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Review Series

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The role of CXC chemokines in pulmonary fibrosis

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The CXC chemokine family is a pleiotropic family of cytokines that are involved in promoting the trafficking of various leukocytes, in regulating angiogenesis and vascular remodeling, and in promoting the mobilization and trafficking of mesenchymal progenitor cells such as fibrocytes. These functions of CXC chemokines are important in the pathogenesis of pulmonary fibrosis and other fibroproliferative disorders. In this Review, we discuss the biology of CXC chemokine family members, specifically as it relates to their role in regulating vascular remodeling and trafficking of circulating mesenchymal progenitor cells (also known as fibrocytes) in pulmonary fibrosis.

Introduction

The body's response to various known and unknown (idiopathic) processes in the lung can lead to pulmonary fibrosis. The most common and devastating form of pulmonary fibrosis is referred to as idiopathic pulmonary fibrosis (IPF). IPF is a chronic, and usually fatal, pulmonary disorder with a mortality rate of approximately 70% five years after diagnosis (1, 2). Most reported cases of IPF seem to be spontaneous, with less than 2% of cases familial in character (3, 4). The prevalence of IPF increases with age (2, 5). The term IPF was previously applied to all cases of pulmonary fibrosis that did not have a recognized cause. However, it is currently reserved for, and is synonymous with, the pathological diagnosis of usual interstitial pneumonia (UIP) following lung biopsy. The hallmark of UIP is temporal heterogeneity (that is, areas of established fibrosis interspersed with areas of relatively normal lung, and gradations between these two extremes) and architectural loss and chronic scarring accompanied by microscopic honeycomb-like structural change in the subvisceral pleura region (Figure 1). The fibrosis is present in the interstitial space (the space between the endothelium and the basement membrane, beneath the epithelium), which includes the alveolar walls. Other distinguishing features of UIP include the relative paucity of inflammation, a hyperplastic epithelium, and the presence of focal collections of fibroblasts, referred to as fibroblastic foci. This has led some investigators to hypothesize that IPF is a disease that is characterized by repetitive epithelial injury and abnormal repair (6). The absence of marked inflammatory infiltrates has led to substantial controversy as to the role of inflammation in IPF. This absence of inflammation does not, however, exclude a role for inflammation in the initiation of the injury that subsequently leads to fibrosis (7, 8). Furthermore, the origin and importance of the fibroblastic foci is controversial. It has recently been suggested that they are not discrete foci but in fact represent an organized reticulum that courses through the lung (9). Interestingly, this reticulum is surrounded by an extensive capillary network, which suggests that vascular remodeling is an important component of pulmonary fibrosis (9). The pathological findings regarding UIP contrast with those regarding cryptogenic organizing pneumonia (COP), which is characterized by airway aggregates of fibroblasts in an immature collagen matrix. The lung architecture is typically preserved in COP, and although there might be interstitial inflammation, there is no interstitial fibrosis. COP typically has an excellent prognosis and does not lead to end-stage fibrosis. Why then do these two patterns of lung injury, each with a substantial presence of fibroblasts and collagen matrices and variable degrees of vascular remodeling, have two different outcomes? It is probable that the preservation of the lung architecture and the intact basement membrane allows repair to proceed normally in COP, as opposed to the aberrant repair that is seen in UIP.

One of the major limitations to pulmonary fibrosis research is the lack of a good animal model of fibrotic lung disease, particularly a model of IPF. Bleomycin has been used in mice to initiate fibrotic lung lesions that have many of the histological components of IPF (10, 11). Bleomycin administration results in epithelial cell necrosis within 24 hours, acute alveolitis 2–3 days following challenge, and intense interstitial inflammation 4–12 days following challenge (10, 11). Fibroblast proliferation and ECM synthesis are initiated 4–14 days after challenge, with collagen content elevated approximately 2-fold 3 weeks following challenge (10, 11). Furthermore, the injury is self limited and begins to resolve after 4–6 weeks. Although these pathologic changes clearly occur in a more rapid fashion than in human IPF, and not withstanding the fact that the injury is self limited and spontaneously resolves with time, the bleomycin model has been widely used as a model of human pulmonary fibrosis and can provide useful insights into the biology of lung injury, fibrosis, and repair.

In this Review, we focus on the role of CXC chemokines in regulating vascular remodeling and extravasation of circulating mesenchymal progenitor cells (also known as fibrocytes) in pulmonary fibrosis. We present data from animal models of fibrosis, particularly the bleomycin model of pulmonary fibrosis, which provide a conceptual framework from which to begin to address the pathogenesis of the human disease IPF.

