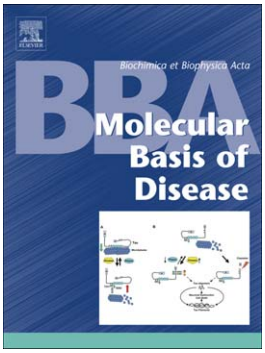

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REVIEW

Chemokines in Tissue Fibrosis

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Abstract

Fibrosis or scarring of diverse organs and tissues is considered as a pathologic consequence of a chronically altered wound healing response which is tightly linked to inflammation and angiogenesis. The recruitment of immune cells, local proliferation of fibroblasts and the consecutive accumulation of extracellular matrix proteins are common pathophysiological hallmarks of tissue fibrosis, irrespective of the organ involved. Chemokines, a family of chemotactic cytokines, appear to be central mediators of the initiation as well as progression of these biological processes. Traditionally chemokines have only been considered to play a critical role in orchestrating the influx of immune cells to sites of tissue injury. However, within the last years, further aspects of chemokine biology including fibroblast activation and angiogenesis have been deciphered in tissue fibrosis of many different organs. Interestingly, certain chemokines appear to mediate common effects in liver, kidney, lung, and skin of various animal models, while others mediate tissue specific effects. These aspects have to be kept in mind when extrapolating data of animal studies to early human trials. Nevertheless, the further understanding of chemokine effects in tissue fibrosis might be an attractive approach for identifying novel therapeutic targets in chronic organ damage associated with high morbidity and mortality.

General Aspects of Tissue Fibrogenesis

Under physiological conditions wound healing is a dynamic process involving four sequential phases: hemostasis, inflammation, proliferation and finally tissue remodelling. The proliferative phase is characterized by angiogenesis, expansion of fibroblasts and re-epithelialization after resolution of the acute tissue injury mechanism [1]. In contrast to acute damage, during chronic tissue injury of different causes (e.g. autoimmune, toxic, viral etc.) the balance between extracellular matrix (ECM) synthesis and its degradation is skewed towards an accumulation of scar tissue [2-3]. Although such process of tissue repair and remodeling is effective in creating a functional barrier within or between organs, aberrant wound healing and continued exposure to chronic injury can result in tissue scarring (fibrosis) and ultimately in loss of function of the affected organ. This chronic pathogenic process involves the recruitment and activation of fibroblasts. Upon activation by resident cells, fibroblasts promote increased tissue remodelling associated with enhanced expression of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloprotinases (TIMPs), which are mainly stimulated by TGF-ß. Other cytokines such as platelet derived growth factor (PDGF), epidermal growth factor (EGF) and angiotensin II support the mitogenic activity of fibrogenic cells. Furthermore, recruited inflammatory cells lead to persistent fibrogenic responses by expression of soluble factors and oxidative stress-related molecules [4]. This appears to be functionally relevant as inflammation is implicated in almost every aspect of chronic tissue diseases [5]. Besides inflammation, angiogenesis is believed to play an important role in tissue remodelling associated with chronic injury. This is exemplified by the fact that fibroblasts respond to hypoxia with expression of cytokines such as fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) and angiopoietins which are intrinsically involved in formation of new blood vessels [6-7].

In recent years, chemokines released by various tissue resident and infiltrating cells, and their cognate receptors have been implicated in the propagation of virtually all of these biological processes [8]. The current review focuses on the role of chemokines and their

receptors in various fibrotic diseases with enhanced fibrosis-associated inflammation and angiogenesis (Figure 1).

Basic Aspects of Inflammation Involved in Fibrotic Processes

Recruitment of inflammatory cells is a fundamental process of the early phases of wound healing. However, certain types of tissue injury can result in dysregulation of inflammatory and wound-healing response, preceding the formation of fibrosis. Initial inflammation is characterized by the rapid infiltration of polymorphonuclear neutrophils (PMN) followed by monocytes which differentiate into macrophages in response to injury. PMNs and macrophages constitute the first line of immune defense [9-10]. Upon activation, neutrophils and macrophages release a multitude of inflammatory and fibrogenic cytokines [1].

Macrophages are divided into classically (M_1) or alternatively (M_2) activated phenotypes dependent on the cytokine environment. M_1-polarized cells are mainly responsive to type 1 inflammatory cytokines, while M_2 has a restricted expression of alternative markers [11]. Moreover, M_2 macrophages are subdivided in the subsets M2a, M2b or M2c depending on their molecule-based induction. Specifically, M2a are activated by IL-4 and IL-13, M2b by immune complexes in combination with IL-1β or LPS, and M2c by IL-10, TGFβ, or glucocorticoids [12]. An increased level of M_2 macrophages has been observed in patients with fibrotic diseases [13-14], indicating that these cells are a potential source of fibrogenic cytokines. Through chemotaxis T and B leukocytes are also recruited to the site of inflammation and further promote secretion of fibrogenic cytokines (TGF-β1, PDGF, IL-1) [1].

