Review

The importance of balanced pro-inflammatory and anti-inflammatory mechanisms in diffuse lung disease

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Abstract

The lung responds to a variety of insults in a remarkably consistent fashion but with inconsistent outcomes that vary from complete resolution and return to normal to the destruction of normal architecture and progressive fibrosis. Increasing evidence indicates that diffuse lung disease results from an imbalance between the pro-inflammatory and anti-inflammatory mechanisms, with a persistent imbalance that favors pro-inflammatory mediators dictating the development of chronic diffuse lung disease. This review focuses on the mediators that influence this imbalance.

Keywords: chemokine, cytokine, fibrosis, inflammation, lung

Introduction

The lungs comprise a unique interface between the body and the environment, presenting an alveolar surface area of ~75 m^2 and only a minimal barrier of 4–8 μm between the alveolar airspace and the microvasculature. While this configuration is ideal for gas exchange, it also increases vulnerability to noxious stimuli and pathogens. Consequently, the pulmonary tissue must be able to generate a rapid innate host defense, characterized by acute inflammation, against both inhaled and hematogenous challenges and to promptly clear the offending agent while not compromising its essential gas exchange function. This acute pulmonary inflammatory response typically results in local increases in vascular permeability and a predominantly early neutrophilic influx followed by mononuclear cell infiltration. Once the noxious agent has been successfully contained, inflammation should then resolve with normal repair, tissue remodeling and a return to homeostasis. Because of its great capacity to initiate the acute inflammation of innate immunity, however, the lung may also be predisposed to tissue injury through excessive reactions generated by both local and distant mediators. In conditions such as interstitial lung disease (ILD), the over-exuberant tissue inflammation may result in severe irreversible lung injury mediated primarily by elicited and activated leukocytes. This review will focus on the balance of pro- and anti-inflammatory mechanisms in diffuse lung disease by describing the various cytokines involved in this balance.

Interleukin-1 family of cytokines

The interleukin-1 (IL-1) family of cytokines consists of two agonists, IL-1α and IL-1β, and one antagonist, IL-1 receptor antagonist (IL-1ra) [1]. The IL-1 agonists are protein isoforms encoded by two distinct genes [1]. IL-1α is predominantly membrane associated whereas IL-1β is secreted [1]. They are both produced by a variety of cells and both bind to the type I IL-1 receptor on target cells, eliciting similar biological functions [1]. Binding of IL-1 to

BALF = bronchoalveolar lavage fluid; CCL = CC ligand; CCR = CC receptor; CXCL = CXC ligand; IFN = interferon; IL = interleukin; IL-13R = IL-13 receptor; IL-1ra = interleukin-1 receptor antagonist; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; MCP = macrophage chemotactic protein; MIP = macrophage inflammatory protein; MMP = matrix metalloproteinase; PDGF = platelet-derived growth factor; TGF = transforming growth factor; TNF = tumor necrosis factor.

the type I IL-1 receptor ultimately leads to release of NF-κB for translocation to the nucleus and subsequent transactivation of several genes (that is, those encoding cyclooxygenase, adhesion molecules, cytokines, NO synthase, acute phase proteins, and other cytokines and chemokines) [2,3]. As IL-1 receptors are present on essentially all immune and non-immune system cells, IL-1 can bind and engage and elicit responses from most cells involved in the inflammatory response.

IL-1 also binds to an IL-1 type II or 'decoy' receptor that does not signal [1]. This may be a mechanism to sequester IL-1 and stop it interacting with the IL-1 type I receptor [1]. The IL-1 type II receptor is cleaved by metalloproteases on the cell surface, which releases a soluble form of the IL-1 type II receptor that can bind IL-1β and inhibit IL-1 signaling.