Angiogenesis: vascular remodeling relevant to pulmonary fibrosis

Angiogenesis is defined as the growth of new blood vessels and is a critical biological event that occurs during various physiologic and pathologic processes (12). Pathological angiogenesis is associated with all chronic inflammatory and chronic fibroproliferative disorders as well as with tumor growth. The terms *angiogenesis* and
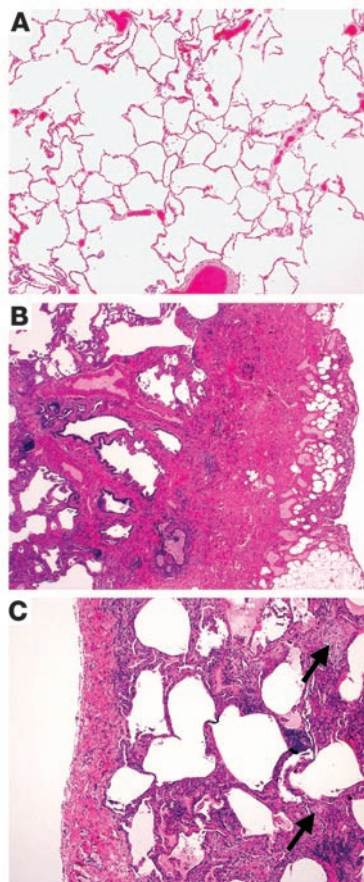
Figure 1
Histopathology of normal lung tissue (**A**) and lung tissue from two patients with IPF (**B** and **C**). Shown are (**A**) normal lung tissue (H&E staining); (**B**) IPF lung tissue with severe end-stage fibrosis and honeycomb changes (trichrome staining); and (**C**) IPF lung tissue with areas of fibrosis and an area compatible with a fibroblastic focus (FF) (H&E staining). Magnification, ×200.

vascular remodeling are often used interchangeably in the context of both pathological and aberrant angiogenesis, as they are here.

Although inflammation and angiogenesis are distinct and separable processes, they are linked and often temporally overlap (13). The histological appearance that is associated with all chronic fibroproliferative disorders is granulation-like tissue that shows prominent vascular remodeling. The metabolic demands of granulation-like tissue that is undergoing hyperplastic and reparative changes are extremely high, and such tissue requires a proportionally greater capillary blood supply than normal tissue to meet these increased demands. Therefore, the vascular remodeling that is associated with chronic fibroproliferative disorders is analogous to the angiogenesis that occurs during tumorigenesis to increase the delivery of metabolic substrates to the proliferating tumor cells.

Vascular remodeling is tightly regulated (14–28). In the local microenvironment, the extent of vascular remodeling is determined by the balance between expression of factors that promote angiogenesis and expression of factors that inhibit angiogenesis. The CXC chemokine family of cytokines is unique in that different family members regulate vascular remodeling in a disparate manner. Each member of this family has four highly conserved cysteine residues, with the first two cysteines separated by a nonconserved aa (29, 30). A second structural domain dictates their functional activity in regulating angiogenesis (30). The amino terminus of several family members contains a 3-aa sequence (Glu-Leu-Arg), known as the ELR motif, immediately before the first cysteine residue (29, 30). ELR+ CXC chemokines promote angiogenesis (29, 30). By contrast, CXC chemokines that are IFN inducible and lack

the ELR motif inhibit angiogenesis (29, 30). ELR+ and ELR– CXC chemokines bind different CXC chemokine receptors (CXCRs) on endothelial cells, which ultimately leads to either promotion or inhibition of angiogenesis, respectively.

Angiogenic ELR+ CXC chemokines. The ELR+ members of the CXC chemokine family that promote angiogenesis are CXC chemokine ligand 1 (CXCL1), CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8 (Table 1) (29, 30). Angiogenic factors in a local microenvironment can function in a direct or serial manner to promote angiogenesis. For example, in a mouse model of Kaposi sarcoma, a serial mechanism is as follows: VEGF activation of endothelial cells leads to upregulation of the antiapoptotic molecule BCL2, which in turn promotes the expression of endothelial cell–derived CXCL8 (31); the upregulated expression of CXCL8 functions in an autocrine and paracrine manner to maintain the angiogenic phenotype of the endothelium (Figure 2) (31). Other serial pathways can also promote CXCL8-dependent angiogenesis, such as the signaling pathways induced by intracellular ROS, EGF, and HGF, which lead to nuclear translocation of NF-κB, expression of CXCL8 in tumor cells, and subsequent tumor-associated angiogenesis (32–35).

Although CXCL12 is not an ELR+ CXC chemokine, it has been implicated in mediating angiogenesis through its receptor, CXCR4 (36–39). This in turn has led to speculation that the predominant function in tumorigenesis of this ligand-receptor pair is to mediate angiogenesis. However, several other studies have shown that low levels of CXCL12 exist in tumors and that CXCR4 is predominately expressed by tumor cells and not endothelial cells (40, 41). In these studies, it was found that CXCL12 did not promote angiogenesis but instead promoted tumor metastasis (41). A possible explanation for these different results is that tumor cells expressing CXCR4 might be able to "out-compete" tumor-associated endothelial cells for any CXCL12 binding due to their higher level of expression of CXCR4. A similar mechanism might exist in chronic fibroproliferative disorders where surrounding parenchymal cells or other stromal cells such as fibrocytes might express CXCR4 and out-compete endothelial cells for CXCL12. This would lead to CXCL12 exerting its profibrotic effects through recruitment of stromal cells or fibrocytes rather than through mediation of angiogenesis (42).