T leukocytes are subdivided in $CD4^+$ helper (T_H) or $CD8^+$ cytotoxic cells. $CD4^+$ helper cells are further divided in T_H1 or T_H2 subsets based on the phenotype of expressed cytokines [15]. T_H1 cells express interferon-γ (IFN- γ), IL2 and IL-12, which induce potent anti-fibrotic activities, while T_H2 cells promote B cell-drived humoral immunity and fibrosis by secreting IL-4, IL-5, IL-10, and IL-13 [16]. In turn, recruited T cells can activate fibroblasts to produce collagen, ultimately resulting in tissue scarring.

During the fibrotic process, a complex interaction of cytokines, chemokines, growth factors, proteases, and ECM proteins, released by altered mesenchymal cells (fibroblasts and myofibroblasts) and resident epithelial cells are considered to amplify the inflammatory infiltrate.

Basic Aspects of Angiogenesis Involved in Fibrotic Processes

Angiogenesis is a crucial biological process under both physiologic and pathologic conditions. Pathologic angiogenesis is widely associated with chronic inflammation and fibroproliferative disorders [17]. The metabolic demands of the affected tissue are extremely high and require enormous capillary blood supply. The process of angiogenesis is divided into two phases, induction and resolution. The inductive phase involves the release of pro-angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), PDGF, TGF-ß1 and epidermal growth factor (EGF) by stromal cells, leading to changes in endothelial cell-cell and cell-matrix interaction, including degradation of vascular basement membrane (VBM) and activation of endothelial cells. The resolution phase comprises the recruitment of perivascular supporting cells, resulting in an assembly of fully functional new blood vessels. Under normal circumstances, the microvasculature is maintained in a quiescent state regulated by a fine tuned balance between various pro- and anti-angiogenic factors [18]. In fibrosis, a dysregulation of this balance with a predominance of inherent pro-angiogenic actions has been shown to contribute to the progression of tissue scarring [9-11]. Besides PDGF and TGF-ß, VEGF has also been identified to play potent pro-fibrogenic role during fibrogenesis [6, 19]. Interestingly, hypoxia is one of the most potent stimuli which can induce the gene expression of VEGF [14], and is therefore considered as an important component of homeostatic mechanisms which stimulates angiogenesis and also fibrosis in tissues [6, 20-21]. Furthermore, pericytes and fibroblasts are very sensitive to hypoxia and play a crucial role in angiogenesis by interacting with endothelial cells through PDGF and VEGF signaling [20].

Chemokine System

Chemokines are a family of small heparin-binding chemotactic cytokines, which are long known for orchestrating the recruitment of leukocytes to sites of inflammation. Apart from immune cell trafficking, chemokines also modulate a number of other crucial biological processes including hematopoiesis, cardiogenesis, vasculogenesis and neuronal development [8]. Members of the chemokine family share similar structures and can be divided into four groups, CXC, CC, C and CX_3C, characterized by the number of amino acids located between the N-terminal cysteine residues [22]. They induce their pleiotropic effects by interacting with G protein-coupled receptors (GPCR) expressed on various target cells (Table 1). The chemokine network, consisting of at least 50 ligands and 19 receptors, is a highly redundant and promiscuous system [23-24]. Moreover, the ability of chemokines to form multimeric and heterodimeric structures further contributes to the biological complexity of chemokine functions. According to these observations, various approaches have been studied to dissect distinct roles of chemokines in different diseases. Specifically, the use of knockout and transgene technologies *in vivo* has offered new avenues to decipher various functional aspects of chemokines in diverse disease models [25].

Liver Fibrosis

Liver fibrosis is characterized by hepatocellular necrosis, inflammation, tissue remodelling and angiogenesis ultimately resulting in cirrhosis or hepatocellular carcinoma [19, 26-27]. The recruitment of leukocytes into the injured liver is one of the main features orchestrated by chemokines during hepatic fibrogenesis. Overall, a large number of chemokines is expressed by hepatic resident cells which directly drive the influx of specific immune cells during injury [28-29]. Among hepatic resident cells, hepatic stellate cells (HSCs) play a pivotal role in the initiation and progression of liver fibrosis by secreting large amounts of extracellular matrix proteins [26]. Stellate cells are also able to express a multitude of chemokines, including the CC chemokines CCL2, CCL3, CCL5, as well as the CXC chemokines CXCL8, CXCL9, CXCL10 [30] and CXCL12 [31]. Interestingly, the CXC family of

chemokines also operate in pathological angiogenesis preceding/perpetuating fibrosis. CXC chemokines containing the ELR motif (ELR^+) induce angiogenesis, while chemokines lacking this motif (ELR^-) suppress the formation of new blood vessels [7, 32]. Many of these chemokines have already been functionally linked to liver fibrosis in murine models and in patients with chronic (mainly viral induced) liver diseases.

