ligands (120, 125). The authors suggested that MCP-5 is the CCR2 ligand that promotes fibrosis in the mouse lung. Mice deficient for MIP-1 alpha or CCR5 remarkably attenuated bleomycin-induced pulmonary fibrosis. The bone marrow recruitment of fibrocytes in mice deficient for MIP-1alpha or CCR5 was significantly reduced compared to wild type mice, suggesting essential role of MIP-1alpha-CCR5 axis in a mouse model of pulmonary fibrosis (126). A recent study demonstrated that CCR7-positive fibrocytes infiltrate the kidney via secondary lymphoid tissue chemokine (SLC/CCL21) -

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positive vessels in a mouse model of renal fibrosis (127). Therefore, at least in mice, some kinds of chemokines are important for the recruitment of fibrocytes to some peripheral tissues (119, 125). Further studies will be needed to clarify that human fibrocytes can traffic to lung or other tissues including the skin in human SSc.

### 6. THE ROLES OF CHEMOKINE IN VASCULAR INJURY

Clinical manifestations of vascular abnormality in SSc include Raynaud's phenomenon, digital ulcers, nailfold bleeding, pulmonary hypertension, and renal crisis (12). Since Raynaud's phenomenon usually antedated to other clinical symptoms and induce tissue hypoxia, endothelium injury may be the first event of the disease. Then, profibrogenic cytokines produced by platelets and inflammatory cells may induce the tissue fibrosis. Raynaud's phenomenon appears a kind of ischemic reperfusion injury. During the ischemic-reperfusion injury, several overlapping pathway including reactive oxygen, cytokines, toll-like receptor-mediated mechanism, and complement cascades can upregulate chemokine expression, leading to the subsequent leukocyte infiltration and inflammatory response (128). Recent findings indicate that toll-like receptor signaling is important for the chemokine upregulation in ischemic organs (128). For example, the rapid breakdown of extracellular matrix by ischemic-reperfusion may result in accumulation of hyaluronan fragments, which can ligate the toll-like receptor-4 and thereby induce chemokine synthesis in macrophages (129) and endothelial cells (130). CXC chemokines regulate the infiltration of neutrophils and Th1 cells and CC chemokines recruit monocytes in the ischemic-reperfusion model of myocardium, kidney, and brain (128). Additionally, these chemokine signaling also induce angiogenic and profibrotic effects. However, the exact roles of chemokines in the pathogenesis of Raynaud's phenomenon or vascular injury of SSc remain unclear.

### 7. CONCLUSIONS AND PERSPECTIVE

This review discussed the putative role of chemokines and chemokine receptors in the pathogenesis of SSc. A number of chemokines and chemokine receptors are involved in the development of SSc, via cell recruitment, cell activation, profibrotic effect, and angiogenesis. It is likely that MCP-1 and its ligand, CCR2 play a central role in the pathogenesis of SSc. However, other CC chemokines, CXC chemokines, and CX3C chemokines may also cooperatively contribute to the development of this disease. Nonetheless, a recent randomized controlled trial failed to improve the efficacy of anti-MCP-1 monoclonal antibody in rheumatoid arthritis despite of amounts data in animal model (131). So far, most therapeutic studies of fibrotic mouse model are targeting a specific chemokine or chemokine receptor. However, further studies blocking for multiple chemokines or chemokine receptors will be needed to clarify the redundant roles of those chemokines and chemokine receptors and to maximize the effect of the therapy. Otherwise, anti-chemokine and anti-chemokine receptor targeting may be

useful by a combination of immunosuppressive therapy in SSc.

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**Abbreviations:** BAL, bronchoalveolar lavage; bFGF, basic fibroblast growth factor; CTACK, cutaneous T-cell attracting chemokine; CTGF, connective tissue growth factor; ENA, epithelial-neutrophil activating protein; GVHD, graft-versus-host disease; GRO, growth-regulated oncogene; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IL, interleukin; IP-10, interferon-gamma-inducible protein; I-TAC, IFN-inducible T cell chemoattractant; MCP-1, monocyte chemoattractant protein-1; MDC, monocyte-derived chemokine; MIG, monokine induced by IFN-gamma; MIP, macrophage inflammatory protein; PARC, pulmonary and activation-regulated chemokine; PDGF, platelet derived growth factor; RANTES, regulated upon activation normal T cell expressed and secreted; SDF-1, stromal cell-derived factor

1; SLC, secondary lymphoid tissue chemokine; SSc, systemic sclerosis; TARC, thymus and activation-regulated chemokine; TGF, transforming growth factor; Th1, type 1 helper T cell; Th2, type 2 helper T cell; TNF, tumor necrosis factor

**Key Words:** Systemic Sclerosis, Chemokine, Leukocyte Recruitment, Tissue Fibrosis, Vascular Injury, Monocyte Chemoattractant Protein-1, Fibrocyte, Review

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