CXCR2 mediates the angiogenic effect of ELR+ CXC chemokines. There are two CXCRs, CXCR1 and CXCR2, that are relevant to ELR+ CXC chemokines. However, only CXCL6 and CXCL8 specifically bind CXCR1, whereas all ELR+ CXC chemokines bind CXCR2 (43). Furthermore, although expression of both CXCR1 and CXCR2 can be detected in endothelial cells (43–45), CXCR2 has been found to be the primary functional chemokine receptor in mediating in vitro human lung microvascular endothelial cell chemotaxis toward ELR+ CXC chemokines (43, 44, 46). Further studies have confirmed the importance of CXCR2 in mediating ELR+ CXC chemokine–induced angiogenesis in human intestinal microvascular endothelial cells (47). Activation of CXCR2 on endothelial cells by CXCL8 induces rapid stress fiber assembly, chemotaxis, enhanced

Table 1

Structural and functional differences of CXC chemokines in the regulation of vascular remodeling

Chemokine	Effect on angiogenesis	Relevant structural motif	Receptor through which effect on angiogenesis is mediated
CXCL1 (also known as GROα)	Angiogenic	A-T-<u>E-L-R</u>-C-Q-C	CXCR2
CXCL2 (also known as GROβ)	Angiogenic	A-T-<u>E-L-R</u>-C-Q-C	CXCR2
CXCL3 (also known as GROγ)	Angiogenic	V-T-<u>E-L-R</u>-C-Q-C	CXCR2
CXCL5 (also known as ENA-78)	Angiogenic	L-R-<u>E-L-R</u>-C-V-C	CXCR2
CXCL8 (also known as IL-8)	Angiogenic	A-K-<u>E-L-R</u>-C-Q-C	CXCR2
CXCL4 (also known as PF4)	Angiostatic	D-G-D-L-Q-C-L-C	CXCR3
CXCL9 (also known as MIG)	Angiostatic	V-R-K-G-R-C-S-C	CXCR3
CXCL10 (also known as IP-10)	Angiostatic	S-R-T-V-R-C-T-C	CXCR3
CXCL11 (also known as ITAC)	Angiostatic	F-K-R-G-R-C-L-C	CXCR3

The underlined ELR motif indicates the conserved aa sequence of Glu-Leu-Arg in the amino terminus of CXC chemokines, which is an important structural motif in dictating binding to the putative CXC chemokine receptor, CXCR2.

proliferation, and phosphorylation of ERK1/2 (47). These in vitro studies, which demonstrate the importance of CXCR2 in mediating the angiogenic effects of ELR⁺ CXC chemokines, have been confirmed in vivo, for example, in studies using an orthotropic lung cancer model, a heterotopic renal cell cancer model, and a mouse model of chronic airway allograft rejection (48–51).

ELR⁻ CXC chemokines are inhibitors of angiogenesis. The angiostatic members of the CXC chemokine family include CXCL4, CXCL9, CXCL10, and CXCL11 (29, 30) (Table 1). CXCL9, CXCL10, and CXCL11 (but not CXCL4) are induced by both type I and type II IFNs (52). Moreover, the relationship among IFNs, IFN-inducible CXC chemokines, and their biological functions are directly relevant to the function of other cytokines, such as Th1 cytokines, that lead to the stimulation of IFN expression. Therefore, through the induction of IFN-γ, Th1 cytokines such as IL-2, IL-12, IL-15, IL-18, and IL-23 and chemokines such as CC chemokine ligand 19 (CCL19) and CCL21 have profound effects on the production of CXCL9, CXCL10, and CXCL11. Furthermore, this cytokine cascade connects the Th1 cytokine profile with angiostasis and creates the concept of *immunoangiostasis* (53). Interestingly, it has recently been shown that the inflammatory lung disease sarcoidosis is associated with an angiostatic environment, as compared with the angiogenic environment that is seen in IPF (54). This is important because sarcoidosis is considered a Th1-mediated disease that resolves spontaneously in many patients (54), whereas IPF is considered more of a Th2-mediated disease.