The role of CC chemokines in liver fibrosis

A well described chemokine pathway in fibrotic liver diseases implicates the chemokine receptors CCR1 and CCR5. CCR1 shares the ligands CCL3 and CCL5 with CCR5. Genetic deficiency of any of these receptors leads to attenuation of experimental liver fibrosis in mice [33], functionally validating genetic studies showing reduced liver scarring in hepatitis C virus (HCV) infected individuals possessing a 32 base pair deletion in *CCR5* (*CCR5Δ32*) [33-34]. Interestingly, there is growing evidence for the functional divergence of these receptors in the liver. While CCR1 induces its pro-fibrotic effects through hematopoietic cells, CCR5 appears to contribute to liver fibrosis through resident liver cells [33]. Their shared ligands CCL3 (own unpublished data) and CCL5 [35], seem to be central regulators of this pathway, as mice lacking either of these chemokines also exhibited reduced experimental hepatic fibrosis associated with decreased stellate cell activation and immune cell infiltration. Importantly, antagonism of CCR1 and CCR5 with Met-CCL5 attenuated liver fibrosis and expedited the regression of fibrosis upon cessation of injury [35]. Also, the inhibition of oligomerization and glycosaminoglycan binding of CCL5 by (44)AANA(47)-CCL5, a mutated CCL5 protein, ameliorated experimental liver fibrosis *in vivo* [36].

The chemokine CCL2, also known as MCP-1, is the first chemokine which has been directly implicated in hepatic fibrogenesis [37]. In patients with chronic hepatitis, CCL2 expression by liver resident cells is increased within the liver [38]. CCL2 interacts with the chemokine receptor CCR2 which is also the prominent receptor for three other CC chemokines, CCL8 (MCP-2), CCL7 (MCP-3) and CCL13 (MCP-4). This receptor is present on different cells including memory T_H1 cells [39], dendritic cells [40], $CD14^{++}$ $CD16^-$ monocytes [41] as well as

on hepatic stellate cells [42]. *CCR2* deficient mice were protected from liver fibrosis in two independent injury models [33, 43] associated with a reduced infiltration of CCR2 expressing inflammatory macrophages. Moreover, *CCR2* deficiency also ameliorated direct pro-fibrotic effects on HSCs [42]. Similarly to CCR2, the lack of another CC chemokine receptor CCR8 ameliorated experimental liver fibrosis in two independent injury models due to reduced CCL1-directed migration of inflammatory macrophages into the liver [44]. Taken together, these data reveal a central role of CC chemokines during the fibrogenic process within the liver.

The role of CXC chemokines in liver fibrosis

In liver fibrosis, a predominant CXC chemokine receptor, which is involved in the positioning of T-helper (T_H) 1 and T-regulatory (T_{REG}) cells into the liver, is CXCR3 [45]. This receptor and its splice variant bind the interferon-γ-inducible chemokines CXCL9, CXCL10, CXCL11, and CXCL4 in humans [46]. Interestingly, T_H1 cells expressing IFN-γ and IL-12 have been shown to inhibit the development of fibrosis, while T_H2 cells producing IL-4 and IL-13 have been found to exacerbate experimental liver fibrosis [16, 47-48]. CXCR3 and CCR5 are preferentially expressed on T_H1 cells, whereas CCR3 and CCR4 are expressed on T_H2 cells [49]. Mice lacking *CXCR3* are more prone to liver fibrosis which is initiated by the loss of anti-fibrogenic and angiostatic effects of CXCL9 on hepatic stellate cells [50] and sinusoidal endothelial cells [51], supporting the hypothesis that angiogenesis promotes the fibrotic process [6]. In contrast, the deletion of the other CXCR3 ligand CXCL10 inhibits experimental liver fibrosis [52]. These results are in line with clinical studies showing a high serum and intrahepatic expression of CXCL10 in severe HCV induced fibrosis [50, 53]. CXCL10 appears to be also involved in early fibrosis recurrence after liver transplantation for hepatitis C [54]. The paradox that cytotoxic $CXCR3^+$ T_H1 cells attracted by CXCL10 fail to clear the hepatitis C virus might be explained by data obtained by Casrouge and colleagues. They showed that the main part of CXCL10 in the serum of patients is cleaved by dipeptidyl

peptidase IV (DPP4). The resulting truncated form indeed antagonizes the CXCR3 pathway leading to suppressed recruitment of $CXCR3^+$ T into the infected livers [55].

In humans, the platelet-derived chemokine CXCL4 is a further ligand of CXCR3. In line with data obtained with *CXCL10* knockout mice, *CXCL4* deficient mice were protected from experimental liver fibrosis due to impeded migration of $CD8^+$ positive T cells and reduced CXCL4 induced pro-inflammatory chemokines, such as CXCL1 and CCL5, within the liver [56]. Moreover, dimerization of CXCL4 with CCL5 may induce enhanced CCL5 driven vascular monocyte accumulation [57].