The role of the IL-1 family of cytokines in the regulation of fibrosis in ILD is interesting, as these cytokines may have dual and opposing functions. Both IL-1α and IL-1β induce the expression of pro-collagen type I and type III from fibroblasts and type IV collagen from epithelial cells, and stimulate the production of glycosaminoglycans and fibronectin by fibroblasts. In addition, these cytokines can behave as indirect mitogens for fibroblast proliferation via the expression of platelet-derived growth factor (PDGF) and the PDGF alpha-receptor on fibroblasts. Furthermore, IL-1α and IL-1β can serve as proximal mediators of chronic inflammation that also promote fibrosis by inducing fibroblasts to produce a variety of cytokines, including additional IL-1, IL-6 and both CXC and CC chemokines. In contrast, both IL-1 agonists mediate the production of tissue collagenase (matrix metalloproteinase (MMP)-1), gelatinase, prostaglandin E_2 and plasminogen activator. Moreover, both can inhibit fibroblast proliferation via the production of prostaglandin E_2. It has been demonstrated recently that transient expression of IL-1β using an adenoviral vector can lead to progressive fibrosis long after the IL-1β levels have declined and the acute inflammatory response has ended [4]. In this study, an early increase in the levels of the pro-inflammatory cytokines IL-6 and tumor necrosis factor (TNF) and the profibrotic cytokine PDGF, and a sustained increase in levels of transforming growth factor (TGF)-β1 [4], suggest that IL-1β has an important role in the initial injury that leads to a self-perpetuating fibrotic response [4].

IL-1ra is a naturally occurring inhibitor of IL-1 binding to its receptor. Thus, a dynamic balance between IL-1 agonists and IL-1ra influences IL-1-dependent inflammation. A variety of immune and non-immune system cells express IL-1ra [1], which acts as a competitive antagonist of IL-1α and IL-1β, attenuating IL-1 activity *in vitro* and *in vivo* [1]. Intracellular isoforms of IL-1ra, released after cellular injury or apoptosis, inhibit IL-1 signaling and may play a role in the resolution of inflammatory responses after injury.

The exogenous administration of IL-1ra can inhibit bleomycin-induced or silica-induced pulmonary fibrosis in mice and infusion of IL-1ra significantly attenuated lung fibrosis [5]. No significant change was noted in the cellularity of the bronchoalveolar lavage fluid (BALF) of the IL-1ra treated animals [5]. The specific mechanism for the decline in collagen deposition, either decreased collagen production, increased collagen degradation or both, was not examined in this study. Moreover, these experiments did not determine the amount of endogenous IL-1ra nor whether depleting endogenous IL-1ra would affect the bleomycin-induced or silica-induced pulmonary fibrosis. This last issue is important since IL-1ra has been found to be significantly elevated in the BALF and interstitium of patients with idiopathic pulmonary fibrosis (IPF) and sarcoidosis [6].

Studies have demonstrated that patients with either sarcoidosis or IPF have elevated levels of IL-1ra protein in cell-free BALF that are 10-fold higher than levels of IL-1β [6,7]; this is inhibitory for IL-1β-dependent biology [1]. IL-1ra was significantly increased in IPF lung tissue compared with normal control tissue [7]. In contrast, interstitial levels of IL-1β were significantly depressed in IPF patients compared with normal controls [7]. Overall, the ratios of IL-1ra to IL-1β were 19 : 1 in IPF patients compared to 1.3 : 1 in normal controls [7]. In a parallel fashion to IL-1ra, TGF-β levels were 10-fold higher in IPF lung than in normal lung, whereas no change was seen in the TNF levels [7]. These disparate findings suggest that IL-1 biology is complex in ILD and that the inhibition of IL-1β may be important in the remodeling phase of diffuse lung disease related to pulmonary fibrosis.