CXCR3 mediates the angiostatic effects of ELR⁻ CXC chemokines. CXCR3 is the receptor that mediates the angiostatic effects of ELR⁻ CXC chemokines. CXCR3 exists as several variants that are generated by alternative splicing (CXCR3A, CXCR3B, and CXCR3-alt), all of which are involved in mediating the recruitment of Th1 cells to a site of tissue damage as well as mediating the inhibition of angiogenesis (55, 56). CXCR3A is the main chemokine receptor expressed by Th1 effector cells, cytotoxic CD8⁺ T cells, activated B cells, and NK cells (55). In addition, mouse endothelial cells were found to express CXCR3 (57). Further studies, using a mouse model of melanoma, have confirmed the observation that expression of CXCR3 by endothelial cells is necessary for the angiostatic effects of CXCR3 ligands, although these studies did not determine which CXCR3 variant is necessary (58). Subsequent studies demonstrated that, through CXCR3B, CXCR3 ligands blocked the migration and proliferation of human microvascular endothelial cells in response

to various angiogenic factors (59). Furthermore, in a mouse model of pulmonary fibrosis, CXCL11 inhibited vascular remodeling in a CXCR3-dependent manner (60). Mice that received bleomycin and were treated with CXCL11 had decreased fibrosis and decreased intrapulmonary angiogenesis, and these decreases could be blocked using an antibody specific to CXCR3 (60).

CXC chemokines in the regulation of angiogenesis associated with fibroproliferation

The lung has two circulatory systems, a bronchial system that arises from the systemic circulation and a pulmonary system that arises directly from the pulmonary artery. Evidence exists for vascular remodeling in the lung in various pathological conditions, including pulmonary fibrosis (61–64). The angiogenic response of the bronchial circulation is a fundamental response related to alterations in the pulmonary vascular resistance, as can be seen following loss of pulmonary vasculature or in pulmonary hypertension (65–69). Although mice lack a bronchial circulation, vascular remodeling of the systemic circulation can supply up to 15% of the normal pulmonary blood flow within five to six days of experimental ligation of the pulmonary artery (65). The angiogenic factors that were instrumental in mediating angiogenesis under these conditions were found to be ELR⁺ CXC chemokines (70). Current dogma is that the pulmonary circulation has limited potential for vascular remodeling; however, Dutly and associates have recently demonstrated that the pulmonary circulation has a major role in contributing to angiogenesis and the creation of a new blood supply into transplanted tissue in the lung (69). Together these findings support the notions that under ischemic and/or hypoxic conditions, ELR⁺ CXC chemokines are involved in promoting angiogenesis in the lung and that both the bronchial and pulmonary circulations of the lung are important in promoting vascular remodeling.

Vascular remodeling in IPF was originally identified by Turner-Warwick, who, when she examined the lungs of patients with widespread interstitial fibrosis, found evidence of vascular remodeling leading to anastamoses between the systemic and pulmonary microvasculatures (61). Renzoni et al. have also observed vascular remodeling in both IPF and the fibrosing alveolitis that is associated with systemic sclerosis (71). Cosgrove et al. provided further support for the concept of vascular remodeling in IPF when they demonstrated a relative absence of vessels in the fibroblastic foci (72). Interestingly,
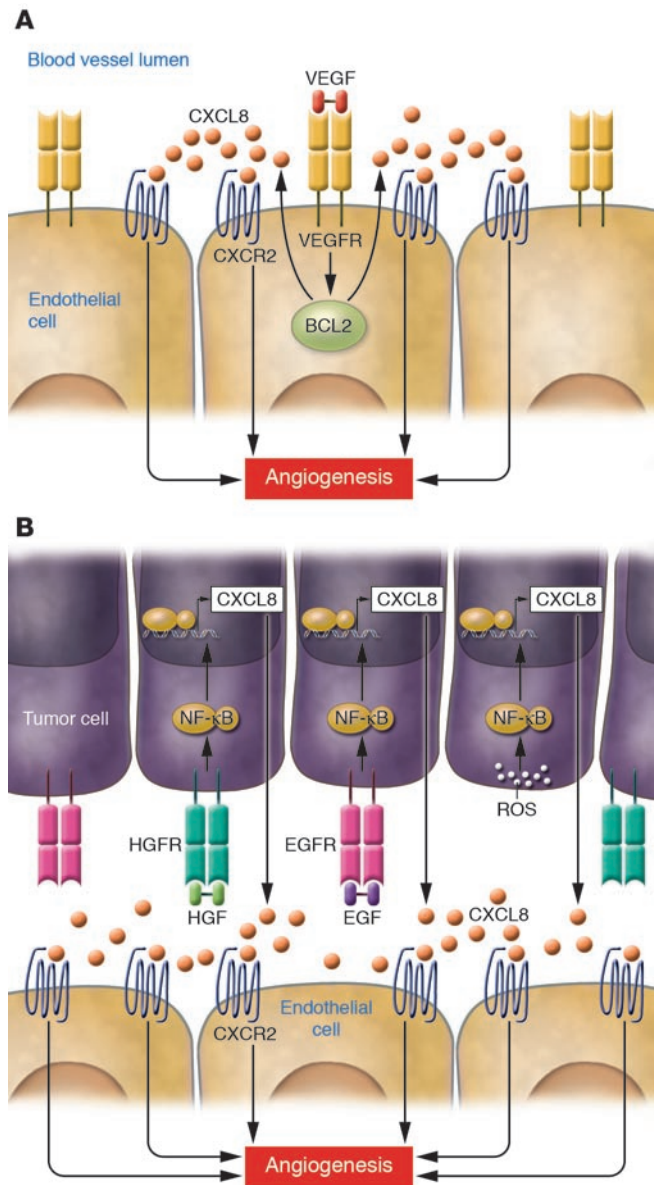
Figure 2
Serial mechanisms of angiogenesis promoted by CXCL8. CXCL8 is an ELR+ member of the CXC chemokine family. The ELR+ members of this chemokine family promote angiogenesis in a direct (not shown) or serial manner. (**A**) In a mouse model of Kaposi sarcoma, a serial mechanism of angiogenesis is the following: VEGF activation of its receptor (VEGFR) on endothelial cells leads to upregulation of the anti-apoptotic molecule BCL2. This in turn promotes the expression of endothelial cell–derived CXCL8, which functions in an autocrine and paracrine manner to promote angiogenesis (31). (**B**) Other serial pathways can also promote CXCL8-dependent angiogenesis, such as the signaling pathways induced by intracellular ROS, EGF, and HGF, which lead to nuclear translocation of NF-κB, expression of CXCL8 by tumor cells, and subsequent tumor-associated angiogenesis (32–35). EGFR, EGF receptor; HGFR, HGF receptor.