The role of CX_3C chemokines in liver fibrosis

An anti-fibrotic role of monocyte-associated chemokine receptor 1 CX_3CR1, the only receptor of the CX3C class of chemokines, and its ligand CX_3CL1 in liver fibrosis was shown by Karlmark *et al.* Accordingly, CX_3CR1 protected against liver fibrosis by regulating differentiation and survival of liver monocytes [58], suggesting that pharmacological modulation of this pathway may offer new therapeutic strategies.

Pulmonary Fibrosis

The pathophysiology of pulmonary fibrosis is a complex biological process which includes features of abnormal inflammatory wound healing, the deposition of extracellular matrix proteins and exaggerated angiogenesis [7]. At advanced stages, scarring of the lung can lead to pulmonary hypertension and respiratory failure which is associated with strongly increased morbidity and mortality in affected patients [59]. Fibroblasts, myofibroblasts and fibrocytes are the main sources of a steady accumulation of the scar tissue [60-61]. These cell types modulate the derangement of alveolar structures and loss of elasticity [60-61]. Pulmonary fibrotic diseases are often associated with arrest of monocytes, neutrophils, mast cells and other leukocytes [59]. The release of CC and CXC chemokines by these pro-inflammatory cells and also by resident cells (alveolar epithelial cells) enhance the inflammatory and fibrotic effects in the lung [62-63].

The role of CC chemokines in pulmonary fibrosis

In murine models, CCL2 and its cognate receptor CCR2 has been implicated in the pathogenesis of pulmonary fibrotic diseases [64]. Besides its specific role as chemoattractant for monocytes, CCL2 mediates direct pro-fibrogenic effects by signalling fibroblasts to express TGF-ß, a known collagen production stimulator [65]. CCR2 is present on monocytes, activated T cells, B cells, NK cells, fibroblasts and mast cells within the lung [66]. Mice lacking CCR2 develop less severe pulmonary fibrosis in different experimental fibrosis models compared to control mice [67]. In this context, CCL2 signal transduction via its cognate receptor CCR2 played an important role in cellular activation rather than immune cell trafficking into the lung [67]. These findings were further supported by studies of Okuma *et al.* showing an attenuation of pulmonary fibrosis in *CCR2* deficient mice due to reduced macrophage mediated MMP-2 and MMP-9 production [68]. These results are in line with human studies showing a high mRNA and protein expression of CCL2 in lung epithelial cells from patients with idiopathic pulmonary fibrosis. Moreover, analysis of bronchoalveolar lavage fluid from patients with idiopathic pulmonary fibrosis showed enhanced levels of CCL2, CCL3, and CCL4, while CCR5 expression was significantly reduced [69]. CCR5, expressed on activated T cells and alveolar macrophages, interacted with the ligands CCL3 and CCL5 [69-70]. The reduction of this receptor, which is preferentially expressed on T_H1 cells, was associated with an increase of IL-4 and a decrease of IFN-γ release in patients with advanced stages of pulmonary fibrosis [69]. Interestingly, IFN-γ is a T_H1 cytokine which suppresses collagen synthesis by fibroblasts, whereas IL-4 is a T_H2 cytokine which stimulates fibroblast proliferation and collagen synthesis. These findings support the hypothesis of an important role of the T_H1/T_H2 balance for the progression of pulmonary fibrosis, as it has been described for liver fibrosis [39, 69]. In contrast to this hypothesis, *CCR5*$^{-/-}$ mice have been shown to be less prone to experimental pulmonary fibrosis compared to wild-type mice [71]. Similarly to CCR5, the CC chemokine receptor CCR1 has also been shown to play an important role in experimental pulmonary fibrosis. Upon injury, *CCR1* mRNA expression peaked at day 7, reflected by augmented expression of its ligands

CCL3 and CCL5. Functionally, blockade of CCR1 led to reduced experimental fibrosis associated with decreased immune cell infiltration into the lung [72]. Another important CC chemokine involved in fibrosis of the lung is the eosinophil chemotactic factor CCL11 (eotaxin-1). It binds to its cognate receptor CCR3, which is highly expressed by eosinophils as well as other immune cells [73-74]. In murine lung fibrosis models, the expression of CCL11 and CCR3 was increased within the lung and was associated with progressive pulmonary infiltration of eosinophilic and neutrophilic granulocytes [73].

