Chemokines and Pulmonary Fibrosis

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The pathogenesis of pulmonary fibrosis is presumably related to initial loss of alveolar type I epithelial cells and endothelial cells. However, the dysregulated repair of pulmonary fibrosis is followed by persistence of inflammation. This is followed by proliferation of type II cells, recruitment and proliferation of endothelial cells and fibroblasts, and deposition of extracellular matrix leading to end-stage alveolar and interstitial fibrosis. Although not all inflammatory disorders result in fibrosis, fibrotic responses are always preceded and potentially perpetuated by chronic inflammation. The maintenance of leukocyte recruitment during inflammation requires intercellular communication between infiltrating leukocytes and the endothelium, resident stromal and parenchymal cells. These events are mediated via the generation of early response cytokines, e.g., IL-1 and TNF, the expression of cell-surface adhesion molecules, and the production of chemotactic molecules, such as chemokines.

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The human CXC, CC, C, and CX3C chemokine families of chemotactic cytokines are four closely related polypeptide families that behave, in general, as potent chemotactic factors for neutrophils, eosinophils, basophils, monocytes, mast cells, dendritic cells, NK cells, T and B lymphocytes. The chemokines display highly conserved cysteine amino acid residues. The CXC chemokine family has the first two NH_2-terminal cysteines separated by one non-conserved amino acid residue, the CXC cysteine motif. The CC chemokine family has the first two NH_2-terminal cysteines in juxtaposition, the CC cysteine motif. The C chemokine has one lone NH_2-terminal cysteine amino acid, the C cysteine motif; and the CX3C chemokine has the first two NH_2-terminal cysteines separated by three non-conserved amino acid residues.

Chemokines have been found to be produced by an array of cells including monocytes, alveolar macrophages, neutrophils, platelets, eosinophils, mast cells, T- and B-lymphocytes, NK cells, keratinocytes, mesangial cells, epithelial cells, hepatocytes, fibroblasts, smooth muscle cells, mesothelial cells, and endothelial cells. The production of chemokines by both immune and non-immune cells supports the contention that these cytokines may play a pivotal role in orchestrating chronic inflammation. We will focus our discussion on the role of the CXC and CC chemokine families in the context of pulmonary fibrosis.

The Role of CXC Chemokines in Pulmonary Fibrosis

The CXC chemokines can be further divided into two groups on the basis of a structure/function domain consisting of the presence or absence of three amino acid residues (Glu-Leu-Arg; ‘ELR’ motif) that precedes the first cysteine amino acid residue in the primary structure of these cytokines (1-3). The ELR^+ CXC chemokines are chemoattractants for neutrophils and act as potent angiogenic factors (4). In contrast, the ELR^- CXC chemokines are chemoattractants for mononuclear leukocytes and are potent inhibitors of angiogenesis (4,5).

Idiopathic pulmonary fibrosis (IPF) is a disease of unknown etiology that is characterized by the accumulation of neutrophils and mononuclear cells, followed by the progressive deposition of collagen within the interstitium and subsequent destruction of lung tissue (6,7). While the mechanisms of cellular injury and the role of classic

inflammatory cells remains unclear, activated alveolar macrophages and neutrophils undoubtedly play a significant role in the pathogenesis of the inflammatory lung lesion of IPF (6-8). While the number or proportion of neutrophils in BALF does not correlate with activity of alveolitis and has limited prognostic value, declines in BALF neutrophils typically occur among patients exhibiting favorable responses to therapy (9). Neutrophilic alveolitis has been described in humans with IPF as well as diverse animal models of pulmonary fibrosis. Our laboratory and others have found that IL-8/CXCL8 is significantly elevated in IPF, as compared to either normal or sarcoidosis patients, and correlates with BALF presence of neutrophils (10). Several of these studies have identified the alveolar macrophage to be an important cellular source of IL-8/CXCL8 in IPF (10). In addition, these studies have suggested that levels of IL-8/CXCL8 in IPF may correlate with a worse prognosis (11).

While studies have suggested an importance for IL-8/CXCL8 in mediating neutrophil recruitment, CXC chemokines have been found to exert disparate effects in regulating angiogenesis (4). This latter issue is relevant to IPF, as the pathology of IPF demonstrates features of dysregulated and abnormal repair with exaggerated angiogenesis, fibroproliferation, and deposition of extracellular matrix, leading to progressive fibrosis and loss of lung function. The existence of neovascularization in IPF was originally identified by Turner-Warwick, who examined the lungs of patients with widespread interstitial fibrosis (IPF), and demonstrated neovascularization leading to anastomoses between the systemic and pulmonary microvasculature (12).