To determine whether the imbalance in the expression of these angiogenic and angiostatic CXC chemokines is relevant to the pathogenesis of pulmonary fibrosis, studies have been extended to the mouse bleomycin model. In this model, there is clear evidence of extensive vascular remodeling during the pathogenesis of pulmonary fibrosis (76). The amounts of CXCL2 and CXCL3 and of CXCL10 and CXCL11 were measured in the lung during bleomycin-induced pulmonary fibrosis and were found to be directly and inversely correlated, respectively, with measures of fibrosis (77, 78). Moreover, when endogenous CXCL2 and CXCL3 were depleted or when exogenous CXCL10 or CXCL11 was administered to the animals during exposure to bleomycin, a marked attenuation of pulmonary fibrosis was observed that was entirely attributable to a reduction in angiogenesis in the lung (60, 77, 78). Taken together, these findings support the notions that vascular remodeling is critical to promote the development of fibrosis and that angiogenic and angiostatic factors, such as CXC chemokines, have an important role in the pathogenesis of this process.

Fibrocytes, a circulating mesenchymal progenitor cell able to induce pulmonary fibrosis

Chronic lung injury is often associated with dysregulated tissue repair because the persistent or recurrent insults over time promote the loss of basement membrane integrity, which in turn leads to failure of normal tissue repair and the development of fibrosis, which is accompanied by loss of normal lung architecture. Recent studies in mouse models have added complexity to this paradigm of tissue injury and repair by indicating that circulating progenitor cells can extravasate and participate, with resident mesenchymal cells, in the repair process (52, 79, 80). The existence of circulating progenitor cells (CPCs) has changed the perspective of the scientific community about lung repair. These cells can behave as progenitor cells that extravasate into the lung and differentiate into different cellular lineages (42, 79, 81–83). CPCs are believed to reside primarily in the BM and can be mobilized to enter the circulation and subsequently to extravasate into a new tissue microniche (42, 52, 79, 80). In their new microniche, CPCs can respond to specific environmental cues, undergo differentiation into specific cellular lineages, integrate into the new microenvironment, and function in a tissue-specific manner (42, 52, 79, 80).

Currently, there are three ideas (one classical and two contemporary) about the origin of the fibroblasts and myofibroblasts in lung tissue that contribute to the pathogenesis of pulmonary fibrosis (42, 84–88). The classical concept is that tissue injury in

they also noted marked vascularity in the areas of fibrosis around the fibroblastic foci, with numerous abnormal vessels in the regions of severe architectural distortion. These findings are similar to those of Renzoni and support the concept of heterogeneity of vascularity in IPF (73). This heterogeneity is not surprising, as IPF is defined by its regional and temporal heterogeneity.

Further studies have found that the bronchoalveolar lavage fluid and lung tissue from patients with IPF have marked angiogenic activity that is almost entirely attributable to overexpression of the angiogenic ELR+ CXC chemokines CXCL5 and CXCL8 and the relative downregulation of the angiostatic ELR− CXC chemokines CXCL10 and CXCL11 (54, 60, 64, 74, 75). Furthermore, it seems that vascular remodeling in IPF is regulated differently than in either sarcoidosis or COP (54, 72). Both COP and sarcoidosis have a better prognosis than IPF, and studies aimed at understanding the differences in the regulation of vascular remodeling in these three diseases might lead to novel insights as to the pathogenesis of IPF.
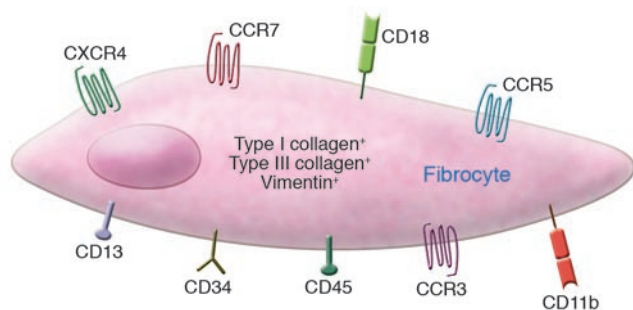
Figure 3
Markers associated with human fibrocytes. Human fibrocytes express ECM components (type I collagen, type III collagen, and vimentin). They also express a range of cell surface markers, including the common leukocyte antigen CD45, the hematopoietic stem cell antigen CD34, the adhesion molecules CD11b and CD18, the pan-myeloid antigen CD13, and the chemokine receptors CCR3, CCR5, CCR7, and CXCR4.