Tumor necrosis factor-α

TNF is primarily a mononuclear phagocyte-derived cytokine that has pleiotropic effects on the inflammatory response. It is a homotrimer that binds to two different cell surface receptors, p55 and p75. These belong to a unique family of receptors, the members of which are transmembrane proteins with an extracellular cysteine-rich domain and a more variable intracellular domain [8]. The p55 receptor and the Fas receptor contain a 60 amino acid domain, known as the 'death domain', that is essential for the transduction of apoptotic signals [8]. It has been suggested recently that ligation of the Fas receptor and the induction of apoptosis may have an important role in the induction of pulmonary fibrosis [9]. Like IL-1 and lipopolysaccharide (LPS), TNF-α signaling occurs through the activation of NF-κB, which in turn enhances the transcription of genes that mediate innate immune responses [10].

TNF has been found to be significantly elevated in bleomycin-induced pulmonary fibrosis and neutralization of TNF results in an attenuation of the cellularity of the

parenchyma of the lung, reduces alveolar septal thickening and decreases the disruption of the alveolar architecture, all accompanied by a reduction in fibrosis [11]. These results are supported by a study in which bleomycin-treated animals received a recombinant soluble TNF receptor at the time of bleomycin instillation [12]. The inhibition of the biological effect of TNF using this strategy resulted in a marked reduction of early as well as established pulmonary fibrosis at both 15 and 25 days post-bleomycin [12]. That TNF has a role in mediating pulmonary fibrosis has been further substantiated using a transient transfer of the gene encoding TNF-α into a rat lung [13]. The overexpression of TNF in this lung induced severe pulmonary inflammation and patchy interstitial fibrosis with induction of TGF-β and increased numbers of myofibroblasts [13]. These studies support the notion that the prolonged expression of TNF may be important in the pathogenesis of fibrosing alveolitis, and attenuation of this cytokine may be useful in improving the lung function of IPF patients.

The Th1/Th2 paradigm and pulmonary fibrosis

The type 1 (Th1; including IFN-γ and IL-2) and type 2 (Th2; including IL-4, IL-5, and IL-10) cytokine patterns of immune response in mice were originally identified using a panel of T helper cell clones. The realization that Th1 and Th2 cytokines are expressed by a variety of cells and that the functions of these cytokines are different suggests that imbalances in the expression of Th1 and Th2 cytokines may be important in dictating different immunopathological responses [14]. For example, Th1 cytokines appear to be involved in cell-mediated immunity associated with autoimmune disorders and allograft rejection, whereas Th2 cytokines are predominately involved in mediating allergic inflammation and chronic fibroproliferative disorders, such as asthma, atopic dermatitis, IPF and systemic sclerosis [14]. Thus, it is more appropriate to define certain diseases in terms of the predominant cytokine profile rather than the predominant T helper cell subset. The strict definition of Th1 and Th2 responses may break down in a scenario where the initial inciting agent triggers an unsuccessful Th1 type response. The subsequent host reaction to the antigen or the chronicity of the disorder may then induce a switch to a response dominated by Th2 cytokines. A manifestation of this latter response is stromal cell/fibroblast proliferation and deposition of extracellular matrix, and ultimately fibrosis. Thus, the cytokine patterns in particular diseases are often predictable and appropriate, whereas severe pathological consequences may result if an inappropriate cytokine phenotype is expressed. This latter situation may play a role in certain chronic inflammatory diseases, such as ILD, where unknown etiologies lead to dysregulated repair with exaggerated chronic inflammation, fibroblast proliferation, deposition of extracellular matrix, angiogenesis, and finally fibrosis that accompanies end-stage pulmonary fibrosis.