The role of CXC chemokines in pulmonary fibrosis

CXC chemokines and receptors also operate in pulmonary fibrosis [75]. CXCR3 and its ligands CXCL9, CXCL10 and CXCL11 act primarily on T helper 1 (T_H1) and natural killer T (NKT) cells [75]. Like in the liver, evidence from mouse and human studies implicates T_H1 response as an anti-fibrogenic and T_H2 response as a pro-fibrogenic event during pulmonary fibrogenesis [16, 76]. Previous studies have suggested that $CXCR3^+$ cells potentiate T_H1 responses [16]. In mouse model, *CXCR3* deficient mice indeed showed pronounced experimental pulmonary fibrosis with decreased expression levels of IFN-γ and a reduction of IFN-γ positive T cells [77], suggesting CXCR3 as a non-redundant receptor limiting lung fibrosis. Notably, such a T_H2 dominant environment has also been associated with severe liver and renal fibrosis in *CXCR3* deficient mice [50, 78]. Interestingly, all ligands of CXCR3, which lack the ELR motif (ELR^-), also have angiostatic actions, whereas ELR^+ CXC chemokines CXCL2, CXCL5 and CXCL12 promotes angiogenesis in patients with idiopathic pulmonary fibrosis (IPF) [79-81]. This seems especially relevant as pulmonary angiogenesis has been strongly linked to progressive fibrogenesis [82]. An imbalance of ELR^+/ELR^- CXC chemokine presence with a predominance of angiogenic ELR^+ chemokines has been shown to contribute to the pathogenesis of IPF [79-81]. Functionally, blocking of CXCL2 [81] or systemic administration of CXCL10 or CXCL11 led to a strongly attenuation of experimental pulmonary fibrosis via reduction of angiogenesis within the lung [83-85]. Moreover, recombinant murine IL-12 also reduced pulmonary fibrosis triggered by IFN-γ [86]. These

findings identify IFN-γ and the CXCR3 ligands CXCL10 and CXCL11 as inhibitors of pulmonary fibrosis.

Renal Fibrosis

Renal fibrosis is characterized by glomerulosclerosis and destructive interstitial fibrosis, and is closely correlated to loss of renal function. The fibrotic process is initiated by cellular activation of resident cells (tubular epithelial cells, vascular endothelial cells and fibroblasts) and by recruitment of activated leukocytes (e.g. macrophages) followed by an excessive expansion of interstitial matrix components by activated renal fibroblasts. Within the last years, a critical role of various chemokines and their receptors has been identified in these biological processes.

The role of CC chemokines in renal fibrosis

Recent studies better defined functions of the chemokine CCL2 as a mediator of experimental renal fibrosis. In various rodent models of renal fibrosis, CCL2 expression was increased by tubular epithelial cells associated with enhanced macrophage infiltration [87]. These findings are congruent with human data showing an increased expression of CCL2 and macrophage infiltration in kidney of patients with diabetic nephropathy [88-89]. The functional aspect of CCL2 in interstitial fibrosis was confirmed by various animal studies using neutralizing antibody or CCL2 deficient mice. Importantly, neutralization of CCL2 reduced interstitial leukocyte and collagen accumulation in mice with crescentic nephritis [90]. In accordance with these findings, mice lacking the *CCL2* gene develop less severe experimental tubulointerstitial fibrosis [91]. Also, the blockade of its receptor CCR2 is associated with reduced interstitial fibrosis after unilateral ureter ligation (UUO) [92]. These findings were further reflected by attenuated interstitial leukocyte and fibroblast accumulation. In a spontaneous mouse model of lupus nephritis, MRL/MpJ Fas$^{lpr/lpr}$ (MRL/*lpr*) mice showed a higher expression of chemokines associated with enhanced mononuclear cell infiltration and expression of chemokine receptors CCR1, CCR2, and CCR5 [93]. In this 'real-

life' model, genetic deletion of *CCL2* (MRL/*lpr*/*CCL2*$^{-/-}$) led to reduced macrophage and T cell influx, proteinuria and also renal damage [94], identifying the CCL2-CCR2 axis as a potential target for therapeutical strategies for certain types of nephritis.

These findings are further supported by human studies in patients with lupus nephritis showing a correlation between urinary CCL2 levels and severity of renal disease as well as macrophage infiltration [95]. Moreover, glucocorticoid treatment of the lupus nephritis led to an attenuation of urinary CCL2 levels confirming *in vitro* results which showed an inhibitory effect of glucocorticoids on CCL2 expression in renal cells [96].

Other CC chemokine receptors involved in chronic renal fibrotic diseases are CCR1 and CCR5. In experimental interstitial fibrosis, their common ligand CCL5 was predominantly expressed by leukocytes and also by interstitial fibroblasts *in vivo* [87, 93]. Along this chemotactic gradient, CCR5^{+} leukocytes migrated to the interstitium and the number of CCR5^{+} leukocytes within the interstitium correlated with serum creatinine levels in human biopsies of various renal diseases [97]. However, in contrast to liver [33] and lung [71] fibrotic disease models, the deletion of *CCR5* in the lupus nephritis model (MRL/*lpr*/*CCR5*$^{-/-}$) led to an unexpected deterioration of kidney damage and was associated with an increase in mononuclear cell infiltration but a decrease in renal T cell accumulation [98]. This apparently protective role of CCR5 has been explained by a negative feedback loop of its own ligands leading to reduced inflammatory cell influx [98]. On the other hand, the antagonism of CCR5 with Met-CCL5 for seven days after renal transplantation in rats ameliorated chronic allograft nephropathy with reduced proteinuria, glomerulosclerosis and interstitial fibrosis after 28 weeks, indicating the importance of CCR5 pathway for the alloimmune response [99]. These results are in line with earlier clinical studies showing improved graft survival in homozygous *CCR5Δ32* individuals compared to either heterozygote or wild-type *CCR5* renal transplant recipients [100]. Importantly, the blockade of the other CCL5 receptor CCR1 also showed beneficial effects on the progression of chronic renal allograft damage [101].