the lung induces the activation and differentiation of a population of resident interstitial fibroblasts into myofibroblasts that migrate into the intraalveolar space, proliferate, and express constituents of the ECM, leading to intraalveolar and interstitial pulmonary fibrosis (86–88). Another idea is that lung injury can induce epithelial cells to transition to a mesenchymal phenotype (that is, to gain the phenotype of fibroblasts and/or myofibroblasts) (84, 88). The third concept is that circulating fibrocytes, derived from BM progenitor cells, home and extravasate to sites of tissue injury and differentiate into myofibroblasts (42, 52, 80, 85).

Although a number of cell types have been implicated in tissue injury and repair, the fibroblast and myofibroblast have a pivotal role in the generation of the ECM. Bucala and associates discovered unique blood-borne fibroblast-like cells that expressed CD34, CD45, and type I collagen and named these cells *fibrocytes* (52). Despite expressing the common leukocyte antigen CD45, fibrocytes are morphologically distinct from leukocytes (52). Fibrocytes can be cultured from a population of CD14$^+$ cells isolated from the peripheral blood (80). Cultured fibrocytes are spindle shaped, express type I collagen, and neither express CD14 nor stain for nonspecific esterase (that is, they do not have the characteristics of monocytes and macrophages); they also lack expression of cell surface markers for epithelial and endothelial cells (42, 52) (Figure 3). Fibrocytes in the circulation and in culture express the fibroblast markers vimentin, collagen I, collagen III, and fibronectin, but they do not express CD3, CD4, CD8, CD16, CD19, CD25, or CD54 (52, 80, 85, 89). In addition, fibrocytes express the adhesion molecules CD11b and CD18, the common leukocyte antigen (CD45), the pan-myeloid antigen (CD13), HLA-DR, and the hematopoietic stem cell antigen (CD34) (42, 52, 79, 80, 85, 89) (Figure 3). Fibrocytes in culture spontaneously express α-SMA, and this expression increases in the presence of either TGF-β or endothelin, compatible with the differentiation of fibrocytes into myofibroblasts (42, 79, 80, 85, 89). This is associated with loss of expression of CD34 (42, 79, 89, 90) and CD45 (42), supporting the notion that with differentiation, these cells lose their stem and common leukocyte markers.

Fibrocytes have been found to be pleiotropic in their behavior and possess several functions that are relevant to fibrosis. They are potent APCs and can recruit and activate T cells that might play a role in the early injury that leads to the development of fibrosis (91). Fibrocytes can promote angiogenesis by producing various angiogenic factors (92). They also produce various cytokines that are potent inducers of collagen production (85, 93) and have been shown to play an important role in the development of fibrosis in animal models of pulmonary fibrosis (42, 82, 83). Interestingly, Rojas and coworkers demonstrated the importance of intact BM in the repair of injured lung, suggesting that there is a population of cells in the BM that are important in the attenuation of lung injury and fibrosis (94). Similarly Ortiz et al. have shown that BM-derived mesenchymal cells have the ability to develop an epithelial phenotype and attenuate bleomycin-induced lung injury (95). The specific conditions that stimulate the release and recruitment of reparative cells as opposed to fibrosis-promoting fibrocytes remain to be determined.

Chemokine receptors in fibrocyte trafficking to the lung. Classic cell trafficking has been well described for leukocytes, but it is an area of relatively new investigation for fibrocytes. The complicated, multi-step process of leukocyte trafficking from the BM into the tissues involves specific combinations of chemokine ligands and chemokine receptors to orchestrate these events (96). In the lung, different expression patterns of chemokine ligands occur at defined points after injury to mediate recruitment of cells including leukocytes and fibrocytes.