Evidence suggests that a cytokine profile of the natural immune/inflammatory response determines the disease phenotype responsible for either resolution or progression to end-stage fibrosis [15,16]. Supporting evidence is derived from studies demonstrating that interferons (IFNs), especially IFN-γ, have profound suppressive effects on the production of extracellular matrix proteins, such as collagen and fibronectin [15,16]. IFN-γ can inhibit both fibroblast and chondrocyte collagen production *in vitro*, as well as decrease the expression of steady state type I and III procollagen mRNA. IFN-γ reduces PDGF-induced lung fibroblast growth but stimulates PDGF production by alveolar macrophages [17]. It also up-regulates stromelysin-1 (the major matrix-degrading metalloproteinase) gene expression by fibroblasts [18]. IFN-γ differentially regulates intercellular adhesion molecule-1 and vascular adhesion molecule-1 expression on fibroblasts [19]. The administration of IFN-γ *in vivo* can cause a reduction of extracellular matrix in animal models of fibrosis [16]. Furthermore, we have recently shown that IL-12 attenuates bleomycin-induced pulmonary fibrosis via the induction of IFN-γ [20]. Moreover, IFN-γ treatment of patients with either systemic sclerosis or IPF for one year resulted in improved pulmonary function [21,22]. This information supports the concept that IFN-γ is one of the major Th1 cytokines possessing profound regulatory activity for collagen deposition during chronic inflammation.

The opposing effects of Th1 and Th2 cytokines in fibrosis are supported by a number of recent investigations demonstrating that IL-4 is an important mediator of fibroblast activation [23]. In contrast to the Th1 cytokine IFN-γ, IL-4 is a major Th2 type cytokine that promotes the production of fibroblast-derived extracellular matrix, including type I and III pro-collagens and fibronectin [23]. It has been identified as a chemotactic factor for fibroblasts and can induce fibroblast proliferation and cytokine production. The scale of IL-4-induced fibroblast collagen synthesis is similar to that induced by equal amounts of TGF-β. Additional studies have demonstrated that fibroblasts possess both membrane bound and soluble forms of the IL-4 receptor. The soluble IL-4 receptor is derived from a truncation of the membrane form and may serve either as an IL-4 binding protein with antagonist activity or as a carrier of IL-4 with its biological properties intact. Interestingly, pulmonary expression of IL-4 in transgenic mice leads to little or no fibrosis, suggesting a disparity between its effect *in vitro* and *in vivo* [24]. Similarly, IL-4 depletion studies and studies with IL-4$^{-/-}$ mice failed to demonstrate an indispensable role for IL-4 in models of Th2-mediated inflammation and fibrosis [25].

Recent work has shown that fibroblast cell lines express IL-4Rα, IL-13Rα1 and IL-13Rα2, but not the IL-2 common γ chain necessary for specific IL-4 signaling. This suggests that many of the fibroblast activation properties of

IL-4 are shared by IL-13. As a Th2 cytokine, IL-13 has similar biological properties to IL-4, and has been implicated in the pathogenesis of fibroproliferative disorders [26]. IL-13 induces the expression of fibroblast-derived type I and III pro-collagens to a similar degree as IL-4 and TGF-β [26]. It inhibits IL-1-induced matrix metalloproteinase (MMP)-1 and MMP-3 production, and enhances tissue inhibitor of metalloproteinase-1 generation in fibroblasts [26]. Recently, IL-13 was selectively expressed in the lungs using a Clara cell promoter [27]. The phenotype of the transgenic mice expressing IL-13 demonstrated airway epithelial cell hypertrophy, mucus cell metaplasia, the hyper-production of neutral and acidic mucus, and subepithelial airway fibrosis [27]. These data demonstrate both the pro-fibrotic effects of IL-13 on collagen homeostasis and the potential differential regulation of collagen homeostasis in fibroblast subtypes by IL-13.

The similarities and overlapping functions of IL-4 and IL-13 have led to an interest in the IL-13 receptor (IL-13R) complex and its relationship to the IL-4 receptor (IL-4R) complex. The IL-4R complex is composed of at least two subunits, IL-4Rα and the IL-2Rγ (common γ chain). The IL-13 receptor complex is composed of various combinations of IL-4Rα, IL-13Rα1, IL-13Rα2 and IL-2Rγ. IL-4Rα appears to be an essential component of both the IL-4 and IL-13 receptors, although it can not by itself bind IL-13. Studies of chronic Leishmaniasis using both IL-4$^{-/-}$ mice and IL-4Rα$^{-/-}$ mice demonstrated that the IL-4$^{-/-}$ mice were able to clear infection but the IL-4Rα$^{-/-}$ mice developed progressive infection and died [28]. This indicates an important and independent role for IL-13 receptor signaling in this model. Similarly, in a schistosomiasis model of hepatic fibrosis, reduction of hepatic fibrosis by inhibition of IL-13, using soluble IL-13Rα2-Fc, was greater than that in IL-4$^{-/-}$ mice.