Taken together, these *in vivo* and *ex vivo* analysis of CC chemokines and their receptors provided further valuable insights into immune cell mediated progressive tubulointerstitial

damage and fibrosis. Thus, antagonism of these proteins may represent a therapeutic option for chronic renal inflammation and fibrosis.

The role of CXC chemokines in renal fibrosis

CXCR3 and its ligand CXCL10 has also been involved in renal fibrotic disorders. The antagonism of the CXCL10-CXCR3 axis by neutralizing antibody promoted renal fibrosis triggered by altered balance of hepatocyte growth factor (HGF) and TGF-ß1 activity. Interestingly, CXCL10 inhibition affected neither macrophage nor T cell influx into the kidney [78]. These findings identify the CXCL10-CXCR3 axis as an important potential target for therapeutical strategies in progressive renal fibrosis.

Dermal Fibrosis – Scleroderma

Scleroderma, also known as systemic sclerosis (SSc), is characterized by three distinct pathologic processes: autoimmune inflammation, fibrosis and angiogenesis. Accordingly, the interplay of leukocytes, endothelial cells and fibroblasts seems to play a central role in the pathogenesis of scleroderma. Chemokines have indeed been recognized as crucial mediators of leukocyte trafficking into the sclerotic skin [102]. Apart from inflammation, chemokines are also involved in other pathologic processes including angiogenesis, fibrogenesis and cell differentiation which contribute to clinical manifestation of SSc.

The role of CC chemokines in skin fibrosis

Among chemokines, CCL2 has been identified as the most critical chemokine for tissue fibrosis and inflammation in systemic sclerosis (SSc). In patients with SSc, CCL2 levels are increased in serum, along with enhanced CCL2 expression in the epidermis, inflammatory mononuclear cells, and endothelial cells [103]. These findings are in line with *in vitro* experiments demonstrating a transendothelial leukocyte migration mediated by SSc fibroblast-released biologically active CCL2 [104]. The functional relevance of CCL2 in tissue fibrosis was further validated by animal models. In a murine sclerodermatous graft-versus-

host disease (GVHD) model, CCL2 has been found to precede the progression of skin and pulmonary fibrosis [105]. Functionally, the lack [106] or neutralization [107] of CCL2 ameliorated experimental dermal sclerosis *in vivo* associated with a decrease of inflammatory cells and collagen content in the skin. Furthermore, pharmacological antagonism of CCL2 and CCL4 with SKL-2841 protected bleomycin-induced dermal fibrosis [108]. These results provide evidence that CCL2 plays a critical role in positioning of leukocytes into the skin in early stages of SSc, which in turn activate resident fibroblasts to produce collagen, ultimately resulting in tissue scarring. The expression of another CC chemokine, CCL5, has also been functionally involved in SSc. In patients with SSc, mRNA and protein levels of CCL5 were increased in bronchoalveolar lavage fluid [109] and also within the skin [110-111]. In a murine sclerodermatous graft-versus-host disease (GVHD) model, cutaneous CCL3 and CCL5 also operate in dermal and pulmonary fibrosis [105]. Recently, significant abnormalities of CCL7 expression have also been shown in SSc. Notably, abundant CCL7 levels in serum of patients with SSc correlated with the extent of skin sclerosis and the severity of pulmonary fibrosis [112]. In line with these human data, CCL7 expression was increased in type 1 tight-skin mice, which spontaneously develop skin fibrosis [113], defining CCL7 as a potential mediator of dermal fibrosis in SSc. Increased T_H2 chemokines, CCL17, CCL22 [114] and CCL27 [115], in serum of patients with SSc has also been linked to the extent of skin sclerosis.