Human fibrocytes express the chemokine receptors CC chemokine receptor 3 (CCR3), CCR5, CCR7, and CXCR4 (42, 80, 89). By contrast, mouse fibrocytes express CCR2, CCR7, and CXCR4 (42, 80, 89, 97). Fibrocytes that express CXCR4 migrate in response to CXCL12 under specific conditions in vitro, and the CXCL12-CXCR4 axis has an important role in mediating fibrocyte extravasation into the lungs so that fibrocytes can contribute to the pathology of pulmonary fibrosis (Figure 4) (42). Fibrocytes also express CCR7, which is a chemokine receptor that is important for DC and T cell migration in response to the CC chemokines CCL19 and CCL21 (98). A population of fibrocytes that express CCR7 and that are distinct from the CXCR4-expressing fibrocytes has been identified in a mouse model of pulmonary fibrosis (42). However, intrapulmonary recruitment of CXCR4$^+$ fibrocytes is markedly greater than the intrapulmonary recruitment of CCR7$^+$ fibrocytes (42). Similarly, CCR2 was shown to play a role in the recruitment of fibrocytes in a model of FITC-induced lung injury, and this seemed to be mediated by CCL12 (82, 83). Interestingly, in a model of renal fibrosis, CCR7 seemed to have an important role in the recruitment of fibrocytes to the kidney (99). Therefore, at least in mice, CXCR4 and CCR2 seem to mediate recruitment of fibrocytes to the lung, whereas CCR7 might be important for the recruitment of fibrocytes to the kidney (42, 83). If indeed these cells can traffic to human lung, become activated, proliferate, and differentiate into myofibroblasts, then preventing their recruitment would impact the pathogenesis of pulmonary fibrosis.

Fibrocytes contribute to pulmonary fibrosis. Depletion of CXCL12 in the bleomycin-induced pulmonary fibrosis mouse model directly correlated with decreased deposition of ECM and decreased detection of cells expressing α-SMA in the lung (42). This suggests that fibrocytes directly contribute to the development of pulmonary fibrosis. In another study, Moore and colleagues examined the contribution of fibrocytes to fibrosis in a FITC-induced mouse model of pulmonary fibrosis (83). In this study, fibrocytes in the bronchoalveolar lavage fluid and lung tissue were analyzed. They found that populations of fibrocytes expressed CCR2, CCR5, CCR7, and CXCR4. The finding of high expression of CCR2 by mouse fibrocytes is in contrast to what is known about human
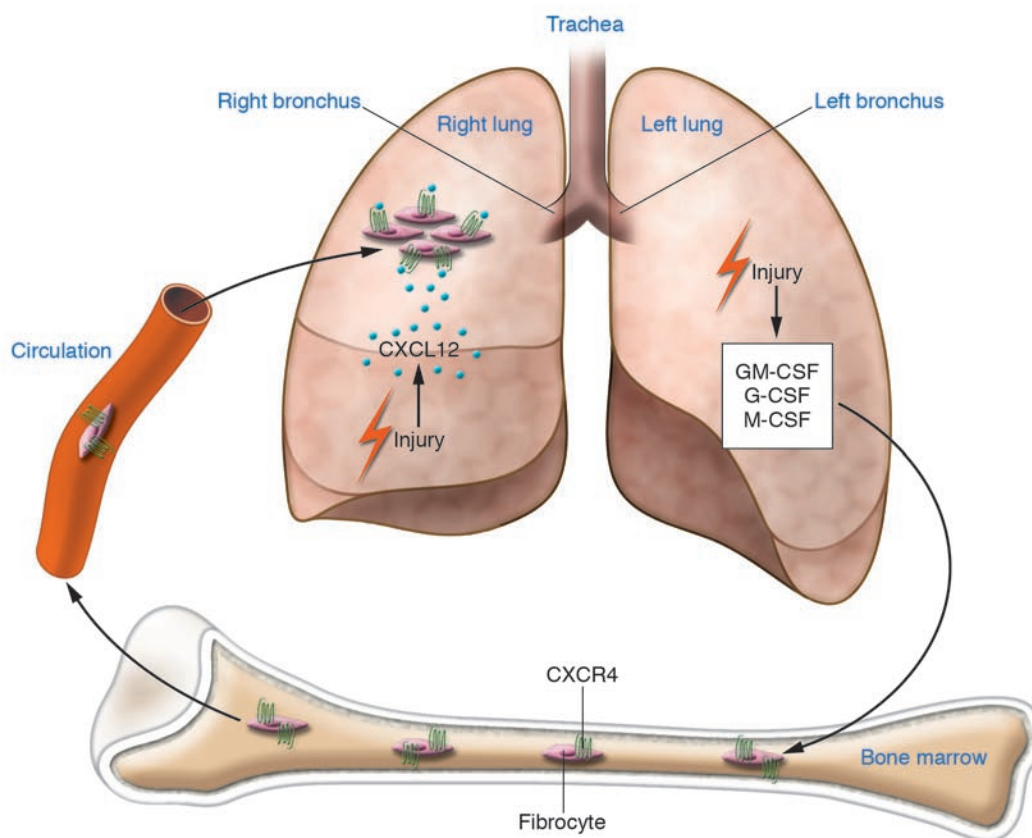
Figure 4
The role of the CXCL12-CXCR4 biological axis in fibrocyte extravasation in pulmonary fibrosis. Lung-derived factors (such as GM-CSF, G-CSF, and M-CSF) generated under conditions of lung injury communicate with the BM to expand the number of fibrocytes in the BM and to mobilize fibrocytes that express CXCR4 into the circulation. CXCR4-expressing fibrocytes traffic through the circulation and extravasate into the lung in response to the CXCR4 ligand CXCL12, which is produced during the pathogenesis of fibrosis.