Our laboratory has demonstrated that in IPF lung tissue there is an imbalance in the presence of CXC chemokines that behave as either promoters of angiogenesis (IL-8/CXCL8) or inhibitors of angiogenesis (IP-10/CXCL10) (13). This imbalance favors augmented net angiogenic activity (13). Lung tissue from IPF patients have elevated levels of IL-8/CXCL8, as compared to control lung tissue, and demonstrate in vivo angiogenic activity that can be significantly attributed to IL-8/CXCL8 (13). Immunolocalization of IL-8 demonstrated that the pulmonary fibroblast was the predominant interstitial cellular source of this chemokine (13). In contrast to the increased angiogenic activity attributable to IL-8/CXCL8, we found a deficiency of the production of the angiostatic factor, IP-10/CXCL10, in IPF, as compared to controls (13).

Relative levels of IL-8/CXCL8 and IP-10/CXCL10 from IPF pulmonary fibroblast conditioned media demonstrated a significant imbalance favoring IL-8/CXCL8-induced angiogenic activity. In contrast, normal pulmonary fibroblasts had greater levels of IP-10/CXCL10 that favored a net inhibition of angiogenesis (13). The difference in expression of IL-8/CXCL8 and IP-10/CXCL10 between IPF and control pulmonary fibroblasts lends further support to the notion of a phenotypic difference between IPF and normal pulmonary fibroblasts which has been well described (14).

We have recently shown that ENA-78/CXCL5 is an additional important regulator of angiogenic activity in IPF (15). We found that lung tissue from patients with IPF expressed greater levels of ENA-78/CXCL5 as compared to normal control lung tissue. These higher levels of ENA-78/CXCL5 were associated with increased angiogenic that was significantly attributable to ENA-78/CXCL5. The predominant cellular sources of ENA-78/CXCL5 were hyperplastic type II cells and macrophages. This is in contrast to our previous findings that pulmonary fibroblasts were the predominant cellular source of IL-8/CXCL8 and suggests that the expression of chemokines with similar biological functions does not necessarily indicate redundancy (13).

To determine whether the imbalance in the expression of these CXC chemokines is relevant to the pathogenesis of pulmonary fibrosis, we used a murine model of bleomycin-induced pulmonary fibrosis (16,17). MIP-2/CXCL2 and IP-10/CXCL10 were temporally measured during bleomycin-induced pulmonary fibrosis from whole lung tissues, and were found to be directly and inversely correlated, respectively, with total lung hydroxyproline levels, a measure of lung collagen deposition (16,17). Moreover, depletion of MIP-2/CXCL2 or exogenous administration of IP-10/CXCL10 during bleomycin exposure, resulted in marked attenuation of pulmonary fibrosis that was attributable to a reduction in angiogenesis in the lung (16,17). These findings support the notion that angiogenesis is a critical biological event that supports fibroplasia and deposition of ECM in the lung during pulmonary fibrosis, and that angiogenic and angiostatic factors, such as CXC chemokines play an important role in the pathogenesis of this process. We have also shown that IL-12 attenuates bleomycin induced pulmonary fibrosis via induction IFN-γ (18). These findings provide further support for IFN-γ, and thereby the interferon inducible chemokines, IP-10/CXCL10 and MIG/CCL9, as inhibitors of fibrosis.

The Role of CC Chemokines in Pulmonary Fibrosis

The CC chemokines are chemoattractants for monocytes, T and B-lymphocytes, NK cells, dendritic cells, basophils, mast cells, and eosinophils (1-3). Animal models, such as bleomycin-induced pulmonary fibrosis have demonstrated the presence and contribution of CC chemokines to the pathogenesis of fibrosis. Time-dependent expression of MCP-1/CCL2 has been reported in response to bleomycin challenge in rodents (19,20). Passive immunization of mice with either neutralizing antibodies to murine MCP-1/CCL2 or MIP-1α/CCL3 resulted in a reduction of infiltrating cells and fibrosis (21,22). Depletion of MCP-1/CCL2 had the greatest effect on mononuclear cells, whereas neutralization of MIP-1α/CCL3 reduced B-lymphocyte, macrophage, and neutrophil infiltration (21,22). In addition to the ability of CC chemokines to modulate leukocyte recruitment in the lung during the pathogenesis of pulmonary fibrosis, MCP-1/CCL2 has been found to be an important cofactor for the stimulation of fibroblast collagen production and induction of the expression of TGF-β_1 (23). These findings support the notion that MCP-1/CCL2 stimulation of pulmonary fibroblasts

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is an important event leading to gene expression of endogenous TGF-β_1 and subsequent gene expression of type I procollagen.