Animal models of pulmonary fibrosis have provided insight into the role of Th2 cytokines in the mediation of pulmonary fibrosis, and recent studies have confirmed that they are also involved in IPF. Lung tissue of patients with IPF was examined to determine whether a Th1 or a Th2 pattern of cytokine expression was present [29]. Although both Th1 (characterized by the expression of IFN-γ) and Th2 (characterized by the expression of IL-4 and IL-5) cytokines are expressed in IPF lung tissue, the expression of Th2 cytokines predominated over that of IFN-γ [29]. This pattern of cytokine expression may be related to the potential role of the humoral response in the pathogenesis of IPF, or be related to the inability of IFN-γ to tilt the balance away from an IL-4/IL-13-dependent pro-fibrotic environment. The finding that IFN-γ levels are inversely related to the levels of type III pro-collagen in the BALF of IPF patients further supports the idea of an imbalance between relatively high levels of Th2 cytokines and low levels of IFN-γ [30]. The levels of IFN-γ correlated especially with patients that demonstrated progression of their pulmonary fibrosis through further deterioration of their pulmonary function [30]. These findings may be further supported by the recent suggestion that treatment with IFN-γ may be beneficial for IPF patients who fail to respond to glucocorticoids [22]. These findings suggest that the persistent imbalance in the expression of Th1 and Th2 cytokines in the lung may be a mechanism by which diffuse pulmonary fibrosis progresses.

IL-10 is a Th2 cytokine that inhibits a variety of innate and adaptive immune activities. It inhibits a number of pro-inflammatory cytokines, including IFN-γ, IL-1, TNF, IL-12, and CXC and CC chemokines. While the exogenous administration of IL-10 may protect the lung from injury in response to either LPS or immune-complex deposition [31], IL-10 can be detrimental to the host under conditions of microorganism invasion [32]. The role of IL-10 in pulmonary fibrosis is controversial. Interestingly, patients with chronic hepatitis C who failed to respond to IFN have shown improved histology and decreased liver fibrosis when treated with IL-10 [33]. Paradoxically, patients that are genetically predisposed to high levels of IL-10 production, determined by genetic analysis of the IL-10 locus, have a poor response to IFN-α [34]. Exogenous administration of IL-10 using a liposomal vector significantly inhibited bleomycin-induced pulmonary fibrosis in a murine model [35]. IL-10 downregulates quartz-induced pulmonary inflammation and cell activation in a rat model [36]. In contrast, silica induces fibrosis in the NMRI strain of mice that display decreased TNF activity and protracted overproduction of IL-10 [37]. Similarly, IL-10$^{-/-}$ mice that have been exposed to silica show increased inflammation but decreased fibrosis, suggesting that IL-10 has both anti-inflammatory and pro-fibrotic activities [38]. With the mounting evidence that IPF involves a Th2-type profile, the possibility of IL-10 exacerbating the disease cannot be excluded.

Interestingly IL-9, a Th2 cytokine that has been implicated in the pathogenesis of asthma, has been shown to attenuate silica-induced pulmonary fibrosis in a murine model [39]. This was demonstrated in both transgenic mice that systemically over-expressed IL-9 and wild-type mice that received systemic IL-9 [39]. Of particular interest is the fact that the over-expression of IL-9 was paradoxically associated with a reduced shift towards a Th2 response [39]. One possible explanation for this may be that the artificial over-expression of IL-9 invokes a different Th2 response than the normal expression of IL-9 [40].