The role of CXC chemokines in dermal fibrosis

CXC chemokines with ELR motif attract neutrophils and mediate pro-angiogenic effects. The ELR^+ chemokines involved in SSc are CXCL1, CXCL8 and CXCL16. Increased serum CXCL1 and CXCL8 levels have been shown in patients with SSc [116]. Moreover, there is growing evidence of genetic association between the CXCL8 gene polymorphism and increased risk of SSc [117]. The other angiogenic CXC chemokine CXCL16 and its receptor CXCR6 were also elevated in SSc serum and on SSc dermal endothelial cells, respectively [118]. CXC chemokines without ELR motif orchestrate $CXCR3^+$ T_H1 cells and mediate

angiostatic effects. These ELR$^-$ CXC chemokines involved in SSc are CXCL9, CXCL10, CXCL11 and CXCL12. Angiostatic CXCL9 and CXCL10 were increased in serum and highly expressed in skin of SSc patients compared to controls [114, 118]. In contrast, the mRNA and protein expression of their receptor CXCR3 was decreased in SSc patients, suggesting a CXC chemokine receptor-regulated angiogenic activity in SSc skin.

Chemokine receptor antagonistic strategies in human fibrotic diseases

The growing evidence that chemokines and their receptors are involved in tissue fibrosis [33, 35], might lead to treatment of patients with fibrotic diseases with chemokine receptor antagonists. Indeed, the CCR5 receptor antagonist maraviroc is under investigation as a target of anti-fibrotic therapies in HCV/HIV co-infected subjects (www.clinicaltrials.gov). Other chemokine receptor antagonists are also actively investigated in early human trials. These include the CC receptor 1 antagonist BAY86-5047 in patients with endometriosis and the CCR2 antagonist MLN1202 in subjects with a risk for cardiovascular diseases (www.clinicaltrials.gov). However, it should always be considered that many chemokine receptor antagonists failed to show efficacy in clinical trials, despite data of successful therapeutical interventions in murine models [119]. Reasons for these negative results might be pharmacokinetic aspects of the drugs, off-target effects and cytotoxicity at increased doses. While these features are applicable to all G-protein coupled receptors, more unique/specific to the chemokine system is its high degree of redundancy and complexity [120]. Nevertheless, although there is a large empirical gap in our knowledge of the chemokine system, the use of chemokine receptor antagonist is considered as a great therapeutic potential for fibrogenic diseases in the future.

Conclusions

Tissue fibrosis is a dynamic progression of aberrant wound healing which is accompanied by chronic inflammation and angiogenesis. This fibrotic process represents a risk for morbidity and mortality associated with organ failure in various fibrotic diseases affecting the liver,

lung, kidney and skin. The trafficking, activation and proliferation of inflammatory cells and their interaction with resident cells are orchestrated by chemokines and their cognate receptors. Importantly, some chemokines such as CCL2, CCL3 and CCL5 have shown common effects across different diseases, while others such as CXCL10 mediate tissue specific effects (Table 2). This fact needs to be taken into account when translating pre-clinical animal data to human clinical trials. Nevertheless, given the complexity and redundancy of the chemokine network, further investigations of chemokine effects in tissue fibrosis are warranted as the search for novel targets for specific fibrotic therapeutic agents is ongoing.

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Conflict of interest

The authors declare no conflict of interest with regards to the products described in this review.

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Tables

Table 1

Table 1 | Chemokine receptors and ligands in fibrosis		
Chemokine receptor	**Chemokine ligand**	**Cell expression**
CC Family		
CCR1	CCL3, CCL5, CCL7, CCL14	fibroblast, monocyte, NK cell, platelet, T_H1 cell
CCR2	CCL2, CCL7, CCL8, CCL13, CCL16	fibroblast, dentritic cell, monocyte, T_H1 cell
CCR3	CCL5, CCL7, CCL8, CCL11, CCL13	basophil, eosinophil, platelet, T_H2 cell
CCR4	CCL17, CCL22	endothelial cell, NK cell, platelet, T_H2 cell
CCR5	CCL3, CCL4, CCL5, CCL11, CCL14, CCL16	fibroblast, macrophage, monocyte, T cell, T_H1 cell
CCR6	CCL20	B cell, dentritic cell, T cell
CCR7	CCL19, CCL21	dentritic cell, T cell
CXC Family		
CXCR3	CXCL4, CXCL9, CXCL10, CXCL11	endothelial cell, NK cell, T_H1 cells, T_{REG} cell
CXCR4	CXCL12	fibroblast, endothelial cell, platelet

Table 2

Table 2 | Pro- and anti-fibrotic chemokines involved in fibrotic diseases affecting the liver, lung, kidney and skin				
Chemokine	**Liver**	**Lung**	**Kidney**	**Skin**
Pro-fibrogenic	CCL1, CCL2, CCL3, CCL5, CXCL1, CXCL4, CXCL10	CCL2, CCL3, CCL5, CCL11, CXCL2, CXCL5, CXCL12	CCL2, CCL5	CCL2, CCL3, CCL5, CCL7, CCL17, CCL22, CCL27, CXCL1, CXCL8, CXCL16
Anti-fibrogenic	CXCL9, CX_3CL1	CXCL10, CXCL11	CXCL10	CXCL9, CXCL10, CXCL11, CXCL12