fibrocytes, which express low levels of CCR2 after isolation (100). Fibrocytes isolated from mouse lungs expressed CCR2, migrated toward the CCR2 ligands, CCL2 and CCL12, and lost expression of CCR2 when cultured in vitro (83). Fibrocyte recruitment has also been shown to be reduced in $Ccr2^{-/-}$ mice exposed to intrapulmonary FITC (83). Recruitment of lung fibrocytes in $Ccr2^{-/-}$ mice was restored if the mice received BM from CCR2-sufficient mice. Conversely, if wild-type mice received a $Ccr2^{-/-}$ BM transplant, the mice were protected from FITC-induced fibrosis (83). Interestingly, the same authors did not find the same results with mice lacking the CCR2 ligand CCL2; they instead found that CCL12 was the most important CCR2 ligand for the recruitment of CCR2+ fibrocytes to the lung in this model of pulmonary fibrosis (82). However, CCL12 is likely to only be relevant to mouse biology, as no human homolog has been identified.

To further confirm that fibrocytes can differentiate into myofibroblasts in vivo, Mori and colleagues studied skin wound healing in chimeric mice in which only BM-derived cells expressed GFP. The GFP+ BM-derived fibrocytes in wounds coexpressed GFP and α-SMA, indicating that fibrocytes were derived from the BM (101). BM-derived progenitor myofibroblasts have also been found in pulmonary fibrosis after lung irradiation in mice (102). Hashimoto et al. also used a GFP chimeric model and found that,

following bleomycin administration, there were abundant GFP+ fibroblasts in the lung (103). Surprisingly, no GFP+ myofibroblasts were detected. Notwithstanding this study, most in vitro and in vivo studies of fibrocytes suggest that fibrocytes recruited from the peripheral circulation ultimately develop an α-SMA+ phenotype and contribute to the development of pulmonary fibrosis in the mouse. This is compatible with the in vitro findings for human fibrocytes, and therefore it is conceivable that fibrocytes contribute to the pathogenesis of pulmonary fibrosis in humans. Interestingly, it has recently been shown that both CXCR4 and CCR7 are expressed in human pulmonary fibrosis specimens (104, 105). Yang et al. demonstrated increased expression of CXCL12 and CXCR4 in both familial and sporadic pulmonary fibrosis as compared with normal specimens (105). By contrast, Choi et al. described increased expression of CCR7 but not CXCR4 in IPF specimens as compared with normal lung tissue adjacent to tumors (104). The difference in the normal specimens used as controls in these two studies (104, 105) might explain the differences in the findings. Therefore, although there is no direct evidence of a role for fibrocytes in the pathogenesis of human pulmonary fibrosis, the presence of the ligands and receptors that are necessary for the recruitment of fibrocytes is circumstantial evidence for their playing an important role.

Conclusions

Normal wound repair requires a coordinated sequence of events that includes angiogenesis and recruitment of fibrocytes, which regress when healing is complete. By contrast, the development of fibrosis is associated with aberrant repair, persistence of collagen deposition, and the development of vascular remodeling. The CXC chemokines are a unique cytokine family that has the potential to regulate both fibrocyte recruitment and vascular remodeling. CXC chemokines can exhibit either angiogenic or angiostatic biological activity, and the balance of their expression seems to be important in the regulation of vascular remodeling associated with chronic fibroproliferative disorders in the lung. Similarly, the ability of fibrocytes to differentiate along the mesenchymal lineage has created a novel paradigm related to their role in mediating pulmonary fibrosis. The CXCL12-CXCR4 biological axis and perhaps other chemokine–chemokine receptor interactions seem to be important for the trafficking and extravasation of fibrocytes into the lung during the pathogenesis of pulmonary fibrosis (Figure 4).

However, several questions remain to be fully answered, such as, What other signals are involved in the recruitment of fibrocytes? Are signals different between humans and mice? What factors and signaling pathways are involved in the differentiation of fibro-cytes into myofibroblasts? Does the microniche in the lung determine whether the fibrocyte differentiates into a myofibroblast or another mesenchymal lineage cell? Furthermore, it remains to be determined whether, similar to the mouse models, there are separate populations of BM-derived cells that are important for repair instead of the promotion of fibrosis. If indeed there are distinct populations of BM-derived mesenchymal cells, what factors are involved in the recruitment of these distinct populations? All of these issues are critical to our understanding of fibrosis and should be addressed in order to design therapeutic strategies to attenuate fibrocyte function and vascular remodeling, thereby preventing them contributing to fibrotic disorders of the lungs.

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