Chemokines

Human CXC, CC, C and CX_3C chemokines are four closely related polypeptide families of potent chemotactic factors for neutrophils, eosinophils, basophils, monocytes, mast cells, dendritic cells, NK cells and T and B lympho-

cytes. CXC chemokines can be further divided into two groups according to whether or not their structure/function domain contains the three amino acid sequence Glu-Leu-Arg (the ELR motif), which, when present, precedes the first cysteine residue in the primary structure. The ELR^+ CXC chemokines are chemoattractants for neutrophils and act as potent angiogenic factors. In contrast, the ELR^- CXC chemokines are chemoattractants for mononuclear leukocytes but are potent inhibitors of angiogenesis. There is ~20–40% homology between the members of the four chemokine families. 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. These cells can produce chemokines in response to a variety of factors that trigger the innate immune response, including pattern recognition, ligands, IL-1, TNF, C5a, leukotriene B4 and IFNs.

The signals responsible for the recruitment of neutrophils to the lung, and the perpetuation of the neutrophilic alveolitis of IPF, have not been fully characterized. Levels of the CXC chemokine IL-8/CXCL8 are significantly elevated in patients with IPF compared to either normal or patients with sarcoidosis, which correlates with the presence of neutrophils in the BALF [41,42]. In addition, these studies have suggested that the levels of IL-8/CXCL8 in IPF may correlate with poor prognosis [42]. These findings suggest that factors that modulate the activity of IL-8/CXCL8 could potentially influence the balance of inflammatory and anti-inflammatory mechanisms during the pathogenesis of ILD.

Several studies have demonstrated the presence of CC chemokines in human ILD [43]. Elevated levels of macrophage inflammatory protein (MIP)-1α/CCL3 have been associated with both sarcoidosis and IPF [43]. Furthermore, pulmonary fibroblasts isolated from patients with IPF produced greater amounts of MIP-1α/CCL3 after challenge with IL-1β than similarly treated pulmonary fibroblasts recovered from patients without fibrotic lung disease. Similar to the findings for MIP-1α/CCL3, macrophage chemotactic protein (MCP)-1/CCL2 has been found to be significantly elevated in ILD [43]. MCP-1/CCL2 has been detected in pulmonary epithelial cells, mononuclear phagocytes, fibroblasts, endothelial cells and vascular smooth muscle cells [43]. In addition, in the presence of either TNF or IL-1β, MCP-1/CCL2 production from isolated pulmonary fibroblasts from patients with IPF is greater than in the same cells from normal controls [43], and pulmonary fibroblasts from patients with IPF demonstrate a reduced ability to downregulate their MCP-1/CCL2 expression in the presence of either prostaglandin E_2 or glucocorticoids [43]. These findings suggest 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 pulmonary fibrosis.

Furthermore, it has been shown that MCP-1/CCL2 can stimulate IL-4 production and its over-expression is associated with defects in cell-mediated immunity, indicating that it might be involved in Th2 polarization [44]. 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 IL-4, IL-5 and IL-10, but normal amounts of IFN-γ and IL-2 [44]. Thus, MCP-1/CCL2 may have both a direct role in the pathogenesis of pulmonary fibrosis through its effects on monocytes, and an indirect role through the control of T helper cell polarization and the profile of cytokine production. Similarly, the murine CC chemokine C10/CCL6 is differentially regulated by Th1 and Th2 cytokines [45]. Bone marrow derived macrophages produce C10/CCL6 in response to IL-4, IL-10 and IL-13 in a dose-dependent manner [45]. In contrast, IFN-γ inhibits IL-3 and granulocyte-macrophage colony-stimulating factor induced expression of C10/CCL6 [45]. This is further evidence for the interaction between CC chemokines and Th2 cytokines and suggests that chemokines may have an important role in the switch towards a pro-fibrotic Th2-type phenotype.