Several studies have demonstrated the presence of CC chemokines in interstitial lung disease (ILD) (24-26). MIP-1α/CCL3 has been found in BALF of patients with IPF and sarcoidosis (25). In addition, these levels correlated with increased monocyte chemotactic activity in the BALF (25). The predominant cellular sources of MIP-1α/CCL3 within the lung of these patients, were both alveolar and interstitial macrophages and pulmonary fibroblasts (25). Minimal to no detectable MIP-1α/CCL3 was expressed in normal subjects. Furthermore, pulmonary fibroblasts isolated from patients with IPF produced greater amounts of MIP-1α/CCL3 after challenge with IL-1β, than did similarly treated pulmonary fibroblasts recovered from patients without fibrotic lung disease. Similar to the findings for MIP-1α/CCL3, MCP-1/CCL2 has been found to be significantly elevated in ILD (24). MCP-1/CCL2 mRNA and protein has been detected in pulmonary epithelial cells, mononuclear phagocytes, fibroblasts, and endothelial cells, and vascular smooth muscle cells (24, 26). Moreover, pulmonary fibroblasts from IPF patients demonstrate a reduced ability to down-modulate their MCP-1/CCL2 expression in the presence of either PGE_2 or the glucocorticoid, dexamethasone (26). These findings suggested that both MIP-1α/CCL3 and MCP-1/CCL2 are expressed in increased amounts within the airspace and interstitium of patients with ILD, and that these chemokines may be important mediators of mononuclear cell recruitment that characterize and perpetuate these diseases.

MCP-1/CCL2 has an important role in the modulation of CD4+ T cell activation during cell-cell interactions with lung fibroblasts and that these interactions may dictate the cytokine profile associated with a Th response (27). MCP-1/CCL2-deficient mice are unable to mount Th2 responses. Lymph node cells from immunized MCP-1/CCL2-/- mice synthesize extremely low levels of interleukin-4, interleukin-5 and interleukin-10, but normal amounts of interferon-gamma and interleukin-2 (28). Thus, MCP-1/CCL2 may have both a direct role in the pathogenesis pulmonary fibrosis through effects on monocytes, and an indirect role through control of T helper cell polarization. Similarly, the murine CC chemokine, C10/CCL6 is differentially regulated by Th1 and Th2 cytokines and promotes bleomycin induced pulmonary fibrosis (29). Bone marrow derived macrophages produce C10/CCL6 in response to IL-4, IL-10 and IL-13 in a dose dependent manner. In contrast, IFN-γ inhibits IL-3 and GM-CSF induced expression of C10/CCL6 (29).

We have shown that IL-13 and C10/CCL6 are elevated in the pathogenesis of bleomycin induced pulmonary fibrosis. Neutralization of IL-13, but not IL-4, attenuated bleomycin induced pulmonary fibrosis and levels of C10/CCL6 suggesting that IL-13 has an important role in the development of pulmonary fibrosis. IL-13 is a potent inducer of C10/CCL6, in vivo, and neutralization of C10 attenuated bleomycin induced pulmonary fibrosis and intrapulmonary macrophage numbers. This suggests that IL-13 has a role in the development of pulmonary fibrosis that is independent of its direct effect on fibroblasts and is evidence for an interaction between Th2 cytokines and specific CC chemokines. Furthermore, the Th2 cytokine, IL-13, has been shown to stimulate eotaxin/CCL11 production from airway epithelial cells (30). This is further evidence for the interaction of CC chemokines and Th2 cytokines and suggests that chemokines may have an important role in the switch towards a pro fibrotic Th2 type phenotype.

CCR1 has been shown to play an important role in the pathogenesis of bleomycin induced pulmonary fibrosis (31). Following the administration of bleomycin the expression of CCR1 mRNA peaked at 7 days. This paralleled the expression of RANTES/CCL5 and MIP-1α/CCL3, the major ligands for CCR1. Treatment with antibodies to CCR1 lead to a reduction in both inflammatory cell infiltrates and the development of fibrosis (31). Similarly CCR2 -/- mice are protected form both bleomycin and FITC induced pulmonary fibrosis (32). Similar effects have been seen in murine model of obliterative bronchiolitis where the fibrotic response associated with this disorder was attenuated in CCR2-/- mice (33). This suggests that targeting chemokine receptors may be an efficient way to inhibit pulmonary fibrosis.

Conclusion

While the role of inflammatory cells in the pathogenesis of pulmonary fibrosis has long been accepted, increasing evidence suggest that nonimmune cells such as the fibroblast and epithelial cell have important roles that may even Chemokines are important mediators of the recruitment and activation of these diverse cells. There is now also evidence for the interaction of chemokines in the polarization of Th1 and Th2 responses supporting the importance of chemokine networks in Th2 polarization and the pathogenesis of pulmonary fibrosis. Similarly the role of angiogenesis is becoming increasingly recognized in chronic inflammation with evidence of imbalances in the mediators of angiogenesis in a variety of chronic inflammatory disorders. Therapeutic interventions directed towards chemokines, their receptors, or alterations in cytokine phenotypic profiles may prove beneficial in the treatment of pulmonary fibrosis.

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