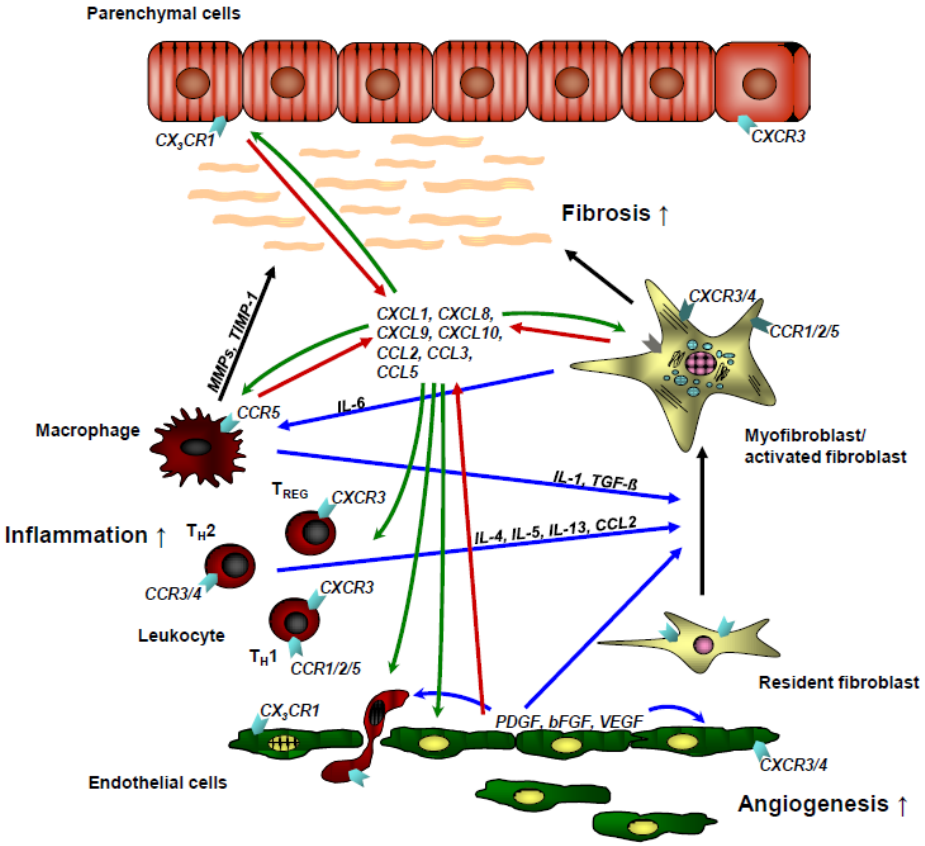
Figure legends

Figure 1

Key features of tissue fibrosis. Fibrogenesis, characterized by excessive deposition and accumulation of ECM, is a central pathologic process of tissue fibrosis. This process is initiated by a multitude of chemokines which induce their pleiotropic effect by interacting with their cognate receptors, expressed by various target cells. Injured endothelial cells induce the release of angiogenic factors such as VEGF, PDGF, bFGF and also chemokines, which in turn alter endothelial permeability and support the recruitment and proliferation of leukocytes. Under normal conditions, these factors and cell types drive the wound healing process. In fibrosis, repetitive vascular and tissue injury and deregulated inflammatory response as well as endothelium repair induce persistent inflammation and angiogenesis. The immunologic response includes the accumulation of macrophages and neutrophils in affected tissue, which amplify the recruitment and activation of leukocytes. In this context, the predominantly represented subset of T cells T_H2 with enhanced pro-fibrogenic cytokine and chemokine release (interleukin-4, interleukin-5, interleukin-13 and CCL2) contribute to the progression of fibrosis by activation of fibroblasts. The angiogenic response includes the release of angiogenic factors leading to changes in endothelial cell-cell and cell-matrix interaction, including degradation of VBM and activation of endothelial cells, finally resulting in new blood vessels. This angiogenic phase is characterized by an imbalance of ELR^+/ELR^- CXC chemokine presence with a predominance of angiogenic ELR^+ chemokines. Endothelial and resident cell-expressed pro-fibrogenic and angiogenic chemokines and growth factors activate resident fibroblasts. Excessive production and deposition of ECM proteins by these cells results ultimately in permanent scarring.

bFGF, basic fibroblast growth factor; IFN, interferon; IL, interleukin; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor β; T_H1, type 1 T helper cells; T_H2, type 2 T helper cells; TIMP, tissue inhibitor of metalloproteinases; T_{REG}, regulatory T cells; VBM, vascular basement membrane; VEGF, vascular endothelial growth factor.

Figure 1



Highlights

- Chemokines are involved in all processes of a wound healing response
- Chemokines display common and organ-specific effects during fibrogenesis
- Chemokines display pro- or anti-fibrotic properties depending on the model used
- Chemokine-antagonistic strategies are being actively developed for human trials