The importance of receptor polymorphisms in various disease states has been demonstrated recently. CCR5 is the major receptor for MIP-1α/CCL3, MIP-1β/CCL4 and RANTES/CCL5. Homozygosity for the CCR5Δ32 mutation has been shown to predict for prolonged renal allograft survival (90% at 20 years), reduced risk of asthma and decreased severity of rheumatoid arthritis [46]. In contrast, there was an increased frequency of the CCR5Δ32 allele in patients with sarcoidosis that was associated with more apparent disease and an increased need for corticosteroids [47]. This suggests that CCR5Δ32 is associated with altered susceptibilty to immmunologically mediated diseases and that the balance between chemokines and their appropriately expressed receptors is necessary for the full manifestation of various diseases.

CXC chemokines in the regulation of angiogenesis and promotion of pulmonary fibrosis

Angiogenesis is a fundamental component of inflammation and wound repair. It has only recently become apparent, however, that dysregulated angiogenesis may be important in the support of fibroplasia and deposition of extracellular matrix that occurs during chronic fibroproliferative disorders such as IPF. The existence of neovascularization

in IPF was originally identified by Turner-Warwick [48] in the lungs of patients with widespread interstitial fibrosis. This idea has been further substantiated with evidence of extensive neovascularization during the pathogenesis of pulmonary fibrosis in a rat model of bleomycin-induced pulmonary fibrosis [49].

Angiogenesis in pulmonary fibrosis is related to the overexpression of ELR$^+$ CXC chemokines relative to the decreased expression of angiostatic (ELR$^-$) interferon-inducible CXC chemokines. Lung tissue from patients with IPF has markedly increased angiogenic activity that is almost entirely attributable to the imbalance caused by the overexpression of the angiogenic ELR$^+$ CXC chemokine IL-8/CXCL8 and the relative downregulation of the angiostatic interferon-inducible CXC chemokine IP-10/CXCL10 [50]. To determine whether the imbalance in the expression of these CXC chemokines is relevant to the pathogenesis of pulmonary fibrosis, the expression and biological activity of murine MIP-2/CXCL2 (an angiogenic ELR$^+$ CXC chemokine) and the angiostatic CXC chemokine IP-10/CXCL10 were correlated with the extent of fibrosis during bleomycin-induced pulmonary fibrosis in a murine model system [51,52]. Increased MIP-2/CXCL2 activity correlated with increased pulmonary fibrosis whereas increased IP-10/CXCL10 activity correlated with attenuated pulmonary fibrosis [51,52]. Moreover, both the depletion of endogenous MIP-2/CXCL2 by passive immunization with neutralizing antibodies and the administration of exogenous IP-10/CXCL10 to the animals during bleomycin exposure resulted in a marked attenuation of pulmonary fibrosis that was entirely attributable to a reduction in angiogenesis in the lung [51,52]. These findings support the notion that angiogenesis is a critical biological event that supports fibroplasia and deposition of extracellular matrix in the lung during pulmonary fibrosis. Furthermore, with the recent demonstration of the efficacy of IFN-γ in the treatment of patients with IPF [22], these studies support the idea that IFN-γ may mediate its effect, in part, by shifting the imbalance in the expression of ELR$^+$ and ELR$^-$ CXC chemokines to favor an angiostatic environment, which would lead to inhibition of dysregulated neovascularization/vascular remodeling, fibroproliferation and deposition of extracellular matrix in IPF patients.

Conclusions

Inflammation in the lung involves a delicate balance between pro-inflammatory and anti-inflammatory mediators, with the balance of cytokines dictating the ultimate response to initial injury. The presence of natural inhibitors of IL-1 and TNF has long been recognized. Increasing evidence suggests that an imbalance in Th1 and Th2 cytokine profiles is important in diffuse lung disease and the fibrotic response. There is now also evidence that chemokines are involved in this polarization, supporting the importance of cytokine networks in the pathogenesis of diffuse lung disease. 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 alterations in cytokine phenotypic profiles may prove beneficial in the treatment of diffuse lung disease.

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